Early Effects of Cladribine Tablets on Immune and Inflammatory Markers in Central and Peripheral Compartments in Relapsing Multiple Sclerosis

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RESEARCH IN CONTEXT

1. **Reductions in autoreactive immune cells involved in the pathophysiology of multiple sclerosis (MS) and an increase in regulatory T (Treg) cells in the cerebrospinal fluid (CSF) and blood were seen as early as weeks 5 and 10 after the initial dose of cladribine tablets (CladT) in the CLOCK-MS study.**

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 blood and CSF.**

OBJECTIVE

To investigate the impact and relevance of cladribine in the central nervous system (CNS) by assessing changes in the CSF and blood levels of lymphocyte subsets, myeloid cells, neuronal injury markers, and soluble immunological markers in patients with relapsing MS (RMS) during treatment with CladT (at weeks 5 and 10 post-CladT initiation).

INTRODUCTION

Increasing evidence suggests that in RMS, early signs of neuronal degeneration are dependent on inflammatory activity in the CNS. Previous investigations have revealed the effects of disease-modifying therapies on the CSF profile of adaptive and innate immune cells and proteins that reflect different aspects of MS immunopathogenesis.[1]

The mechanisms facilitating the benefit of CladT in RMS, particularly with respect to its effects on compartmentalised inflammation within the CSF, remain unclear.

METHODS

- The CLOCK-MS study (CladT: Collaborative Study to Evaluate Impact on CNS Biomarkers in MS) is an open-label, randomised, multicentre phase 4 study exploring the MoA of CladT in RMS (NCT03963375)
- Overall, 47 patients were recruited across 5 sites and randomised 1:2:2:1 to a lumbar puncture (LP) at baseline (BL) and a second LP at either 5 weeks, 10 weeks, 1 year, or 2 years post-CladT initiation. All patients received CladT (3.5 mg/kg cumulative dose over 2 years) according to the approved United States prescribing information[2]
- CSF samples from patients completing testing at weeks 5 (n=9) and 10 (n=15) post-treatment and blood samples at weeks 5 and 10 from all patients were analysed. Data were analysed using a non-parametric Wilcoxon signed rank test. The data were one-sample and paired for each week's cohort
- Key study endpoints reported here:
  - Changes in **CSF and blood of CD3+ T cells, CD19+ B cells, and lymphoid and myeloid immune cell subsets** from baseline LP to second LP
  - Changes in **CSF neurofilament light chain (NfL) levels** from baseline LP to second LP

Please refer to **Supplementary Figure 1** for study design, and inclusion/exclusion criteria, and **Supplementary Table 1** for detailed study endpoints.

