Relationship between CSF Cellular Populations and MRI Biomarkers of MS Disease Severity: Insights from the CLOCK-MS Study

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RESEARCH IN CONTEXT

1. **Reduction of central nervous system (CNS) inflammation with cladribine tablets (CladT), which is a CNS penetrant, may ameliorate CNS damage in patients with multiple sclerosis, which is increasingly recognised as a mechanism of progression independent of relapse activity (PIRA).**
2. **Specific cell subtypes measured in the cerebrospinal fluid (CSF) are strongly associated with acute inflammation (Gd-enhancing magnetic resonance imaging [MRI] lesions) and neurodegeneration in patients at baseline.**

OBJECTIVE

To explore the relationship between brain MRI biomarkers and cellular immune biomarkers of multiple sclerosis (MS) in patients with relapsing MS (RMS)

INTRODUCTION

Pivotal studies showed the effectiveness of CladT in reducing new or enhancing MS lesions.[1] Subsequent studies demonstrated the effects of CladT on CSF immune cells and proteins, reflecting CladT's role in regulating immune cells involved in MS.[2-4]

The impact of CladT on the interaction between imaging and cellular biomarkers of MS is not understood. Both imaging and cellular changes from pre-treatment baseline may inform the mechanism by which CladT reduces MS-related damage.

The CLOCK-MS Study (CladT: Collaborative Study to Evaluate Impact on central nervous system [CNS] Biomarkers in MS) is an open-label, randomised, multi-centre phase 4 study exploring the mechanism of action of CladT in RMS with an emphasis on the CNS compartment (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 and at either 5 weeks, 10 weeks, 1 year, or 2 years after starting CladT (3.5 mg/kg over 2 years as per US prescribing information[5]) (**Supplementary Figure 1**).
- MRIs were performed at baseline and MRI measures included lesion volume, thalamic volume, total grey matter volume, and white matter volume.
- Gadolinium-enhancing (Gd+) lesions were manually assessed as a marker of acute inflammation.
- Immune cell populations in the blood and in the CSF were characterised by flow cytometry. Absolute cell counts were adjusted based on patient age and prior disease modifying therapy status.
- Spearman correlation coefficients were calculated between number of Gd+ lesions and adjusted cell counts.
- Liquid Chromatography-Tandem Mass Spectrometry was used to calculate the concentration of cladribine in the serum and CSF from patients at week 5 after starting CladT.
- Canonical correlation, the multivariate generalization of standard correlation, identifies relationships between two sets of variables and produces correlated components, similar to Principal Component Analysis (PCA) (Please refer to **supplementary section 'Canonical correlations for the non-statistician'** for more details).

RESULTS

Table 1. Correlation between cell count and Gd+ lesions at baseline screening visit

Cerebrospinal Fluid			Blood		
Cell Line	r	p-value	Cell Line	r	p-value
Transitional B cells	**0.371**	**0.011**	**CD4+ Tcm**	**0.296**	**0.048**
CD4+ Treg	**0.362**	**0.013**	CD4+ Treg	0.226	0.135
CD4+ Tcm	**0.320**	**0.030**	NK cells	0.213	0.161
CD8+ T cells	**0.312**	**0.035**	Activated B cells	0.190	0.212
DCs	**0.309**	**0.037**	CD4+ T cells	0.145	0.343
CD88+ DCs	0.282	0.057	Naïve B cells	0.144	0.344
CD19+ B cells	0.265	0.075	Th2 CD4+ T cells	0.139	0.362
CD4+ T cells	0.251	0.092	Naïve CD8+ cells	0.134	0.379
Monocytes	0.241	0.106	CD8+ Tcm	0.125	0.413
CD4+ Tem	0.219	0.143	Neutrophils	0.119	0.435
Memory B cells	0.201	0.179	Th17 CD4+ T cells	0.108	0.482
Naïve CD4+ cells	0.195	0.194	CD56high NK cells	0.103	0.502
T cells	0.194	0.197	Naïve CD4+ cells	0.038	0.803
CD4+ T cells	0.167	0.268	Transitional B cells	0.020	0.894
Naïve CD8+ cells	0.160	0.287	Monocytes	0.019	0.900
B cells	0.157	0.297	T cells	-0.010	0.949
CD8+ Tem	0.141	0.349	TEMRA	-0.050	0.744
CD8+ Tcm	0.134	0.374	B cells	-0.056	0.714
CD8+ T cells	0.124	0.411	CD8+ T cells	-0.095	0.534
CD20+ T cells	-0.006	0.966	Plasmablasts	-0.097	0.527
CD8+ Treg	-0.088	0.559	Classical monocytes	-0.104	0.498
			Th1 CD4+ T cells	-0.106	0.488
			Eosinophils	-0.121	0.427
			Memory B cells	-0.144	0.346
			CD8+ Treg	-0.146	0.338
			CD4+ Tem	-0.177	0.245
			CD8+ Tem	-0.209	0.169

