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Immune Cell and Inflammatory Marker Dynamics in Cerebrospinal Fluid After Treatment of Multiple Sclerosis With Cladribine Tablets: Results From the CLOCK-MS Study

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RESEARCH IN CONTEXT

1. Reductions in pathological immune cells implicated in multiple sclerosis (MS) in cerebrospinal fluid (CSF) and blood were seen through 1 year (Yr) after cladribine tablets (CladT) initiation in patients with MS reconfirming its central and peripheral effects in the treatment of MS.

2. The findings of this study shed light on the proposed mechanism of action (MoA) of CladT, including its impact on pathological B and T cell subsets, and inflammatory and neurodegeneration biomarkers in the CSF and blood.

OBJECTIVES

To assess longitudinal changes in lymphocytes and soluble markers of intrathecal inflammation from US-based patients with relapsing multiple sclerosis (RMS) post CladT treatment initiation.

INTRODUCTION

Previous studies[1,2] show how CladT affects the CSF immune cells and proteins, reflecting its overall role in regulating immune cells involved in MS immunopathogenesis. However, the effect of CladT on the markers of intrathecal inflammation in patients with RMS is yet to be fully understood.

The CLOCK-MS study (CladT: Collaborative Study to Evaluate Impact on CNS Biomarkers in MS) is an ongoing open-label, randomized, multicenter phase 4 study exploring the MoA of CladT in RMS (NCT03963375)

METHODS

- 47 patients from 5 sites in the US were randomly assigned in a 1:2:2:1 ratio to undergo a lumbar puncture at baseline (BL), and at either 5 weeks (Wks), 10 Wks, 1 Yr, or 2 Yrs after starting CladT (**Supplementary Figure 1**)
- All patients received CladT at a cumulative dose of 3.5 mg/kg over 2 years per US prescribing information[3]
- Changes in the CSF and blood levels of CD3+ T cells, CD19+ B cells, and lymphoid and myeloid immune cell subsets from BL to Yr 1 were analyzed using a non-parametric Wilcoxon signed rank test
- Single-cell RNA sequencing (scRNA-seq) was performed on a subset of CSF and blood samples

RESULTS

Baseline demographics and disease characteristics

42.9 (12.3) years old **Age**, mean (SD)

59.6% (n=28) **Females**

N=47

2.0 (1.5, 4.0) **EDSS**, median (IQR)

6.0 (1.0, 17.0) **Years since MS diagnosis** median (IQR)

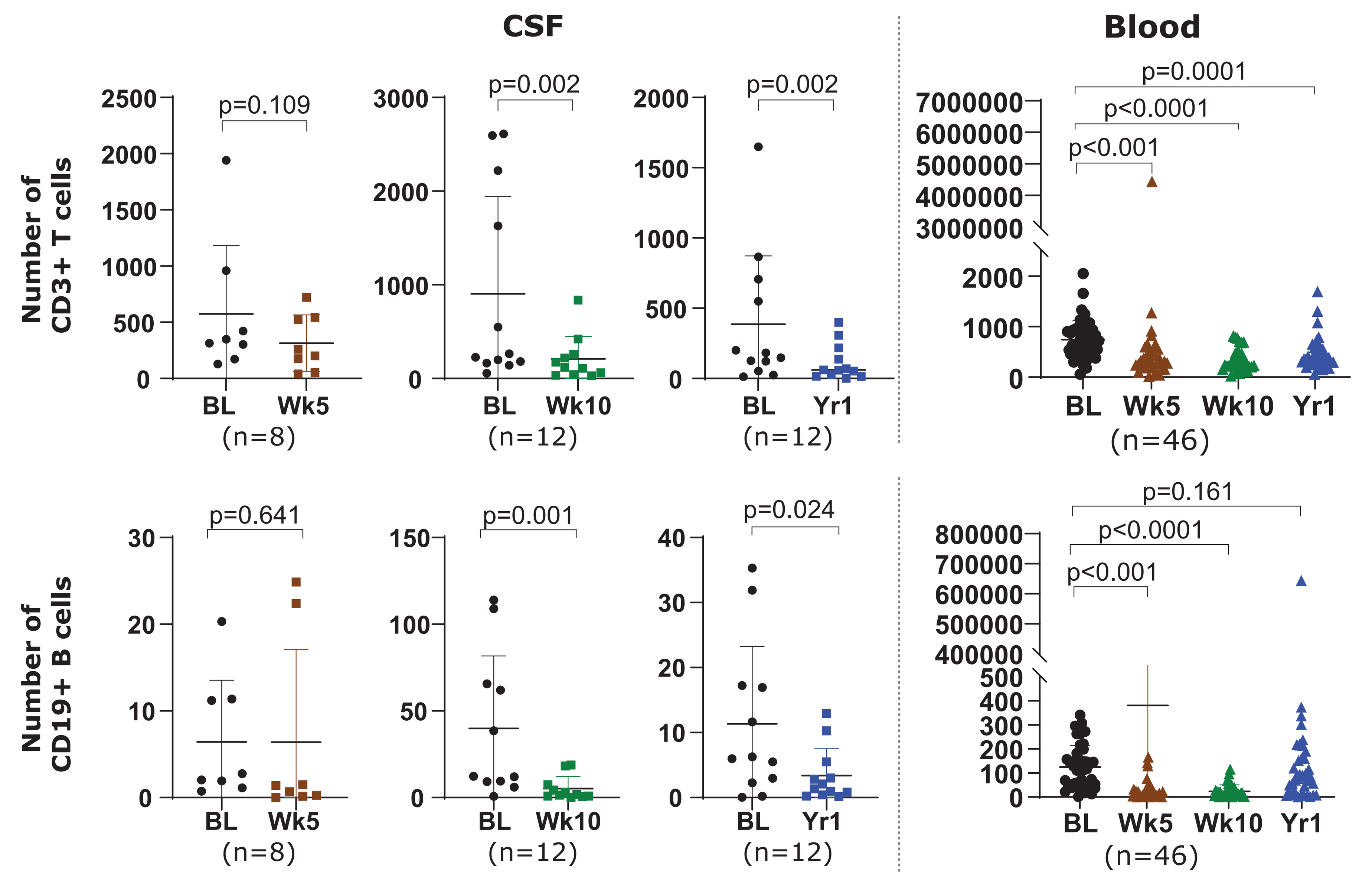
EDSS, Expanded Disability Status Scale; IQR, interquartile range; SD, standard deviation

Please refer to **Supplementary Table 1** for detailed BL demographics and disease characteristics, and **Supplementary Table 2** for prior disease-modifying therapy use.

