Abstract no. 44

Increase of Naïve B Cells, M2 Macrophages and Reduction of Memory B/T Cells During Immune Repopulation at 96 Weeks in CLARITY Assessed by Immune Cell Deconvolution

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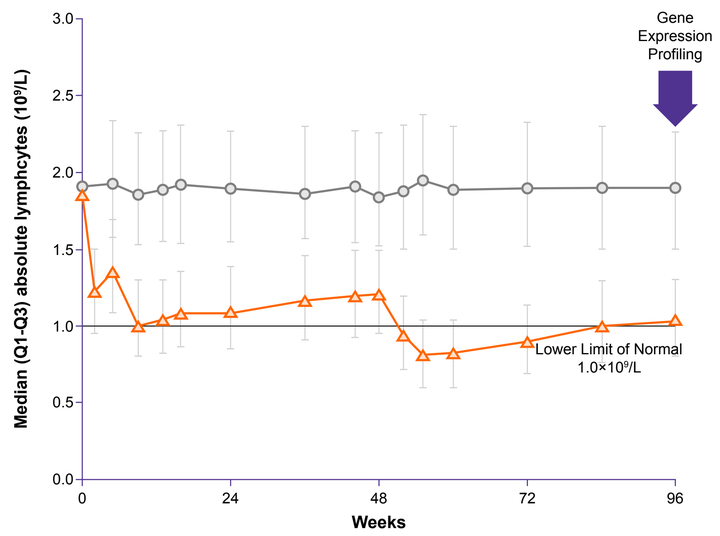
INTRODUCTION

- Cladribine tablets 10 mg at a cumulative dose of 3.5 mg/kg are administered as two short oral courses at the beginning of Years 1 and 2.
- Total lymphocyte counts are transiently reduced following dosing, with median values returning to within normal range within 9 months in year 2 (**Figure 1**) and B cell median counts returning to within normal range by 6 months in year 1.[1-3]
- Flow cytometric observations suggest a long-lasting reduction in memory B cells.[1]

Figure 1. Median Absolute Lymphocyte Counts Over Time in the Cladribine Tablets 3.5 mg/kg and Placebo Groups



Visits with sample size ≥30 are displayed.

OBJECTIVE

- To characterise immune cell type abundance in peripheral blood from patients with relapsing-remitting multiple sclerosis (RRMS) in the CLARITY study using advanced computational algorithms.

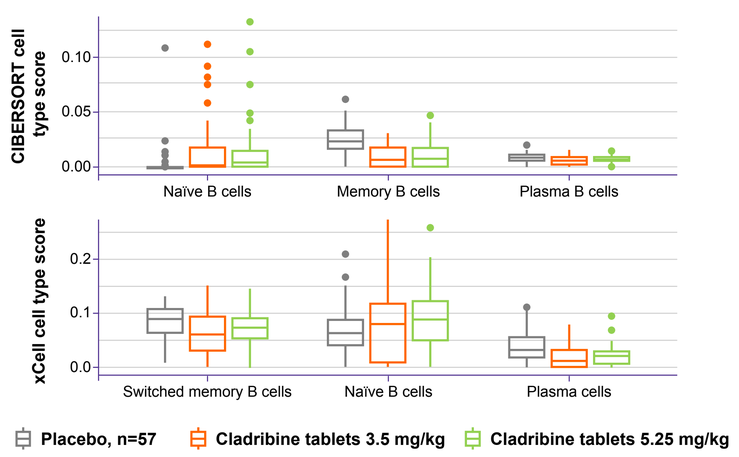
METHODS

- The CLARITY trial (NCT00213135) was designed as a double-blind, placebo-controlled study to evaluate the efficacy and safety of oral cladribine in patients with RRMS.[4]
- Expression profiling of the whole blood of 189 patients collected at week 96 post-baseline was performed using Human Array U133 Plus 2.0 (Affymetrix).
- There were 57 placebo, 62 3.5 mg/kg cladribine tablets, and 70 5.25 mg/kg cladribine tablets patients.
- These samples were analysed with the CIBERSORT[5] deconvolution algorithm and the xCell[6] signature-based method for immune cell subsets (**Figure 2**).
- CIBERSORT uses support vector regression to estimate absolute fractions of 22 immune cell subtypes and xCell performs cell type enrichment analysis for 43 immune cell types.
- Comparison between arms was done using a Wilcoxon Rank Sum test and results with *P* values less than 0.05 were considered nominally significant.

