Early selective depletion of Lymphocyte B subsets and Immune Tolerance restoration in Highly Active Relapsing Multiple Sclerosis patients during Cladribine treatment

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Background: Cladribine (Clad) was approved for Highly Active Relapsing Multiple Sclerosis (HA-RMS). It is well known that Clad depletes lymphocyte subsets (LS) *in vivo* with a predilection for B cells[1], but these effects are not fully understood.

Aim: We investigated in vivo Clad effects on memory B cells (Bmem), regulatory B cells (Bregs), regulatory T cells (Tregs), cytotoxic T cells (Tc) and helper T cells (Th) in blood-samples from HA-RMS patients.

Methods: Thirty HA-RMS patients started Clad (Early Sales Program) from July 2018. Blood samples were collected at the beginning of treatment (W0) and after 8, 24, 48 weeks and were analyzed by flow cytometry. Wilcoxon signed-rank test was used to comparison continuous variables between the different time points.

Figure 1. B memory (CD19+/CD27+) trend from W0 to W24 represented by dot plots (A) and line chart (B).

* p-value < 0.05;
** p-value ≤ 0.001;
*** p-value ≤ 0.0001.

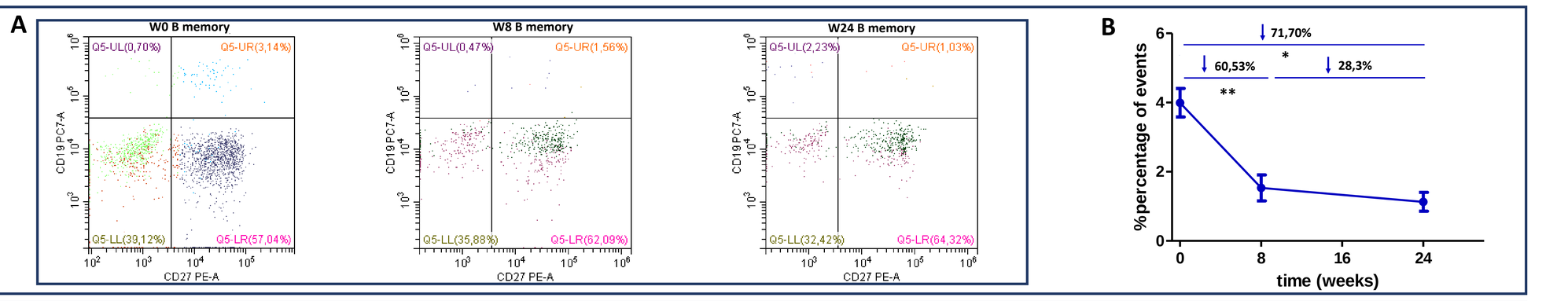


Figure 2. B regulatory (CD19+/CD38+) trend from W0 to W24 represented by dot plots (A) and line chart (B).

* p-value < 0.05;
** p-value ≤ 0.001;
*** p-value ≤ 0.0001.

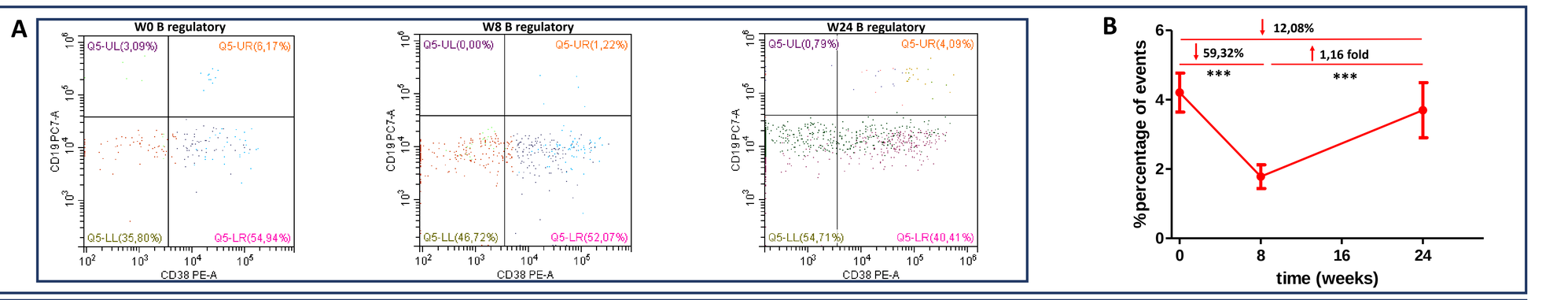


Figure 3. B regulatory (CD19+/CD25+) trend from W0 to W24 represented by dot plots (A) and line chart (B).

