Blood Biomarker Dynamics in Highly Active Relapsing Multiple Sclerosis Patients Treated with Cladribine Tablets: Results of the 2-Year MAGNIFY-MS Study

H. Wiendl[1], N. De Stefano[2], F. Barkhof[3,4], X. Montalban[5], A. Achiron[6], T. Derfuss[7], A. Chan[8], S. Hodgkinson[9], A. Prat[10], L. Leocani[11], K. Schmierer[12,13], F. Sellebjerg[14,15], P. Vermersch[16], H. Jin[17], E. Järvinen[17], A. Chudecka[18], L. Gardner[19]

[1]Department of Neurology, Institute of Translational Neurology, University of Münster, Münster, Germany; [2]Department of Medicine, Surgery and Neuroscience, University of Siena, Siena, Italy; [3]Department of Radiology, VU University Medical Center, Amsterdam, The Netherlands and [4]UCL Institute of Neurology, London, UK; [5]Department of Neurology-Neuroimmunology, Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Hospital Universitario Vall d'Hebron, Universitat Autonoma de Barcelona, Barcelona, Spain; [6]Multiple Sclerosis Center, Sheba Academic Medical Center, Ramat Gan, Israel; [7]Department of Neurology, University Hospital Basel, Basel, Switzerland; [8]Department of Neurology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland; [9]Ingham Institute for Applied Medical Research, University of New South Wales Medicine, Sydney, NSW, Australia; [10]Department of Neurosciences, Université de Montréal, Montréal, QC, Canada; [11]Experimental Neurophysiology Unit, Vita-Salute San Raffaele University, Milan, Italy; [12]The Blizard Institute, Centre for Neuroscience, Surgery & Trauma, Barts and The London School of Medicine & Dentistry, Queen Mary University of London, London, UK and [13]Clinical Board Medicine (Neuroscience), The Royal London Hospital, Barts Health NHS Trust, London, UK; [14]Danish MS Center, Department of Neurology, Copenhagen University Hospital - Rigshospitalet, Glostrup, Denmark and [15]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark; [16]Univ. Lille, Inserm U1172 LilNCog, CHU Lille, FHU Precise, Lille, France; [17]the healthcare business of Merck KGaA, Darmstadt, Germany; [18]Cytel Inc., Geneva, Switzerland; [19]EMD Serono, Billerica, MA, USA

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CONCLUSIONS

- **Multifaceted immune changes were detected over a 2-year observation period following cladribine tablets treatment, including a novel observation regarding increase in CD16^{low}CD56^{bright} and NKp46 cells**
- **Reduction in pro-inflammatory B-cell (IL-6$^+$) and T-cell (GMCSF, TNFα, IFNγ) cytokines, and an increase in anti-inflammatory T-cell (IL-4$^+$) cytokines above baseline level were observed**
- **Serum IgG and IgM levels remained within normal limits for most patients**
- **Serum NfL levels decreased, indicating a positive response to cladribine tablets treatment**

INTRODUCTION

- The MAGNIFY-MS (NCT03364036) blood biomarker (BB) analysis aimed to characterize the effect of cladribine tablets (3.5 mg/kg cumulative dose over 2 years) on BBs in patients with highly active relapsing multiple sclerosis (RMS).
- Previously published data characterized the specific patterns of immune cell dynamics over 12 months following the first course of treatment with cladribine tablets in a subset of MAGNIFY-MS patients (n=57), and highlighted the pronounced reduction of immune cell subtypes from Month 2 onwards.[1]

OBJECTIVE

To describe BB dynamics over 2 years of treatment with cladribine tablets, including immune cell subsets, serum proteins, and intracellular cytokines.

