Investigation of Changes in Lymphocyte Subset Counts and Their Relationship With MRI Outcomes in the MAGNIFY-MS Study

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CONCLUSIONS

Patients with detectable CUA lesions at baseline had a reduction in CUA lesions over 2 years in the study

Most patients treated with cladribine tablets had no detectable CUA lesions at M24

Differences in immune subset counts in relation to MRI outcomes were detected, including:

- **Memory B cell counts in the residual CUA group were higher at M12 compared with the other groups. The difference in B-cell counts between the groups was no longer detected at M24, after the second dose of treatment**
- **CD4⁺ naïve cell counts were higher in the residual CUA lesion group, the differences were also evident at M12 and M24. For CD8⁺ naïve cell counts, the difference was no longer detected at M24**

INTRODUCTION

- Currently it is unclear how lymphocyte subsets in the peripheral blood are associated with magnetic resonance imaging (MRI) outcomes in patients with multiple sclerosis (MS) treated with cladribine tablets 10 mg (3.5 mg/kg cumulative dose over 2 years)
- MAGNIFY-MS (NCT03364036) was a 2-year, phase IV, open-label, single-arm study in which eligible patients (n=270) with relapsing MS received cladribine tablets
- Previously, we saw no significant correlation between changes in memory and regulatory B cells and MRI outcomes at month (M)3 and M6 in the overall MAGNIFY-MS population[1]
- In this *post-hoc* analysis, we analysed immune cell kinetics based on MRI activity, where patients were stratified by the response of combined unique active (CUA) lesions to cladribine tablets treatment

OBJECTIVE

To investigate changes in lymphocyte subset counts and their association with MRI outcomes in patients receiving cladribine tablets for highly active relapsing MS in the MAGNIFY-MS study

METHODS

- Patients were stratified by the presence of annualised CUA MRI lesions at baseline (BL) and at M24 after initiating treatment using the following criteria:
  - No CUA lesions (BL CUA=0, M24 CUA=0)
  - Resolved CUA lesions (BL CUA>0, M24 CUA=0)
  - Residual CUA lesions (BL CUA>0, M24 CUA>0)
- Lymphocytes* were analysed at BL, M3, M6, M12, M15, M18, and M24
- Patients were excluded from the analysis if they were missing MRI or lymphocyte subset data. All statistical analyses were *post-hoc* and exploratory. No correction for multiple testing was performed

*Lymphocytes and the specific markers in this analysis are listed in the supplementary material

Figure 1. MAGNIFY-MS study design

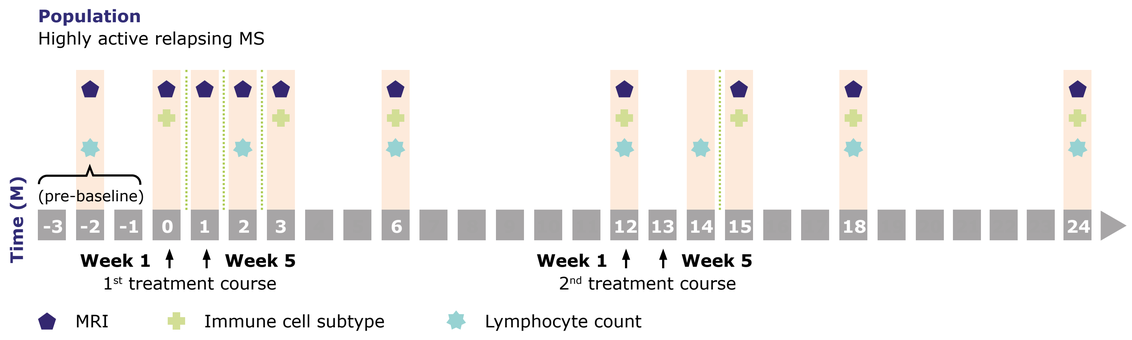


Figure 1: **M**, month; **MRI**, magnetic resonance imaging; **MS**, multiple sclerosis

RESULTS

- A total of 211 patients were included in the analysis. The majority of cladribine tablets treated patients were in the no CUA lesions or resolved CUA lesion groups (**Table 1**)

