less severe inflammation in lupus. However, we still need to determine the mechanism behind the changes in the migration of B cells to the gut immune tissue, the production of IgA, and the microbiome composition as well as the role of P. distasonis in lupus inflammation.

S01.3 RNA-SEQ IN PERIPHERAL BLOOD IMMUNE CELLS IDENTIFIES MODULAR NETWORKS PREDICTIVE AND PROTECTIVE FOR PROGRESSION FROM ANA POSITIVITY TO CLASSIFIABLE SYSTEMIC AUTOIMMUNE DISEASE

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ANA-positivity represents a complex 'At-Risk' state for development of connective tissue disease (CTD). While ANA may become positive years in advance of clinical CTD, they are also positive in up to 25% of the population, of whom only a small fraction ultimately develop symptoms. Complex immune disturbances iare evident even among ANA-positive individuals who do not ultimately progress to overt disease [1]. In a prospective observational cohort of ANA positive individuals 'At-Risk' for CTD we have shown that a validated blood IFN-Score was predictive of progression to classifiable SLE [2]. However, the wider transcriptional fingerprint of the 'At-Risk' state and other factors modifying risk of progression are not known. We hypothesise that diverse immune processes, both independent and interacting with IFN pathway activation, could modulate risk of progression.

Purpose To investigate how peripheral blood immune cell transcriptional signatures derived by RNA Seq associate with progression or non-progression from At-Risk ANA positivity to clinically apparent CTD.

Methods Peripheral blood mononuclear cells (PBMCs) were isolated at baseline from ANA-positive At-Risk individuals demonstrating ≤1 clinical criterion for classifiable CTD, symptom duration <12 months and naive of glucocorticoid or immunosuppressive therapy. Progression was prospectively adjudicated at 12 months and defined as accrual of clinical/immunological criteria sufficient to meet classification for SLE (SLICC 2012) or other relevant CTDs. Bulk RNASeq was performed on PMBCs from 16 Progressors and 19 non-Progressors. Weighted gene co-expression network analysis (WCGNA) was performed using WCGNA package and gene ontology (GO) enrichment was envaluated using ClusterProfiler, in R Bioconductor. The top 20% genes ranked by connectivity were defined as hub genes. Major cell subsets were quantified in parallel by multiparameter flow cytometry.

Results 29 modules were identified by WCGNA. Eigengenes for 3 modules were significantly associated with progression status. A single, 152 gene module showed strong positive correlation with progression ($R=0.55$, $p<0.001$). Hub genes were significantly enriched for type I IFN-siganlling pathway and included established interferon stimulated genes such as IFI44 and IRF7.

Two further modules had a negative, ie protective, association with progression; a smaller 37 gene module, correlated negatively with both blood interferon score ($R=-0.46$, $p=0.005$) and with progression ($R=-0.43$, $p=0.01$). A larger 252 gene module was also negatively related to progression ($R=-0.43$, $p=0.009$) and demonstrated significant pathway enrichment for regulation of cell morphogenesis and actin cyctoskeleton organisation.

Conclusions We identify novel modular transcriptomic signatures implicated in SLE disease initiation. We show (i) IFN-pathway activation is the single strongest transcriptomic risk marker of progression from the 'At Risk state' and (ii) we identify 2 novel protective signatures in peripheral blood immune cells for which further functionally characterization is ongoing.

S01.4 BELIMUMAB DISRUPTS MEMORY B-CELL TRAFFICKING IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Purpose Belimumab (BEL), a recombinant human monoclonal antibody directed against B-cell activating factor (BAFF), is the first approved biological agent for patients with systemic lupus erythematosus (SLE) and a high level of disease activity or lupus nephritis (LN). BEL inhibits primary humoral immune responses by depleting naive B-cells that are dependent on BAFF for their survival while secondary humoral immune responses by memory B cells (MBCs) remain intact. Indeed, some studies reported an increase of circulating MBCs following neutralization of BAFF1,2. So far these effects of BEL on the MBC compartment in SLE patients have not been investigated. This study aimed to establish the dynamics of circulating MBCs in patients with SLE treated with BEL and to perform an in-depth analysis of the impact of BEL on the MBC compartment.

Methods First, extensive B cell subset phenotyping was performed prospectively by employing high-sensitivity flow cytometry (HSFC) based on EuroFlow protocols3 in severe SLE/LN patients treated with BEL4. Additionally, in-depth characterisation of surging MBCs in circulation was performed by single-cell RNA sequencing (scRNA-seq).

Results HSFC established that the increase in MBCs was non-specific and observed in a broad range of MBC immunoglobulin subclasses peaking as early as 2 weeks after BEL initiation. Subsequent scRNA-seq analysis of the emerging MBCs revealed a non-proliferating phenotype with a prominent decrease in activation status. In these circulating MBCs, a large amount of migration and adhesion genes were downregulated suggesting that the accumulation of MBCs following BEL treatment was related to their impaired cell-cell adhesion, disrupting cell-trafficking and preventing extravasation.

Conclusions After initiation of BEL treatment, a substantial increase of circulating MBCs was firmly established in patients with SLE/LN. The surge of circulating MBCs appeared to be associated with disrupted lymphocyte trafficking of MBCs, thereby suggesting a new potential therapeutic mechanism of BEL on MBCs in SLE. These findings have important implications to our understanding and consequent improvement of B-cell targeted treatment strategies in patients with active SLE and LN as MBC accumulation in circulation might allow for more efficient targeting of the B-cell compartment.