Supplementary Materials

MRI scanners, acquisition and MTR.

The MS-SMART trial involved 13 UK sites with multiple MRI scanners, including Philips 3T and 1.5T (Philips Healthcare, Best, the Netherlands), Siemens 3T and 1.5T (Siemens Healthineers, Erlangen, Germany), GE 3T (General Electric Healthcare, Chiago, IL). The MRI acquisition parameters are reported in Table 1. London and Edinburgh site provided also the magnetization transfer ratio (MTR) acquisition, as part of the advanced MRI sub-study.

Table 1. MRI acquisition parameters

Sequences	Repetition time (ms)	Echo time (ms)	Flip angle (α)	Field of view (mm^2)	Voxel size (mm^3)	No. of slices
PD/T2 weighted 2D TSE	3500	19/85	90°	240x240	1x1x3	50
T1 weighted 3D	7.0	3.1	8°	180x256	1x1x1	256
MTR 3D FFE	6.4 35*	2.7/4.3 4.07/9.49*	9°	180x256 256X256*	1x1x1	256 176*

Abbreviations**:** TSE=turbo spin-echo; MTR= magnetisation transfer ratio; FFE= fast field echo. MTR parameters are referred to London site, while * denotes the parameters used at Edinburgh site.

Table 2. Demographic, clinical, and radiological characteristics of the patients excluded from the SEL study.

Demographics and clinical metrics	Number of patients	100
	Age, median [y] (IQR)	54.6 (48.8 – 58.2)
	Female n (%)	68 (68%)
	Baseline disease duration, median [y] (IQR)	18 (14 – 26)
	Baseline progression duration, median [y] (IQR)	6 (3 – 9)
	EDSS at baseline, median (range)	6.0 (6.0 – 6.5)
	EDSS change from baseline to week 96, mean (SD)	0.25 (0.78)
	Patients with disability progression over time, number (%)	72 (72%)
	MSFC z-score at baseline, mean (SD)	-0.26 (1.16)
	MSFC z-score change, mean (SD)	-0.53 (1.64)
	NHPT at baseline, mean [sec^{-1}] (SD)	0.03 (0.01)
	NHPT z-score change, mean (SD)	0.05 (0.59)
	T25FW at baseline, median [sec] (IQR)	11.8 (8.8 – 23.8)
	T25FW z-score change, mean (SD)	-1.53 (4.71)
	PASAT score at baseline, mean (SD)	37.43 (14.33)
	PASAT z-score change, mean (SD)	-0.11 (0.70)
	SDMT score at baseline, mean (SD)	43.31 (12.16)
	SDMT change, mean (SD)	-0.37 (10.61)
MRI metrics	T2 lesion volume at baseline, mean [ml] (SD)	16.52 (16.50)
	T2 lesion volume at week 96, mean [ml] (SD)	17.92 (19.49)
	New/enlarging T2 lesions at week 96, mean number (SD)	3.80 (8.53)
	New PBH at week 96, mean number (SD)	0.28 (0.67)
	NBV at baseline, mean [ml] (SD)	1430 (79)
	CGM at baseline, mean [ml] (SD)	788 (44)
	DGM at baseline, mean [ml] (SD)	45 (4)
	WM at baseline, mean [ml] (SD)	596 (42)
	PBVC, mean [%] (SD) week 24 to week 96 baseline to week 96	 -0.57% -1.34%

Abbreviations: IQR=interquartile range; SD=standard deviation; EDSS=expanded disability status scale; MSFC=multiple sclerosis functional composite; NHPT=nine-hole peg test; T25FW=timed 25-foot walk test; PASAT=paced auditory serial addition task; SDMT=symbol digit modalities test; PBH=persistent black holes (manually detected); NBV=normalised brain volume; CGM=cortical grey matter volume; DGM=deep grey matter volume; WM=white matter volume; PBVC=percent brain volume change.

All the changes in the clinical metrics were calculated as the difference between the week 96 and the baseline relative values.

Table 3. SEL-derived metrics at the patient level by treatment allocation arm.

Panel A

		Treatment allocation				
		Fluoxetine (n=90)	Riluzole (n=85)	Amiloride (n=86)	Placebo (n=84)	p value*
Lesion numbers per patient, mean (range)						
Total T2 lesions		65.3 (6 – 176)	66.6 (3 – 201)	68.2 (4 – 352)	68.7 (7 – 266)	p=0.94
SEL-derived	non-SEL	37.4 (4 – 97)	41.5 (1 – 158)	43.5 (2 – 234)	42.5 (4 – 206)	p=0.52
	possible SEL	7.2 (0 – 28)	6.2 (0 – 27)	6.4 (0 – 36)	5.9 (0 – 25)	p=0.45
	definite SEL	20.7 (0 – 76)	18.9 (2 – 51)	18.3 (0 – 94)	20.3 (1 – 47)	p=0.56

