PD-1, CCR7, CXCR5, CD45RA antibodies. All patients fulfilled the ACR 1982 classification criteria for SLE. Cellular changes were analyzed in the context of clinical information.

Results: In the present cohort, the SLE patients were mainly female (86.6%) and of median age of 36.7 (29.8-49.4) with a disease duration of 6.1 (1.6-11.8) years, and active disease with SLEDAI-2K at baseline 12.0 (8.0-16.0). The frequency of age-associated B cells (ABCs; CD27-IgD-CD11c+ CD21-) decreased by 13% ($p=0.03$) in the first two to four months after rituximab start, while globally the DN (IgD-CD27-) B cells transiently increased by around 3% ($p=0.15$) at the first follow-up. This increase could not be attributed to the DN1 (CXCR5+CD11c-) or DN2 (CXCR5-CD11c+) subsets but to the CD11c-CXCR5- DN (DN3) B cells (increase= 6.7%, $p=0.03$). In parallel, T effector cells (CCR7- CD45RA+) and TEMRA (CD45RA+ CCR7-) frequencies increased after first follow up in both CD4+ and CD8+ T cells. The frequency of T_{FH} (CXCR5+ PD-1+) cells did not change after rituximab, however a decrease of PD-1^{high} CD4+ cells was observed in most patients, although not significant, after 2-4 month of treatment. In most patients the frequency of PD-1^{high} CD4+ cells either reduce or stay the same after RTX treatment (reduction= 0.53, $p=0.28$). After 11-15 months of RTX treatment the frequency of PD-1^{high} CD4+ T cell reduces by a -0.5% in comparison to 2-4 months ($p=0.039$). The SLEDAI at baseline did not correlate with the frequency of PD-1^{high} CD4+ T cells ($r=0.03$, $p=0.9$).

Conclusion: the importance of T cell - B cell interactions in SLE pathogenesis was recently strengthened by the identification of the lymphocyte subsets T_{FH} / T_{PH} and ABCs respectively. Here, in the context of rituximab treated SLE, we could detect a reduction in the frequencies of both ABCs and PD-1^{high} T cells after treatment with rituximab, while the DN3 and effector memory T cells frequencies increased. Our data suggests that anti-CD20 mediated B-cell depletion affects both B-cell and T-cell subsets frequencies, and that monitoring these specific cell subsets may be clinically relevant.

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POS0004

T-CELL REPERTOIRE OF SYNOVIAL FLUID IN SPONDYLOARTHROPATHIES EXHIBITS HALLMARKS OF HLA-DEPENDENT CLONAL EXPANSIONS AND REMAINS STABLE OVER 1.5 YEARS

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Background: Different studies show involvement of T-cells in pathogenesis of spondyloarthropathies (SpAs) - a group of rheumatic diseases strongly associated with presence of several MHC-I alleles (HLA-B*27, -B*38, -B*39, etc). Recently we and others identified a specific T-cell receptor motif in blood and synovial fluid of HLA-B*27+ AS patients that reinforces the "arthritogenic peptide" hypothesis of AS pathogenesis [1-2]. However, common characteristics of clonal T-cell repertoire of synovial fluid in SpAs remain poorly investigated.

Objectives: We aimed to investigate synovial fluid T-cell repertoires of SpA patients with different HLA-genotypes and stability of the clonal composition in recurring flares of the disease.

Methods: Mononuclear cells were isolated from paired peripheral blood (PB) and synovial fluid (SF) samples of SpA patients (ankylosing spondylitis and psoriatic arthritis, n=27). For 3 patients additional SF samples were collected during relapse of the synovitis (after 9-15 months). CD4 and CD8 T-cells were isolated by immunomagnetic separation. Deep sequencing of UMI-tagged TCR beta cDNA libraries was used to accurately reconstruct clonal T-cell repertoires. HLA class I and II were typed for each donor using an in-house NGS-based system.

Results: We observed restricted T-cell clonal composition in synovial fluid: on average only 6% of PB T-cell clonotypes were detected in SF of the same donor. T-cell repertoires of both CD4 and CD8 SF subsets compared to PB were highly oligoclonal (index Gini PB vs SF: CD4 0.36±0.10 vs 0.68±0.08, CD8 0.57±0.17 vs 0.81±0.12) in all patients. Number of identical amino acid CDR3 sequences between two repertoires correlated with the number of identical HLA-alleles for the donors. This trend was exhibited more strikingly in SF compared to PB,

suggesting that common antigens may play a role in accumulation of identical T-cell clonotypes in the inflamed joint. Using several bioinformatic approaches we identified groups of highly similar SF clonotypes linked to HLA-B*27 and/or HLA-B*38 genotype.