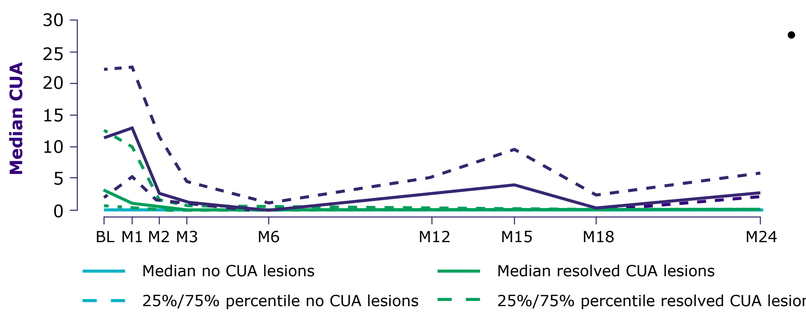
Table 1. Patient characteristics

	No CUA lesions N=96 (45.5%)	Resolved CUA lesions N=91 (43.1%)	Residual CUA lesions N=24 (11.4%)
Female, n (%)	64 (66.7)	64 (70.3)	15 (62.5)
Age >40 years, n (%)	51 (53.1)	38 (41.8)	5 (20.8)
Time since onset of MS (Months), mean ± SD	87.72 ± 85.72	78.85 ± 84.32	67.65 ± 76.11
Time since diagnosis (Months), mean ± SD	59.14 ± 73.35	54.36 ± 67.71	49.83 ± 73.44
Time since first relapse (Months), mean ± SD	54.28 ± 74.86	50.01 ± 69.27	44.48 ± 66.44
≥1 relapses within 12 months prior to Baseline, n (%)	96 (100)	89 (97.8)	24 (100)
EDSS score ≤3 at Baseline, n (%)	66 (68.8)	70 (76.9)	23 (95.8)
Prior DMT use, n (%)	52 (54.2)	52 (57.1)	13 (54.2)
High relapse activity*, n (%)	63 (65.6)	51 (56.0)	17 (70.8)

*High relapse activity defined as 2 or more relapses in the previous year regardless of prior DMT use

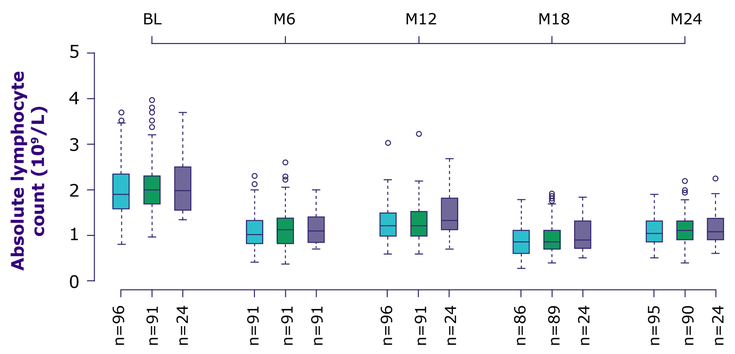
Table 1: **CUA**, combined unique active; **DMT**, disease-modifying therapy; **EDSS**, Expanded Disability Status Scale; **MS**, multiple sclerosis; **SD**, standard deviation

Figure 2. Evolution of annualised CUA lesions during 24M



- Both resolved and residual groups showed reduction in median CUA lesions from BL after treatment initiation and post-2nd-year dosing, up to M24 (**Figure 2**). Patients with residual CUA lesions at M24 had higher overall levels of CUA lesions than patients in the no CUA or resolved CUA groups during the treatment period

Figure 3. Absolute lymphocyte counts



- Absolute lymphocyte counts were decreased in all groups; no between-group differences were observed (**Figure 3**)

Figure 4. Changes in lymphocyte subset counts

*p<0.05 in comparison to no CUA lesions

†For visualization purposes, a total of 6 outliers were removed at BL with memory B-lymphocytes >150 cells/µL