- CD3+ T cells were significantly reduced post CladT treatment at Wk 5, Wk 10, and Yr 1 in the blood, and Wk 10 and Yr 1 in the CSF (vs BL) (**Figure 1**)
- CD19+ B cells were significantly reduced at Wks 5 and 10 vs BL and recovered to BL levels at Yr 1 in the blood, and were significantly reduced at Wk 10 and Yr 1 in the CSF (vs BL) (**Figure 1**)
- Memory B cells were significantly reduced in the blood and CSF at Wk 5, Wk 10, and Yr 1 (**Figure 2**)
- There were significant reductions in CD4+ T regulatory (Treg) cells in the CSF at Wk 10 and Yr 1. However, an increase in the proportion of CD4+ Treg cells was observed at Wks 5 and 10 in the CSF (**Supplementary Table 3**)
- Serum neurofilament light chain levels were significantly reduced at Yr 1 vs BL. Serum glial fibrillary acidic protein levels remained stable through Yr 1 (**Supplementary Figure 2**)
- Emergence of CD4+ Treg memory and naïve cell signatures through Yr 1 were observed in the CSF but not in the blood (**Figure 3**)
- The annualized relapse rate was low at Yr 1 (0.06 [95% CI: −0.01, 0.12]). EDSS scores remained stable from BL to Yr 1 (median [IQR] 2.3 [1.5, 4.0])
- Numbers of gadolinium-enhancing lesions were unchanged at Yr 1, and T2 lesions were significantly reduced. There was also a significant increase in the median subcortical gray matter volume and a significant reduction in the median cerebral white matter volume (**Supplementary Table 4**)

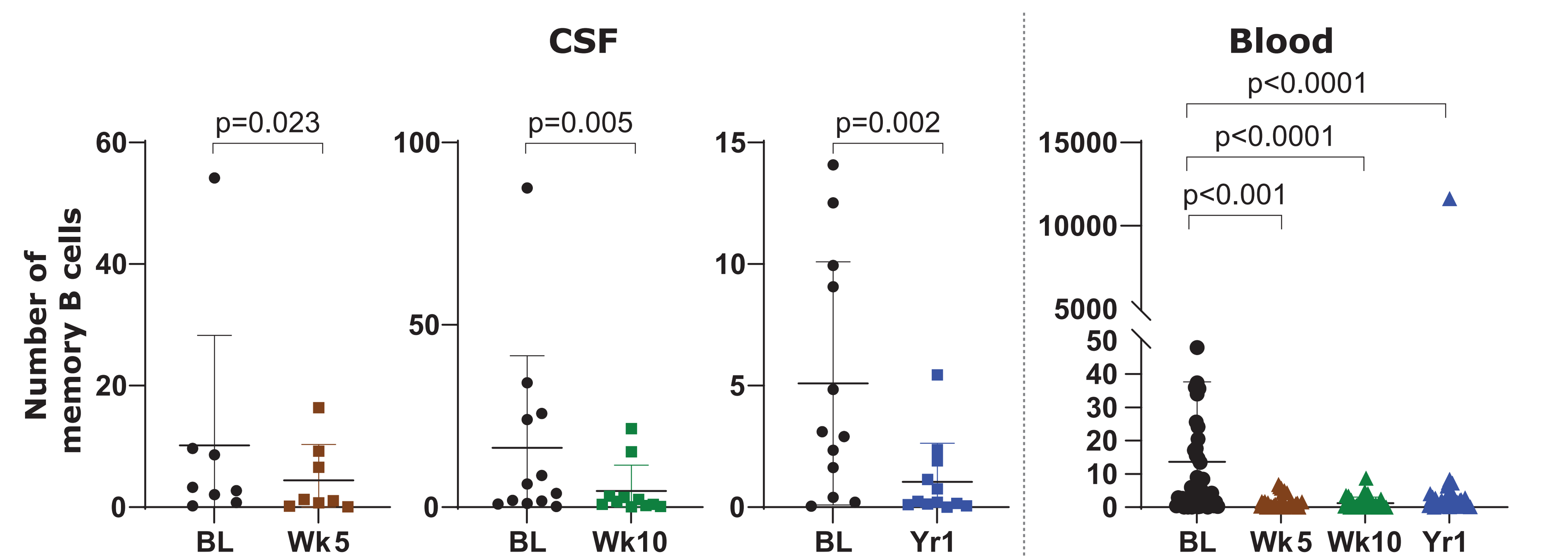
Please refer to **Supplementary Table 4** for efficacy outcomes data at Yr 1 (vs BL).

Figure 1: Changes in CSF and blood CD3+ T cells and CD19+ B cells at BL, Wk 5, Wk 10, and Yr 1 post-CladT initiation