METHODS

- Blood samples from patients in MAGNIFY-MS were collected at baseline and Months (M) 3, 6, 12, 15, 18, and 24 after treatment initiation for immune cell characterization, measurement of immunoglobulins (Ig), and serum neurofilament (sNfL) analysis.
- Intracellular cytokine analyses were performed for a subset of patients in the BB sub-study and required blood collection at additional time points (M1, 2, and 14).
- All analyses were performed without adjustment for multiplicity.
- Correlation analysis between change in immune cell subsets from Baseline to the M3/6 visit versus presence of MRI lesions during the Baseline period to M3/6 was assessed with Cramer's V, and included patients with both B-cell and MRI assessments from the main study (N=270).

Cladribine tablets 10 mg (3.5 mg/kg cumulative dose over 2 years) are indicated for the treatment of patients with MS in the United States (relapsing forms of MS, including relapsing-remitting disease and active secondary progressive disease, in adults).

RESULTS

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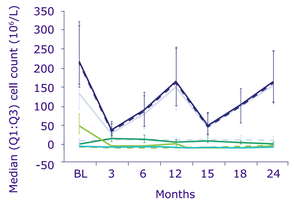
- A total of 270 patients enrolled in the main study were analyzed (BB sub-study n=57, **Supplementary Table 1**).
- Quantitative immune cell changes were observed following each yearly treatment course of cladribine tablets, as summarized below.

B-cell Panel (FAS)



Median Percentage Change from Baseline

	M3	M6	M12	M15	M18	M24
CD19⁺	-80.14	-60.60	-26.88	-77.24	-55.30	-27.65
CD20⁺	-80.50	-60.32	-24.56	-77.11	-54.21	-24.77
Memory	-92.69	-91.56	-86.90	-96.47	-94.67	-89.29
Activated CD69⁺	-74.02	-60.91	-28.82	-73.02	-51.87	-15.95
Plasma cells CD38⁺	-66.62	-59.00	-54.75	-78.02	-72.39	-62.47
Short-lived plasma	-68.18	-56.55	-56.70	-82.96	-79.54	-70.10
Naïve	-75.87	-45.87	1.63	-69.17	-39.73	10.85
Transitional	-4.06	14.82	11.92	28.69	11.27	6.30
Regulatory	110.73	92.95	30.64	91.57	33.83	1.62

In the table, purple shading signifies value above Baseline level; green shading, highest value above Baseline level; blue shading, nadir value.
BL, baseline; **CD**, cluster of differentiation; **FAS**, full analysis set; **Q1:Q3**, quartile range; **M**, month

- Median percentage change from baseline at M24: regulatory (+1.62%), transitional (+6.3%), naïve (+10.85%), memory (-89.29%) B-cells; and total plasma cells: -62.47%.

T-cell Panel (FAS)

Median Percentage Change from Baseline

	M3	M6	M12	M15	M18	M24
CD4⁺	-48.60	-47.18	-40.16	-69.04	-66.98	-57.51
CD4⁺ CM	-49.83	-47.31	-39.97	-70.18	-65.91	-56.96
CD4⁺ EM	-28.62	-26.81	-19.81	-46.05	-48.69	-38.56
CD4⁺ reg	-25.98	-29.84	-25.60	-48.40	-48.73	-40.30
CD4⁺ naïve	-60.43	-57.74	-50.50	-83.66	-78.50	-68.85
CD4⁺ Th1	-44.35	-43.20	-35.55	-63.68	-63.01	-52.86
CD4⁺ Th2	-50.89	-52.31	-47.80	-75.96	-71.82	-62.72
CD4⁺ Th17	-33.09	-30.26	-18.39	-44.57	-44.77	-31.74
CD8⁺	-42.33	-39.42	-36.28	-57.08	-54.44	-45.93
CD8⁺ CM	-40.92	-43.30	-32.26	-62.74	-56.93	-43.26
CD8⁺ EM	-26.20	-23.09	-22.26	-44.11	-44.18	-35.05
CD8⁺ naïve	-67.58	-65.44	-58.31	-84.66	-77.74	-67.54

In the table, blue shading signifies nadir value.
CM, central memory; **EM**, effector memory; **FAS**, full analysis set; **Q1:Q3**, quartile range; **reg**, regulatory; **Th**, T helper cell type

- Reduction in most T cells is seen from M3 onward; no repopulation to baseline levels was observed during the trial.