- Memory B cell counts were reduced in all analysed groups from M3 to M24 (**Figure 4**)
- However, in the residual CUA lesion group memory B cell counts were higher at M12 ($p<0.05$) compared with resolving or no CUA lesion groups (not at M24)
- Patients with residual CUA lesions had higher CD19⁺ counts at M12 ($p<0.05$)
- Patients with residual CUA lesions had consistently higher CD4⁺ naïve T-cell counts, which were significant at M3 and M12–M24 ($p<0.05$)
- Patients with residual CUA lesions had higher CD8⁺ naïve T-cell counts at M3, M12 and M15 ($p<0.05$), a difference which was no longer detected at M24

For supplementary material scan the QR code

Figures 2, 3, 4: **BL**, baseline; **CUA**, combined unique active; **M**, month — No CUA lesions; Resolved CUA lesions; Residual CUA lesions

REFERENCE
1. Wiendl H, et al. *Neurology*. 2023;100(17. Suppl. 2):3016.

DISCLOSURES
SH serves on advisory boards for Bayer, Biogen, Merck, Novartis, Roche, and Sanofi. She has received money for travel and speaker honoraria from Bayer, Biogen, Merck, Novartis, Roche, and Sanofi. **TD** serves on scientific advisory boards for Bayer, Actelion (Janssen/J&J), Biogen, Celgene (BMS), GeNeuro, MedDay, Merck, Mitsubishi Pharma, Novartis, Roche, and Sanofi; has received funding for travel and/or speaker honoraria from Bayer, Biogen, Merck, Novartis, Roche, and Sanofi; and receives research support from Biogen, the European Union, Novartis, Roche, the Swiss MS Society, and the Swiss National Foundation. **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 Research & Development Institute, Inc., Billerica, MA, USA, an affiliate of Merck KGaA, Merck, Novartis, Roche, Sanofi, and Teva. **PV** has received honoraria or consulting fees from AB Science, Biogen, Celgene (BMS), Imcyse, Merck, Novartis, Roche, Sanofi, and Teva; and research support from Novartis, Roche, and Sanofi. **FB** is supported by the NIHR Biomedical Research Centre at UCLH and is a steering committee or Data Safety Monitoring Board member for ATRI/ACTC, Biogen, Merck and Prothena. Consultant for Celltrion, Combinostics, IXICO, Janssen, Merck, Rewind Therapeutics, Roche. Research agreements with Biogen, GE Healthcare, Merck, Roche. Co-founder and shareholder of Queen Square Analytics Ltd. **KS** has received research support from Biogen, Merck, and Novartis; speaking honoraria from, and/or served in an advisory role for, Amgen-Gensenta, Biogen, EMD Serono Research & Development Institute, Inc., Billerica, MA, USA, an affiliate of Merck KGaA, Merck, Novartis, Roche, Sanofi, and Teva; and remuneration for teaching activities from AcadeMe, Medscape, and the Neurology Academy. **NDS** is a consultant for Bayer, Biogen, Merck, Novartis, Roche, Sanofi, and Teva; has grants or grants pending from FISM and Novartis, is on the speakers' bureaus of Biogen, Merck, Novartis, Roche, Sanofi, and Teva; and has received travel funds from Merck, Novartis, Roche, Sanofi, and Teva. **AA** has received honoraria or consulting fees from Bayer, Biogen, Celgene (BMS), Merck, Novartis, Roche, and Sanofi; and research support from Bayer, Biogen, Merck, Roche, and Sanofi. **LL** has received honoraria for consulting services or speaking activities from Biogen, Merck, Novartis, and Roche; and research support from Biogen, Merck, and Novartis. **AC** has received speakers'/board honoraria from Actelion (Janssen/J&J), Almirall, Bayer, Biogen, Celgene (BMS), Merck, Novartis, Roche, Sanofi, and Teva, all for hospital research funds. He received research support from Biogen, Sanofi, and UCB, the European Union, and the Swiss National Foundation. He serves as associate editor of the European Journal of Neurology, on the editorial board for Clinical and Translational Neuroscience, and as topic editor for the Journal of International Medical Research. **XM** has received speaking honoraria and travel expenses for participation in scientific meetings, has been a steering committee member of clinical trials or participated in advisory boards of clinical trials in the past years with AbbVie, Actelion (Janssen/J&J), Alexion, Biogen, Celgene (BMS), EMD Serono Research & Development Institute, Inc., Billerica, MA, USA, an affiliate of Merck KGaA, Immunic, Janssen, MedDay Merck, Mylan, Nervgen, Novartis, Roche, Sandoz, Sanofi, Teva, TG Therapeutics, Excemed, MSIF, and NMSS. **AP** has received honoraria and operating grants from pharmaceutical companies. **AK** and **HJ** are employees of Merck Healthcare KGaA, Darmstadt, Germany. **LG** is an employee of employee of EMD Serono Research & Development Institute, Inc., Billerica, MA, USA, an affiliate of Merck KGaA. **EJ** is an employee of Merck OY, Espoo, Finland, an affiliate of Merck KGaA. **HW** is member of scientific advisory boards/steering committees for Bayer, Biogen, Merck, Novartis, Roche, Sanofi, and Teva. He received speaker honoraria and travel support from Bayer, Biogen, CSL Behring, EMD Serono Research & Development Institute, Inc., Billerica, MA, USA, an affiliate of Merck KGaA, Fresenius Medical Care, Merck, Omniamed, Novartis, Sanofi, and Teva. He received compensation as a consultant from Biogen, Merck, Novartis, Omniamed, Roche, and Sanofi. He has received research support from Bayer, Biogen, Merck, Novartis, Sanofi, and Teva, as well as German Ministry for Education and Research (BMBF), German Research Foundation (DFG), Else Kröner Fresenius Foundation, Fresenius Foundation, Hertie Foundation, Merck, Novartis, NRW Ministry of Education and Research, Interdisciplinary Center for Clinical Studies (IZKF) Münster, and RE Children's Foundation.