RESULTS

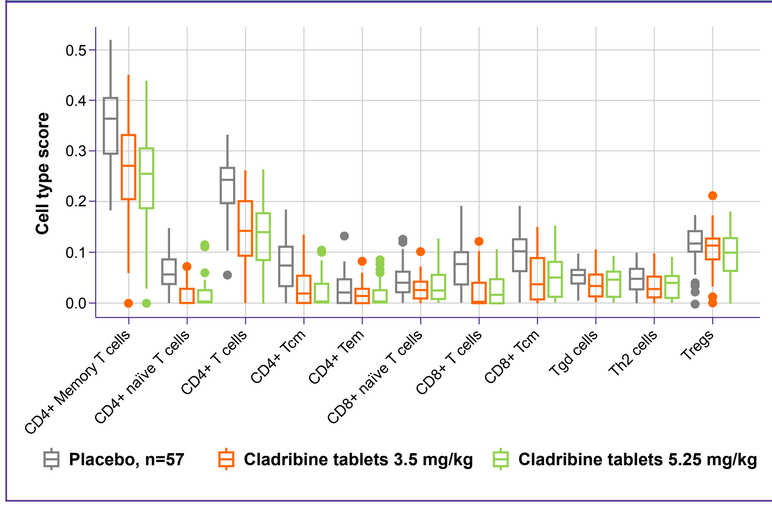
- At 96 weeks, the relative abundance of naïve B cells in cladribine treated patients was significantly higher than in placebo (**Figure 3**).
- Although total mature B cells were not changed anymore at the 48 week time point, memory B cells and plasma cells were significantly reduced with cladribine tablets vs placebo (**Figure 3**).
- Cell abundance of naïve and memory CD4$^+$, CD8$^+$ and TH2 T cells was significantly reduced with cladribine tablets vs placebo (**Figure 4**).
- The M2 macrophage signature was significantly enhanced with cladribine tablets vs placebo (**Figure 5**).
- There was no significant difference between natural killer cells, basophils, platelets, dendritic cells, mast cells, CD8$^+$ T effector memory or pro B cells with cladribine tablets vs placebo (data not shown).

Figure 3. B Cell Abundance



Supplementary Appendix 1: Abundance significance tables and median CD19+ B lymphocyte counts at 24 months obtained through QR code

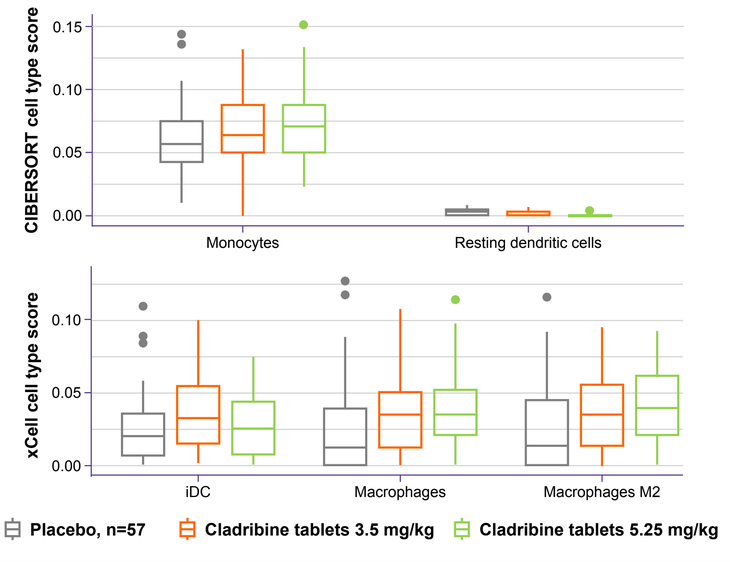
Figure 4. CD4$^+$ and CD8$^+$ T Cell Abundance



Tcm, T central memory; **Tem**, T effector memory; **Tgd**, T gamma delta; **Th2**, T helper 2; **Tregs**, regulatory T cells

Supplementary Appendix 2: Abundance significance tables and median CD4$^+$ and CD8$^+$ T lymphocyte counts at 24 months obtained through QR code

