Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods.

Additional MRI sequence parameters

In vivo 7T MRI

On the Siemens 7T MRI scanner (equipped with a birdcage-type transmit coil and a 32-channel receive coil), Magnetization-Prepared 2 RApid Gradient Echoes (MP2RAGE) sequence was acquired in 23 individuals participating to the longitudinal lesion evolution cohort.

- 7T 3D MP2RAGE had the following sequence parameters: TR= 6000 ms; TE= 3.02 ms; FA=0°; echo-train length=1; AT= 8 min; 223 sagittal slices; 0.7 mm isotropic voxels with voxel size 0.34 µL.

Lesions T1 times. At the time of each scan, T1 maps were directly reconstructed on the scanner from the MP2RAGE images. Lesion segmentation on T1 maps was performed using a semi-automatic threshold approach (Jim 7.0 software, Xinapse Systems) and included the lesion core and the edge for lesions both with and without rims.

Postmortem 7-tesla MRI

Postmortem MRI was performed on the same 7T MRI scanner with the following sequences:

- 3D T1-weighted magnetization-prepared rapid gradient echo (T1-MPRAGE) with repetition time (TR) = 2200 ms; echo time (TE) = 3.04 ms; inversion time (TI) = 1050 ms; flip angle (FA) = 7°; nominal resolution = 0.6x0.6x0.6 mm; and 176 coronal slices. The acquisition time for the sequence was 6 min 35 sec.
- 3D high-resolution multigradient-echo (GRE) T2* sequence providing both T2*-weighted magnitude contrast and susceptibility-weighted phase contrast: TR = 60 ms; TE = 6.09, 15.99, 25.89, 35.79 ms; 4 averages; 88 slices; FA = 10°; acquisition time = 2 hours 15 min per 36 mm slab; nominal resolution = 0.42x0.42x0.42 mm. Four coronal slabs with 20% overlapping slices were acquired to cover the whole brain.

Images acquired in multiple slabs covering the whole brain were stitched together using the embedded information in the image files and post-processing algorithms developed using the AFNI software package.

Neuropathology

Diaminobenzidine (DAB)-enhanced Turnbull staining protocol (iron). Sections were immersed in 2% ammonium sulfide solution for 90 min, then washed with dH2O. Sections were incubated with 10% potassium ferrocyanide solution and 0.5% hydrochloric acid solution at temperature 37C (oven) for 15 minutes. After washing in dH2O five times x3 minutes, sections were incubated in 0.3% H2O2 in methanol for blocking endogenous peroxidase. After washing in PBS, iron staining was amplified by DAB solution (Abcam, #ab94665). After washing, sections were counterstained with 10% hematoxylin.

Immunostaining. After deparaffinization, sections were processed for antigen retrieval and protein blocking solution for 20 minutes. Sections were then incubated with the primary antibodies overnight at 4C or 1 hour room temperature (RT), then rinsed and incubated with secondary antibodies for 30 minutes. Either the immunoperoxidase (DAB) or the alkaline phosphatase (AP) methods was used. After washing, sections were counterstained with 10% hematoxylin.

Antigen	Target	Primary antibody type	Antigen retrieval	Dilution	Incubation	Source (catalogue #)
Myelin/PLP	Myelin	Mouse monoclonal	Steamer 20 min	1:500	1 hour RT	BioRad (MCA839G)
CD68-PGM1	Macrophages/ activated microglia	Mouse IgG3 monoclonal	Steamer 20 min	1:100	1 hour RT	Dako (M087629-2)
TMEM119	Microglia	Rabbit polyclonal	Steamer 20 min	1:100	Overnight 4C	MilliporeSigma (HPA051870)
MRP-14	Early activated macrophages	Mouse monoclonal	Steamer 20 min	1:100	Overnight 4C	Dako (MAC387)
IBA-1	Macrophages/ microglia	Rabbit polyclonal	Steamer 20 min	1:200	1 hour RT	Wako (019-19741)
GFAP	Astrocytes	Rabbit polyclonal	Proteinase K	1:100	1 hour RT	Dako (Z033401-2)
CD8	CD8 T lymphocytes	Mouse monoclonal	Steamer 45 min	1:100	Overnight 4C	Dako (M710301-2)
Fibrinogen	Blood-brain barrier opening	Mouse monoclonal	Proteinase K	1:100	Overnight 4C	Abcam (ab58207)
SMI32	Axonal non-phosphorylated neurofilaments H	Mouse monoclonal	Steamer 45 min	1:100	1 hour RT	Biolegend (801701)