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RESULTS

Supplementary Table 1. Lymphocytes and markers

Population	Phenotype
Memory B cells	$CD45^{bright}$, SSC^{low}, $CD3^-$, $CD14^-$, $CD56^-$, $CD19^+$, $CD20^+$, $CD27^+$
$CD19^+$ B cells	$CD45^{bright}$, SSC^{low}, $CD3^-$, $CD14^-$, $CD56^-$, $CD19^+$
$CD4^+$ naïve cells	$CD3^+$, $CD8^-$, $CD4^+$, $CD45RA^+$, $CCR7^+$
$CD8^+$ naïve cells	$CD3^+$, $CD8^+$, $CD4^-$, $CD45RA^+$, $CCR7^+$
$CD4^+$ cells	$CD45^{bright}$, SCC^{low}, $CD3^+$, $CD4^+$

Data collection and analysis was sponsored by Merck (CrossRef Funder ID: 10.13039/100009945)

RESULTS

Supplementary Table 2. Full patient characteristics

		No CUA lesions N=96 (45.5%)	Resolved CUA lesions N=91 (43.1%)	Residual CUA lesions N=24 (11.4%)
Sex	Female, n (%)	64 (66.7)	64 (70.3)	15 (62.5)
	Male, n (%)	32 (33.3)	27 (29.7)	9 (37.5)
Age	Age >40 years, n (%)	51 (53.1)	38 (41.8)	5 (20.8)
	Age ≤40 years, n (%)	45 (46.9)	53 (58.2)	19 (79.2)
Time since onset of MS (Months), mean ± SD		87.72 ± 85.72	78.85 ± 84.32	67.65 ± 76.11
Time since diagnosis (Months), mean ± SD		59.14 ± 73.35	54.36 ± 67.71	49.83 ± 73.44
Time since first relapse (Months), mean ± SD		54.28 ± 74.86	50.01 ± 69.27	44.48 ± 66.44
≥1 relapses within 12 months prior to Baseline, n (%)		96 (100)	89 (97.8)	24 (100)
EDSS score at Baseline	EDSS score ≤3, n (%)	66 (68.8)	70 (76.9)	23 (95.8)
	EDSS score >3, n (%)	30 (31.2)	21 (23.1)	1 (4.2)
Prior DMT use	Prior DMT, n (%)	52 (54.2)	52 (57.1)	13 (54.2)
	Treatment naïve, n (%)	44 (45.8)	39 (42.9)	11 (45.8)
High relapse activity*	High relapse activity, n (%)	63 (65.6)	51 (56.0)	17 (70.8)
	Non high relapse activity, n (%)	33 (34.4)	40 (44.0)	7 (29.2)