Panel B

		Treatment allocation				
		Fluoxetine (n=90)	Riluzole (n=85)	Amiloride (n=86)	Placebo (n=84)	p value*
Lesion volume per patient, mean [ml] (range)						
Total T2 lesions		13.6 (0.1 – 71.4)	11.3 (0.1 – 37.9)	12.5 (0.2 – 51.6)	11.6 (0.2 – 51)	p=0.49
SEL-derived	non-SEL	5.1 (0.1 – 37.7)	5.9 (0.1 – 31.6)	5.9 (0.2 – 29.5)	5.5 (0.1 – 27.8)	p=0.47
	possible SEL	2.9 (0 – 38.8)	2.0 (0 – 35.3)	2.3 (0 – 25.7)	1.9 (0 – 23.9)	p=0.32
	definite SEL	5.6 (0 – 38.2)	3.3 (0.1 – 19.6)	4.2 (0 – 39.7)	4.3 (0.1 – 25.5)	p=0.09

Panel A describe the conventional MRI measures, i.e. total T2 lesions and in the rows below the sub-categories of lesions classified based on the SEL-derived metrics, for each one of the three treatment arms and the placebo group. Similarly, panel B shows the T2 lesion volume and below the SEL-derived volumes for each one of the treatments and placebo arms.

As shown in the last column of each panel, no overall differences between treatment arms were observed in terms of either counts or volumes of T2 lesions and their subcategories.

*p value corresponding to the test that checked whether treatment allocation as a whole had an effect on the dependent variable (SEL-derived metrics).

Table 4. Intraclass correlation coefficients (ICC) to analyse the effect of the multicentre trial

Evaluated outcome*	ICC - Level centre
T2 lesion volume	<0.001
PBVC	0.04
Definite SEL log-volume	0.001
Possible SEL log-volume	<0.001
Non-SEL log-volume	0.01
EDSS	0.07
MSFC z-score	<0.001
NHPT z-score	0.003
T25FW z-score	<0.001
PASAT z-score	0.05
SDMT	0.02

Intraclass correlation coefficients (ICC) retrieved from the mixed-effects regression models between the evaluated outcome variables, with the variable of the centre nested in the model.

*Covariates in the models included age at baseline, sex.

Abbreviations: EDSS=Expanded Disability Status Scale; MSFC=Multiple Sclerosis Functional Composite; NHPT= Nine-Hole Peg Test, T25FW=Timed 25-Foot Walk Test; PASAT=Paced Auditory Serial Addition Task; SDMT=Symbol Digit Modalities Test.

Table 5. Multiple linear regression using a multiple imputation model to retrieve SEL-derived measures for patients not included in our analysis

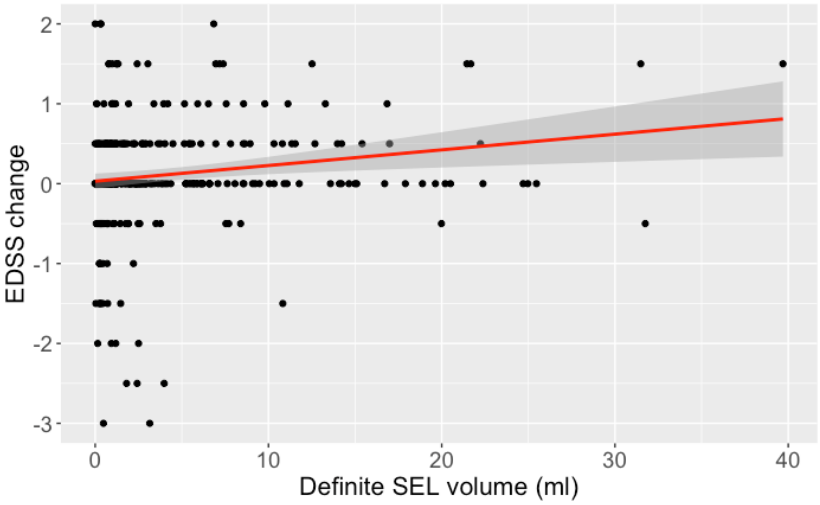
	Non SEL log-volume beta (95% CI) * p value	**Possible SEL log-volume** beta (95% CI) * p value	**Definite SEL log-volume** beta (95% CI) * p value
EDSS	-0.03 (-0.22, 0.16) p=0.76	0.10 (-0.12, 0.32) p=0.35	**0.23 (0.03, 0.42) p=0.03**
MSFC z-score	-0.14 (-0.61, 0.33) p=0.57	-0.26 (-0.81, 0.29) p=0.36	**-0.64 (-1.13, -0.15) p=0.001**
NHPT z-score	-0.15 (-0.34, 0.05) p=0.15	-0.15 (-0.38, 0.09) p=0.22	-0.11 (-0.32, 0.10) p=0.31
T25FW z-score	-0.15 (-1.56, 1.25) p=0.83	-0.97 (-2.61, 0.65) p=0.24	**-1.71 (-3.17, -0.25) p=0.02**
PASAT z-score	**-0.20 (-0.39, -0.02) p=0.03**	-0.17 (-0.38, 0.05) p=0.12	**-0.37 (-0.56, -0.18) p=0.001**
SDMT	**-4.31 (-6.86, -1.78) p=0.001**	-2.41 (-5.33, 0.52) p=0.11	**-2.95 (-5.60, -0.29) p=0.03**

To retrieve the values of the SEL-derived volumes for the missing subjects, a multivariate imputation by chained equations (predictive mean matching) was used in order to include all patients enrolled in the trial (n=445).