Statistical analysis
Clinical cohort

For the clinical cohort analysis, individuals were classified into three groups according to the number and distribution of chronic lesions with paramagnetic rims. To examine the association of the demographic and clinical variables with rim lesions (categorized to three groups: 0, 1-3, >=4), different test methods were used according to the variable type. Statistical comparisons were made using Mann-Whitney U-test, ANOVA with correction for multiple comparisons, Fisher's exact tests, and Spearman correlations as appropriate. ANOVA was applied to quantitative (continuous or discrete) variables with normal distribution. For the variables with abnormal distribution (EDSS, lesion volume, thalamus and putamen volumes, number of chronic rims, rim lesion volume, PASAT), ANOVA was applied to the natural logarithm variables (based on Box-cox procedure). Multiple comparisons with Tukey's method were followed if p-value for F-test was less than 0.05. Shapiro-Wilk test was applied to residuals for checking normality assumption. For the categorical variables, Fisher's exact test was used and followed by multiple comparisons with Bonferroni method if the p-value is less than 0.05. Forward stepwise

regression was performed to look for a set of independent variables (predictors) which could predict the disability outcome (EDSS). In the procedure, natural logarithm-transformed EDSS was a dependent variable, the significant level was specified as 0.1 for both entry into and staying in the model, and the model residuals had normal distribution.

Longitudinal lesion evolution

The compound annual growth rate (CAGR=[(final/initial lesion volume)^(1/years)]-1) of adjusted lesion volume was obtained for each lesion, and the Mann-Whitney U-test was applied to assess CAGR differences between lesions with and without 7T paramagnetic rim. Since there was no evidence for bimodal distribution, we arbitrarily classified lesions as shrinking (CAGR ≤-1%), steady (between -1 and 1%) or expanding (≥1%); Fisher's exact test (3x2 contingency table) was used to assess differences among rim lesion groups.

A mixed-effects model was used to study lesion volume change over time. Since the volume distribution was right-skewed, a natural logarithm transformation was applied. Since 19 of 24 individuals had 2 (14 individuals) or more lesions, the model included random effects for both lesion (intercept and slope) and subject (intercept only). Age at baseline, sex, clinical phenotype, and history of treatment were considered as covariates, and a significance level of 0.1 was used for model selection.

Log-transformed adjusted lesion volume data were also analyzed by modeling the lesion volume change over time through linear mixed-effects models, where age at scan and presence of the 7T rim were included as fixed effects. Variables considered as covariates included age at baseline, sex, clinical phenotype, and history of treatment; these were dropped from the final model due to lack of association. Multiple lesions from the same individual were treated as independent since the subject effect was not significant (p=0.14). The same analysis was also repeated excluding 8 expanding lesions with evident MRI inflammatory activity during the follow-up (Table 2).