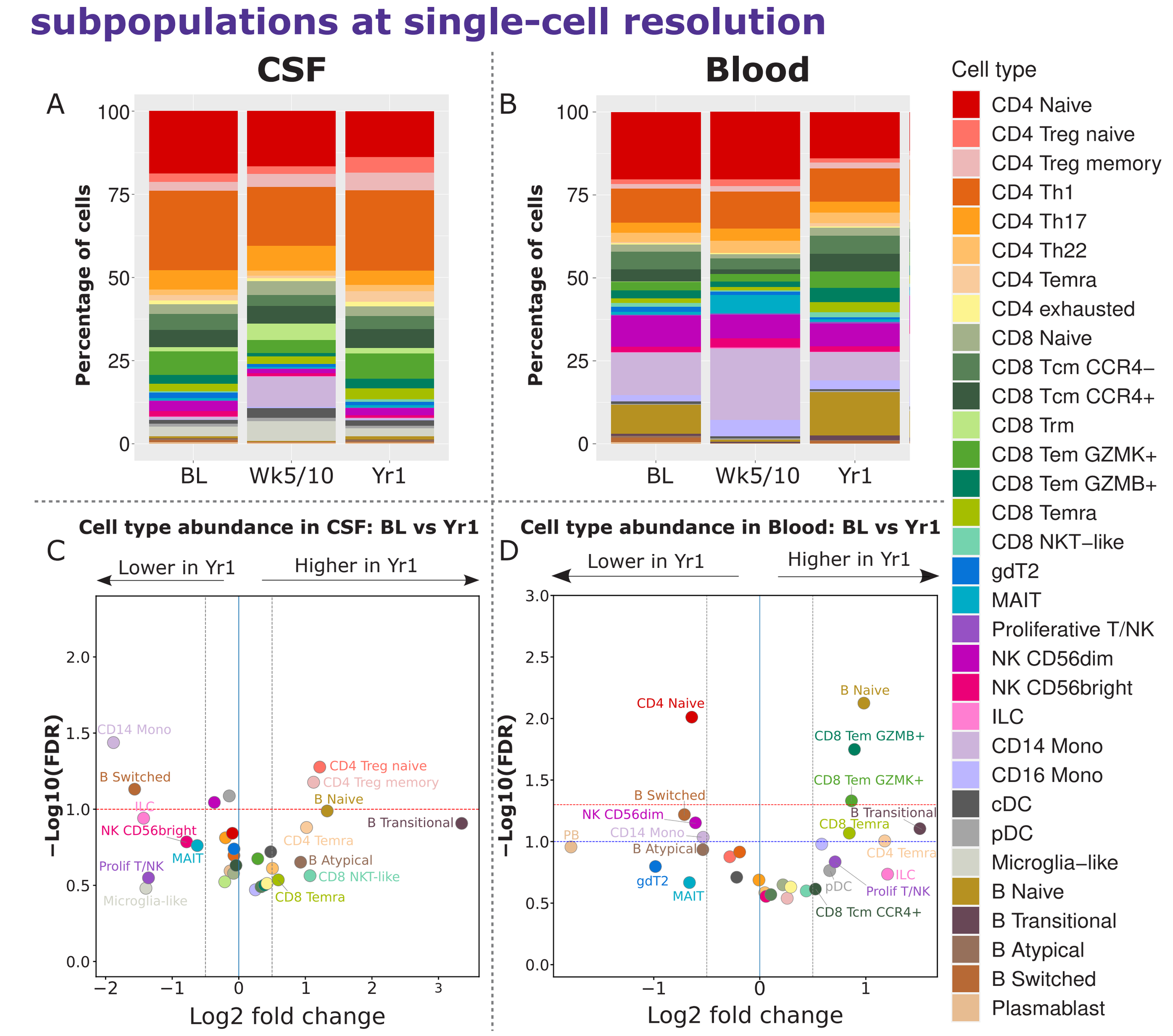
BL, baseline; CD, cluster of differentiation; CladT, cladribine tablets; CSF, cerebrospinal fluid; Wk, week; Yr, year.

Figure 2: Changes in CSF and blood memory B cells at BL, Wk 5, Wk 10, and Yr 1 post-CladT initiation



BL, baseline; CD, cluster of differentiation; CladT, cladribine tablets; CSF, cerebrospinal fluid; Wk, week; Yr, year.

Figure 3: CladT-mediated changes in the CSF and blood subpopulations at single-cell resolution



BL, baseline; CSF, cerebrospinal fluid; Treg, regulatory T cells; Wk, week; Yr, Year.

A and **B** represent percentage of each cluster throughout CladT treatment in CSF and blood, respectively. **C** and **D** are volcano plots showing differences in cluster abundance at BL vs Yr 1 (sccomp methodology). The blue and red horizontal dashed lines indicate significance thresholds of 0.1 and 0.05, respectively. Please refer to **Supplementary Figure 3** for further details on UMAP and volcano plots for Wk 5/10 in CSF and blood from all patients.

CONCLUSIONS

- **Early and sustained effects of CladT through Yr 1 were detected in the CSF, including overall reductions in the number of B and T cells**
- **scRNA-seq revealed the emergence of CD4+ Treg naïve and memory subsets within the CSF after CladT therapy while flow cytometric analysis also showed early increases in the abundance of Tregs within the CSF**
- **Lower absolute numbers of Tregs found in the CSF by flow cytometric analysis following CladT therapy suggests proportionally less depletion relative to other immune cells**
- **The shift in CSF immune cell populations, including Tregs, after CladT therapy highlights potential mechanisms for its therapeutic benefit in the treatment of RMS**

SCAN FOR AFFILIATIONS, DISCLOSURES, AND SUPPLEMENTARY MATERIALS

References: 1. Ammoscato F, et al. *Clin Immunol.* 2024;269:110380.
2. Wiendl H, et al. Oral presentation at ECTRIMS 2024, 18-20 September, Copenhagen, Denmark (O037).
3. MAVENCLAD. US Prescribing information. EMD Serono, Inc; 2024.