Serum proteins (FAS)

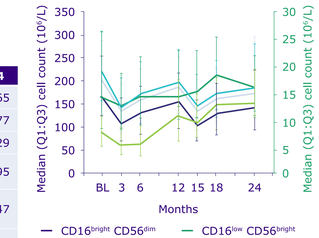
Table 1. Median Serum IgM and IgG Levels Over Time (FAS)

	BL	M3	M6	M12	M15	M18	M24
IgM absolute value, g/L	1.14	1.1	1.01	0.95	0.79	0.8	0.77
IgM percentage change from BL	-	-6.98	-13.38	-19.61	-27.33	-30.65	-31.49
IgG absolute value, g/L	10.3	10.6	10.4	10.35	10.6	10.7	10.6
IgG percentage change from BL	-	5.00	3.71	1.05	4.90	2.40	2.99

BL, baseline; **FAS**, full analysis set; **Ig**, immunoglobulin; **M**, month

- Serum IgM levels decreased throughout the study (**Table 1**). There were no clinically relevant changes in IgG levels. The majority of patients had their individual values of IgM and IgG within reference ranges (IgG: 5.65-17.65 g/L; IgM: 0.40-2.30 g/L).
- Compared with baseline, sNfL was reduced at M12 (-25.22%) and M24 (-23.23%) (**Supplementary Figure 1**).

NK-cell Panel (FAS)



Median Percentage Change from Baseline

	M3	M6	M12	M15	M18	M24
CD16^{bright} CD56^{dim}	-36.13	-25.61	-11.05	-35.05	-24.99	-12.94
CD16^{low} CD56^{bright}	-8.94	3.72	2.56	4.77	30.13	17.21
NKp46	-20.85	-22.38	29.49	28.42	71.73	77.70
CD16⁺	-32.50	-21.78	-8.10	-28.56	-21.47	-13.76
NK cells	-31.15	-22.18	-7.10	-27.70	-18.38	-13.05

In the table, purple shading signifies value above Baseline level; green shading, highest value above Baseline level; blue shading, nadir value.
BL, baseline; **CD**, cluster of differentiation; **FAS**, full analysis set; **M**, month; **NK**, natural killer; **Q1:Q3**, quartile range

- Increased levels above baseline from M12 onwards were detected for CD16^{low} CD56^{bright} (M24, +17.21%) and NKp46 cells (M24, +77.7%).

Intracellular Cytokines

- In the BB sub-study, reductions in *pro-inflammatory* cytokine-producing IL-6$^+$ B-cells and GMCSF$^+$, TNFα$^+$ and IFNγ$^+$ T-cells were observed. There were also increases in *anti-inflammatory* cytokine-producing IL-10$^+$ B cells and IL-4$^+$ CD4$^+$ and CD8$^+$ T cells (**Tables 2** and **3**).

Table 2. Median Absolute Change (%) from Baseline for Cytokine Producing B-cells (BB Sub-study)

MFI B-cell subset	Time point (months)					
	M3	M6	M12	M15	M18	M24
IL-10⁺ B cells	1.9	1.11	-0.94	-1.6	-1.57	-0.78
IL-10⁺ Memory B cells	-0.93	-1.35	-1.47	-2.49	-1.52	-1.44
IL-6⁺ B cells	-7.77	-9.02	-16.33	-15.31	-15.62	-12.69
IL-6⁺ Memory B cells	-8.72	-9.53	-12.02	-10.63	-11.56	-10.88

In the table, purple shading signifies value above Baseline level; green shading, highest value above Baseline level; blue shading, nadir value.
BB, blood biomarker; **IL**, interleukin; **M**, month; **MFI**, mean fluorescence intensity; **Q1:Q3**, quartile range

Table 3. Median Absolute Change (%) from Baseline for Cytokine Producing T-cells (BB sub-study)

