Several other targets did not reach statistical significance, but trended downwards with inhibition such as IL-1 β , IL-12 IL-18 and IL-33. Following Enteseal T-cell activation by anti-CD3 stimulation, 1 μ M MK2i suppressed TNF and IL-17A.

Conclusion: MK2i suppressed LPS driven production of several disease relevant mediators such as TNF. MK2i also suppressed anti-CD3 induced T-cell derived mediators such as TNF and IL-17A. MK2 inhibition is a promising novel therapeutic target in the treatment of Spondyloarthritis.

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POS0409

MOLECULAR PROFILING OF THE REMISSION STATE IN PSORIATIC ARTHRITIS: IS THE FIRE EXTINGUISHED?

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Background: The most important goal of therapy in psoriatic arthritis (PsA) is to reach the remission state of disease. Nowadays, data on molecular players of clinical remission and effective disease inactivation are scarce: gene expression analysis could highlight markers characteristic of PsA remission state.

Objectives: Our aim was to analyze the gene expression profile of patients with clinically inactive (drug-induced remission) PsA versus healthy controls and PsA active state.

Methods: From a cohort of 300 patients affected by PsA according to CASPAR criteria, we selected 40 patients (peripheral arthritis subset) with >1-year remission by TNF α antagonism assessed by DAPSA $\leq$ 4 (R) and 40 PsA with active disease state by DAPSA > 14 score (no bDMARDs ongoing) (A), and 40 healthy controls (HC) matching for mean age and gender ratio. Both PsA groups were not on corticosteroid treatment. Each condition has been profiled first using pooled RNAs from peripheral blood (biological duplicates) by Affymetrix Human GeneChip HTA 2.0. To identify a transcript as differentially expressed in both duplicates, a fold change (FC) 1.5 and *p*-value 0.05 has been set. Then, from the comparative list of differential expressed genes (DEGs) in R vs HC state, coding mRNAs were selected and interactions analyzed by STRING software, biological functions and annotations by Gene Ontology (GO). From these analysis, 12 genes were validated in the whole PsA cohort (R+A) and HCs, by quantitative real-time PCR (RT-qPCR) in triplicate (TaqMan chemistry, GAPDH housekeeping, $2^{-\Delta\Delta Ct}$ for relative quantification, FC cutoff $\pm$ 1.5 for differential analysis).

Results: The transcriptomic analysis generated 3 comparative lists of mRNAs (R vs HC= 125; R vs A= 1184; A vs HC= 378 transcripts). Then, we focused on remission state analyzing the mRNAs list R vs HC. Filtering data for coding DEGs, was made a list of 24 genes further analyzed for functions and interactions: STRING analysis created one interactions network and GO highlighted in which biological processes these DEGs are involved (Fig panels 1A, 1B). Validation analysis by rt-qPCR in the PsA cohort (R+A) and HCs confirmed the down-regulation of c-FOS and the upregulation of CCDC50 (alias YMERY) genes in the R vs HC conditions (*p*value= 0.004). The dysregulation of further genes in other comparisons was measured, as shown in Tab and Fig panel 1C.

Conclusion: Observing the amount of DEGs, is evident that the drug-induced remission state is more similar to healthy condition, however this trend of "similarity" does not mean "identical". The bioinformatic analysis showed that DEGs in the R vs HC conditions are primarily involved in biological processes related to immune system as well as correlates in an interactions network. Our molecular characterization of clinical remission revealed that effective disease inactivation appears to be molecularly-driven by two key genes, c-FOS and CCDC50 (YMERY), both involved in the NF- κ B signalling pathway modulation.

Coding DEGs mis-regulated in the remission state. Differentially expressed genes (DEGs) in the Remission (R) state vs Healthy Controls (HC) analyzed for multiple-proteins interaction by STRING software V10.5 (panel A) and biological functions by Gene Set Enrichment Analysis (panel B). DEGs validations by RT-qPCR, confirmed the CCDC50 and c-FOS dysregulation in the R state (panel C, RQ= Relative Quantification, t test RvsHC *p*value=0,004).

GENE SYMBOL	Gene chip Array R vs HC	Remission vs HC	Active vs HC	Remission vs Active
ALPL	-1.7	-1.1	1.3	-1.25
ANPEP	-1.68	1.0	1.1	-1.1
BPI	-1.57	-1.1	1.27	-1.5
CCDC50	1.5	1.5	-1.25	1.8
CEACAM8	-2.3	-1.2	1.2	-1.5
CHI3L1	-1.59	-1.1	-1.0	-1.0
DEFA1B	-2.3	-1.4	1.9	-2.7
FCAR	-1.56	-1.1	1.1	-1.3
FOS	-1.51	-2.0	-1.5	-1.4
KLRB1	1.52	-1	-1.6	1.6
PADI2	-1.54	-1.1	1.0	-1.1
TNFSF14	-1.51	1.1	1.2	-1.1

DEGs by RT-qPCR. Validated differentially expressed genes (DEGs) in the remission (R) state vs healthy controls (HC) selected from the microarray analysis comparative list (FC cutoff $\pm$ 1.5). Differential analysis (40 R vs 40 HC vs 40 Active) was made by FoldChange (FC) cutoff $\pm$ 1.5 (overexpressed $\geq$ 1.5; - 1.5 $\geq$ downregulated).

