Effect of Cladribine Tablets on CSF Immune Cell Subsets in Patients with Relapsing Multiple Sclerosis (CLOCK-MS study)

Gregory F. Wu, Amjad Samara, Bradley Judge, Amber Salter, Samantha Lancia, Claudia Cantoni, Roman Smirnov, Kenneth Lee, Rea Agnihotri, Dana C. Perantie, Dmitriy A. Yablonskiy, Alexander Sukstanskii, Robert C. Axtell, *Julie Korich, Christina Caon,* Brian T. Edelson, Maxim N. Artyomov, Gabriel Pardo, Olaf Stuve, Ahmed Z. Obeidat, Amit Bar-Or, Matthew R. Brier, and Anne H. Cross

This work was sponsored by EMD Serono (CrossRef Funder ID: 10.13039/100004755). Authors in italics are employees of the healthcare business of Merck KGaA, Darmstadt, Germany, or its affiliates. Please scan the QR code below for full affiliations.

SCAN FOR POSTER PDF
For personal use only and may not be reproduced without written permission of the authors

RESEARCH IN CONTEXT

1. **CLOCK-MS supports the central and peripheral effects of cladribine tablets (CladT) in patients with multiple sclerosis, via reductions in pathological B and T cell subsets, and inflammation and neurodegeneration markers.**
2. **The findings of this study highlight the value of integrating profiles of cereborspinal fluid (CSF) and blood to fully elucidate the mechanism of action of CladT. The continued depletion and slower reconstitution of immune cells in the CSF may have contributed to its therapeutic effect.**

OBJECTIVES

To assess changes in cerebrospinal fluid (CSF) and blood levels of leukocyte subsets and neuronal injury markers up to 2-years post-cladribine tablets (CladT) treatment in patients with relapsing multiple sclerosis (RMS) from the US-based CLOCK-MS study.

INTRODUCTION

Previous studies[1,2] demonstrated the impact of CladT on CSF immune cells and proteins, highlighting its overall role in regulating immune cells involved in MS immunopathogenesis. However, the extent to which CladT impacts the composition of immune cells has not been determined.

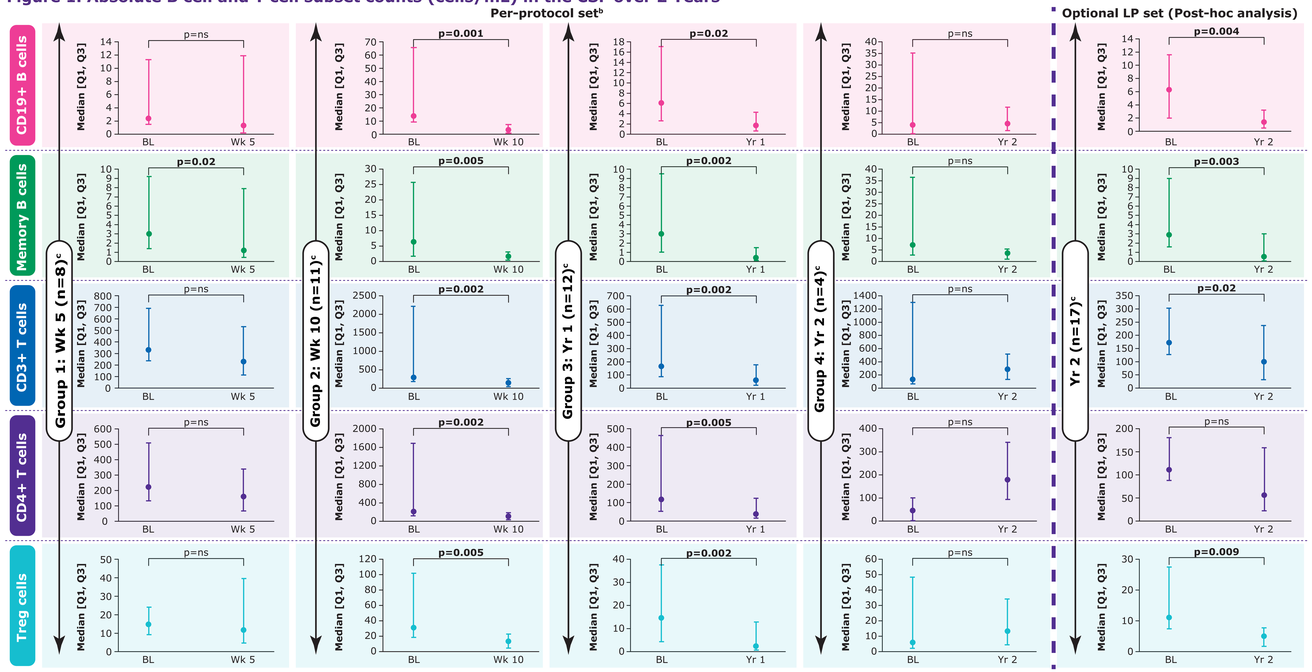
The CLOCK-MS study (CladT: Collaborative Study to Evaluate Impact on central nervous system [CNS] Biomarkers in multiple sclerosis [MS]) is an open-label, randomized, multicenter, Phase 4 study in patients with RMS aimed at enhancing the understanding of the action of CladT in the CNS (NCT03963375).