Figure 5. Monocyte and Macrophage Abundance



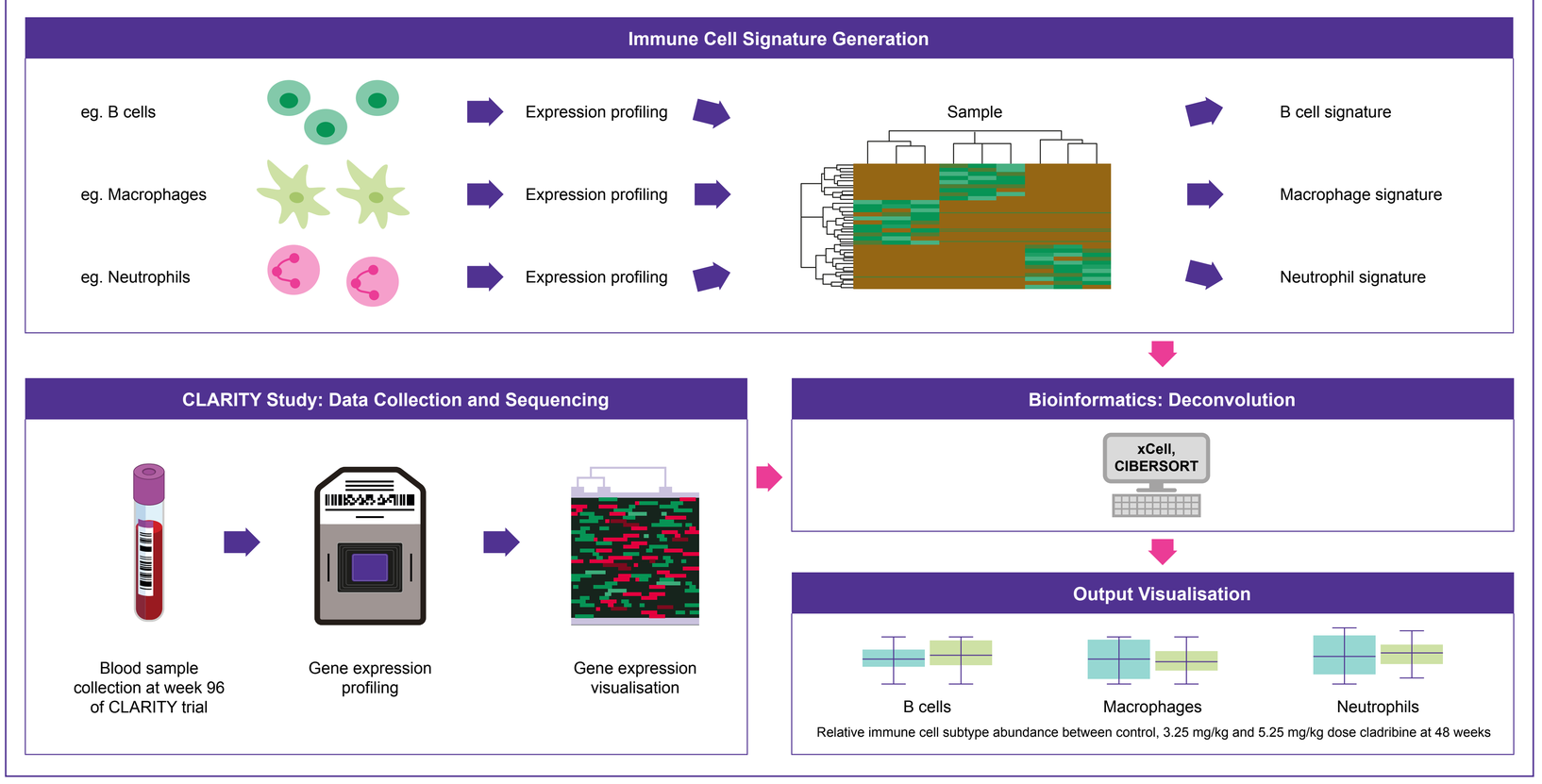
iDC, immature dendritic cells.

Supplementary Appendix 3: Abundance significance tables obtained through QR code

CONCLUSIONS

- **At 96 weeks following treatment with cladribine tablets, early reductions in memory B cells are maintained, while naïve B cell numbers appear to have recovered. We also observed significant reductions in plasma cells and both memory and naïve T cell subsets at 2 years. Importantly, there was a relative increase in M2 macrophages, a cell type that is not directly targeted by cladribine.**
- **To our knowledge, neither of the bioinformatic computational techniques described in this study have been previously applied to microarray data in a multiple sclerosis clinical study.**
- **The changes in leukocytes observed are suggestive of a qualitative and quantitative shift in the immune environment of patients with MS towards an anti-inflammatory phenotype following treatment with cladribine tablets.**

Figure 2. Deconvolution Method



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DISCLOSURES

GG has received speaker honoraria and consulting fees from Abbvie, Actelion, Atara Bio, Almirall, Bayer Schering Pharma, Biogen Idec, FivePrime, GlaxoSmithKline, GW Pharma, Merck & Co., Merck KGaA, Pfizer Inc, Protein Discovery Laboratories, Teva Pharmaceutical Industries Ltd, Sanofi-Genzyme, UCB, Vertex Pharmaceuticals, Ironwood, and Novartis; and has received research support unrelated to this study from Biogen Idec, Merck & Co., Novartis, and Ironwood. **TL** has received consultancy fees or clinical research grants from Biogen, EMD Serono, Novartis, Genentech, Chugai, Alkermes. **PSS** has served on advisory boards for Biogen, Merck KGaA, Novartis, Teva, MedDay Pharmaceuticals, and GSK; on steering committees or independent data monitoring boards in trials sponsored by Merck KGaA, Teva, GSK, and Novartis; has received speaker honoraria from Biogen Idec, Merck KGaA, Teva, Sanofi-Aventis, Genzyme, and Novartis. His department has received research support from Biogen, Merck KGaA, Teva, Novartis, Roche, and Genzyme. **IK**, **JDM** and **AR** are employees of EMD Serono Research & Development Institute Inc., a business of Merck KGaA Darmstadt Germany. **UB** is an employee of Merck Serono SA, Eysins, Switzerland, a business of Merck KGaA, Darmstadt, Germany.

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