The concentration of soluble IL-6 receptor increased after BCD-089 administration in a dose-dependent manner. A raise in the concentration of IL-6 was seen at doses >1.0 mg/kg. Membrane IL-6R saturation of 90%>100% was seen at doses ≥0.6 mg/kg. Regardless to the dose, serum CRP decreased below the limit of detection in most volunteers within the first week after injection. All tested PD markers returned to baseline at the end of the follow-up.

Conclusions: Single SC administration of BCD-089 was well tolerated, showed favourable safety profile and low immunogenicity at all tested doses in healthy volunteers. PK/PD assessment revealed non-linear PK and significant capacity to inhibit the IL-6 signalling pathway. These findings support further clinical development of BCD-089.

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SAT0024

IL-36 AXIS IS AN EMERGING THERAPEUTIC TARGET IN PSORIATIC ARTHRITIS SYNOVIAL TISSUE

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Background: The IL-36 family of cytokines includes three agonists, IL-36 α , IL-36 β and IL-36 γ , and two established or hypothetical antagonists, respectively IL-36Ra and IL-38. IL-36 agonists are pro-inflammatory cytokines highly expressed in skin and involved in the pathogenesis of psoriasis. A recent study demonstrated that only a subset of patients with rheumatoid arthritis (RA) had an elevated IL-36 agonists/antagonists ratio within the synovium and could potentially respond to IL-36 inhibition strategies but little is known about the expression and biologic functions of the IL-36 axis in synovial tissue of psoriatic arthritis (PsA) so far hardly been studied.

Objectives: In this study, we aimed to comparatively investigate the expression and role of IL-36 cytokines in synovial tissue of early RA and PsA patients.

Methods: Synovial tissue samples were collected from patients with early RA and PsA (disease duration <12 months) DMARDs (Disease Modifying Anti-Rheumatic Drugs) and steroids-naïve. All patients underwent an ultrasound-guided synovial biopsy before starting the treatment; the procedure was repeated following six months of treatment with conventional DMARDs. The expression of IL-36 family members was investigated in synovial tissue at gene level by RNA-Sequencing (87 RA, 15 PsA), at protein level by immunohistochemistry (IHC) and immunofluorescence (IF) (20 RA, 26 PsA) and in plasma by ELISA (22 RA, 38 PsA). RA and PsA-fibroblasts-like-synoviocytes (FLS) and peripheral blood mononuclear cells (PBMCs) were treated *in vitro* to assess their response to IL-36 stimulation.

Results: Gene and protein expression of IL-36 agonists was comparable between RA and PsA synovial tissue; conversely, the antagonists IL-36Ra and IL-38 were significantly lower in PsA compared to RA. Accordingly, the agonists/antagonists ratio was considerably higher in PsA synovium, suggesting an activation of the IL36 pro-inflammatory pathway. Among the immune cells infiltrating the PsA synovium, macrophages (CD68+), T lymphocytes (CD3+) and plasma cells (CD138+) were the primary IL36 α -expressing cells. At baseline, the synovial expression of IL-36 α was significantly higher in PsA patients who did not respond to DMARDs treatment at 12 months; this differential synovial expression of IL-36 α between responders and non-responders was also maintained at six months. In keeping with this observation, we showed that treatment with methotrexate or sulfasalazine did not reduce the expression of IL-36 in PsA cells *in vitro*. Finally, we observed that PsA-FLS and PsA-PBMCs produced significantly higher levels of IL-8 upon stimulation with IL-36 α in comparison with cells isolated from RA patients.

Conclusions: The expression of the anti-inflammatory IL-36 cytokines antagonists are differently regulated in early RA and PsA, being significantly lower in the latter. Moreover, the pro-inflammatory IL-36 α is up-regulated in synovial tissue of PsA non-responders to conventional DMARDs. The impaired balance between agonists and antagonists might contribute to the persistent inflammation characterising the diseased tissue. The exogenous replacement of the IL-36 antagonists may be a novel promising therapeutic target for PsA patients.

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SAT0025

CXCL4 ALTERS TRANSCRIPTOMIC AND EPIGENETIC IMPRINTING OF DENDRITIC CELLS THEREBY DRIVING FIBROSIS THROUGH EXTRACELLULAR MATRIX FORMATION

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Background: Aberrant accumulation of extracellular matrix (ECM) in multiple organs or fibrosis is one of the three hallmarks that characterises the pathogenesis of systemic sclerosis (SSc), together with immune dysregulation and small vessel vasculopathy. Recent studies have shown that CXCL4 (Chemokine CXC motif ligand 4) levels are increased in patients with SSc and correlated with skin and lung fibrosis.¹ CXCL4 plays key role in physiological processes, although it has been also implicated in several pathological conditions such as autoimmune diseases and cancer. We and others have shown that CXCL4 modulates the phenotype and function of immune cells², suggesting a critical role of this chemokine in innate and adaptive immune responses. However, how CXCL4 exactly modulates immune cell responses remains unclear.