METHODS

- 47 patients from 5 sites in the US were randomly assigned in a 1:2:2:1 ratio to undergo a lumbar puncture (LP) at baseline (BL), and at either Week 5 (Wk), Wk 10, Year (Yr) 1, or Yr 2 after starting CladT and all patients had blood drawn at all timepoints (**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 through Yr 2 were analyzed using a non-parametric Wilcoxon signed rank test.
- **Post-hoc analysis:** Patients undergoing LP at Wk 5, Wk 10, and Yr 1 were also given the option to volunteer for a third, optional LP at the end of Year 2 (**Supplementary Figure 1**). The post-hoc analysis was performed in all patients with a Yr 2 LP (i.e Group 4 + optional LPs).

RESULTS

Figure 1. Absolute B cell and T cell subset counts (cells/mL) in the CSF over 2 Years[a]



[a]p-values are represented as Signed Rank test for the median difference. Bold values indicate statistical significance at a level of 0.05. [b]Randomized for LP per protocol (**Supplementary Figure 1**). [c]'n' numbers at the respective BL and time points are same.
BL, baseline; CD, cluster of differentiation; CSF, cerebrospinal fluid; LP, lumbar puncture; ns, not significant; Q, quartile; Treg, T regulatory; Wk, week; Yr, year.
Note: At each timepoint the total number reflects the number of patients randomized minus the number of patients with missing data.
Please refer to **Supplementary Table 1** for baseline demographics and disease characteristics, and **Supplementary Table 2** for details on prior disease-modifying therapies.

CSF - Per-protocol set:

- Statistically significant reductions in CD19+ B cells, CD3+ T cells, CD4+ T cells and Treg cells were observed at Wk 10 and Yr 1 (vs. BL). Similarly, significant reductions in memory B cells were observed at Wks 5 and 10, and Yr 1 (vs. BL) (**Figure 1**; **Supplementary Table 3**. Please refer **Supplementary Table 4** for percentage changes in immune cell subtypes).
- Neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) levels significantly decreased at Yr 1 and Wk 10, respectively. Numerical decreases in kappa free light chain (kFLC) index levels were observed at Wks 5, 10 and Yr 2 (**Table 1**).

CSF - Optional LP at Yr 2 (Post-hoc analysis):

- Statistically significant reductions in CD19+ B cells, memory B cells, CD3+ T cells, Treg cells, and NfL and GFAP levels were observed at Yr 2 (vs BL) (**Figure 1**; **Table 1**).

Note: Please refer to **Supplementary Table 5** for changes in the number of CSF oligoclonal bands over 2 yrs post-CladT initiation.
Supplementary Tables 6, 7 and 8 for changes in blood immune cell subsets and changes in serum biomarkers.