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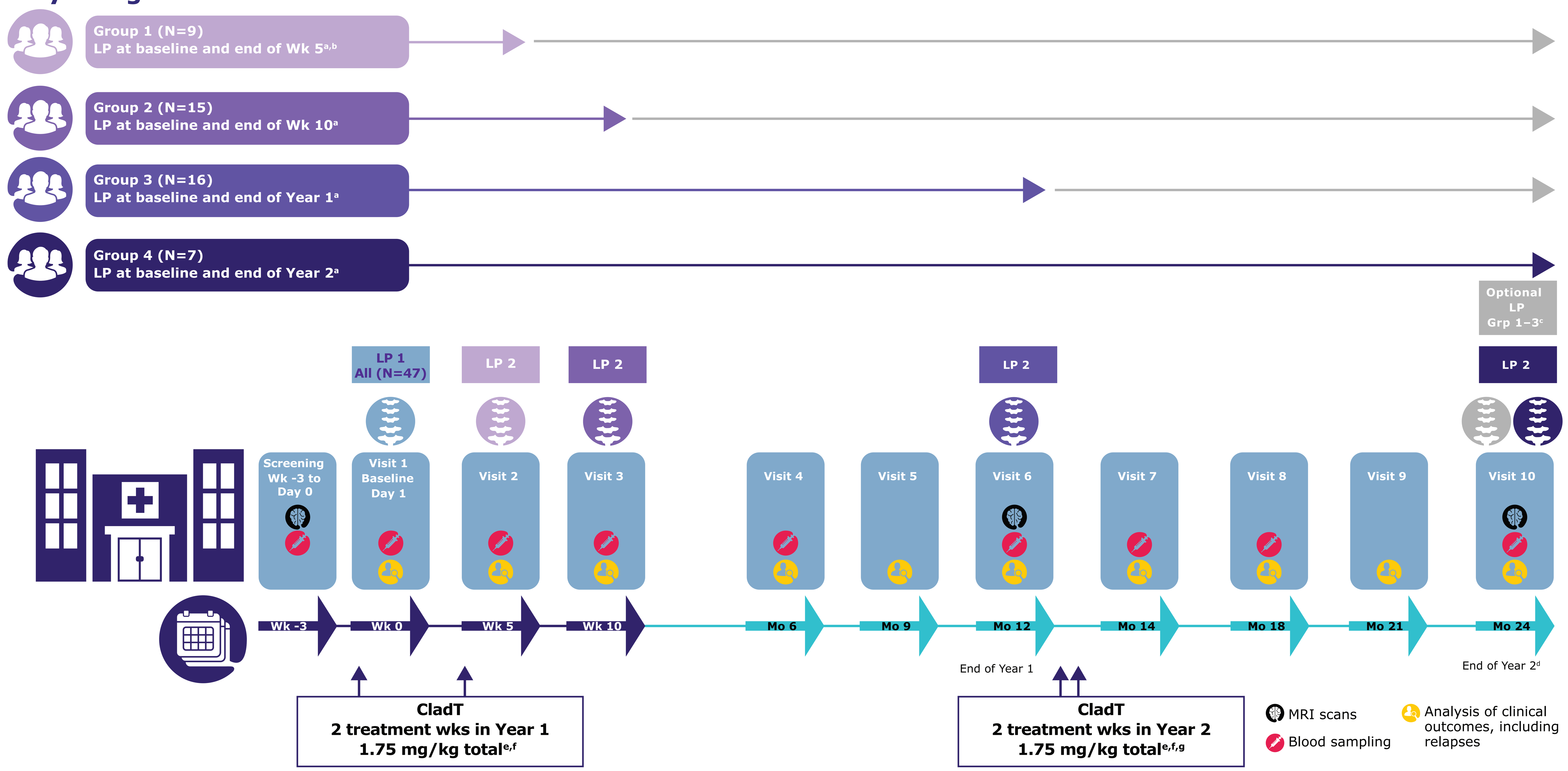
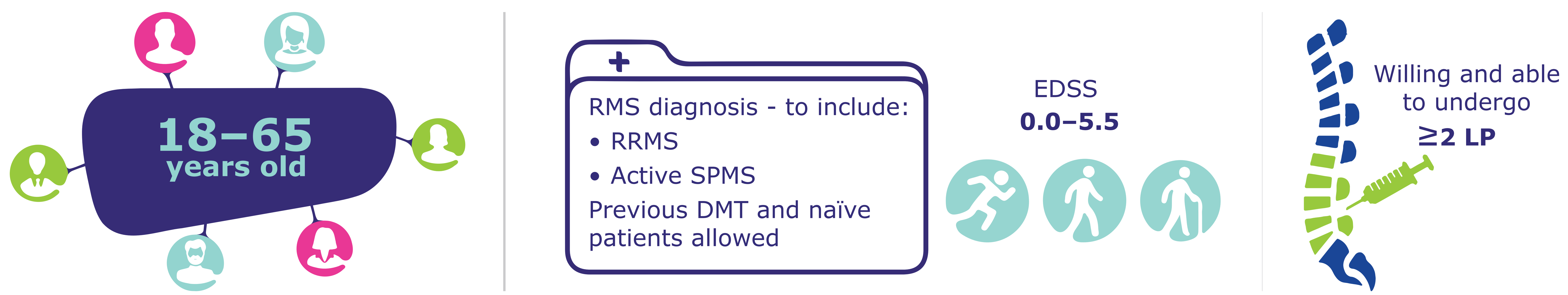
SUPPLEMENTARY MATERIAL

Supplementary Figure 1: CLOCK-MS study design, key inclusion and exclusion criteria, and study endpoints

Study design



Key inclusion criteria



Key exclusion criteria

- ✘ Have any contraindication for LP
- ✘ Have received natalizumab during the last 6 months
- ✘ Have malignancy, infections, signs/symptoms suggestive of PML
- ✘ Have been previously treated with ocrelizumab, alemtuzumab, or daclizumab

CladT, cladribine tablets; DMT, disease-modifying therapy; EDSS, Expanded Disability Status Scale; LP, lumbar puncture; Mo, month; MRI, magnetic resonance imaging; PML, progressive multifocal leukoencephalopathy; RMS, relapsing multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; Wk, week.

[a]For patients assigned to a LP, the LP-aligned blood sample needs to be obtained on the same day as the LP. [b]For patients in Group 1, the LP and aligned blood samples need to be obtained on the same day and following the last intake of Week 5 CladT treatment course. [c]All patients randomized to Groups 1–3 will have the option to volunteer for a third, optional LP at the end of Year 2, the timing of which would be aligned with the second LP of Group 4. [d]If the start of the second treatment year needs to be delayed (e.g., to allow for lymphocyte recovery to ≥800 cells/µL), to maintain the protocol-specified time interval between start of CladT Year 2 treatment and all following assessments, Year 2 visits and procedures for this participant will be delayed accordingly (for up to 6 months). [e]Patients to receive CladT 3.5 mg/kg per the United States Prescribing information.[1] [f]Each treatment week consists of daily treatment over 4–5 consecutive days. The second treatment week is considered 23 to 27 days after the last dose of the first treatment week.[1] [g]The second treatment year is considered at least 43 weeks after the last dose of the first treatment year and can be delayed for up to 6 months.[1]

1. Mavenclad. Prescribing information. EMD Serono, Inc; 2024.

Primary endpoints

Levels of lymphocyte subsets and neuronal injury marker in the CSF:

- CD3+ T lymphocytes, CD19+ B lymphocytes
- NfL

Secondary endpoints

Levels of lymphocyte subsets and soluble immunological and neurological injury markers in the peripheral blood[a]

- CD3+ T lymphocytes, CD19+ B lymphocytes
- NfL

Levels of other lymphocyte subsets and immune cells in the CSF and peripheral blood[a]

- CD4+ T cells and subsets
- CD8+ T cells, plasmablasts, NK cells, CD14+ cells

Clinical outcomes

- ARR over 24 months
- Change in EDSS from baseline at Months 12 and 24
- 6-month confirmed EDSS progression