RESULTS

Table 1: Baseline demographics and disease characteristics

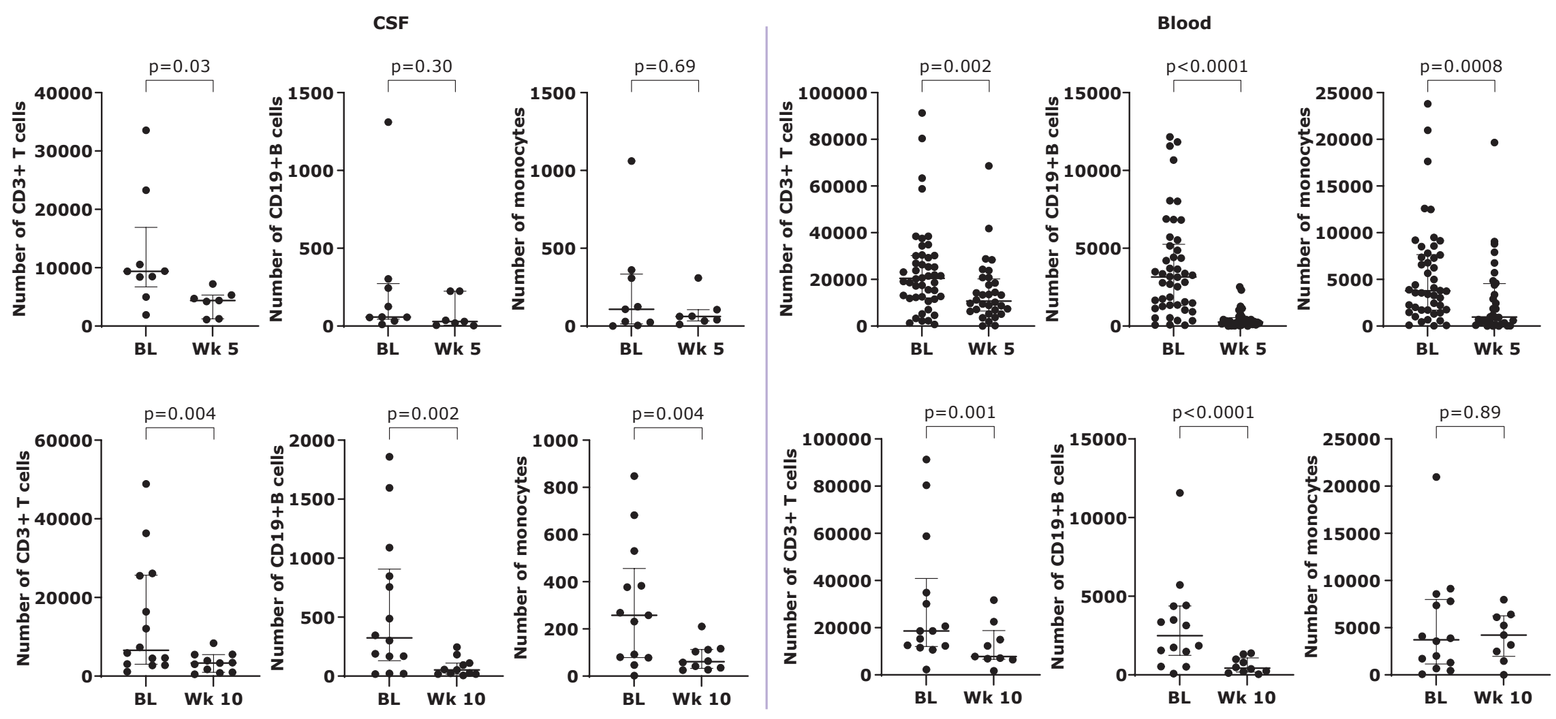
Characteristics	Group 1 (Wk 5 LP) (N=9)	Group 2 (Wk 10 LP) (N=15)
Age, years, mean±SD	45.3±15.6	44.7±11.6
Female, n (%)	6 (66.7)	12 (80.0)
Race, n (%)		
White or Caucasian	8 (88.9)	13 (86.7)
Black or African American	1 (11.1)	2 (13.3)
Ethnicity, n (%)		
Hispanic or Latino	0 (0.0)	1 (6.7)
Non-Hispanic or Latino	9 (100.0)	14 (93.3)
MS type: RRMS, n (%)	9 (100.0)	15 (100.0)
Disease duration, years, mean±SD	11.3±10.9	8.7±9.0
EDSS, median (range)	2.0 (1.5, 3.0)	2.5 (1.5, 4.0)

EDSS, Expanded Disability Status Scale; LP, lumbar puncture; MS, multiple sclerosis; RRMS, relapsing-remitting MS; SD, standard deviation; Wk, week.

Please refer to **Supplementary Table 2** for details on prior disease-modifying therapy use.

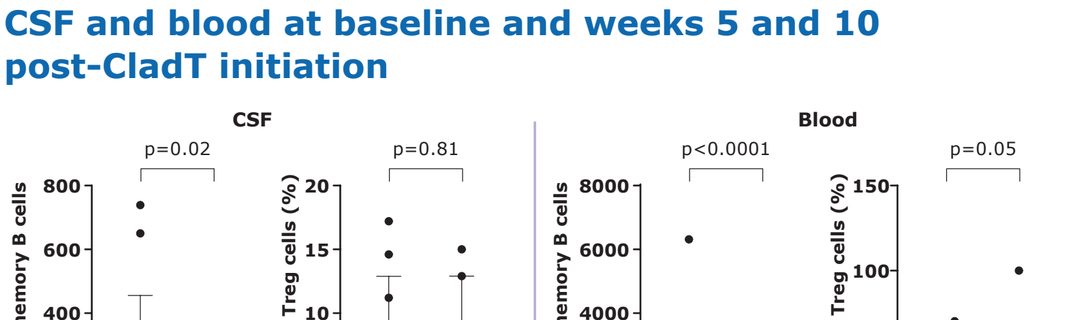
- A significant reduction in the levels of CD3+ T cells was observed at weeks 5 and 10 (vs baseline; p<0.05) post-CladT initiation in the CSF and blood (**Figure 1**)
- CD19+ B cells in the blood were significantly reduced at weeks 5 and 10 (vs baseline; p<0.0001) post-CladT therapy, whereas significant reductions in CSF CD19+B cells were observed only at week 10 (p=0.002; **Figure 1**)
- Among additional immune cell changes observed in the CSF at week 10, there were significant reductions in the numbers of monocytes (p=0.004) and memory B cells (p=0.004), along with an increase in the proportion of Treg cells (p=0.05; **Figures 1** and **2**)
- The number of monocytes in blood were also reduced at week 5 (p=0.0008) followed by recovery at week 10 (p=0.89; **Figure 1**)
- No significant changes in CSF NfL levels were observed at weeks 5 and 10 post-CladT treatment vs baseline (**Figure 3**)