Table 1. Changes in CSF biomarkers over 2 Years

Timepoints[a]	kFLC index; median [Q1,Q3]	NfL (pg/mL); median [Q1,Q3]	GFAP (pg/mL); median [Q1,Q3]
Per-protocol set			
BL (n=8)	11.9 [6.1,76.7]	1250.0 [536.0,1550.0]	4510.0 [4200.0,9470.0]
Wk 5 (n=8)	11.1 [5.9,53.5]	988.0 [913.0,1240.0]	5620.0 [3450.0,11600.0]
Difference[b]	−0.81 [−1.9,0.16]	7.0 [−310.0,260.0]	900.0 [−1060.0,1420.0]
p-value	0.30	0.81	0.58
BL (n=11)	29.0 [16.8,126.3]	1090.0 [543.0,1310.0]	6070.0 [3970.0,7810.0]
Wk 10 (n=11)	27.5 [18.7,88.8]	850.0 [663.0,1080.0]	3490.0 [2310.0,4880.0]
Difference[b]	−5.0 [−18.0,3.4]	−91.0 [−512.0,9.0]	−1040.0 [−3660.0,−480.0]
p-value	0.10	0.07	**0.002**
BL (n=12)	20.2 [5.5,53.8]	928.0 [535.0,1410.0]	5050.0 [1700.0,8210.0]
Yr 1 (n=12)	26.6 [4.1,41.1]	419.0 [300.0,882.0]	4220.0 [2810.0,6810.0]
Difference[b]	−0.53 [−9.8,4.6]	−294.0 [−991.0,−129.0]	−440.0 [−1200.0,560.0]
p-value	0.52	**0.01**	0.35
BL (n=4)	20.8 [9.5,53.2]	1000.0 [926.0,1000.0]	10100.0 [4680.0,10500.0]
Yr 2 (n=4)	18.7 [8.6,65.9]	627.0 [608.0,936.0]	5730.0 [3620.0,6020.0]
Difference[b]	−0.91 [−2.1,−0.84]	−299.0 [−609.0,−4.0]	−4370.0 [−4400.0,−1060.0]
p-value	0.63	0.19	0.13
Optional LP set (Post-hoc analysis)			
BL (n=18)[c]	14.8 [9.5,36.9]	927.0 [543.0,1090.0]	6720.0 [4510.0,10100.0]
Yr 2 (n=18)	13.9 [8.6,22.8]	492.0 [391.0,793.0]	5105.0 [3310.0,6520.0]
Difference[b]	−1.7[−7.0,−0.56]	−243.0[−690.0,15.0]	−1030.0[−2010.0,−170.0]
p-value	0.11	**0.005**	**0.008**

[a]At each timepoint the total number (n) reflects the number of patients randomized minus the number of patients with missing data. [b]Differences were calculated for each individual person then the median were calculated for all the results. [c]missing patients: kFLC and GFAP, n=2; NfL, n=1.
BL, baseline; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; kFLC, kappa free light chain; LP, lumbar puncture; NfL, neurofilament light chain; Q, quartile; Wk, week; Yr, year.

CONCLUSIONS

- **Significant reductions in CSF immune cell subsets were seen following CladT therapy for RMS at Wk 10, Yr 1 and at Yr 2 post-hoc analysis performed on a larger cohort.**
- **Significant reductions in CSF biomarkers including NfL and GFAP were observed during the Yr 2 post-hoc analysis.**

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 (0037).
3. MAVENCLAD. US Prescribing information. EMD Serono, Inc; 2024.

Presented at the Consortium of Multiple Sclerosis Centers (CMSC) | May 27–29, 2026 | Charlotte, NC, US

SUPPLEMENTARY MATERIAL

Author Affiliations

Gregory F. Wu[1], Amjad Samara[1], Bradley Judge[1], Amber Salter[2], Samantha Lancia[2], Claudia Cantoni[3], Roman Smirnov[1], Kenneth Lee[1], Rea Agnihotri[1], Dana C. Perantie[1], Dmitriy A. Yablonskiy[1], Alexander Sukstanskii[1], Robert C. Axtell[4], Julie Korich[5], Christina Caon[5], Brian T. Edelson[1], Maxim N. Artyomov[1], Gabriel Pardo[4], Olaf Stuve[2], Ahmed Z. Obeidat[6], Amit Bar-Or[7], Matthew R. Brier[1], and Anne H. Cross[1]

[1]Washington University in St. Louis School of Medicine, MO, USA; [2]UT Southwestern Medical Center, Dallas, TX, USA; [3]Barrow Neurological Institute, Phoenix, AZ, USA [4]Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; [5]EMD Serono, Boston, MA, USA; [6]Medical College of Wisconsin, Milwaukee, WI, USA; [7]University of Pennsylvania School of Medicine, PA, USA.

Author disclosures

GFW has received consulting fees from Genentech and Sangamo, research support from Biogen, EMD Serono, and Genentech, and speaker fees from EMD Serono.