MRI outcomes at Months 12 and 24

- T1 Gd+ and new/enlarging T2 lesions
- Changes in quantitative gradient echo contrast imaging (R2t*) in cerebral cortex, white and deep grey matter

Safety

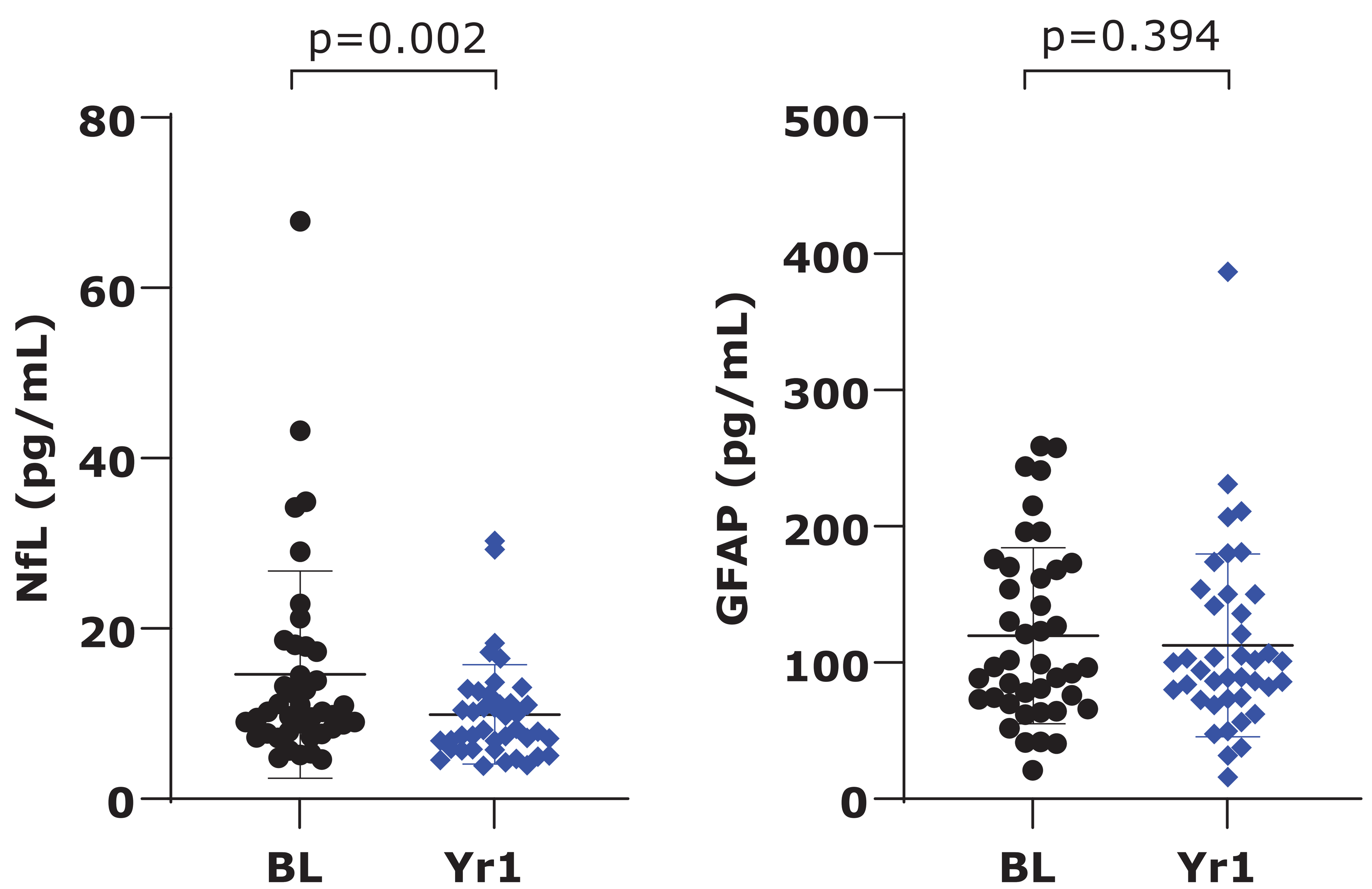
- TEAEs, SAEs, and TEAEs leading to treatment discontinuation
- ALC
- Complete blood cell count

ALC, absolute lymphocyte count; ARR, annualized relapse rate; CSF, cerebrospinal fluid; EDSS, Expanded Disability Status Scale; Gd+, gadolinium-enhancing; MRI, magnetic resonance imaging; NfL, neurofilament light chain; NK, natural killer; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

[a]Change from baseline to second LP (end of 5 weeks, 10 weeks, 1 year, and/or 2 years).

