Title: 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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Short title to be displayed on app: Immune Cell Deconvolution in CLARITY

Background: Cladribine tablets (CT) 10 mg (cumulative dose of 3.5mg/kg [CT3.5]) are administered as two short oral courses over 2 years. Total lymphocyte counts are transiently reduced following dosing, with median values returning to normal range within 11 months and B-cell median counts within 6 months. Clinical efficacy of CT is sustained beyond lymphocyte recovery. Flow cytometric observations suggest a long-lasting reduction in memory B cells. Objectives were to apply advanced computational algorithms to characterise immune cell transcriptomic signatures in relapsing-remitting multiple sclerosis patients during immune repopulation at 96 weeks in the CLARITY study.

Methods: Gene expression data (U133 Plus 2.0 array) in whole blood samples at 96 weeks were available from patients randomised to placebo (n=57), CT3.5 (n=62) or CT 5.25 mg/kg (CT5.25,n=70). These were analysed with the CIBERSORT deconvolution algorithm and the xCell signature-based method for immune cell subsets. CIBERSORT uses support vector regression to estimate absolute fractions of 22 immune cell subtypes, xCell performs cell type enrichment analysis for 43 immune cell types. Comparison between arms were done using a Wilcoxon Rank Sum test. P-values <0.05 were considered nominally significant.

Results: At 96 weeks, the relative abundance of naïve B-cells in CT treated patients was significantly higher vs placebo. Plasma cells and class-switched memory B-cells were significantly reduced in CT vs placebo. No significant difference in mature B-cells between placebo and CT was detected. The M2 macrophage signature was significantly enhanced in CT vs placebo. Cell abundance of both naïve and memory $CD4^+$ and $CD8^+$ was significantly reduced in CT vs placebo.

Conclusions: To our knowledge, neither of the bioinformatic computational techniques described in this study have been previously applied to microarray data in an MS clinical study. At 96 weeks following CT treatment, changes in leukocytes suggestive of a shift towards an anti-inflammatory phenotype were detected.

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Author disclosures:

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Irina Kalatskaya, Julie DeMartino and **Alex Rolfe** are employees of EMD Serono Research & Development Institute a business of Merck KGaA Darmstadt Germany.

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