AS, BJ, SL, CC, RS, KL, RA, DCP, DAY, ASu, and **MNA** have nothing to disclose.

ASa has received research funding from CMSC, NMSS, and the US DOD and is a member of the editorial board for *Neurology.* She serves as a consultant for Gryphon Bio LLC, Sora Neuroscience, and Abata Therapeutics. She has equity in Owl Therapeutics. She is a member of the DSMB for PREMOD2, CAVS-MS, and MARCH.

RCA has consulted for EMD Serono, Biogen, and Roche and is on the advisory board for Progentec Diagnostics Inc.

JK was an employee of EMD Serono, Boston, MA, USA at the time of the study.

CC is an employee of EMD Serono, Boston, MA, USA.

BTE has received research funding from the NMSS.

GP has received speaker honoraria and/or consulting fees from Alexion, Biogen, Celgene/BMS, EMD Serono, Horizon/Amgen, Novartis, Roche/Genentech, Sanofi-Genzyme and TG Therapeutics; received research support (to the institution) from Abbvie, Adamas, Alkermes, Biogen, EMD Serono, Roche/Genentech, Sanofi-Genzyme, Novartis, and Teva, and is on the advisory board for Progentec Diagnostics and Cadenza Bio.

OS is funded by a Merit Review grant (FAIN: BX005664-01) from the US Department of Veterans Affairs, Biomedical Laboratory Research and Development, and receives grant support from EMD Serono, Exalys and Sanofi-Genzyme. He serves on the editorial board of Therapeutic Advances in Neurological Disorders, has served on Data Monitoring Committee for Pfizer, Roche/Genentech, and TG Therapeutics without monetary compensation, and has advised Celgene/BMS, EMD Serono, Roche/Genentech, Sanofi-Genzyme, and TG Therapeutics.

AZO received speaking or consulting fees from Alexion, Banner Life Sciences, Biogen, Biologix, Celgene/BMS, EMD Serono, Roche/Genentech, GW Pharma, Horizon/Amgen, Jazz Pharma, Novartis, Sanofi-Genzyme, Sandoz Pharmaceuticals, TG Therapeutics, and VielaBio as well as honoraria from Medscape and MJH Life Sciences.

AB-O has received fees for advisory board participation and/or consulting from Accure, Atara Biotherapeutics, Biogen, Celgene/BMS, GlaxoSmithKline, Gossamer, Janssen/Actelion, Medimmune, EMD Serono, Novartis, Roche/Genentech, and Sanofi-Genzyme as well as received grant support to the University of Pennsylvania from Biogen, Novartis and Roche/Genentech.

MRB has received consulting honoraria from Biogen, BMS, EMD Serono, Roche/Genentech, Novartis, Sanofi, and TG Therapeutics. He receives funding from the NIH and CMSC.

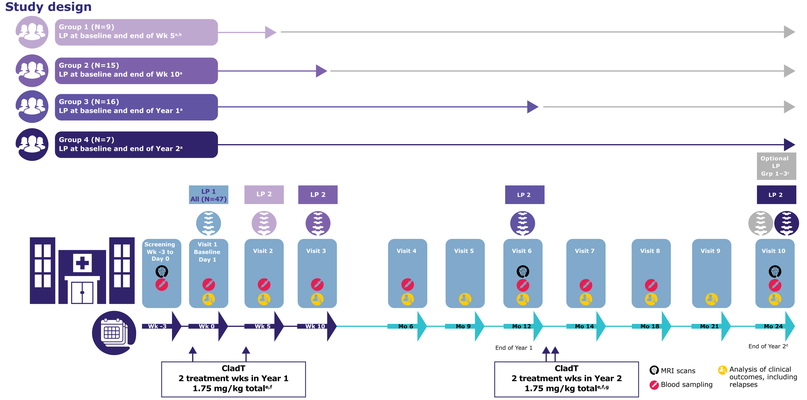
AHC has received fees for advisory board participation and/or consulting for Biogen, Bristol Myers Squibb, EMD Serono, F. Hoffmann-La Roche Ltd, Genentech, Horizon Pharmaceuticals, Janssen, Novartis, Octave, Sanofi-Genzyme, and TG Therapeutics.