MFI T-cell subset	Time point (months)					
	M3	M6	M12	M15	M18	M24
GMCSF⁺ CD4⁺ T cells	-3.25	-1.61	-5.71	-6.67	-5.41	-3.93
IFNγ⁺ CD4⁺ T cells	-3.63	-4.10	-6.13	-9.20	-6.97	-5.15
TNFα⁺ CD4⁺ T cells	-12.15	-16.30	-25.85	-46.30	-48.40	-28.45
IL-4⁺ CD4⁺ T cells	0.79	0.84	0.61	0.92	2.30	1.70
IL-10⁺ CD4⁺ T cells	1.78	0.53	-0.30	-0.87	-1.16	-0.94
GMCSF⁺ CD8⁺ T cells	-3.77	-2.80	-10.48	-11.60	-9.63	-4.70
IFNγ⁺ CD8⁺ T cells	-0.3	-3.05	-14.72	-18.78	-15.06	-11.85
TNFα⁺ CD8⁺ T cells	-13.93	-15.40	-27.65	-44.65	-48.48	-28.05
IL-4⁺ CD8⁺ T cells	0.40	0.74	0.18	0.52	1.19	0.61
IL-10⁺ CD4⁺ T cells	-0.01	-0.23	-0.68	-1.19	-1.01	-0.89

In the table, purple shading signifies value above Baseline level; green shading, highest value above Baseline level; blue shading, nadir value.
BB, blood biomarker; **CD**, cluster of differentiation; **GMCSF**, granulocyte-macrophage colony-stimulating factor; **IFNγ**, interferon gamma; **IL**, interleukin; **M**, month; **MFI**, mean fluorescence intensity; **TNFα**, tumor necrosis factor alpha

Correlations

- No correlation was found between memory or regulatory B cells and CUA, T1 Gd+ and active T2 lesions at M3 and M6 (**Supplementary Figure 2**).

For patient demographics, sNfL, and correlation data please scan QR code

Reference: 1. Wiendl H, et al. *Neurol Neuroimmunol Neuroinflamm.* 2023;10(1):e200048.

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FS has served on scientific advisory boards, been on the steering committees of clinical trials, served as a consultant, received support for congress participation, received speaker honoraria, or received research support for his laboratory from Biogen, Celgene (BMS), EMD Serono, the healthcare business of Merck KGaA, Darmstadt, Germany, Novartis, Roche, Sanofi, and Teva. **PV** has received honoraria or consulting fees from AB Science, Biogen, Celgene (BMS), Imcyse, the healthcare business of Merck KGaA, Darmstadt, Germany, Novartis, Roche, Sanofi, and Teva; and research support from Novartis, Roche, and Sanofi. **HJ** and **EJ** are employees of the healthcare business of Merck KGaA, Darmstadt, Germany. **ACHu** is an employee of Cytel Inc., Geneva Branch, Switzerland, funded by the healthcare business of Merck KGaA, Darmstadt, Germany. **LG** is an employee of EMD Serono, Billerica, MA, USA

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Supplementary Table 1. Patient Demographics

	Main study population (N=270)	Sub-study population (n=57)
Female, n (%)	180 (66.7)	35 (61.4)
Age >40 – <65 years, n (%)	118 (43.7)	22 (38.6)
Time since onset of MS in months, mean ± SD	84.90 ±85.472	84.94 ± 93.385
Time since diagnosis in months, mean ± SD	60.87 ±74.489	52.54 ± 67.413
Time since first relapse in months, mean ± SD	54.44 ±72.583	52.63 ± 80.704
Number of relapses within 12 months prior to Baseline, n (%)		
0	3 (1.1)	2 (3.5)
1	102 (37.8)	15 (26.3)
2	133 (49.3)	29 (50.9)
>2	32 (11.9)	11 (19.3)
EDSS score ≤3 at Baseline, n (%)	204 (75.6)	42 (73.7)
Median EDSS at Baseline (range)	2.0 (0.0–5.0)	2.5 (0.0–5.0)
Number of previous DMTs, n (%)		
0	117 (43.3)	30 (52.6)
1	88 (32.6)	13 (22.8)
2	50 (18.5)	10 (17.5)
>2	15 (5.6)	4 (7.0)