A linear mixed-effects model was also implemented to assess the rate of change in normalized supratentorial brain volume over time, and Pearson's correlation was used to study the association between annual changes in lesion and supratentorial brain volume based on the estimated slopes for each lesion derived from the random coefficient model estimates. Statistical analysis used Prism and SAS version 9.3.

eTable 1. Cohort characteristics of 7T patients with archival 3T yearly NIH scans for ≥10 years

# patient	Age (years)	Disease duration (years)	Sex	Clinical phenotype at final time point	# analyzed lesions with 7T rim	# analyzed lesions without 7T rim	Lesion follow up duration (years)
1	55	24	M	progressive	3	1	21
2	66	29	F	progressive	1	1	22
3	57	27	M	relapsing	1	1	21
4	52	27	F	progressive	4	0	17
5	63	25	M	relapsing	1	1	19
6	47	16	M	relapsing	2	1	13
7	48	10	M	relapsing	2	1	6
8	44	12	F	relapsing	2	1	9
9	38	14	M	relapsing	1	1	12
10	53	22	F	progressive	2	1	6
11	53	16	F	progressive	1	1	9
12	43	11	F	progressive	2	1	11
13	66	26	M	progressive	2	0	14
14	45	18	M	progressive	1	1	13
15	44	11	F	relapsing	1	1	7
16	66	27	F	progressive	1	1	15
17	62	30	M	progressive	0	2	18
18	65	26	F	relapsing	0	2	19
19	65	12	F	relapsing	0	2	10
20	73	15	F	relapsing	0	2	14
21	44	18	M	relapsing	0	2	10
22	50	29	F	progressive	0	1	23
23	50	20	F	relapsing	0	2	14
Summary	Mean±SD 54±10	Mean±SD 20±7	10 M/12F	13 relapsing/ 10 progressive	27	27	Mean±SD 14±5

Abbreviations: SD=standard deviation; M=male; F=female.

eTable 2. Cohort characteristics by MRI scanner

Demographic and clinical data of 192 MS cases by MRI scanner						
Patient groups	no rims		1-3 rims		≥ 4 rims	
MRI scanner	7T	3T	7T	3T	7T	3T
#	34	50	35	31	23	19
Sex (female, %)	27 (79%)	32 (64%)	26 (74%)	19 (61%)	16 (70%)	12 (63%)
Clinical phenotype	CIS/RR 24 SP 8 PP 2	CIS/RR 37 SP 8 PP 5	CIS/RR 23 SP 10 PP 2	CIS/RR 23 SP 4 PP 4	CIS/RR 14 SP 4 PP 5	CIS/RR 10 SP 6 PP 3
Mean age, years (SD)	50 (16.6)	45.5 (12.8)	48.8 (11.4)	45.3 (11.3)	45.1 (9.5)	43.3 (13)
Mean disease duration, years (SD)	16.7 (13.9)	11.1 (11)	14.3 (9.5)	11.1 (10.1)	11.9 (7.5)	12.6 (9.4)
# patients never been treated (%)	7/34 (21%)	20/50 (40%)	2/25 (8%)	9/31 (29%)	3/23 (13%)	2/19 (10%)
African Americans (%)	3 (8.8%)	7 (14%)	5 (55%)	7 (22%)	5 (21%)	5 (26%)
HLA-DRB1*15:01 (%)	15 (44%)	18 (36%)	8 (23%)	5 (16%)	7 (30%)	8 (42%)
Median EDSS (range)	1.5 (0-7.5)	1.5 (0-7.5)	2 (0-7)	2 (0-8)	3 (1-7.5)	4 (1-7)
Mean MSSS (SD)	2.5 (2.2)	3.4 (2.6)	3.1 (2.3)	3.7 (2.7)	5 (2.3)	4.9 (2.7)
Mean PASAT (SD)	50 (9)	50 (9)	49 (8)	48 (12)	48 (11)	41 (12)
Mean SDMT (SD)	53 (15)	54 (11)	48 (13)	49 (14)	45 (18)	42 (18)

Abbreviations: CIS=clinical isolated syndrome; RR=relapsing-remitting; SP=secondary progressive; PP=primary progressive; SD=standard deviation; EDSS=Expanded Disability Status Scale; MSSS=Multiple Sclerosis Severity Score; PASAT=Paced Auditory Serial Addition Test; SDMT=Symbol Digit Modalities Test.