Acknowledgements

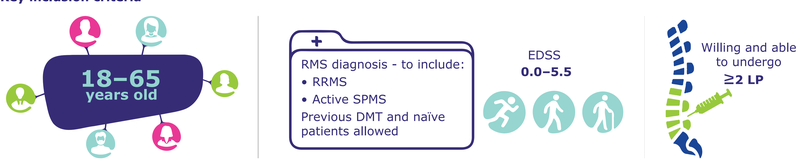
This study was sponsored by EMD Serono (CrossRef Funder ID: 10.13039/100004755). Medical writing support was provided by Ramya T of Merck Specialities Pvt. Ltd., Bengaluru, India, an affiliate of Merck KGaA, Darmstadt, Germany.

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

[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[c].

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

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.

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 and serum GFAP

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

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

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

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Baseline demographics and disease characteristics

Characteristics	Total (N=47)
Age, years, mean±SD	42.9±12.3
Female, n (%)	28 (59.6)
Race, n (%)	
White or Caucasian	39 (83.0)
Black or African American	6 (12.8)
Asian (all types)	2 (4.3)
Ethnicity, n (%)	
Hispanic or Latino	2 (4.3)
Non-Hispanic or non-Latino	45 (95.7)
MS type, n (%)	
RRMS	44 (93.6)
SPMS	3 (6.4)
Disease duration from MS diagnosis, years, median (IQR)	6.0 (1.0,17.0)
EDSS, median (IQR)	2.0 (1.5,4.0)

EDSS, Expanded Disability Status Scale; IQR, interquartile range; MS, multiple sclerosis; RRMS, relapsing-remitting MS; SD, standard deviation; SPMS, secondary progressive MS.

Supplementary Table 2. Prior DMTs

Characteristics	Total (N=47)
Received prior DMT, n (%)	
No	14 (29.8)
Yes	33 (70.2)
Last Prior DMT, n (%)	
None	14 (29.8)
Natalizumab	2 (4.3)
Interferon beta-1a	3 (6.4)
Fingolimod	6 (12.8)
Dimethyl fumarate	8 (17.0)
Teriflunomide	6 (12.8)
Diroximel fumarate	1 (2.1)
Other	7 (14.9)

DMT, disease-modifying therapy.

Supplementary Table 3. CSF levels of immune cell subsets at BL, Wk 5, Wk 10, Yr 1 and Yr 2 post-CladT initiation

	Per-protocol set															Optional LP set (Post-hoc analysis)			
Absolute numbers (cells/mL) median [Q1,Q3]	**Baseline (n=8)**	**Week 5 (n=8)**	**Difference[a]**	**p-value**	**Baseline (n=11)**	**Week 10 (n=11)**	**Difference[a]**	**p-value**	**Baseline (n=12)**	**Year 1 (n=12)**	**Difference[a]**	**p-value**	**Baseline (n=4)**	**Year 2 (n=4)**	**Difference[a]**	**p-value**	**Baseline (n=17)**	**Year 2 (n=17)**	**Difference[a]**	**p-value**
CD19+ B cells	2.4 [1.5,11.3]	1.05 [0.20,11.9]	−0.57 [−1.5,0.80]	0.64	12.4 [9.3,65.7]	1.9 [0.86,7.4]	−11.8 [−62.6,−5.1]	**0.001**	6.1 [2.6,17.1]	1.7 [0.63,4.3]	−4.3 [−14.2,0.40]	**0.02**	3.9 [0.24,35.2]	4.6 [1.6,11.7]	−0.86 [−30.6,8.5]	0.63	6.3 [2.0,11.6]	1.4 [0.50,3.2]	−2.9 [−9.2,−0.83]	**0.004**
Memory B cells	3.0 [1.4,9.2]	1.2 [0.45,7.9]	−1.5 [−2.6,−0.36]	**0.02**	6.4[1.7,25.7]	1.6 [0.45,3.1]	−4.8 [−25.0,−0.70]	**0.005**	3.0 [1.02,9.5]	0.22 [0.12,1.5]	−2.8 [−6.5,−0.19]	**0.002**	7.2 [2.8,36.5]	3.7 [1.07,5.4]	−1.9 [−32.8,−0.05]	0.25	2.9 [1.6,9.0]	0.43 [0.07,3.0]	−1.7 [−3.7,−0.75]	**0.003**
CD3+ T cells	330.9 [237.5,690.4]	230.0 [113.7,533.1]	−146.6 [−348.6,−28.3]	0.11	263.1 [180.1,2219.8]	118.5 [41.6,257.8]	−201.9 [−1775.1,−106.8]	**0.002**	165.6 [87.2,627.9]	60.6 [21.6,177.3]	−121.9 [−445.5,−46.9]	**0.002**	134.4 [65.1,1300.8]	287.2 [134.2,517.0]	−0.26 [−1116.2,401.4]	0.88	172.0 [126.8,303.0]	100.0 [31.6,236.8]	−111.8 [−248.0,47.3]	**0.02**
CD4+ T cells	222.9 [133.7,509.2]	161.2 [67.6,340.0]	−117.4 [−279.3,−15.2]	0.08	182.9 [120.1,1689.0]	67.3 [34.8,186.7]	−137.0 [−1388.5,−87.1]	**0.002**	117.5 [52.7,463.2]	38.3 [16.1,123.9]	−84.2 [−336.9,−32.3]	**0.005**	45.5 [1.4,100.9]	179.1 [94.0,340.9]	135.0 [−6.9,339.5]	0.38	111.1 [88.2,181.0]	55.9 [22.1,159.6]	−89.1 [−173.9,16.6]	**0.08**
CD4+ Treg cells	14.8 [9.2,24.1]	11.8 [4.7,39.6]	0.61 [−10.4,15.7]	0.64	31.3 [18.6,102.0]	13.4 [4.6,22.8]	−22.5 [−54.5,−5.3]	**0.005**	14.6 [4.3,37.6]	2.4 [0.70,12.8]	−9.6 [−32.0,−4.0]	**0.002**	6.0 [2.1,48.4]	13.3 [4.4,34.2]	3.9 [−42.2,30.3]	1.00	11.1 [7.4,27.5]	5.0 [1.7,7.7]	−8.4 [−19.4,−4.7]	**0.009**