Total SF repertoires of relapsing synovitis of the same donor showed huge clonal overlap, and the most frequent clonotypes remained almost unchanged (Morisita's overlap index for total SF repertoires 0.69±0.26; for top 1000 clonotypes 0.79±0.19, n=3).

Conclusion: We report HLA-dependent sharing of identical and similar T-cell clonotypes in SF of patients with ankylosing spondylitis and psoriatic arthritis and high stability of SF repertoire during several flares that support antigen-driven accumulation of T-cells in the site of inflammation.

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POS0005

THE EFFECT OF LKB1 INHIBITION IN RA PATHOGENESIS

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Background: Liver kinase B1 (LKB1) is known as a tumor suppressor gene and also inhibits reactive oxygen species (ROS) levels. Intracellular ROS are catalyzed by the enzyme complex nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX). We previously reported that NOX4 induced the migration and invasion of fibroblast-like synoviocytes (FLS) in rheumatoid arthritis (RA). Although LKB1 is expected to alleviate synovial inflammation through ROS regulation, the role of LKB1 in RA has not been examined.

Objectives: To explore whether LKB1 affects RA inflammation, we transfected LKB1 siRNA and analyzed related gene expressions in RA FLS.

Methods: Synovial tissues were obtained from RA patients who were undergoing synovectomy or joint replacement. The isolated cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin and maintained in a 5% CO₂ incubator at 37 °C. FLS were used for experiments after four to six passages. Cells were transfected with lipofectamine transfection reagent and LKB1 siRNA duplex targeting constructs. After incubation for 24h, downregulation of target expression was evaluated by real-time PCR and western blot analysis.

Results: RA FLS was transfected with LKB1 siRNA and 90% of LKB1 mRNA expression was decreased. LKB1 knock-down also caused the decreased expression of mechanistic target of rapamycin (mTOR; 0.38 fold) and serine/threonine kinase (AKT) 2 (0.40 fold), which are downstream targets of LKB1. NOX4 was significantly increased (4.94 fold) by LKB1 inhibition. On the other side, the down regulated NOX4 induced significantly elevated LKB1 mRNA expression in RA FLS. When the expressions of proinflammatory cytokines were examined, IL-1β, IL-6, TNF-α were highly increased by LKB1 deficiency. FLS migration-related chemokines, IL-8 and MMP-3 were also enhanced compared to control.

Conclusion: There was a negative correlation between NOX4 and LKB1 in RA FLS. As LKB1 deficiency induced the expression of proinflammatory cytokines and migration related chemokines, LKB1 could play a critical role in RA pathogenesis.

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POS0006

NX-5948, A SELECTIVE DEGRADER OF BTK, SIGNIFICANTLY REDUCES INFLAMMATION IN A MODEL OF AUTOIMMUNE DISEASE

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Background: Aberrant activation of B cells and autoantibody mediated tissue damage are hallmarks of autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Therefore, novel treatments that prevent autoantibody generation or antibody-mediated end organ tissue damage are of high interest. Bruton's tyrosine kinase (BTK) transduces signals downstream of the B cell receptor (BCR), toll-like receptors, and Fc receptors in B cells and myeloid cells [1]. Overexpression of BTK in B cells leads to hyperactive BCR signaling, plasma cell generation, autoantibody secretion, and an SLE-like disease in mice [2]. Conversely, reducing BTK expression in B cells can ameliorate disease in Lyn-deficient mice.[3] BTK inhibitors, such as evobrutinib, have entered clinical studies for the treatment of autoimmune diseases.[4]

Objectives: Small molecule-induced protein degradation offers a unique approach to target BTK; this approach simultaneously eliminates both BTK kinase activity and BTK-mediated scaffolding interactions in the signalosome. Chimeric Targeting Molecules (CTMs) are small molecules that catalyze ubiquitylation and proteasomal degradation of target proteins and are comprised of a ubiquitin ligase binding element ("harness"), a linker, and a target binding element ("hook"). NX-5948 is a CTM that contains a BTK hook linked to a cereblon (CRBN) harness. We examined the activity of NX-5948 in a collagen-induced arthritis model as part of an assessment of its potential as a drug candidate for autoimmune disease.

Methods: Cellular degradation of BTK, Aiolos and Ikaros as well as induction of CD69 and CD86 was determined using flow cytometry. Degradation of BTK in CD-1 mice or cynomolgus monkey was determined using flow cytometry analysis. In a collagen-induced arthritis (CIA) model, mice were vaccinated with type II collagen and treated before the onset of symptoms. Serum cytokine and anti-type II collagen antibody levels were determined using Luminex and ELISA, respectively.