Figure 1: Levels of CD3+ T cells, CD19+ B cells and myeloid cells at baseline and weeks 5 and 10 post-CladT initiation in the CSF and blood



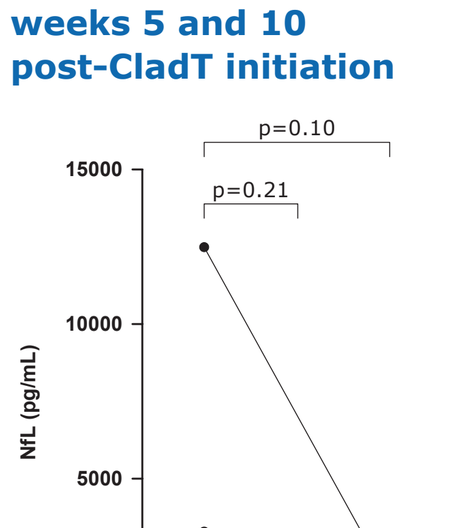
BL, baseline; CladT, cladribine tablets; CSF, cerebrospinal fluid; Wk, week.

Figure 2: Levels of memory B cells and Treg cells in CSF and blood at baseline and weeks 5 and 10 post-CladT initiation



BL, baseline; CladT, cladribine tablets; CSF, cerebrospinal fluid; Treg, regulatory T cell; Wk, week.

Figure 3: CSF NfL levels at baseline and weeks 5 and 10 post-CladT initiation



	BL	Wk 5	Wk 10
NfL (pg/mL), mean±SD	2062.37± 3016.58	1402.39± 725.04	819.69± 487.11

BL, baseline; CladT, cladribine tablets; CSF, cerebrospinal fluid; NfL, neurofilament light chain; Wk, week.

CONCLUSIONS

- **Overall, CladT exerts early effects in the CNS compartment and in the periphery, including reductions in B and T cells, which may contribute to its therapeutic benefit in RMS**

SCAN FOR AFFILIATIONS, DISCLOSURES AND SUPPLEMENTARY MATERIALS

Reference: 1. Novakova L, et al. *Neurology.* 2017;89(22):2230–2237; 2. Mavenclad. Prescribing information. EMD Serono, Inc; 2024.

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Supplementary Materials

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Author Disclosures

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CC, SL, KL, RA, and **DP** have nothing to disclose.

AS has received research funding from CMSC, Multiple Sclerosis Society of Canada, 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 CELLO.

RA has consulted for EMD Serono Research & Development Institute, Inc., Billerica, MA, USA, an affiliate of Merck KGaA, Biogen Idec, and Roche and is on the advisory board for Progentec Diagnostics Inc.

JK and **EE** are employees of EMD Serono, Inc., Rockland, MA, USA, an affiliate of Merck KGaA.