[a]Differences are calculated as baseline minus the follow-up time point.
BL, baseline; CD, cluster of differentiation; CladT, cladribine tablets; CSF, cerebrospinal fluid; LP, lumbar puncture; Treg, T regulatory; Wk, week; Yr, year.
Note: At each timepoint the total number (n) reflects the number of patients randomized minus the number of patients with missing data.

Supplementary Table 4. Percent change from baseline in immune cell subsets post-CladT initiation in CSF

	CSF				
	Per-protocol set				Optional LP set (Post-hoc analysis)
Percentage change from baseline, median [Q1,Q3]	**Week 5 (n=8)**	**Week 10 (n=11)**	**Year 1 (n=12)**	**Year 2 (n=4)**	**Year 2 (n=17)**
CD19+ B cells	−44.5% [−83.8,−8.6]	−83.5% [−95.1,−79.9]	−83.0% [−90.8,36.1]	−46.7% [−84.0,4273.2]	−84.9% [−91.7,−39.6]
Memory B cells	−60.7% [−67.5,−42.1]	−75.4% [−91.1,−18.6]	−85.5% [−94.1,−44.9]	−52.5% [−80.5,−20.1]	−81.7% [−94.5,−52.3]
CD3+ T cells	−43.8% [−69.7,8.5]	−76.8% [−83.8,−68.0]	−69.2% [−83.0,−37.7]	4.5% [−82.0,9941.0]	−69.1% [−86.3,32.1]
CD4+ T cells	−46.4% [−69.7,0.11]	−78.3% [−83.6,−67.3]	−62.5% [−83.7,−41.8]	86.1%[a] [−78.9,17011.1]	−68.5%[b] [−85.2,6.5]
CD4+ Treg cells	6.6% [−70.9,62.6]	−63.7% [−84.8,−22.1]	−72.0% [−88.3,−49.9]	32.8% [−82.9,49686.7]	−78.2% [−91.6,−45.8]

CD, cluster of differentiation; CladT, cladribine tablets; CSF, cerebrospinal fluid; LP, lumbar puncture; Treg, T regulatory.
Note: At each timepoint the total number (n) reflects the number of patients randomized minus the number of patients with missing data. [a]n=3; [b]n=16.