* p-value < 0.05;
** p-value ≤ 0.001;
*** p-value ≤ 0.0001.

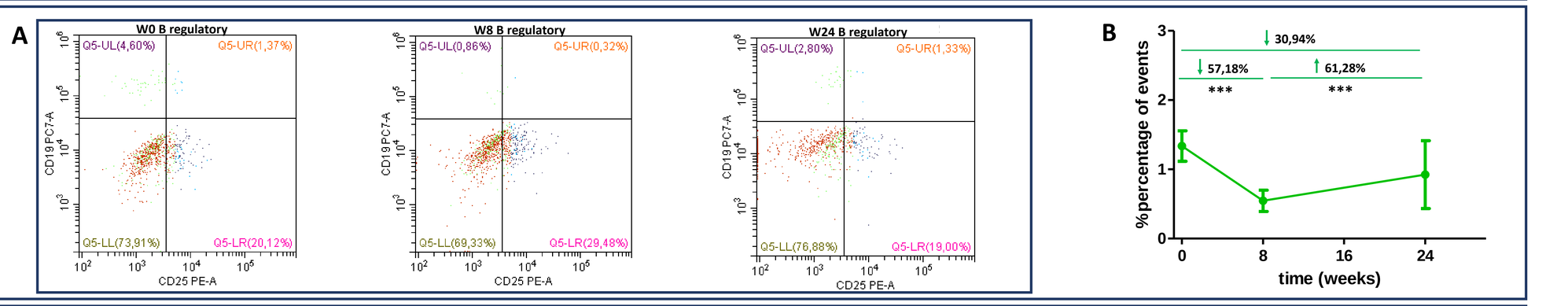


Figure 4. T regulatory (CD4+/CD25+) trend from W0 to W24 represented by dot plots (A) and line chart (B).

* p-value < 0.05;
** p-value ≤ 0.001;
*** p-value ≤ 0.0001.

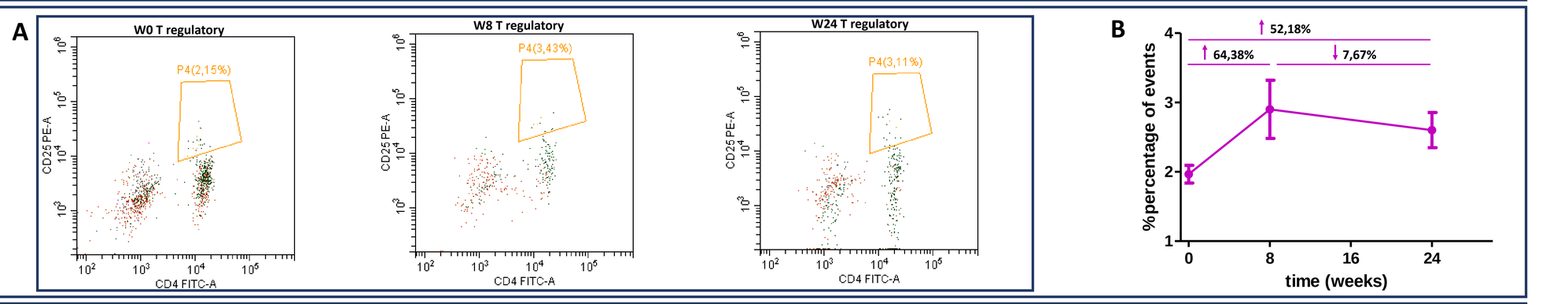


Figure 5. T helper (CD3+/CD4+) trend from W0 to W24 represented by dot plots (A) and line chart (B).

* p-value < 0.05;
** p-value ≤ 0.001;
*** p-value ≤ 0.0001.