DMT, disease-modifying therapy; **EDSS**, Expanded Disability Status Scale; **MS**, multiple sclerosis; **SD**, standard deviation

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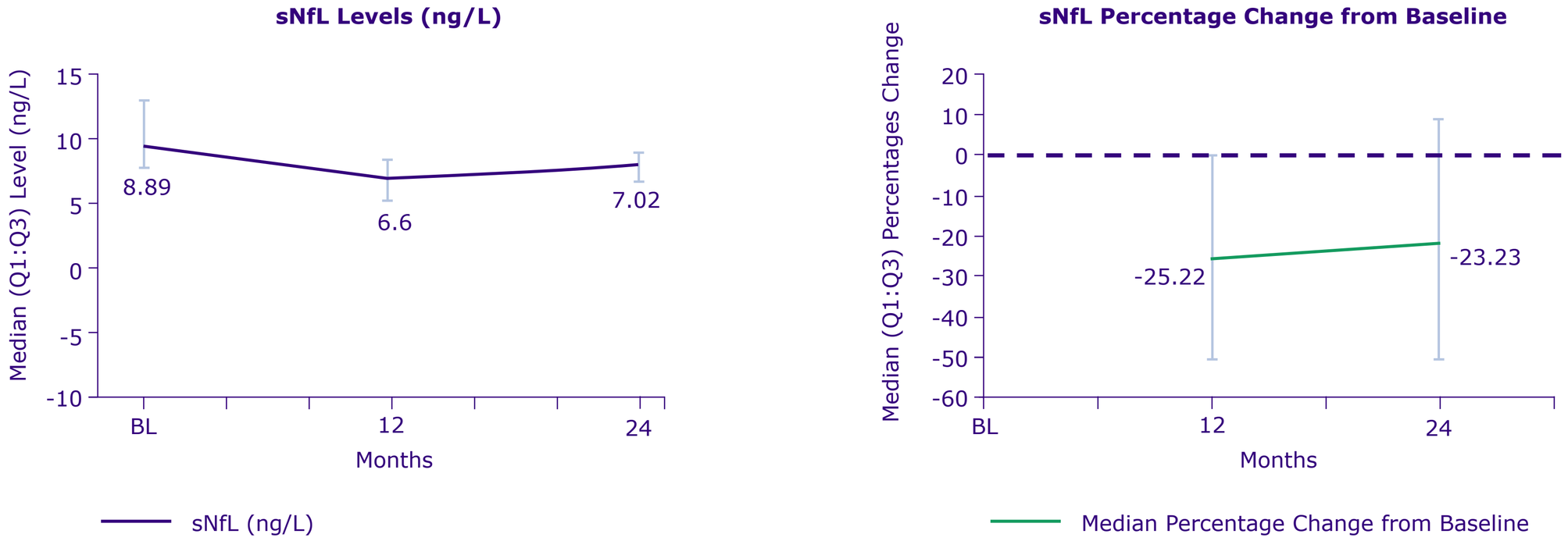
Supplementary Figure 1. sNfL Levels Over Time (FAS)



BL, baseline; **FAS**, full analysis set; **M**, month; **Q1:Q3**, quartile range; **sNfL**, serum neurofilament light chain

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Supplementary Figure 2. Correlation of B-cells with MRI Findings

Cramer's V is a measure of association between two nominal variables. It ranges from 0 to 1, where 0 indicates no association between the two variables and 1 indicates a perfect association between the two variables. $p < 0.05$ is considered significant.

BL, baseline; **CUA**, combined unique active; **Gd+**, gadolinium enhancing; **M**, month; **MRI**, magnetic resonance imaging; **n**, number of patients with both B-cell and MRI assessments; **r**, Cramer's V correlation coefficient

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