Supplementary Table 5. Changes in CSF OCBs in Yr 1 and Yr 2 post-CladT initiation

	Per-protocol set				Optional LP set (Post-hoc analysis)	
	Baseline (n=11)	**Year 1 (n=11)**	**Baseline (n=5)**	**Year 2 (n=5)**	**Baseline (n=18)**	**Year 2 (n=18)**
OCBs (IgG) present or absent in the CSF						
Absent, n (%)	0 (0.0)	1 (9.1)	—	—	—	—
Present, n (%)	11 (100.0)	10 (90.9)	5 (100.0)	5 (100.0)	18 (100)	18 (100)
Number of bands[a]						
2	—	—	0 (0.0)	1 (20.0)	—	3 (16.7)
3	0 (0.0)	1 (10.0)	—	—	—	1 (5.6)
4	1 (10.0)	0 (0.0)	0 (0.0)	1 (20.0)	—	1 (5.6)
>5	9 (90.0)	9 (90.0)	5 (100.0)	3 (60.0)	18 (100)	13 (72.2)

There was 1 patient who had OCB present = Yes at baseline, and OCB = Absent at Year 1, but the number of bands was not noted when present at baseline.
CSF, cerebrospinal fluid; IgG, immunoglobulin G; LP, lumbar puncture; OCBs, oligoclonal bands; Yr, year.

SUPPLEMENTARY MATERIAL

Supplementary Table 6. Blood levels of immune cell subsets at BL, Wk 5, Wk 10, Yr 1 and Yr 2 post-CladT initiation

Per-protocol set															
Absolute numbers (cells/mL) median [Q1,Q3]	**Baseline (n=36)**	**Week 5 (n=36)**	**Difference[a]**	**p-value**	**Baseline (n=36)**	**Week 10 (n=36)**	**Difference[a]**	**p-value**	**Baseline (n=39)**	**Year 1 (n=39)**	**Difference[a]**	**p-value**	**Baseline (n=37)**	**Year 2 (n=37)**	**Difference[a]**	**p-value**
CD19+ B cells	122.4 [49.9,172.4]	7.9 [4.0,23.3]	−109.6 [−154.4,−37.5]	**<0.0001**	116.5 [48.2,198.8]	14.7 [5.8,39.2]	−114.4 [−172.9,−32.2]	**<0.0001**	128.3 [51.6,182.5]	85.3 [39.8,159.2]	−28.5 [−59.6,18.5]	0.07	114.3 [51.6,198.8]	97.3 [62.5,137.1]	−20.3 [−59.5,45.7]	0.46
Memory B cells	5.6 [1.4,17.4]	0.72 [0.25,1.7]	−4.8 [−16.0,−1.2]	**<0.0001**	5.2 [1.3,20.5]	0.47 [0.19,1.8]	−4.7 [−17.3,−1.2]	**<0.0001**	5.2 [1.3,17.7]	1.5 [0.42,2.7]	−4.4 [−15.5,−0.26]	**<0.0001**	5.2 [1.4,20.5]	0.97 [0.55,2.5]	−4.6 [−16.6,−0.71]	**<0.0001**
CD3+T cells	727.9 [455.9,953.3]	301.3 [186.9,558.4]	−340.7 [−550.8,−194.6]	**<0.0001**	665.0 [444.7,936.3]	291.6 [202.3,493.6]	−263.0 [−520.6,−80.9]	**<0.0001**	722.8 [517.1,936.3]	332.3 [239.6,487.3]	−366.1 [−547.3,−175.0]	**<0.0001**	793.0 [533.8,937.2]	234.6 [155.1,346.7]	−480.0 [−700.8,−269.8]	**<0.0001**
CD4+ T cells	486.6 [295.8,595.1][b]	161.9 [78.0,301.2][b]	−257.4 [−362.9,−116.5]	**<0.0001**	468.4 [284.9,592.3][b]	179.1 [115.2,292.9][b]	−193.7 [−381.9,−48.5]	**<0.0001**	474.1 [327.4,592.3][c]	218.6 [128.6,310.9][c]	−232.0 [−351.9,−128.3]	**<0.0001**	479.0 [332.7,595.1]	116.3 [69.2,181.8]	−335.3 [−444.5,−177.5]	**<0.0001**
CD4+ Treg cells	32.3 [22.6,43.4][b]	22.6 [8.2,31.6][b]	−10.1 [−23.2,0.09]	**0.0001**	30.8 [19.6,43.2][b]	26.0 [13.0,37.3][b]	−5.6 [−18.1,5.1]	**0.05**	32.1 [22.7,43.2][c]	20.6 [10.2,28.9][c]	−14.8 [−19.8,−2.3]	**<0.0001**	33.8 [22.7,43.4]	14.2 [5.3,20.7]	−17.1 [−29.1,−9.8]	**<0.0001**

[a]Differences are calculated as baseline minus the follow-up time point.
BL, baseline; CladT, cladribine tablets; CD, cluster of differentiation; Treg, T regulatory; Wk, week; Yr, year.
Note: At each timepoint the total number (n) reflects the number of patients randomized minus the number of patients with missing data. [b]n=37; [c]n=40.