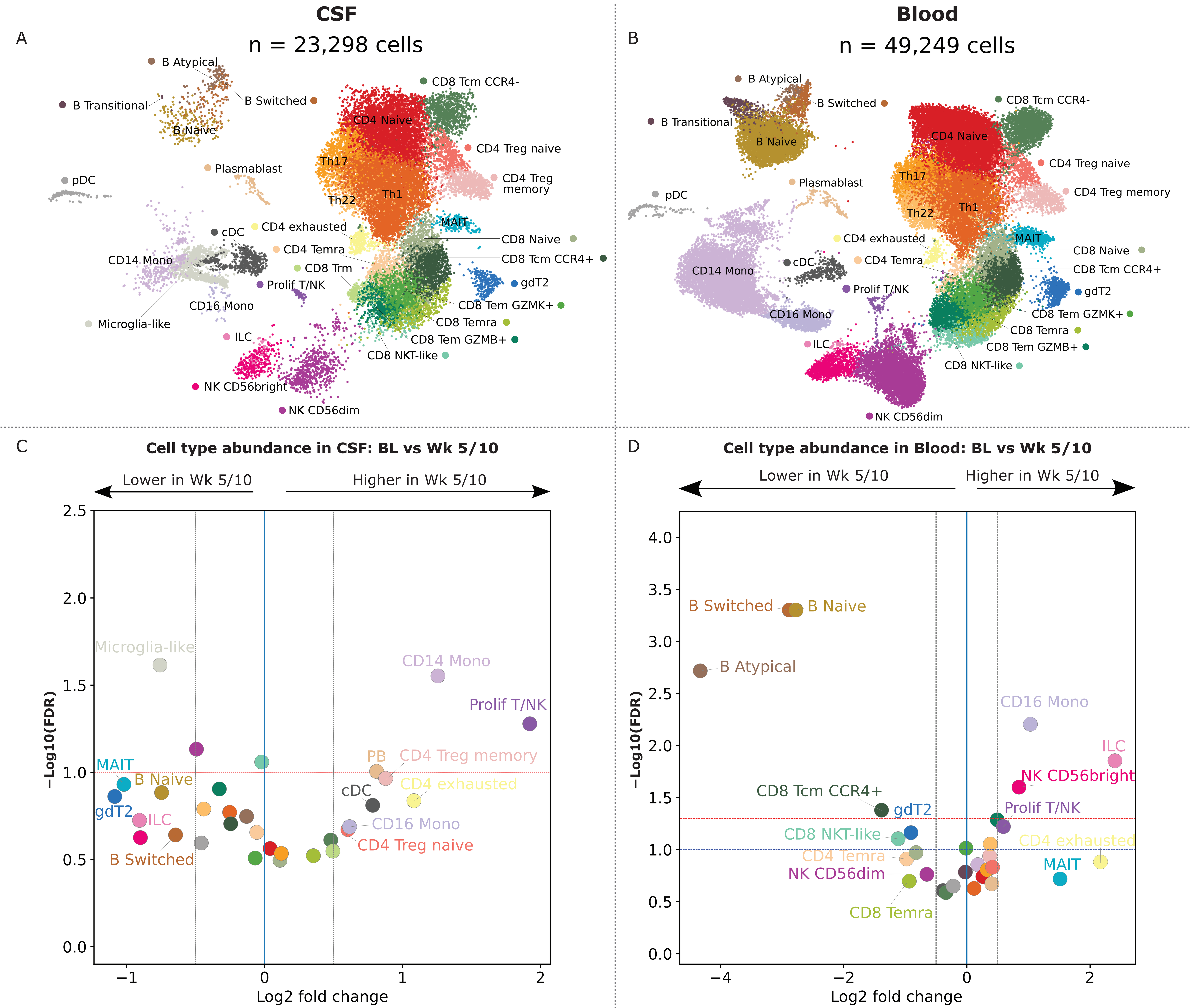
SUPPLEMENTARY MATERIAL

Supplementary Figure 2: Serum NfL and GFAP levels at BL and Yr 1 post-CladT initiation



BL, baseline; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; Yr, Year.

Supplementary Figure 3: CladT-mediated changes in CSF and blood subpopulations at single-cell resolution



BL, baseline; CSF, cerebrospinal fluid; Treg, regulatory T cells; UMAP, Uniform Manifold Approximation and Projection; Wk, week.

A and **B** are UMAP depicting CSF and blood from all patients, respectively. **C** and **D** shows volcano plots depicting differences in cluster abundance from CSF and blood at BL compared to Wk 5/Wk 10 revealed by fold change (log2) versus p value (−log10) based on sccomp methodology. The blue and red horizontal dashed lines indicate significance thresholds of 0.1 and 0.05, respectively

SUPPLEMENTARY MATERIAL

Supplementary Table 1: Baseline demographics and disease characteristics

Characteristics	Total (N=47)	Group 1 (Wk 5) (n=9)	Group 2 (Wk 10) (n=15)	Group 3 (End of Yr 1) (n=16)
Age, years, mean±SD	42.9±12.3	45.3±15.6	44.7±11.6	39.3±10.7
Female, n (%)	28 (59.6)	6 (66.7)	12 (80.0)	7 (43.8)
Race, n (%)				
White or Caucasian	39 (83.0)	8 (88.9)	13 (86.7)	13 (81.3)
Black or African American	6 (12.8)	1 (11.1)	2 (13.3)	2 (12.5)
Asian (all types)	2 (4.3)	0	0	1 (6.3)
Ethnicity, n (%)				
Hispanic or Latino	2 (4.3)	0	1 (6.7)	1 (6.3)
Non-Hispanic or non-Latino	45 (95.7)	9 (100.0)	14 (93.3)	15 (93.8)
MS type, n (%)				
RRMS	44 (93.6)	9 (100.0)	15 (100.0)	14 (87.5)
SPMS	3 (6.4)	0	0	2 (12.5)
Disease duration from MS diagnosis, years, median (IQR)	6.0 (1.0, 17.0)	8.0 (1.0, 21.0)	7.0 (0.0, 17.0)	4.0 (1.0, 8.5)
EDSS, median (IQR)	2.0 (1.5, 4.0)	2.0 (1.5, 3.0)	2.5 (1.5, 4.0)	2.0 (1.5, 3.5)

EDSS, Expanded Disability Status Scale; IQR, interquartile range; MS, multiple sclerosis; RRMS, relapsing-remitting MS; SD, standard deviation; SPMS, secondary progressive MS.
Data for Group 4 (n=7) are not presented.

Supplementary Table 2: Prior-DMTs

Characteristics	Total (N=47)	Group 1 (Wk 5) (n=9)	Group 2 (Wk 10) (n=15)	Group 3 (End of Yr 1) (n=16)
Received prior DMT				
No	14 (29.8)	2 (22.2)	5 (33.3)	5 (31.3)
Yes	33 (70.2)	7 (77.8)	10 (66.7)	11 (68.8)
Last Prior DMT				
None	14 (29.8)	2 (22.2)	5 (33.3)	5 (31.3)
Natalizumab	2 (4.3)	1 (11.1)	0	0
Interferon beta 1a	3 (6.4)	2 (22.2)	1 (6.7)	0
Fingolimod	6 (12.8)	1 (11.1)	3 (20.0)	1 (6.3)
Dimethyl fumarate	8 (17.0)	2 (22.2)	0	4 (25.0)
Teriflunomide	6 (12.8)	0	3 (20.0)	3 (18.8)
Diroximel fumarate	1 (2.1)	0	0	1 (6.3)
Other	7 (14.9)	1 (11.1)	3 (20.0)	2 (12.5)

DMT, disease-modifying therapy.
Data for Group 4 (n=7) are not presented.

