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POSTER ABSTRACTS

641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

High Dimensional Detection of Non-Malignant B-Cells and Its Clinical Implications in Patients with Chronic Lymphocytic Leukaemia (CLL) Undergoing Frontline Therapy

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Introduction: The analysis of non-malignant B-cells in chronic lymphocytic leukaemia (CLL) is challenging due to their scarcity and paucity of reliable identification methods. However, given the well-defined immunophenotypic characteristics of CLL cells, it is feasible to distinguish and quantify non-malignant B-cells by incorporating an extensive panel of B-cell markers into unsupervised high-dimensional analysis. In this study, we performed high dimensional mass cytometry analysis of circulating B-cells in patients enrolled in the NCRI phase III RIAltO trial, which compared frontline chemoimmunotherapy (CIT) regimens in CLL.

Methods: Mass cytometry was performed on samples obtained from CLL patients enrolled in the RIAltO trial (NCT01678430). Patients were randomly assigned to receive bendamustine or chlorambucil plus ofatumumab, with or without idelalisib. Samples were collected before treatment and at early (median 11.2 [IQR 11 - 11.7] months) and late (18.5 [16.1 - 22.6] months) post-treatment timepoints, as well as at disease relapse. Healthy controls (HC) were included for comparison. $CD19^+$ B cells were analysed using a customised antibody panel with 29 surface-antigen markers.

Results: A total of 198 samples from 79 patients and three HC were analysed. Unsupervised analysis using uniform manifold approximation and projection (UMAP) and FlowSOM identified two distinct groups of $CD19^+$ B-cell clusters. Malignant clusters were identified based on established immunophenotypic markers of CLL, while non-malignant clusters were identified using the inverse co-expression of these markers ($CD5^-CD43^-ROR1^-CD20^+CD79b^+CD81^+$). In keeping with the assumption that non-malignant clusters comprised physiological B-cells, they were the majority cell type among HC, rare before treatment or at relapse, and variably detected following therapy. When relating non-malignant clusters with clinical outcomes, their re-emergence following therapy correlated with an increase in markers of haematopoietic recovery, including haemoglobin concentration ($R^2 = 0.4$, $p = 0.002$), neutrophil count ($R^2 = 0.39$, $p = 0.002$) and platelet count ($R^2 = 0.41$, $p = 0.002$). Furthermore, re-emergence of non-malignant, $CD27^+$ memory B-cells the at late post-treatment timepoint positively correlated with serum immunoglobulins (Ig): IgA ($R^2 = 0.41$, $p = 0.012$), IgM ($R^2 = 0.37$, $p = 0.027$) and IgG ($R^2 = 0.42$, $p = 0.01$) levels. Notably, stratification of patients based on post-treatment median cluster size showed that a higher frequency of non-malignant B-cells correlated with significantly longer time to progression (TTP; median 49.9 [95% CI: 28.5 - NR] versus 9.7 [7.2 - 26.8] months, $p < 0.001$). This correlation remained significant after adjusting for baseline characteristics, CLL prognostic factors, treatment allocation, minimal residual disease (MRD) status, and haematopoietic recovery. Subsequently, a Boolean gating strategy which prospectively defines non-malignant B-cells ($CD5^-CD43^-ROR1^-CD20^+CD79b^+CD81^+$) was devised. Despite a weak correlation with MRD ($R^2 = -0.27$, $p = 0.084$), the proportion of non-malignant B cells at the end of treatment was found to be an independent determinant of TTP and overall survival (OS) among patients with $\geq 0.1\%$ residual CLL cells.

Conclusion: To our knowledge, this is the first study to identify correlations between the re-emergence of non-malignant B-cells following anti-CLL therapy and specific clinical endpoints such as haematopoietic recovery, serum Ig levels, TTP and OS. Although further validation is required in the context of targeted agents, our findings suggest that therapy-induced re-emergence of non-malignancy B-cells may have important biological and clinical implications in CLL.

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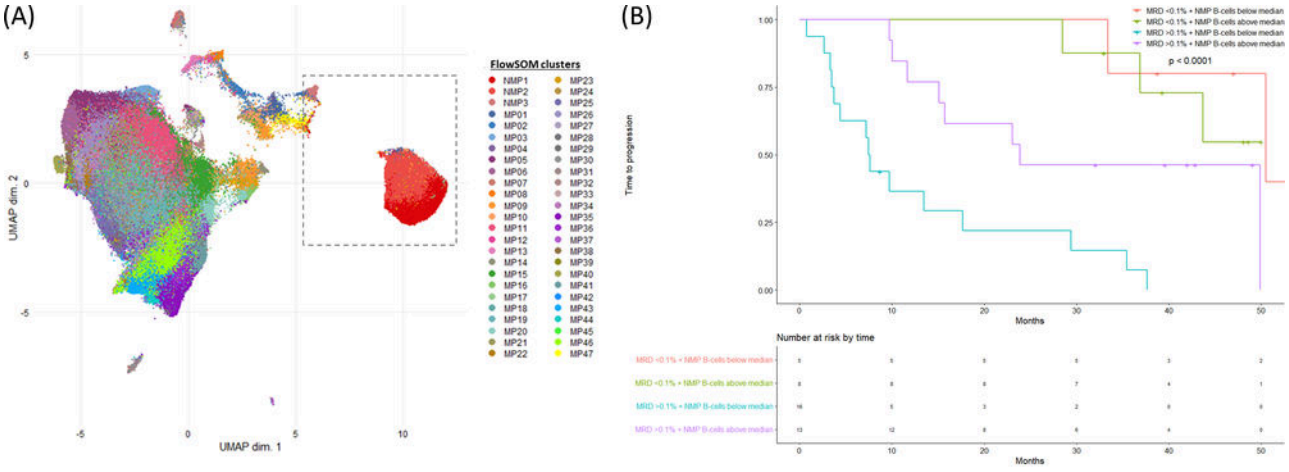

Figure 1: (A) UMAP representing CD19⁺ B cells in all analysed samples. Each event was coloured by their FlowSOM cluster assignment, and clusters with a non-malignant phenotype (NMP) were boxed together. (B) Kaplan-Meier plot and log-rank test of time to progression (TTP) among patients with positive or negative MRD3 disease status, as well as high (≥0.17%) or low (<0.17%) CD5⁻CD43⁻ROR1⁻CD20⁺CD79b⁺CD81⁺ B cells at the early post-treatment timepoint.

Figure 1

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