Background: Patients with systemic lupus erythematosus (SLE) display an aberrant DNA methylation (DNAm) pattern with a predominant interferon signature. However, the DNAm patterns in purified immune cell populations are not well described.

Objectives: To examine genome-wide DNAm changes in sorted CD4+ T cells, monocytes, granulocytes and B cells in SLE patients compared to healthy controls (HC).

Methods: Genome-wide analysis was performed in 20 SLE patients with active lupus nephritis (LN) and 20 age-, sex- and ethnicity-matched HCs. Peripheral blood was processed using gradient density centrifugation for the granulocyte fraction, and CD4+ T-cells, monocytes and B cells were isolated from the mononuclear cell fraction using a RoboSep device (Stemcell Technologies). DNA was extracted using the DNA/RNA/miRNA Universal kit (Qiagen). Genome-wide DNAm was evaluated using Infinium MethylationEPIC BeadChips (Illumina Inc.). Top differentially methylated CpG sites (DMC) were validated using pyrosequencing, and further analysed in follow-up samples, as well as extended to another 36 SLE patients with inactive LN, 36 SLE with never-LN and 20 HCs. Patients and HCs were compared using a paired t-test stratified by cell type. A false discovery rate (FDR) <0.05 was considered statistically significant. Clustering of non-gene annotated CpGs was defined as distance <5000 bp with proximity to neighbouring genes <1x10^5 bp, identified through mapping of Entrez Gene Identifiers.

Results: Overall, SLE patients with active LN compared to HCs exhibited DMCs in 22, 46, 312 and 78,068 probes in CD4+ T cells, monocytes, granulocytes and B cells, respectively. In CD4+ T cells, monocytes and granulocytes, the majority of DMCs were hypomethylated and related to interferon-regulated genes. In B cells, the majority of DMCs were hypermethylated with 1960 genes in the promoter region restricted to differential methylation >10% and ≥2 DMCs, of which 1074 were hypermethylated. Of these, the most significantly hypermethylated CpGs in the promoter region included CAGYR, TMPRSS5, SLC6A2, KLK10, KLK11, WIZ, LPAR1, ZNF385B and ILT1R1 (Figure 1). Top hypomethylated genes included IFI44L and MX1. Ingenuity Pathway Analysis (IPA) predicted top upstream regulators in B cells to be IL4, TNF and IFNG. In addition, we identified 1437 DMCs associated with ncRNA and 4626 DMCs with no genomic association. The non-gene associated DMCs were related to 275 non-coding clusters. Top non-coding clusters were in proximity to BMP7, ARHGFE10, PRDM1, RIN2 and CCR6, which were differentially methylated. IPA predicted IL2, CD40LG and NFKB1 as the most important upstream regulators for neighbouring genes of non-coding clusters.

Pyrosequencing confirmed B cell hypermethylation in SLE patients is widespread, and aberrant DNAm patterns in CD4+ T cells, monocytes, and granulocytes in all but monocytes, as well as hypermethylation at the non-coding cluster in chromosome 3 (p=0.05) and MTA3 (p=0.03) in B cells.

Conclusion: B cell hypermethylation in SLE patients is widespread, and may indicate a novel mechanism for SLE pathogenesis. Differential methylation of several interferon-regulated genes may be associated with disease activity.
Conclusion: SGECs in the inflammatory milieu of pSS are characterized by induction of autophagy and pro-survival mechanisms, and by expression of adhesion molecules. These changes correlate with SG infiltration with immune cells and with histologic disease severity. Among clinically available therapies, the JAK/STAT inhibitor baricitinib effectively reduced autophagy, countered the state of maladaptive activation of SGECs, and restored epithelial cell homeostasis. Transcriptomics and metabolomics studies are ongoing to dissect the specific mechanisms responsible for these beneficial effects.