eTable 3. Detailed cohort characteristics of relapsing-remitting MS patients (131 cases)

Demographic and clinical data				
Rim category	no rims	1-3 rims	≥ 4 rims	Statistical analysis
#	61 (47%)	46 (35%)	24 (18%)	-
Sex (female, %)	46 (75%)	34 (74%)	18 (75%)	Fisher 2x3 p=0.9 n.s.
Mean age, years (SD)	43.5 (14.1)	45.0 (10.5)	40.9 (9.0)	ANOVA p=0.3 n.s.
Mean disease duration, years (SD)	9.5 (10.3)	9.4 (8.1)	8.7 (6.9)	ANOVA p=0.9 n.s.
African Americans (%)	7 (11%)	9 (20%)	7 (29%)	Fisher 2x3 p=0.1 n.s.
HLA-DRB1*15:01 (%)	20/61 (33%)	7/46 (15%)	8/24 (33%)	Fisher 2x3 p=0.9 n.s.
Median EDSS (range)	1.5 (0-3.5)	1.5 (0-5)	2 (1-5)	ANOVA p=0.01
Mean MSSS (SD)	2.2 (1.9)	2.4 (1.9)	3.2 (1.5)	ANOVA p=0.08 n.s.
Mean PASAT (SD)	52 (6)	49 (10)	46 (10)	ANOVA p=0.009
Mean SDMT (SD)	58 (10)	52 (13)	52 (16)	ANOVA p=0.06 n.s.
MRI data				
Mean normalized brain volume (SD)	0.708*$ (0.025)	0.690$ (0.032)	0.683* (0.068)	ANOVA p=0.009
Mean normalized cortical volume (SD)	0.355 (0.031)	0.352 (0.027)	0.361 (0.032)	ANOVA p=0.6 n.s.
Mean normalized WM volume (SD)	0.278*$ (0.018)	0.264$ (0.022)	0.257* (0.018)	ANOVA p<0.0001
Mean total lesion volume (SD) [mm^3]	3744 (3279)	10369 (9337)	14396 (10195)	ANOVA p<0.0001
Mean rim lesion volume (SD) [mm^3]	-	3236 (4639)	5521 (6391)	N/A
% rim/total lesion volume	-	29.4%	33.7%	N/A
Mean normalized thalamic volume (SD)	0.013*$ (0.003)	0.009$ (0.003)	0.010* (0.006)	ANOVA p<0.0001
Mean normalized caudate volume (SD)	0.006*$ (0.002)	0.004$ (0.001)	0.004* (0.002)	ANOVA p<0.0001
Mean normalized putamen volume (SD)	0.009*$ (0.002)	0.007$ (0.001)	0.007* (0.001)	ANOVA p<0.0001
Mean normalized ventricular CSF volume (SD)	0.0172*$ (0.0075)	0.0218$ (0.0104)	0.0247* (0.0127)	ANOVA p=0.005
Mean normalized sulcal CSF volume (SD)	0.2749 (0.0215)	0.2889 (0.0270)	0.2947 (0.0629)	ANOVA p=0.03
# patients with ≥1 Gad-lesions	13/61 (21%)	9/46 (20%)	5/42 (21%)	Fisher 2x3 p=0.9 n.s.

Abbreviations: n.s.=not significant; SD=standard deviation; EDSS=Expanded Disability Status Scale; MSSS=Multiple Sclerosis Severity Score; PASAT=Paced Auditory Serial Addition Test; SDMT=Symbol Digit Modalities Test; GM=gray matter; WM=white matter. Statistical significance at the p<0.05 level in Bonferroni-corrected post-hoc analysis was referred with the following symbols: * for the comparison no rim vs. ≥4 rims group; $ for the comparison no rim vs. 1-3 rims group; & for the comparison 1-3 rims vs. ≥4 rims group.

