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Increased Naïve B Cells, M2 Macrophages, and Decreased Memory B and T Cells during Immune Repopulation in CLARITY Assessed by Immune Cell Deconvolution

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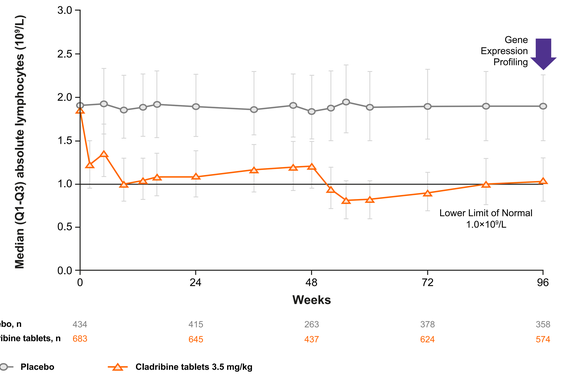
INTRODUCTION

- 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.

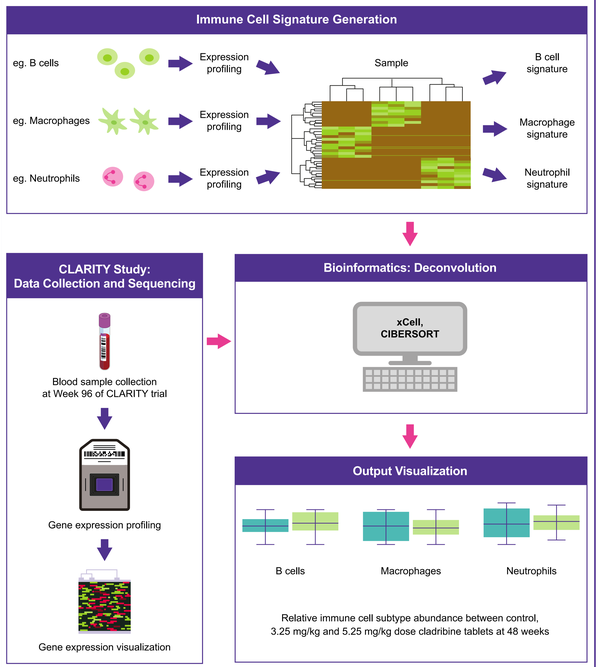
OBJECTIVE

- To characterize 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 cladribine tablets 3.5 mg/kg, and 70 cladribine tablets 5.25 mg/kg patients
- These samples were analyzed 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

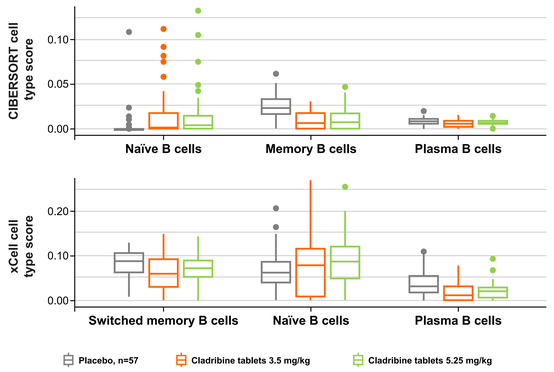
Figure 2. Deconvolution Method



RESULTS

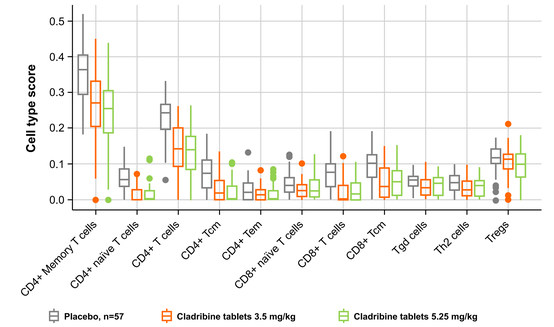
- At 96 weeks, the relative abundance of naïve B cells in cladribine treated patients was significantly higher than in placebo (**Figure 3**)
- Although total mature B cells were not changed anymore at the 48 week time point, memory B cells and plasma cells were significantly reduced with cladribine tablets versus placebo (**Figure 3**)
- Cell abundance of naïve and memory CD4$^+$, CD8$^+$ and TH2 T cells was significantly reduced with cladribine tablets versus placebo (**Figure 4**)
- The M2 macrophage signature was significantly enhanced with cladribine tablets versus 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 versus 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

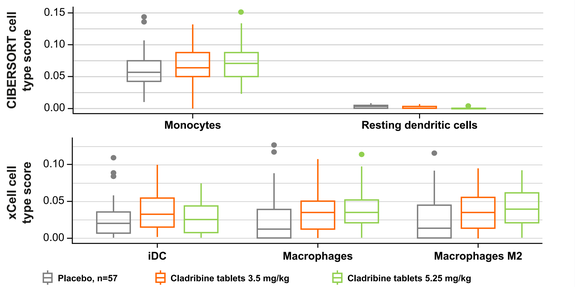
Figure 4. CD4$^+$ and CD8$^+$ T Cell Abundance



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 & 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 were maintained, while naïve B cell numbers appeared 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 mutiple sclerosis towards an anti-inflammatory phenotype following treatment with cladribine tablets**

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ACKNOWLEDGMENTS

This study was sponsored by EMD Serono Inc, a business of Merck KGaA, Darmstadt, Germany (in the United States), and Merck Serono SA, Geneva, an affiliate of Merck KGaA Darmstadt, Germany (ROW). The authors would like to thank patients and their families, investigators, co-investigators, and the study teams at each of the participating centres and at Merck KGaA, Darmstadt, Germany. Medical writing assistance was provided by Ella Palmer and Farah Johnson-May of inScience Communications, Springer Healthcare Ltd, Chester, UK, and was funded by Merck KGaA, Darmstadt, Germany.

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, a business of Merck KGaA Darmstadt Germany. **UB** is an employee of Merck Serono SA, Eysins, Switzerland, a business of Merck KGaA, Darmstadt, Germany. **DJ** is an employee of Merck KGaA, Darmstadt, Germany.

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