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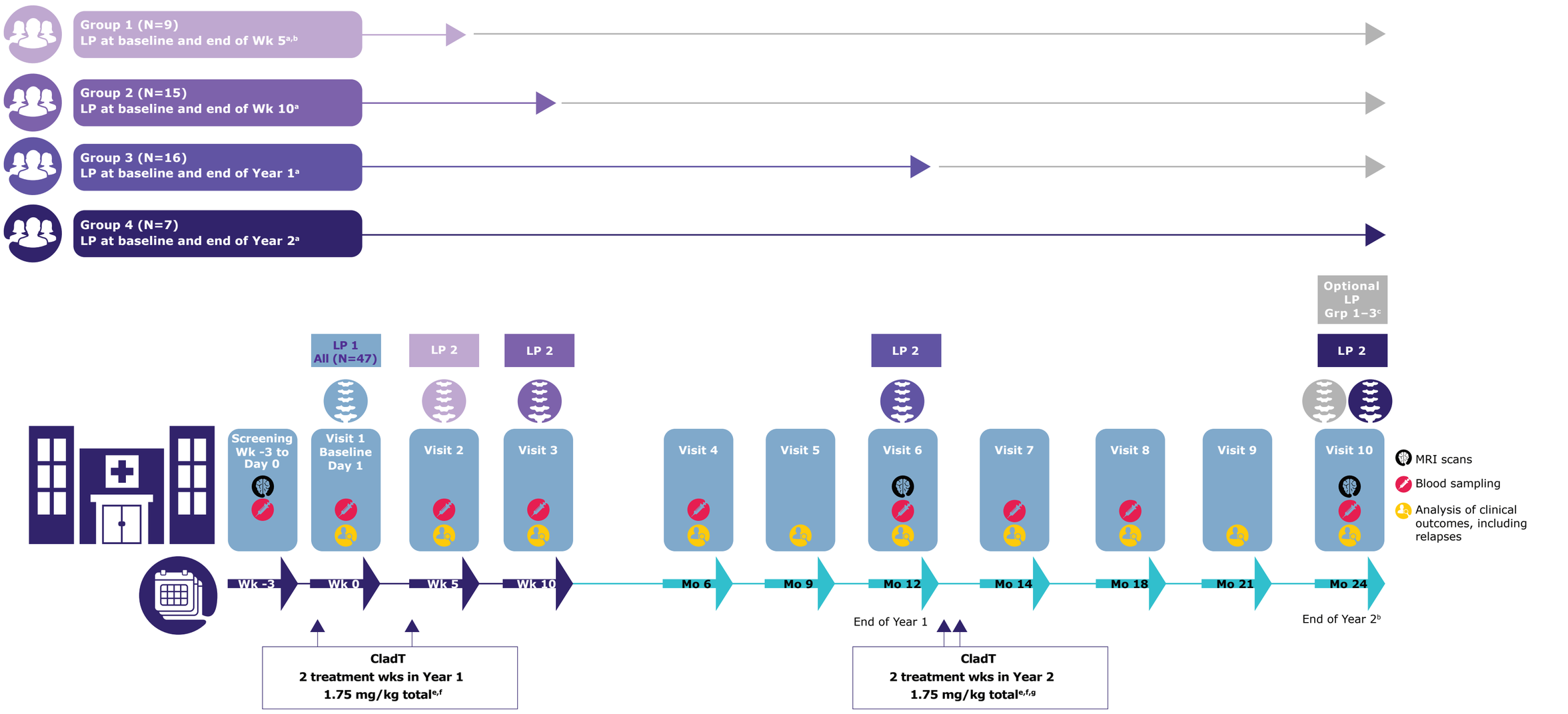
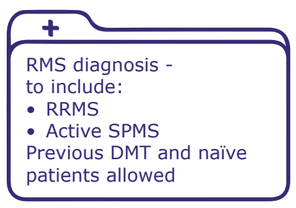
Supplementary Materials

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

Study design



Key inclusion criteria



Key exclusion criteria

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

[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 randomised 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]

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.

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

Supplementary Table 1: CLOCK-MS study endpoints

Primary endpoints	
	Levels of lymphocyte subsets and neuronal injury maker in the CSF: • CD3+ T lymphocytes, CD19+ B lymphocytes • NfL
Secondary endpoints	
	Levels of lymphocyte subsets and soluble immunological and neurological injury makers 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

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

ALC, absolute lymphocyte count; ARR, annualised 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.

Supplementary Materials

Supplementary Table 2: Prior-DMTs

Factor	Group 1 (Week 5 LP) (N=9)	Group 2 (Week 10 LP) (N=15)	Total (N=24)
Received DMT			
No	2 (22.2)	5 (33.3)	7 (29.2)
Yes	7 (77.8)	10 (66.7)	17 (70.8)
Last prior-DMT			
None	2 (22.2)	5 (33.3)	7 (29.2)
Natalizumab (Tysabri)	1 (11.1)	0	1 (4.2)
Interferon beta 1a (Avonex)	2 (22.2)	1 (6.7)	3 (12.5)
Glatiramer acetate (Copaxone/Glatopa)	1 (11.1)	3 (20.0)	4 (16.7)
Fingolimod (Gilenya)	1 (11.1)	3 (20.0)	4 (16.7)
Dimethyl fumarate (Tecfidera)	2 (22.2)	0	2 (8.3)
Teriflunomide (Aubagio)	0	3 (20.0)	3 (12.5)
Prior-DMTs taken:[a]			
Rituximab (Rituxan), ocrelizumab (Ocrevus), alemtuzumab (Campath), or daclizumab (Zinbryta)	0	0	0
Peginterferon beta-1a (Plegridy)	1 (11.1)	0	1 (4.2)
Natalizumab (Tysabri)	1 (11.1)	2 (13.3)	3 (12.5)
Interferon beta-1a (Avonex)	4 (44.4)	3 (20.0)	7 (29.2)
Interferon beta-1a (Rebif)	1 (11.1)	3 (20.0)	4 (16.7)
Interferon beta-1b (Betaseron/Betaferon)	1 (11.1)	2 (13.3)	3 (12.5)
Glatiramer acetate (Copaxone/Glatopa)	1 (11.1)	6 (40.0)	7 (29.2)
Fingolimod (Gilenya)	1 (11.1)	3 (20.0)	4 (16.7)
Dimethyl fumarate (Tecfidera)	2 (22.2)	1 (6.7)	3 (12.5)
Teriflunomide (Aubagio)	0	3 (20.0)	3 (12.5)

[a]Note: More than one option can be selected per patient.

DMT, disease-modifying therapy; **LP,** lumbar puncture; **SD,** standard deviation.