eTable 4. Simple correlations with EDSS

	Spearman Correlation Coefficients	
	EDSS	
	p	r
Log lesion volume	<0.0001	0.423
Log rim-lesion volume	0.0003	0.270
Log number of rim lesions	0.0005	0.248
Normalized brain volume	0.59	0.041
Normalized cortex volume	0.12	-0.120
Normalized WM volume	0.0007	-0.256
Normalized thalamus volume	<0.0001	-0.336
Normalized caudate volume	<0.0001	-0.344
Normalized putamen volume	0.09	-0.128
Normalized ventricular CSF volume	<0.0001	0.369
Normalized sulcal CSF volume	0.0003	0.270

Abbreviations: EDSS=Expanded Disability Status Scale; WM=white matter; CSF=cerebrospinal fluid.

eTable 5. Dichotomous evolution of chronic MS lesions with and without 7T rim in the longitudinal cohort: linear regression model

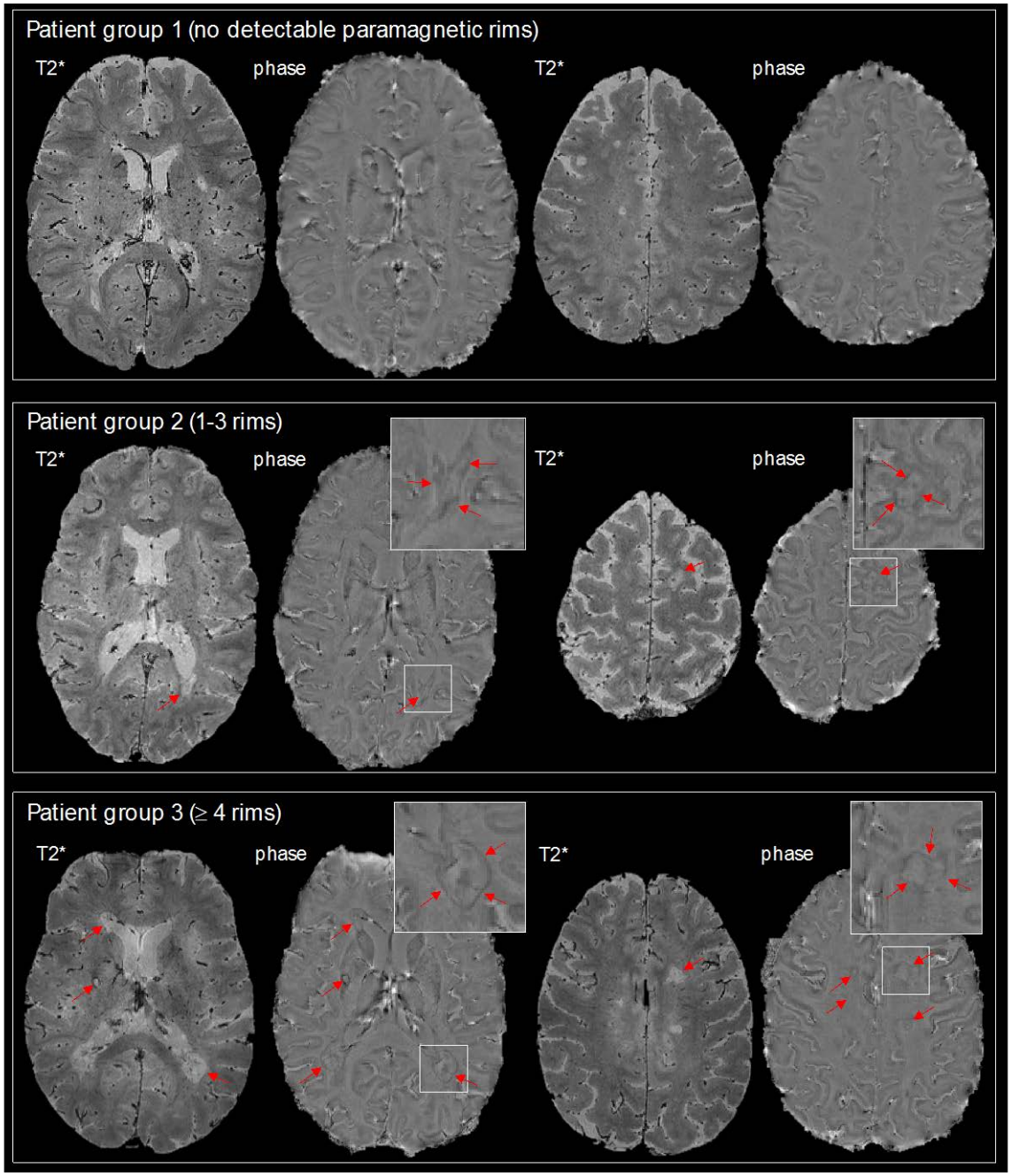
	7T rim+ lesions	7T rim- lesions	Statistical analysis
Mean estimated slope of adjusted log-volume change over time			
All data (617 yearly time-points)	+2.2%/year (expansion)	-3.6%/year (shrinkage)	p<0.0001
Exclusion of 8 expanding lesions with evidence of MRI inflammatory activity (re-enhancement or inclusion of small adjacent WM abnormalities) during the follow-up	-0.002%/year (steady)	-3.5%/year (shrinkage)	p=0.0027