SUPPLEMENTARY MATERIAL

Supplementary Table 3A: Levels of immune cell subsets at BL, Wks 5 and 10, and Yr 1 post-CladT initiation in the CSF

	BL (N=8)	Wk 5 (N=8)	Difference (N=8)	p-value	BL (N=12)	Wk 10 (N=12)	Difference (N=12)	p-value	BL (N=12)	Yr 1 (N=12)	Difference (N=12)	p-value
T cells												
%	87.6 [82.7,90.4]	89.1 [84.2,92.5]	−0.65 [−10.5,5.8]	0.64	86.6 [78.7,89.7]	78.9 [76.6,86.5]	2.3 [0.40,8.6]	0.04	84.1 [81.3,87.5]	78.3 [71.2,83.2]	7.6 [0.50,13.4]	**0.01**
Absolute numbers	330.9 [237.5,690.4]	230.0 [113.7,533.1]	146.6 [28.3,348.6]	0.11	244.4 [172.5,1924.5]	118.5 [41.6,257.8]	201.9 [106.8,1775.1]	**0.002**	165.6 [87.2,627.9]	60.6 [21.6,177.3]	121.9 [46.9,445.5]	**0.002**
CD4+ T cells												
%	74.3 [59.1,80.0]	66.6 [57.9,70.0]	1.7 [−3.0,9.5]	0.46	40.5 [25.7,58.1]	19.6 [4.7,42.4]	16.1 [5.4,29.3]	**0.005**	32.0 [7.7,60.2]	14.9 [1.5,33.5]	10.2 [0.83,43.5]	0.06
Absolute numbers	222.9 [133.7,509.2]	161.2 [67.6,340.0]	117.4 [15.2,279.3]	0.08	450.2 [193.5,1618.2]	72.3 [56.6,210.8]	300.8 [136.9,1094.4]	**0.001**	191.8 [72.6,439.7]	38.2 [25.0,135.8]	140.2 [47.9,317.9]	**0.003**
CD4+ Treg cells												
%	6.0 [4.4,12.9]	8.5 [6.7,11.8]	−0.65 [−3.2,2.6]	0.84	6.9 [6.0,9.8]	11.6 [6.8,13.9]	−4.5 [−5.3,0.16]	**0.02**	9.4 [5.5,14.2]	5.6 [4.1,9.5]	0.97 [−1.8,5.1]	0.27
Absolute numbers	14.8 [9.2,24.1]	11.8 [4.7,39.6]	−0.61 [−15.7,10.4]	0.64	31.3 [18.6,102.0]	13.4 [4.6,22.8]	22.5 [5.3,54.5]	**0.005**	14.6 [4.3,37.6]	2.4 [0.70,12.8]	9.6 [4.0,32.0]	**0.002**
B cells												
%	1.08 [0.15,1.5]	0.46 [0.05,1.7]	0.03 [−0.60,0.45]	0.95	2.7 [0.87,3.2]	0.49 [0.26,0.78]	1.8 [0.55,2.1]	**0.001**	1.05 [0.57,1.6]	0.48 [0.06,0.92]	0.52 [−0.03,1.3]	0.15
Absolute numbers	2.4 [1.5,11.3]	1.05 [0.20,11.9]	0.57 [−0.80,1.5]	0.64	12.4 [9.3,65.7]	1.9 [0.86,7.4]	11.8 [5.1,62.6]	**0.001**	6.1 [2.6,17.1]	1.7 [0.63,4.3]	4.3 [−0.40,14.2]	**0.02**
Memory B cells												
%	54.6 [42.8,64.4]	45.3 [28.6,64.2]	−0.45 [−3.6,16.6]	0.95	53.6 [29.6,72.6]	56.1 [31.3,73.1]	−0.50 [−15.3,14.5]	0.83	42.7 [30.9,52.9]	31.3 [14.4,42.5]	14.1 [5.5,20.6]	**0.01**
Absolute numbers	3.0 [1.4,9.2]	1.2 [0.45,7.9]	1.5 [0.36,2.6]	0.02	5.1 [1.4,24.9]	1.6 [0.45,3.1]	4.8 [0.70,25.0]	**0.005**	3.0 [1.02,9.5]	0.22 [0.12,1.5]	2.8 [0.19,6.5]	**0.002**
Monocytes												
%	1.2 [0.37,2.9]	1.9 [0.82,2.7]	−0.47 [−2.0,0.57]	0.46	2.8 [0.64,7.1]	0.86 [0.28,2.8]	0.62 [−0.19,4.2]	0.08	0.80 [0.30,4.2]	1.2 [0.10,3.9]	−0.25 [−1.8,2.6]	0.97
Absolute numbers	4.6 [0.58,11.9]	3.4 [1.9,10.7]	0.74 [−1.9,7.9]	0.64	11.8 [3.9,20.1]	2.1 [1.5,4.0]	7.8 [1.9,16.8]	**0.01**	3.9 [2.1,9.7]	2.3 [1.5,7.7]	1.2 [−1.4,8.2]	0.30

BL, baseline; CladT, cladribine tablets; CSF, cerebrospinal fluid; Q, quartile; Treg, T regulatory cell; Wk, week; Yr, year.
Values are presented as median [Q1, Q3]. Absolute cell counts are in cells/mL.

SUPPLEMENTARY MATERIAL

Supplementary Table 3B: Levels of immune cell subsets at BL, Wks 5 and 10, and Yr 1 post-CladT initiation in the blood