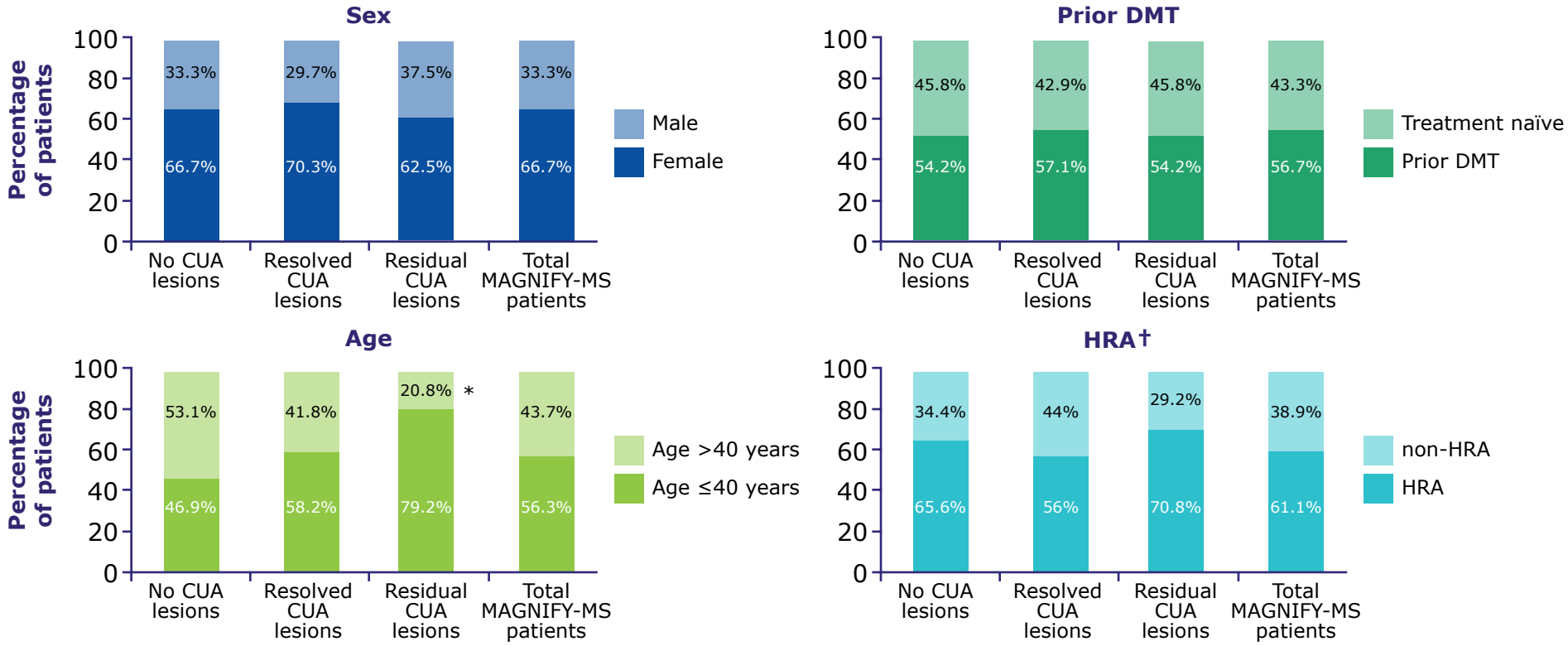
*High relapse activity defined as 2 or more relapses in the previous year regardless of prior DMT use

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

Data collection and analysis was sponsored by Merck (CrossRef Funder ID: 10.13039/100009945)

RESULTS

Supplementary Figure 1. Patient characteristics



No CUA lesions (n=96) Resolved CUA lesions (n=91) Residual CUA lesions (n=24) Total MAGNIFY-MS patients (n=270)

- There were no differences in demographics distribution, except a significantly higher proportion of patients with residual CUA lesions were aged ≤40 years