Cell counts determined by flow cytometry. Correlation between cell count and number of Gd+ lesions at baseline and nominal p value is shown. DCs, dendritic cells; Gd+, gadolinium-enhancing; NK, natural killer; r, correlation coefficient; Tcm, central memory T cells; Tem, effector memory T cells; TEMRA, Terminal effector memory T cells; Th, T helper cells; Treg, regulatory T cells.
Please refer to **Supplementary Table 1** for detailed baseline demographics and disease characteristics, and **Supplementary Table 2** for prior disease-modifying therapy use.

- Overall, a stronger relationship was observed between CSF (vs blood) immune cells and Gd+ lesion number (**Table 1**).
- Increased number of transitional B cells, CD4+ regulatory T cells, CD4+ central memory T cells, CD8+ T cells, and dendritic cells correlated with more Gd+ lesions.

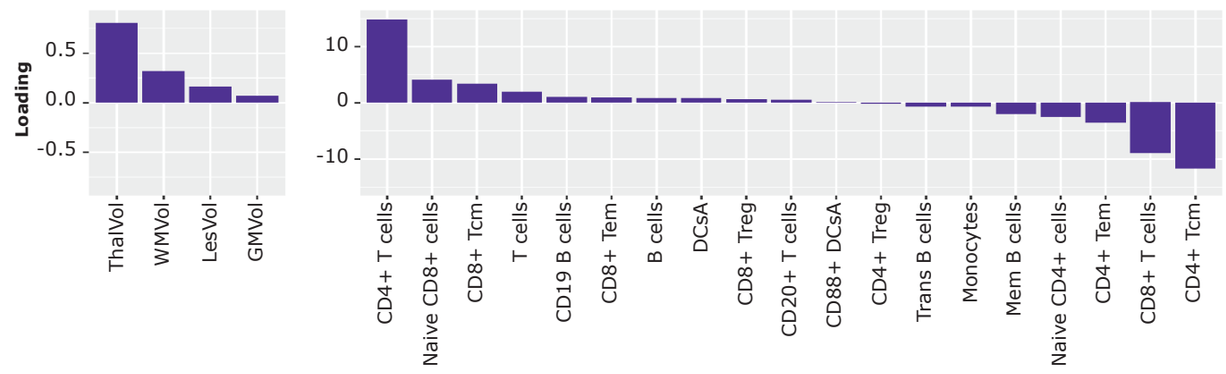
Table 2. Canonical correlation exists between CSF and MRI measures, but not blood and MRI measures

	Statistical Significance of 1st Canonical Correlation	
	F value	p-value
CSF	1.45	0.045
Blood	1.04	0.44

CSF, cerebrospinal fluid; MRI, magnetic resonance imaging.

- Canonical correlation identifies an optimised correlation between two (weighted) multivariate distributions. F tests determine whether the observed correlation occurs by chance or not (**Table 2**).
- There was no statistically significant canonical correlation between blood and MRI markers. CSF cell counts and MRI measures were significantly related.

Figure 1. Factor Loadings Identify Related MRI and CSF Cellular Biomarkers

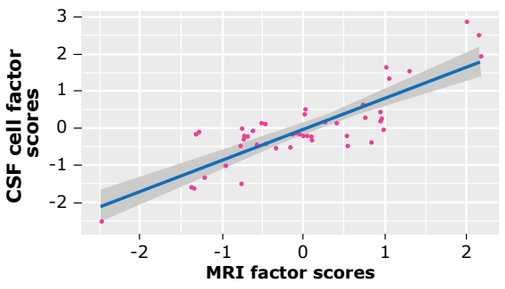


Factor loadings are shown. Scales are arbitrary. Positive loadings represent positive correlations between MRI and CSF markers. Negative loading indicate anti-correlation.