Abbreviations: WM=white matter.

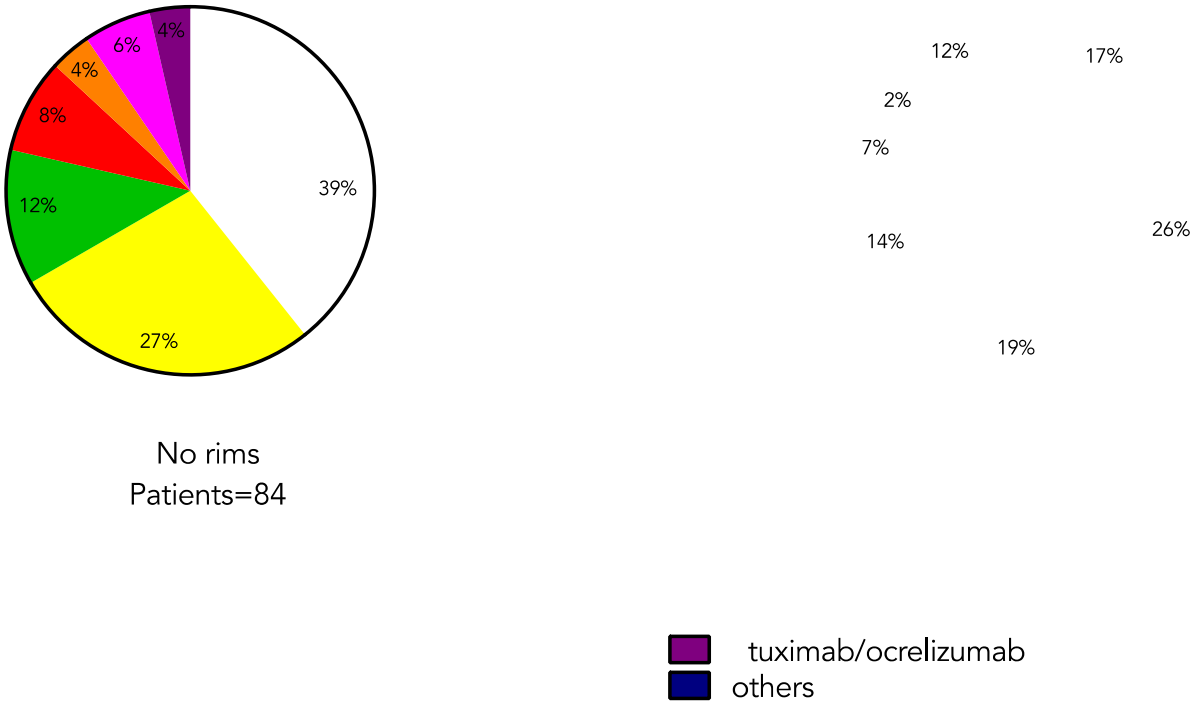
eFigure 1. Representative susceptibility-based 3T MRI images of patients classified in 3 groups based on the number of chronic non-enhancing lesions with paramagnetic rims.