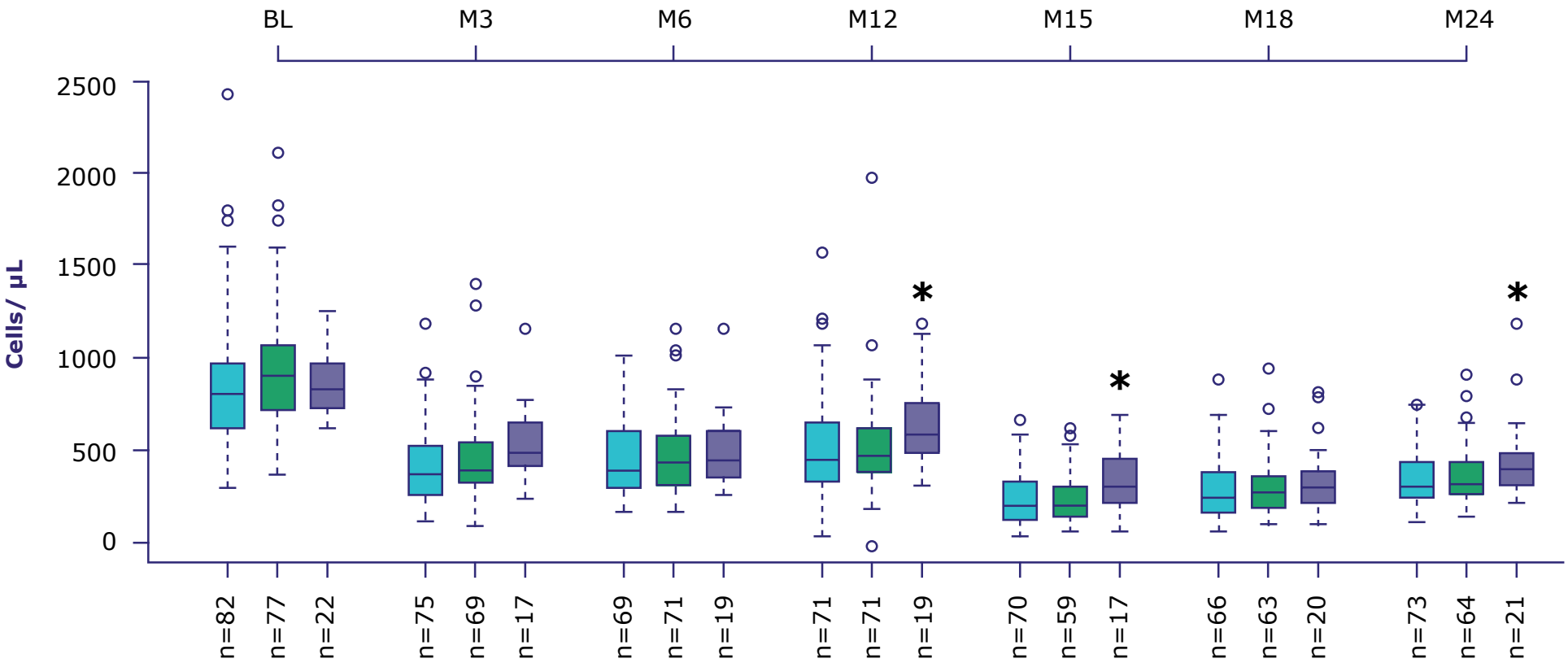
*Statistical difference ($p<0.05$) observed in age stratification for residual vs no CUA lesions
†Defined as 2 or more relapses in the previous year regardless of prior DMT use

CUA, combined unique active; **DMT**, disease-modifying therapy; **HRA**, high relapse activity

Data collection and analysis was sponsored by Merck (CrossRef Funder ID: 10.13039/100009945)

RESULTS

Supplementary Figure 2. Changes in lymphocyte subset counts – CD4⁺ T cells



- Patients with residual CUA lesions had higher CD4⁺ T-cell counts at M12, M15, and M24 ($p<0.05$)

*$p<0.05$ in comparison to no CUA lesions

BL, baseline; **CUA**, combined unique active; **M**, month

No CUA lesions | Resolved CUA lesions | Residual CUA lesions

Data collection and analysis was sponsored by Merck (CrossRef Funder ID: 10.13039/100009945)