CSF, cerebrospinal fluid; DCsA, dendritic cell–associated; GMVol, gray matter volume; LesVol, lesion volume; Mem B cells, memory B cells; MRI, magnetic resonance imaging; Tcm, central memory T cells; Tem, effector memory T cells; Treg, regulatory T cells; ThalVol, thalamic volume; Trans B cells, transitional B cells; WMVol, white matter volume.

- Using canonical correlation, we identified the weighted average of MRI measures maximally correlated with the weighted average of CSF measures. This yields weights similar to PCA 'loadings' **(Figure 1)**.
- The positive relationship between CD4+ T cells and thalamic volume suggests a relationship between immune function and inflammatory and neurodegenerative changes in the thalamus.

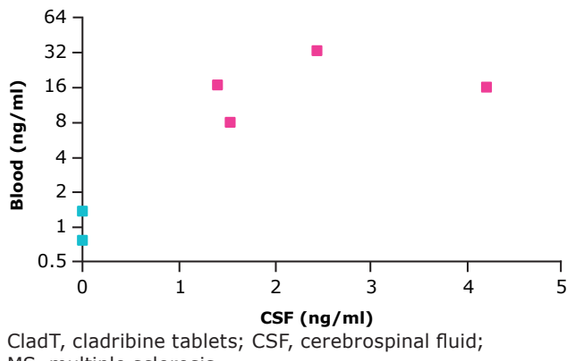
Figure 2. Relationship between MRI and CSF Cellular Factor



CSF, cerebrospinal fluid; MRI, magnetic resonance imaging.

- The original data are projected onto the defined factor loadings to define the 'canonical correlation'. There is a strong positive association in these data across subjects at baseline (**Figure 2**).
- This multi-variate relationship defines an association at baseline which can now be examined longitudinally following initiation of CladT.

Figure 3. Cladribine is present in the serum and CSF in MS patients after initiation of CladT and is cleared quickly



CladT, cladribine tablets; CSF, cerebrospinal fluid; MS, multiple sclerosis.

- Cladribine was detected in the CSF of four patients who received a lumbar puncture (LP) on the same day they took their last dose of CladT in Week 5 (shown as pink dots in **Figure 3**).
- In 2 patients whose LP was done 2 and 4 days after their last dose in week 5, a much lower concentration of cladribine was detected in the serum, and cladribine was undetectable in the CSF (shown as teal dots in **Figure 3**).

CONCLUSIONS

- **Gd+ lesions are associated with a specific inflammatory fingerprint in the blood and CSF at baseline; the association in the CSF is stronger than in blood.**
- **Unlike the blood immune cell profile, the CSF immune cell profile, exhibits a multivariate relationship with brain imaging biomarkers, particularly thalamic volume.**
- **As CladT is present in the CSF of MS patients and depletes immune cells in the CSF, we expect that treatment with CladT will modulate these MRI-CSF relationships.**

SCAN FOR AFFILIATIONS, DISCLOSURES AND SUPPLEMENTARY MATERIALS

Reference: 1. Giovannonii G, et al. NEJM. 2010; 362:416-426; 2. Ammoscato F, et al. Clin Immunol. 2024;269:110380; 3. Wiendl H, et al. Oral presentation at ECTRIMS 2024, 18-20 September, Copenhagen, Denmark (O037); 4. Wu GF, et al Poster at ACTRIMS 2025, February 27–March 1, West Palm Beach, Florida (P148); 5. MAVENCLAD. US Prescribing information. EMD Serono, Inc; 2024.

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Supplementary Materials

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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.