Objectives: Here we investigated the impact of CXCL4 exposure on the transcriptome and DNA methylation of monocyte-derived dendritic cells (moDCs), and the consequence on their function.

Methods: We differentiated human moDCs in the presence of CXCL4. After 6 days differentiation, cells were stimulated with a TLR3 ligand (polyI:C) as described in our previous study.² RNA sequencing and DNA methylation profiling was performed at various time points during differentiation and stimulation.

Results: Integration of high-throughput analyses of RNA sequencing and DNA methylation reveals that CXCL4 drives to dramatic changes on the transcriptome and epigenome levels. This is reflected in the dysregulation of critical innate and adaptive immune pathways, like antigen presentation, and cytokine signalling. For the first time, we show that CXCL4 potentiates a novel function to dendritic cells, namely, the production of ECM molecules, such as fibronectin (FN1) and osteopontin (OPN). Furthermore, we also found that CXCL4 exposure results in epigenetic imprinting during moDC differentiation. Using novel bioinformatic methods, we have found that CXCL4 mediates the altered cell function via key transcriptional regulators.

Conclusions: This study provides better understanding how CXCL4 affects moDCs through several immune and non-immune pathways and shows for the first time the direct implication of CXCL4 on the production of ECM by inflammatory cells, thereby underscoring the pivotal role of CXCL4 in inflammatory and fibrotic conditions such as SSc.

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SAT0041

COMPARISON OF DIFFERENT TYPE 1 IFN SIGNATURES DEMONSTRATES CONCORDANCE IN A REAL WORLD, HOME MONITORED SYSTEMIC LUPUS ERYTHEMATOSUS COHORT

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Background: Type 1 Interferon (IFN) expression has been shown to correlate with disease severity in systemic lupus erythematosus (SLE) patients¹ and anti-IFN biologics are being evaluated in a clinical setting.² The 4-gene IFN response signature of IFI27, IFI44, IFI44L and RSAD2² is frequently used to determine IFN expression. However, numerous studies have reported on the use of different gene signatures.^{3, 4, 5} The impact of using different IFN gene signatures to stratify SLE patients is unclear.

Objectives: The present study compares the relative performance of 4 IFN gene signatures in a cohort of 687 participants with self-reported SLE.

Methods: A centralised site, IRB-approved, SLE cohort was recruited using social media. Qualified participants with self-reported SLE were consented electronically and asked to provide medical record review consent, complete an online questionnaire regarding their disease as well as provide 3 fingerstick blood samples over approximately a 6 week period. Blood samples from 687 participants were tested using a multi-modular gene expression assay containing 11 IFN response genes (primarily from the IFN- α response pathway). Normalised gene expression values were calculated, and the resulting data analysed to determine concordance between IFN gene signatures.

Results: 10 of the 11 IFN response genes were highly correlated with one another ($\rho \geq 0.80$). The 4-gene signature of IFI27, IFI44, IFI44L, and RSAD2² identified 36.5% of the participants as IFN high. Three other literature reported IFN signatures^{3, 4, 5} provided similar classification results with participants being assigned to the same IFN sub-group over 90% of the time, and nearly identical patient distributions.

Abstract SAT0041 – Table 1

Signature	Genes	IFN High (%)	IFN Low (%)	% Agreement (95% CI) to Furie et al.
Furie et al. ²	IFI27, IFI44, IFI44L, RSAD2	36.5	63.5	N/A
Niewold et al. ³	EIF2AK2, IFIT1, MX1	36.3	63.7	94.8% (93.4–95.9)
Kirou et al. ⁴	EIK2AK2, IFIT1, IFI44	36.0	64.0	95.1% (93.7–96.2)
Westra et al. ⁵	IFI6, HERC5, IFIT1, MX1	36.0	64.0	94.8% (93.4–95.9)

Conclusions: The study demonstrated that commonly used IFN gene signatures provide similar IFN subtyping to the 90th percentile. The use of social media to engage patients directly along with self-collection of blood samples provides new opportunity for testing clinical study participants and potentially patients without requiring an office visit.