- Group 1 (no detectable rims): a 50-year-old woman affected by relapsing MS;
- Group 2 (detection of 1-3 rims): a 55-year-old woman affected by relapsing MS; 3 rim lesions were seen on 3T T2*/phase images;
- Group 3 (detection of ≥4 rims): a 57-year-old man affected by progressive MS; 9 rim lesions were seen on 3T T2*/phase images.

Lesions with rims are indicated with arrows; insets show representative magnified view of lesions with rim.

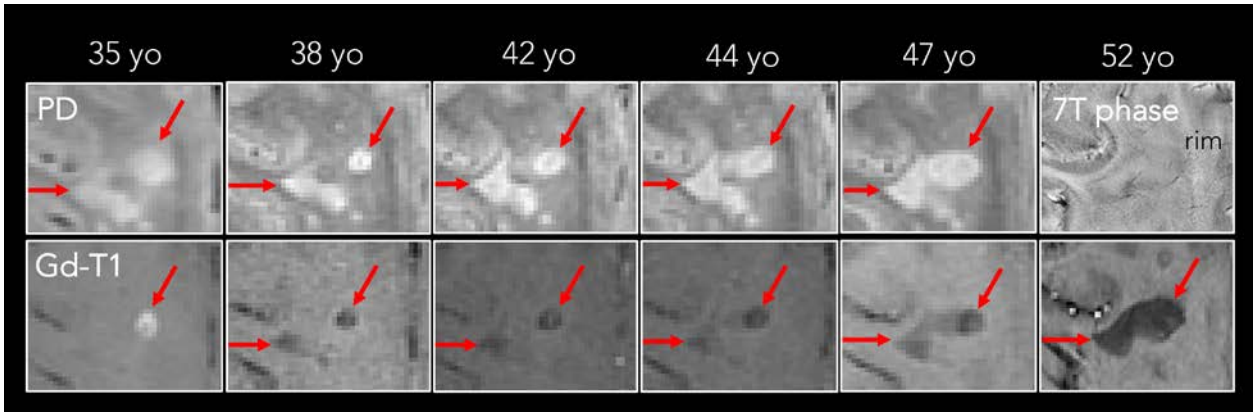


eFigure 2. Distribution of disease-modifying treatments at the time of MRI by patient groups.



Abbreviations: IFN=interferon-beta 1a or interferon-beta 1b; others= bone marrow transplant, cyclophosphamide, teriflunomide.

eFigure 3. Representative examples of slowly expanding lesions (red arrows) on MRI snapshots (proton-density and postcontrast T1-weighted images) in a 52-year-old woman with MS (27 years of disease at the last time point; EDSS=5). By the time of the 7T MRI (last available time point), the two lesions have become completely confluent and show a paramagnetic rim.



eFigure 4. Different evolution and pathology of chronic MS lesions with and without 7T rim.

Lesion #10, derived from the confluence over time of two demyelinated areas as shown on in vivo coregistered T1-weighted scans from age 52 to 59. At the time of autopsy, the edge of one area had a rim (red outline on postmortem 7T phase images), but the other edge did not (blue outline). The rim portion of the lesion (box B) had higher density of iron-laden phagocytes (iron and CD68 staining) and expansion over time (graph) than the rimless edge (box A). Additional staining is shown in Figure 3.