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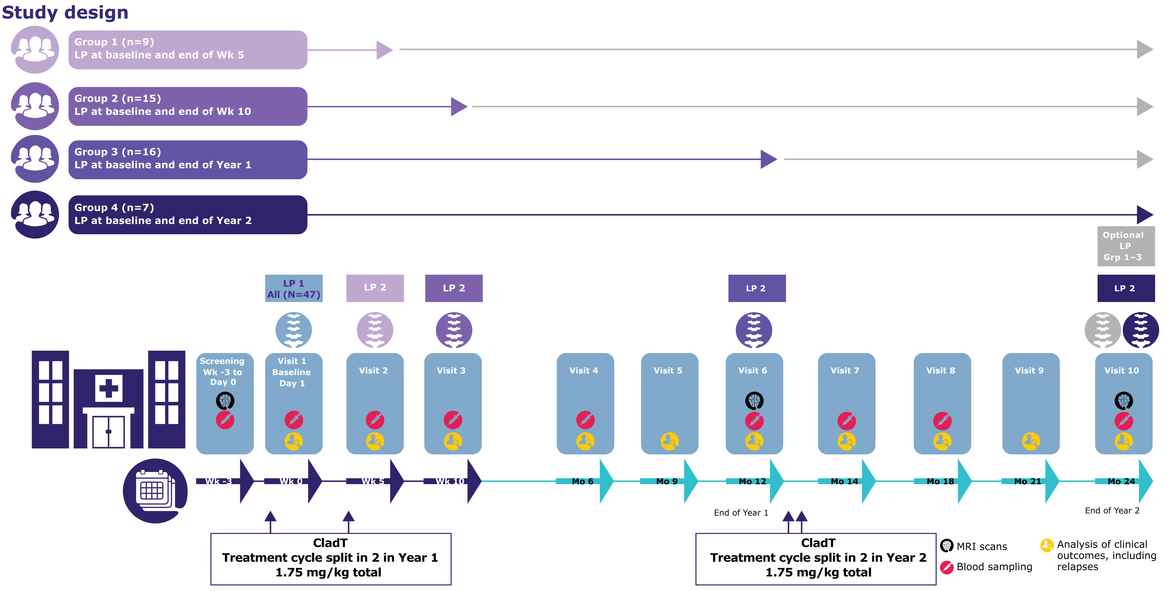
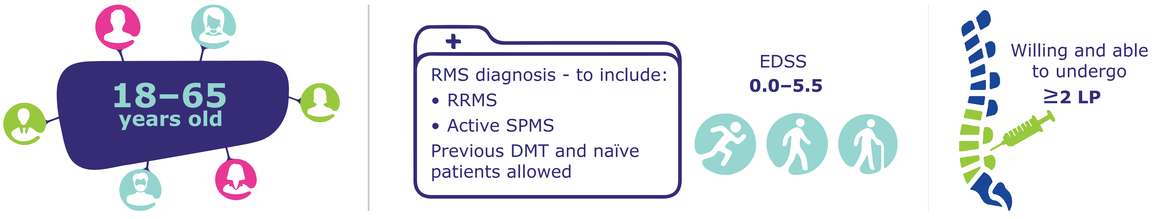
Supplementary Materials

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



Key inclusion criteria



Key exclusion criteria

- ✗ Have any contraindication for LP
- ✗ Have malignancy, infections, signs/symptoms suggestive of PML
- ✗ Have received natalizumab during the last 6 months
- ✗ 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[1].

CladT, cladribine tablets; DMT, disease-modifying therapy; 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. 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)
Number of patients with Gd+ T1 lesions, n (%)	17 (36.2)
Number of Gd+ T1 lesions per patient, median (IQR)	0.00 (0.00, 1.5)

EDSS, Expanded Disability Status Scale; Gd+, gadolinium-enhancing; 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)

Supplementary Materials

Canonical correlations for the non-statistician

- Canonical correlation analysis (CCA) is a statistical method used to explore relationships between two sets of variables, much like how bivariate correlation examines the relationship between two individual variables. In this study using MRI biomarkers (e.g., thalamic volume) and fluid cellular biomarkers (e.g., blood cell counts), CCA helps identify how these two groups of measurements relate to each other as a whole.
- Given two datasets: one set of MRI-derived measures (like volumes of different brain segmentations) and one set of fluid biomarkers (cell counts in the blood or CSF). Each set has multiple variables, and you want to understand how these sets are connected. Bivariate correlation could only compare one MRI measure to one fluid biomarker at a time, which might miss the bigger picture. CCA, on the other hand, looks at the entire sets simultaneously.
- CCA works by finding pairs of linear combinations (called canonical variates) from each set that are maximally correlated with each other. This process is similar to principal component analysis (PCA), where PCA reduces a single dataset into key components that capture the most variation. In CCA, instead of one dataset, the goal is to find combinations of MRI biomarkers and fluid biomarkers that correlate the most. For example, one canonical variate might combine several MRI measures and pair them with a combination of fluid biomarkers to show a strong link between, e.g., brain atrophy and inflammation.
- The output of CCA includes canonical correlations (similar to correlation coefficients in bivariate analysis; Figure 2), which tell you how strongly the two sets are related, and weights that show which variables in each set contribute most to the relationship (Figure 1). Inference is performed to determine if the isolated component is statistically significant.
- F value: The F value is a number used in statistical testing, and it is related to the p-value, which helps us determine if the findings are significant. If the F value is just above one, it usually means the results aren’t significant ($p < 0.05$).