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SAT0042 EVIDENCE OF NETOSIS IN CIRCULATING NEUTROPHILS AND SKIN LESIONS WITH VASCULITIS AND PANNICULITIS IN BEHÇET'S DISEASE

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Background: Behcet's disease (BD) pathophysiology is poorly understood. It is characterised by recurrent episodes of acute inflammation consisting of neutrophil infiltration in affected organs and around blood vessels. Recently, NETosis, trapping and killing pathogens by activated neutrophils, has been described. These Neutrophil Extracellular Traps (NETs) were shown to promote inflammation in a number of autoimmune diseases such as Systemic Lupus Erythematosus, ANCA-Associated Vasculitis and Rheumatoid Arthritis. We hypothesise that in BD, neutrophils trigger inflammation and vasculitis via NETosis.

Objectives: The aim of this study is to demonstrate the implication of NETosis in the pathophysiology of this disease.

Methods: Blood specimens from BD patients and matched healthy volunteers were collected at the American University of Beirut Medical Centre. Circulating neutrophils were isolated and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium. Cultured cells were treated with colchicine, dexamethasone or corresponding vehicle, and stimulated with serum from BD patients, controls or left unstimulated. The kinetic and amount of NET formation was assessed by cell counting using Hoechst 33 342 and Sytox green staining and fluorescent microscopy. Moreover, previously described NET-bound proteins (elastase, myeloperoxidase, citrullinated histone-3 and PR-3) were identified by immunolabeling and mRNA expression levels of PAD4, a key enzyme that promotes citrullination of arginine residues on chromatin, was evaluated in neutrophils from BD compared to controls. Finally, evidence of NETosis *in vivo* was assessed on paraffin embedded specimens from damaged organs of BD patients using immunolabeling and confocal microscopy.

Results: The percentage of unstimulated neutrophils undergoing NETosis *in vitro* was significantly higher in BD patients compared to controls. Treatment of unstimulated BD neutrophils with colchicine and dexamethasone resulted in significant decrease in NETs formation compared to controls. Interestingly, the percentage of NETs increased upon exposure of neutrophils to serum from BD patients. Moreover, PAD4 mRNA expression was 3 times higher in BD patients compared to controls. Immunolabeling assay demonstrated that NET-bound proteins were present in NETs scaffold of unstimulated neutrophils from BD patients *in vitro*. Additionally, NETs were detected in skin tissues of BD patients focally distributed in proximity to small vessels in vasculitis patients and to inflamed adipose tissue in panniculitis patients. These were associated with elastase and citrullinated histone-3 proteins.

Conclusions: We show here for the first time that circulating neutrophils from BD patients are prone to NETosis *in vitro*. NET formation was inhibited by the addition of colchicine and dexamethasone reflecting their therapeutic benefits in BD. The increase of NETosis upon serum stimulation suggests the presence of soluble factors and cytokines triggering NETs in BD. Combined, these results suggest a major role of NETs in the pathophysiology of BD.

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SAT0043 IL-33/ST2-MEDIATED INFLAMMATION IN ENDOTHELIAL CELL IS DIRECTLY AGGRAVATED BY IL-6 DURING LUPUS NEPHRITIS

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Background: "Alarmins" are prototypic endogenous pro-inflammatory factors as they are released from necrotic cells and provoke local damage or systemic inflammation. Evidences are accumulating to support the inclusion of "Alarmins" as targets of autoreactivity as well as inducers in the pathogenesis of Systemic Lupus Erythematosus (SLE). Interleukin (IL)–33 is a novel member of the family of "Alarmins" because of its characteristics and functions in mediating host immune responses. On this background, we sought to determine the role of IL-33/ST2 axis in lupus pathogenesis. The role of IL-33/ST2 axis has not previously been described in lupus nephritis.

Objectives: This project will study the followings:¹ To determine whether IL-33 was present in renal glomerular endothelial cells;² To assess the functional and intracellular signal transduction mechanisms regulating the link between IL-33/ST2-mediated innate immunity and inflammation in human umbilical vein endothelial cells (HUVECs).

Results: This study, for the first time, showed that IL-33 was pathologically expressed in the kidney tissue of patients with lupus nephritis and not in that of subjects with relative normal renal tissues from atrophy. However, no significant difference was observed between patients with lupus nephritis and kidney cancer. Immunofluorescence (IF) for IL-33 in kidney was performed in lupus patients. IL-33 was clearly seen in glomeruli and also in peritubular areas. To determine whether the IL-33 staining in glomeruli area was in endothelium, multiple staining for IL-33, CD34 (a marker for endothelial cells) and 4',6-diamidino-2-phenylindole