*Standardised regression coefficients (beta) and 95% confidence intervals (95% CI) of multiple linear regressions performed with the SEL-associated volumes as predictors (definite SEL, possible SEL and non-SEL log-volumes) and the clinical measure (at baseline and week 96) as response variable. All models were adjusted for age, gender, T2 lesion volume change and percentage brain volume change. In bold p-values <0.05.

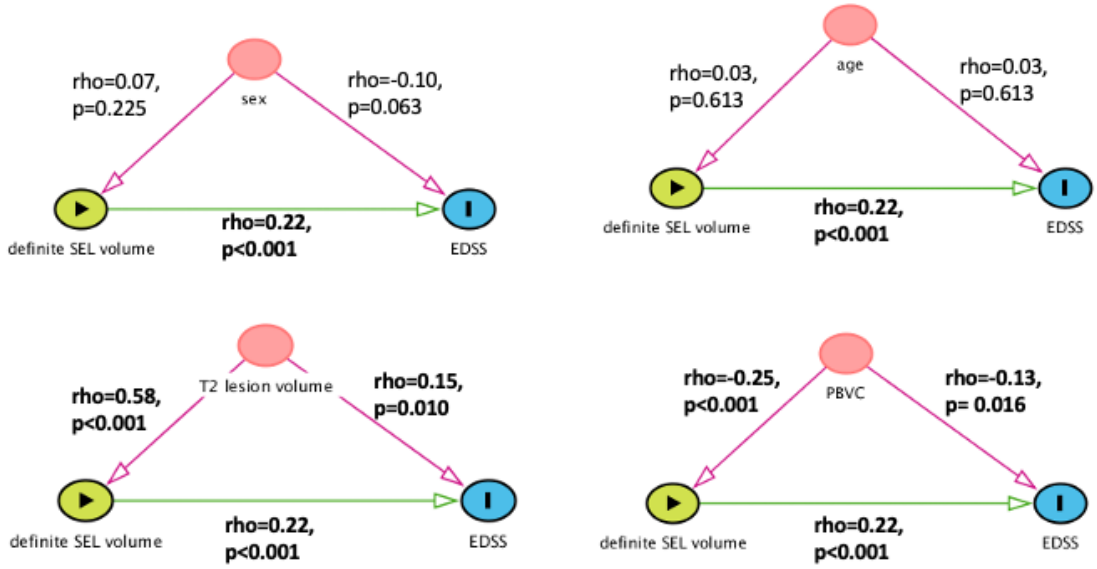
Abbreviations: EDSS=Expanded Disability Status Scale; MSFC=Multiple Sclerosis Functional Composite; NHPT= Nine-Hole Peg Test, T25FW=Timed 25-Foot Walk Test; PASAT=Paced Auditory Serial Addition Task; SDMT=Symbol Digit Modalities Test.

Figure 1. Regression line of the EDSS change as a function of definite SEL volume.



Plot showing the relationship between Expanded Disability Status Scale (EDSS) change from baseline to week 96 and baseline definite SEL volume (ml). A regression line (linear model) is shown in red, along with a 95% confidence interval (grey area) (beta=0.02, 95% CI [0.007;0.03], p=0.003).

Figure 2. Direct Acyclic Graphs (DAGs) showing the relationship between the variables included in the statistical models



Selection process used to identify the confounders to include in the statistical models. This example shows the procedure used for the definite SEL log-volume and the EDSS (at final follow-up of the trial), which were assessed as the exposure and the outcome variables, respectively. The relationship between these two was then observed in concomitance to possible confounders, by analysing the Spearman correlation coefficients (shown on the connecting arrows). The same analyses were replicated for all the other SEL-derived variables and clinical outcomes (not shown).

In the bottom DAGs, the variables T2 lesion volume (at final follow-up) and percentage brain volume change (PBVC) are significantly associated to both exposure (definite SEL volume) and outcome (EDSS), thus those two variables can be considered confounders and they were included in our models.

In the top DAGs, the two demographic variables, sex and age, did not show significant correlation with either the exposure or outcome variable. However, in consideration of extensive literature data of their association to the outcomes, we opted to keep them as confounders in all our models.