B-cell depletion therapy with Belimumab (BLM) is known to significantly decrease total B cells, although the mechanisms are not fully understood. Flow cytometry observations suggested a rapid reduction in B cells, particularly in patients with SLE or LN treated with SOC (n=8) or SOC+BLM (n=11). University Medical Center (LUMC), Nephrology, Leiden, Netherlands

**Objectives:** To investigate dynamics of B-cell subsets in SLE patients treated with or without BLM, with a focus on assessing MBC characteristics.

**Methods:** Extensive B cell subset phenotyping was performed by high-sensitivity flow cytometry (acquisition of 10^7 leukocytes; per EuroFlow protocols) on samples from active LN or SLE patients with major organ involvement treated with standard of care (SOC) consisting of high dose steroids and mycophenolate mofetil combined with or without the addition of BLM. MBC gene expression profiles were characterized with single-cell RNA and V(D)J sequencing (scRNA-SEQ).

**Results:** By employing HS flow cytometry, we established that the absolute increase in circulating MBC in SLE and LN patients was significant for patients who initiated BLM (Figure 1). The increase was observed in a broad range of MBC subsets (Unswitched, IgG1+, IgG2+, IgA1+, IgA2+) at 2 and 4 weeks following initiation of BLM treatment. This rise in MBC could hypothetically be attributed to either proliferation of blood MBC, BLM induced migration of tissue-resident MBCs or BLM related retention of tissue-resident MBC in the blood. scRNA-SEQ analysis of cell cycle gene expression was performed and established in both groups a non-proliferating phenotype in approximately ~94% of MBC post-treatment, including absence of MKI67 as active proliferation marker. Clonal diversity analysis comparing week 2 with baseline revealed an unexpected decrease of MBC or BLM related retention of tissue-destined MBC in the blood. ScRNA-SEQ analysis of cell cycle gene expression was performed and established in both groups a non-proliferating phenotype in approximately ~94% of MBC post-treatment, including absence of MKI67 as active proliferation marker. Clonal diversity analysis comparing week 2 with baseline revealed an unexpected decrease of MBC or BLM related retention of tissue-destined MBC in the blood.

**Conclusions:** The addition of BLM to SOC significantly increases MBCs in patients with SLE independently of proliferation, accompanied by a strong modulation of gene expression, including reduced expression of migration markers pointing towards disrupted lymphocyte trafficking. These data may have important implications for improving treatment strategies in patients with LN or severe SLE, as a deeper depletion of autoreactive MBCs could be established by adding B-cell-depleting therapy after the initiation of BLM.

**REFERENCES:**