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This study was sponsored by Merck KGaA, Darmstadt, Germany

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CONCLUSION

The immune cell changes detected in the CLARITY trial at 2 years are suggestive of a shift towards a more anti-inflammatory phenotype following treatment with cladribine tablets.

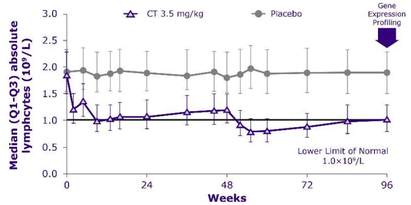
At 2 years, memory B and plasma cells were reduced, and naive B cells and M2 macrophages were increased with cladribine tablets.

The correlation found in the deconvolution vs flow cytometry data is useful to support the interpretation of the deconvolution findings for cell types not analysed by flow cytometry.

INTRODUCTION

- Cladribine tablets 10 mg (cumulative dose of 3.5 mg/kg) are administered as two short oral courses at the beginning of Years 1 and 2 for treatment of multiple sclerosis (MS).
- Total lymphocyte counts are reduced following dosing with cladribine tablets.[1-3]
  - Median values return to the lower limit of normal within 11 months (and median B cells by 6 months) (**Figure 1**, pooled data from CLARITY, CLARITY Extension and the PREMIERE registry).[1]
  - However, clinical efficacy of cladribine tablets is sustained beyond lymphocyte recovery.
  - Data show that clinical efficacy is sustained for up to 4 years after cladribine dosing.[4]

Figure 1. Median absolute lymphocyte counts over time in the Cladribine Tablets 3.5 mg/kg and Placebo Groups



Placebo, n=	434	415	263	378	358
Cladribine tablets, n=	**683**	**645**	**437**	**624**	**574**

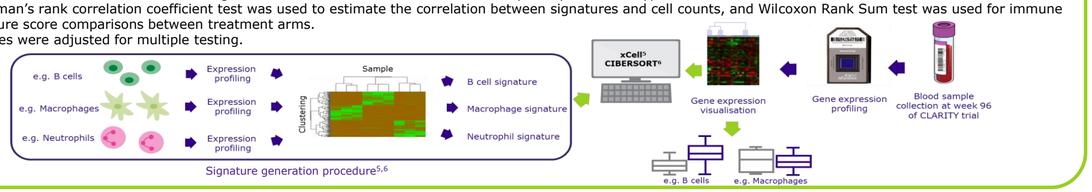
OBJECTIVES

- **Apply immune cell signatures based on gene expression profiling of peripheral blood from a subset of patients with relapsing-remitting MS during immune repopulation at 96 weeks from baseline in the CLARITY study (NCT00213135).**
- **Compare immune cell estimates in cladribine and placebo-treated patients.**
- **Validate immune cell signatures by comparing them with corresponding flow cytometry data of main lymphocyte subtypes.**

METHODS

Figure 2. Expression profiling and deconvolution

- Gene expression profiling of a preselected cohort of patients from the CLARITY trial was done using an Affymetrix U133 microarray (patients randomized to placebo [n=57], cladribine tablets 3.5mg/kg [n=62], or cladribine tablets 5.25mg/kg [n=70]).
- xCell[5] and CIBERSORT[6] deconvolution algorithms were used to estimate the relative quantity of cell subtypes at 96 weeks.
- Spearman's rank correlation coefficient test was used to estimate the correlation between signatures and cell counts, and Wilcoxon Rank Sum test was used for immune signature score comparisons between treatment arms.
- *P*-values were adjusted for multiple testing.



Signature generation procedure[5,6]

RESULTS

Figure 3. B cell enrichment and abundance score at 2 years

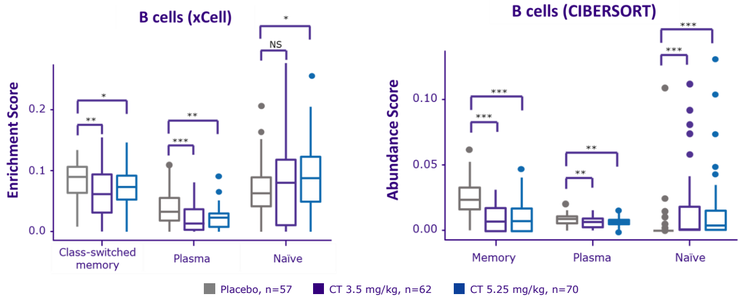


Figure 5. Macrophage enrichment at 2 years (assessed by xCell)

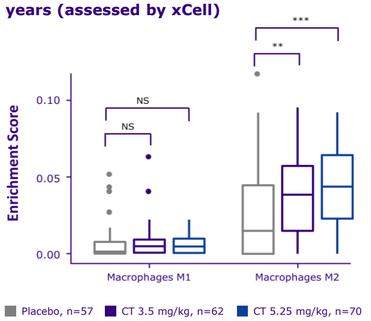
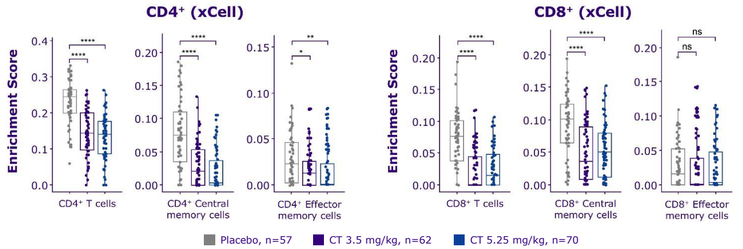
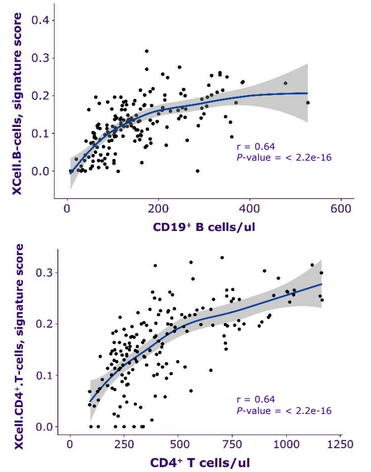


Figure 4. CD4⁺ and CD8⁺ T cell enrichment at 2 years



p-values: *<0.05; **<0.01; ***<0.001; **ns**, not significant

Figure 6. Flow cytometry vs deconvolution at 2 years



- The relative abundance of naïve B cells was nominally significantly higher with cladribine tablets vs placebo (**Figure 3**).
- Plasma cells and class-switched memory B cells were nominally significantly reduced with cladribine tablets vs placebo (**Figure 3**).
- Central memory CD4⁺ and CD8⁺ T cells and CD4⁺ effector memory cells were nominally significantly reduced with cladribine tablets vs placebo (**Figure 4**).
- The M2 macrophage signature was nominally significantly enhanced with cladribine tablets vs placebo (**Figure 5**).
- There was positive and nominally significant correlation of CD19+ B cells and CD4+ T cells assayed by flow cytometry and by the deconvolution assay (**Figure 6**).

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