Supplementary Table 7. Percent change from baseline in immune cell subsets post-CladT initiation peripheral blood

Blood				
Percentage change from baseline, median [Q1,Q3]	**Week 5 (n=37)**	**Week 10 (n=37)**	**Year 1 (n=43)**	**Year 2 (n=40)**
CD19+ B cells	−88.8 [−96.9,−71.4]	−87.2 [−94.2,−76.6]	−27.2 [−53.4,24.0]	−14.5 [−46.7,54.9]
Memory B cells	−90.6 [−96.8,−75.8]	−90.0 [−96.7,−77.3]	−82.3 [−91.1,−55.5]	−74.1 [−92.6,−40.8]
CD3+ T cells	−46.3 [−68.1,−30.6]	−48.9 [−69.5,−13.2]	−52.4 [−61.6,−33.9]	−70.3 [−80.3,−40.1]
CD4+ T cells	−53.5 [−73.3,−33.5]	−53.9 [−72.7,−20.6]	−55.3 [−59.6,−37.0]	−71.8 [−83.1,−58.8]
CD4+ Treg cells	−37.4 [−59.2,0.62]	−24.3 [−49.6,22.6]	−45.1 [−63.8,−13.5]	−55.8 [−82.0,−39.8]

CD, cluster of differentiation; CladT, cladribine tablets; CSF, cerebrospinal fluid; Treg, T regulatory.
Note: At each timepoint the total number (n) reflects the number of patients randomized minus the number of patients with missing data.

Supplementary Table 8. Serum biomarkers at BL, Wk 5, Wk 10, Yr 1 and Yr 2 post-CladT initiation

Per-protocol set																
Cells/mL	**Baseline (n=37)**	**Week 5 (n=37)**	**Difference**	**p-value**	**Baseline (n=35)**	**Week 10 (n=35)**	**Difference**	**p-value**	**Baseline (n=41)**	**Year 1 (n=41)**	**Difference**	**p-value**	**Baseline (n=39)**	**Year 2 (n=39)**	**Difference**	**p-value**
NfL level (pg/mL; median [Q1,Q3])	11.0 [8.3,17.3]	11.8 [8.8,17.5]	0.70 [−0.86,2.6]	0.1	11.0 [7.7,18.1]	11.8 [8.9,14.7]	0.33 [−0.35,2.2]	0.21	10.2 [7.9,17.3]	8.1 [5.9,11.2]	−1.8 [−4.6,0.48]	**0.002**	10.2 [7.7,17.9]	10.2 [7.1,14.0]	−0.22 [−6.2,2.0]	0.23
GFAP level (pg/mL; median [Q1, Q3]	97.0 [74.3,173.0]	114.0 [77.7,156.0]	1.2 [−7.3,18.5]	0.31	97.0 [73.1,170.0]	96.9 [69.1,169.0]	0.75 [−17.8,20.4]	0.97	96.7 [71.4,166.0][a]	97.3 [74.0,146.0][a]	−2.6 [−24.5,17.2]	0.39	98.9 [74.3,170.0]	101.0 [71.1,129.0]	−3.7 [−45.0,11.1]	0.12
NfL Z-Score (mean±SD)	1.22±1.20	1.39±1.23	0.16±0.51	0.06	1.28±1.27	1.37±1.11	0.08±0.62	0.44	1.23±1.19	0.62±1.03	−0.61±1.03	**0.001**	1.23±1.22	0.85±0.83	−0.38±1.2	**0.05**

BL, baseline; CladT, cladribine tablets; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; Q, quartile; SD; standard deviation; Wk, week; Yr, year.
Note: At each timepoint the total number (n) reflects the number of patients randomized minus the number of patients with missing data. [a]n=40.