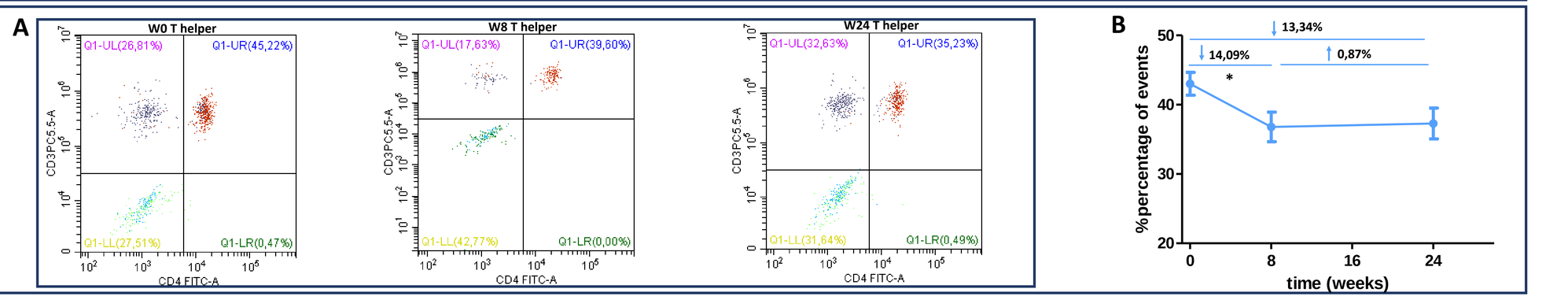
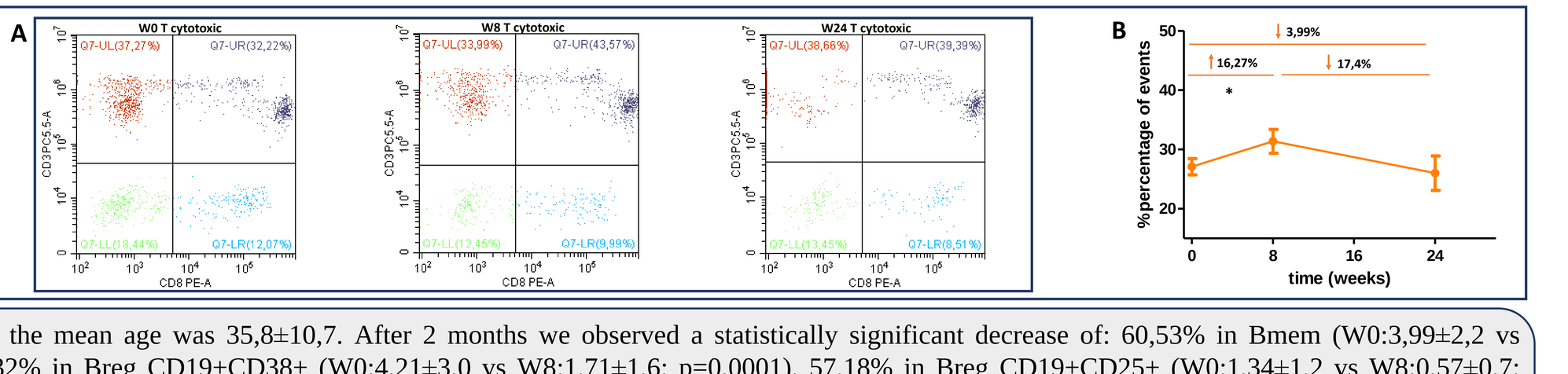


Figure 6. T cytotoxic (CD3+/CD8+) trend from W0 to W24 represented by dot plots (A) and line chart (B).

* p-value < 0.05;
** p-value ≤ 0.001;
*** p-value ≤ 0.0001.



Results: At Clad beginning, the mean age was 35,8±10,7. After 2 months we observed a statistically significant decrease of: 60,53% in Bmem (W0:3,99±2,2 vs W8:1,58±1,8; p=0,001), 59,32% in Breg CD19+CD38+ (W0:4,21±3,0 vs W8:1,71±1,6; p=0,0001), 57,18% in Breg CD19+CD25+ (W0:1,34±1,2 vs W8:0,57±0,7; p=0,0001) and 14,09% in Th (W0:43,02±7,4 vs W8:36,96±9,4; p=0,004). Furthermore, we observed an increase of 64,38% in Tregs CD4+CD25+ (W0:2,06±0,7 vs W8:3,40±2,1; p=0,27), 16,25% in Tc (W0:27,08±8,9 vs W8:31,48±10,1; p=0,015). After 6 months, patients showed a 28,30% further decrease of Bmem; moreover, a 1,16-fold increase and a 61,28% increase respectively for the two Breg subsets compared to W8 was observed. Treg unchanged at W24 but they were higher than the pre-treatment. Tc at W24 returned to basal value while Th remained unchanged and still lower than W0 [**Figures 1–6**].

Conclusions: Our results supported the hypothesis that Clad selectively depletes B lymphocytes especially Bmem; the increase of T and B regulatory subsets suggests a putative role of Clad in restoring immune tolerance. A longer follow-up in a large cohort of patients is necessary to confirm these findings.

References:
1. Jacobs BM, et al. Cladribine: mechanisms and mysteries in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2018; 89(12):1266-1271

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