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OP0237 DISTINCT IMMUNE NETWORKS STRATIFY ORGAN INVOLVEMENT AND RESPONSE TO B CELL TARGETED THERAPIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

M. Shira1, L. Santos Ribeiro1, D. Nguyen1, A. Embleton-Thirsk2, M. Parvaz1, D. Isenberg1, C. Gordon3, M. Ehrenstein1.

1University College London, Rheumatology, London, United Kingdom; 2University College London, Comprehensive Clinical Trials Unit, London, United Kingdom; 3University of Birmingham, Rheumatology Research Group- Institute of Inflammation and Ageing, Birmingham, United Kingdom

Background: The results from the double-blind BEAT-lupus trial comparing belimumab vs placebo, both after rituximab in systemic lupus erythematosus (SLE) have recently been reported (1). We sought to identify biomarkers of response to belimumab after rituximab to aid a personalised approach to therapy for SLE.

Objectives: To identify biomarkers of clinical response to belimumab after rituximab in the BEAT-lupus trial.

Methods: We constructed a model utilising a range of clinical, routine and exploratory laboratory data, from the BEAT-lupus trial to identify variables at baseline (screening) that could predict a major clinical response (MCR, defined as reduction to BILAG C in all domains, steroid dose of ≤7.5mg/day & SLEDAI≤2, without anti-dsDNA antibody component) at 24 weeks. Relevant serum autoantibodies and cytokines were assayed by ELISA/Simoa, and interferon signatures and BAFF expression measured by RT-PCR. A linear mixed model was applied to longitudinal data collected during the trial stratified by treatment and clinical response. An independent cross-sectional lupus cohort was recruited to validate biomarker association with organ involvement.

Results: A major clinical response (MCR) was achieved in 48% (10 responders, 11 non-responders) of patients who received belimumab after rituximab compared to 35% (8 responders, 15 non-responders) in the placebo group (i.e. rituximab alone), added to tapered standard of care, at 52 weeks. Baseline serum IgA2 anti-dsDNA antibody levels emerged as the only positive predictor of attaining MCR in belimumab treated patients (AUROC 0.8, 95% confidence interval [CI] 0.7-0.9), but negatively predicted MCR in the placebo arm (AUROC 0.2, CI 0.1-0.4). At baseline, 77% and 85% of patients were positive for serum IgA2 anti-dsDNA antibodies in belimumab and placebo arms respectively, which reduced to 30% at 52 weeks in the belimumab group but remained unchanged with placebo (Fisher exact test, p=0.007). In striking contrast, the percentage of patients who remained IgG anti-dsDNA antibody positive from baseline to 52 weeks were similar between the belimumab and placebo group, despite the serum levels significantly falling in the belimumab group (1). A significant reduction in serum IgA2 anti-dsDNA antibody levels at 24 and 52 weeks from baseline was only observed in belimumab responders (Figure 1).

The number of circulating IgA2-secreting (but not total) plasmablasts (p=0.032) and T follicular helper cells (p=0.031) were significantly reduced at 52 weeks in the belimumab treated arm compared to placebo. Elevated serum IgA2 anti-dsDNA antibody levels were also associated with active renal disease irrespective of treatment arm (odds ratio, OR 3.2, CI 1.7-5.8, p<0.001). In contrast, serum IgA1 anti-dsDNA antibody (OR 1.3, CI 1.0-1.7, p=0.042) and interferon-alpha levels (OR 1.4, CI 1.0-2.0, p=0.041), and interferon transcriptional signature (OR 1.1, CI 1.0-1.3, p=0.027) showed a modest association with mucocutaneous disease activity; but did not predict response to B cell targeted therapy. Patients with a high baseline serum IL-6 were less likely to achieve an MCR irrespective of therapy (OR 0.4, CI 0.2-0.9, p=0.033). The associations between serum IgA2 and IgA1 anti-dsDNA antibody levels and active renal and mucocutaneous disease respectively were confirmed in an independent cross-sectional lupus cohort.

Conclusion: IgA2 anti-DNA autoantibodies is a biomarker of response to belimumab after rituximab, and of active renal disease, in systemic lupus erythematosus. Our study reveals distinct molecular networks associated with renal and mucocutaneous involvement, and response to B cell targeted therapies, which could guide precision targeting of current therapies for this heterogeneous disease.

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Figure 1. Percentage change in serum IgA2 anti-dsDNA antibody levels through to 52 weeks stratified by clinical response to belimumab (after rituximab) and placebo (after rituximab) at 52 weeks.