Results: In human PBMCs, NX-5948 degrades BTK at sub-nanomolar concentrations and inhibits BCR signaling as measured by CD69 and CD86 induction in anti-IgM-stimulated B cells with similar potency. Oral administration of NX-5948 in mice leads to BTK degradation to <10% of baseline levels in circulating and splenic B cells. NX-5948 also promotes potent BTK degradation in cynomolgus monkeys, and it can suppress BTK levels to <10% of baseline levels after a single oral dose as low as 10 mg/kg.

Unlike IMiD drugs such as lenalidomide, the CRBN harness of NX-5948 was designed to avoid the degradation of known CRBN neo-substrates Aiolos (IKZF3) and Ikaros (IKZF1). In primary human T cells, NX-5948 induces minimal degradation of Aiolos and Ikaros and does not promote IL-2 secretion suggesting that NX-5948 does not convey IMiD activity associated with agents such as lenalidomide.

We examined the activity of NX-5948 in a mouse CIA model compared to that of the BTK inhibitor ibrutinib or dexamethasone as a positive control. In mice treated with NX-5948, symptoms of arthritis were resolved, and a significant reduction in arthritis clinical score was observed. Treatment with NX-5948 resulted in a reduction in anti-type II collagen titer and serum levels of the pro-inflammatory cytokine IL-6. Treatment with NX-5948 yielded superior anti-inflammatory activity relative to ibrutinib and similar activity to dexamethasone. Treatment with NX-5948 was well-tolerated and, unlike dexamethasone, did not promote body weight loss.

Conclusion: Degradation of BTK by NX-5948 shows robust activity in a CIA model compared to existing agents tested as controls. These findings provide support for further investigation of NX-5948 in additional models of autoimmune disease to inform plans for clinical development.

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POS0007

LOSS OF BALANCE BETWEEN PROTECTIVE AND PRO-INFLAMMATORY SYNOVIAL TISSUE T CELL POLYFUNCTIONALITY PREDATES CLINICAL ONSET OF RHEUMATOID ARTHRITIS

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Background: Effective treatment of Rheumatoid arthritis (RA) patients is achievable within a short window of opportunity following diagnosis. T-cells are early drivers of synovial inflammation of RA, therefore, identification of pathogenic T-cell subsets at the synovial tissue of pre-RA, arthralgia subjects, would greatly improve our understanding of disease pathogenesis. Comparative analysis of healthy control, arthralgia subject and RA-patient derived synovial tissue T-cell responses will lead to the identification of pathogenic as well as protective cytokine milieu, thus enabling the identification of early therapeutic targets to help steer the immune response towards resolution.

Objectives: Characterization of T-cell polyfunctionality in the periphery and synovial tissue of 'at-risk' subjects (Arthralgia) RA-patients and healthy controls (HC).

Identification of specific, pathogenic, synovial tissue T-cell subsets.

Methods: Synovial biopsies from RA, AR and HC were obtained by arthroscopic surgery followed by RNAseq analysis (Guo et al., PLoS One, 2018). Single cell synovial tissue cell suspensions from RA, AR and HC and paired PBMC were stimulated *in vitro* and polyfunctional synovial T-cell subsets examined by flow cytometric analysis, SPICE visualization and FlowSom clustering. Flow-Imaging, was utilised to confirm specific T-cell cluster identification. Fluorescent Lifetime Imaging Microscopy (FLIM) was used to visualise metabolic status of specific T-cell populations.

Results: T-cell associated pro-inflammatory gene pathways were increased in RNAseq analysis of RA-patient and arthralgia subject compared to HC synovial tissue biopsies. Flow cytometric analysis of pro-inflammatory cytokine (TNF- α , IFN- γ , IL-2, GM-CSF, IL-17A, IL-22) production and SPICE analysis of *ex vivo* stimulated T-cells revealed marked polyfunctionality of arthralgia subject synovial T-cells, thus providing evidence for a dysregulated synovial T-cell response that pre-dates clinical onset of disease. Importantly, HC synovial tissue harbours a small, albeit surprisingly polyfunctional, CD4 T-cell population characterised

Figure 1. Identification of highly polyfunctional and pro-inflammatory synovial DP T-cells. A. Cluster analysis of RA-patient synovial tissue T-cells (asterisks indicate DP T-cell clusters). B. Flow imaging of CD4⁺, CD8⁺ and DP synovial T-cells. C. SPICE flow cytometric data visualization of DP arthralgia subject and RA-patient synovial T-cells. D. Correlation between the frequency of RA-patient synovial DP T-cells and disease severity.