	BL (N=46)	Wk 5 (N=46)	Difference (N=46)	p-value	BL (N=46)	Wk 10 (N=46)	Difference (N=46)	p-value	BL (N=44)	Yr 1 (N=44)	Difference (N=44)	p-value
T cells												
%	38.4 [27.6,59.0]	31.8 [15.8,49.1]	0.50 [−7.2,16.1]	0.19	38.4 [27.6,59.0]	29.9 [17.2,54.9]	6.6 [−0.50,20.7]	**0.003**	40.4 [27.0,60.1]	28.1 [20.9,44.9]	5.2 [−4.6,20.5]	**0.01**
Absolute numbers	694.9 [453.4,927.6]	305.9 [194.8,578.3]	305.7 [168.6,497.5]	**<0.001**	694.9 [453.4,927.6]	284.4 [201.2,474.6]	262.6 [89.7,496.6]	**<0.0001**	722.8 [500.4,935.4]	346.1 [252.7,555.0]	334.1 [97.1,525.9]	**0.0001**
CD4+ T cells												
%	63.6 [56.4,71.4]	63.6 [54.1,72.7]	−1.00 [−4.4,4.1]	0.76	63.6 [56.4,71.4]	61.2 [50.2,72.9]	3.5 [−0.80,9.0]	**0.004**	63.6 [56.4,72.1]	61.5[53.2,71.2]	2.9 [−2.1,7.1]	0.11
Absolute numbers	455.9 [291.2,585.4]	213.3 [123.4,407.9]	232.1 [95.4,325.8]	**<0.001**	455.9 [291.2,585.4]	186.5 [114.3,275.3]	187.8 [50.1,337.0]	**<0.0001**	470.3 [322.1,589.6]	231.0 [159.2,347.6]	223.2 [45.5,345.1]	**<0.0001**
CD4+ Treg cells												
%	7.4 [5.7,9.4]	10.8 [7.3,14.2]	−4.4 [−6.4,−1.2]	**<0.001**	7.4 [5.7,9.4]	11.3 [10.0,16.7]	−5.3 [−7.9,−2.5]	**<0.0001**	7.4 [5.3,10.0]	9.6 [7.1,11.3]	−1.6 [−3.6,0.08]	**0.001**
Absolute numbers	31.0 [20.7,40.9]	24.2 [12.0,33.1]	8.5 [−1.1,21.1]	0.07	31.0 [20.7,40.9]	26.0 [13.0,37.4]	5.5 [−5.5,14.7]	0.12	32.1 [22.6,42.9]	23.0 [12.9,30.1]	14.0 [1.4,19.7]	**0.01**
B cells												
%	14.1 [7.8,24.4]	1.09 [0.16,6.1]	7.5 [1.1,17.6]	**<0.001**	14.1 [7.8,24.4]	2.6 [0.55,9.5]	11.2 [4.7,18.0]	**<0.0001**	15.0 [7.8,24.6]	11.4 [3.1,23.1]	0.60 [−3.4,9.6]	0.56
Absolute numbers	114.3 [48.2,162.3]	7.9 [4.0,29.0]	109.2 [31.6,153.1]	**<0.001**	114.3 [48.2,162.3]	14.1 [5.2,29.1]	114.6 [38.8,191.9]	**<0.0001**	116.5 [51.6,182.5]	87.5 [20.1,202.4]	28.5 [−38.0,60.4]	0.16
Memory B cells												
%	5.2 [2.2,11.0]	10.2 [4.0,20.3]	−3.1 [−10.1,0.58]	**0.001**	5.2 [2.2,11.0]	3.7 [1.2,9.5]	1.3 [−1.4,4.4]	0.31	4.8 [2.0,11.2]	2.2 [1.2,3.9]	2.5 [0.02,7.3]	**0.0001**
Absolute numbers	4.9 [1.3,17.1]	0.87 [0.26,1.7]	3.9 [0.94,15.5]	**<0.001**	4.9 [1.3,17.1]	0.47 [0.15,1.8]	4.7 [1.2,17.3]	**<0.0001**	5.2 [1.3,17.7]	1.7 [0.42,3.5]	4.4 [0.23,15.5]	**<0.0001**
Monocytes												
%	84.6 [70.0,90.1]	73.4 [57.6,89.9]	3.9 [−3.2,24.1]	0.02	84.6 [70.0,90.1]	83.0 [66.0,89.0]	2.2 [−2.0,9.4]	0.22	85.6 [71.9,90.1]	82.7 [66.3,88.4]	2.1 [−6.4,12.4]	0.19
Absolute numbers	103.0 [69.2,184.6]	23.2 [6.1,98.9]	61.3 [31.1,115.0]	**<0.001**	103.0 [69.2,184.6]	97.5 [30.3,242.8]	14.5 [−43.4,74.1]	0.42	113.5 [69.2,185.1]	105.2 [73.9,178.3]	5.3 [−59.6,61.8]	0.70

BL, baseline; CladT, cladribine tablets; Q, quartile; Treg, T regulatory cell; Wk, week; Yr, year.
Values are presented as median [Q1, Q3]. Absolute cell counts are in cells/μL.

SUPPLEMENTARY MATERIAL

Supplementary Table 4: Efficacy outcomes at Yr 1

Characteristics	BL (N=44)	Yr 1 (N=44)	Difference (N=44)	p-value
EDSS	2.3 [1.5, 4.0]	2.3 [1.5, 3.5]	0.00 [−0.50, 0.50]	0.84
Number of Gd+ lesions	0.00 [0.00, 1.5]	0.00 [0.00, 0.00]	0.00 [0.00, 1.00]	0.57
Number of T2 lesions	16.5 [10.0, 27.0]	15.5 [7.5, 24.0]	1.00 [−1.00, 4.0]	**0.005**
Volume of reported lesions (mm³)	2112.0 [807.0, 4507.0]	2044.5 [720.0, 4363.0]	0.50 [−273.0, 253.0]	0.11
Thalamic volume (mm³)	12880.2 [11766.5, 14349.3]	12881.6 [11815.2, 14543.8]	1.4 [−525.0, 397.0]	0.89
Cerebral white matter volume (mm³)	433633.0 [392122.0, 470249.0]	428853.0 [401156.0, 463603.0]	3432.5 [−1384.0, 7892.5]	**0.004**
Cerebral grey matter volume (mm³)	451320.0 [427260.4, 508932.2]	448250.1 [419667.6, 488886.1]	4250.2 [−665.7, 10508.1]	0.45
Estimated total intracranial volume (mm³)	1392984.0 [1316168.0, 1478139.0]	1405288.0 [1345886.0, 1473240.0]	2016.5 [−9814.5, 14353.5]	0.71
Sub−cortical grey matter volume (mm³)	53208.0 [48051.0, 58556.0]	53638.0 [49144.0, 56893.0]	66.5 [−876.0, 1501.5]	**0.01**

CladT, cladribine tablets; EDSS, Expanded Disability Status Scale; Gd+, gadolinium-enhancing; IQR, interquartile range; Yr, year.
Values presented as Median [IQR], p-value: Wilcoxon Signed Rank Test. At baseline N=44 as 3 patients dopped out of the study prior to Yr 1 (lost to follow-up [n=1], unable to continue CladT in Yr 2 due to low lymphocyte count [n=2]). Baseline data not available for all patients.
Missing values (n= number of patients): number of T2 lesions (n=2), volume of reported lesions (n=2), thalamic volume (n=1), cerebral white matter volume (n=1), cerebral grey matter volume (n=1), estimated total intracranial volume (n=1), sub-cortical grey matter volume (n=1).

SUPPLEMENTARY MATERIAL

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