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POS0410

BIOMARKERS REFLECTING DISTURBED GUT BARRIER DIFFER IN PATIENTS WITH SPONDYLOARTHRITIS, CROHN'S DISEASE AND ACUTE ANTERIOR UVEITIS

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Background: Spondyloarthritis (SpA) are characterized by frequent extra-musculoskeletal manifestations (EMM) among them acute anterior uveitis (AAU) and Crohn's disease (CD). Vice versa, about 50% of AAU and 20% of CD patients have concomitant SpA. SpA patients show gut dysbiosis together with frequent subclinical gut inflammation. Biomarkers reflecting disturbed gut barrier (intestinal-fatty acid binding protein (iFABP), lipopolysaccharide binding protein (LBP) and zonulin) were previously found to be elevated in patients with radiographic axial SpA (r-axSpA) (1).

Objectives: To evaluate whether biomarkers reflecting leaky gut are altered in patients with AAU, CD and SpA compared to healthy controls and whether they differ between patients with EMM with and without concomitant SpA.

Methods: A total of 100 patients from the German Spondyloarthritis Inception Cohort (GESPIC) were included – among them 20 patients with r-axSpA without EMM, 40 patients with CD and 40 patients with non-infectious AAU – out of which 19 and 20 patients, respectively, had concomitant SpA (11/8 and 20/0 axial/peripheral SpA, respectively). The GESPIC patients were compared to 20 age- and

sex-matched healthy donors (HD). The following five serum biomarkers were analyzed with ELISA: calprotectin, iFABP, LBP, soluble CD14 (sCD14) and zonulin.

Results: Patient characteristics are shown in Table 1. Serum levels of calprotectin, LBP, sCD14 and zonulin differed significantly between patients with r-axSpA, AAU and CD with and without concomitant SpA and HD (Figure 1). When comparing patients with EMM with and without underlying SpA, calprotectin serum levels were significantly elevated in CD patients with SpA (8.6 µg/ml (SD 5.5 µg/ml)) compared to CD patients without SpA (5.7 µg/ml (SD 4.1 µg/ml)); Mann-Whitney U Test, $p=0.031$). Serum levels of the analyzed biomarkers did not differ between AAU patients with and without axSpA. Spearman rank correlation revealed a significant association between CRP and calprotectin (correlation coefficient $r=0.230$; $p=0.012$), LBP ($r=0.596$; $p<0.0001$), sCD14 ($r=0.428$; $p<0.0001$) and zonulin ($r=0.221$; $p=0.016$), respectively. Furthermore, LBP and zonulin serum levels correlated positively ($r=0.208$; $p=0.023$); as well as LBP and sCD14 levels ($r=0.418$; $p<0.0001$).

Table 1. Patient characteristics. Mean values (standard deviation) or absolute numbers are shown.

	CD + SpA	CD	r-axSpA	AAU + axSpA	AAU	HD
N	19	21	20	20	20	20
Age	39.1 (11.3)	38.7 (14.4)	38.4 (10.3)	39.6 (12.0)	39.2 (12.5)	38.6 (12.9)
Male (%)	9 (47%)	9 (43%)	9 (45%)	9 (45%)	9 (45%)	9 (45%)
HLA-B27 positive (%)	5 (26%)	3 (14%)	17 (85%)	17 (85%)	13 (65%)	2 (10%)
CRP in mg/l	14.3 (25.6)	18.0 (41.8)	9.1 (11.3)	6.7 (9.9)	2.3 (3.4)	0.6 (0.7)
ASDAS	2.8 (1.1)	3.2 (0.6)	3.2 (0.6)	2.2 (1.0)		
BASDAI	4.1 (2.2)		5.4 (1.2)	3.2 (2.4)

Figure 1. Biomarkers reflecting disturbed gut barrier show distinct signatures in patients with acute anterior uveitis, Crohn's disease and axial Spondyloarthritis. Kruskal Wallis Test; p values shown. Dunn-Bonferroni Post-Hoc analyses, significant pairwise differences are marked; * $p<0.05$; ** $p<0.01$; *** $p<0.0001$ Conclusion:

We found substantial differences in biomarkers reflecting disturbed gut barrier between with SpA, CD, AAU and healthy controls. The presence of SpA was associated with higher calprotectin serum levels in CD as compared to CD without SpA.

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POS0411

TARGETING JAK-STAT SIGNALLING ALTERS THE PHENOTYPIC CHARACTERISTICS OF PsA SYNOVIAL FIBROBLASTS IN RESPONSE TO THE JAK/STAT ACTIVATOR ONCOSTATIN M

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Background: Psoriatic arthritis (PsA) is a chronic inflammatory arthritis associated with psoriasis. The JAK/STAT pathway has been linked to the pathogenesis of PsA. Recently, JAK/STAT inhibitors (JAKi) have emerged as an encouraging class of drugs for the treatment of PsA. Only a few of these inhibitors have been approved for use in PsA patients with others currently in clinical trials.

Objectives: The aim of this study was to examine the effect of JAKi on primary PsA synovial fibroblasts (FLS) function.

Methods: Primary PsA FLS were isolated and cultured with JAKi (Peficitinib, Filgotinib, Baricitinib and Upadacitinib) in the presence of the pro-inflammatory JAK/STAT activator - Oncostatin M (OSM). The effect of JAKi on these cells was

determined by Migration and Invasion Assays, ELISA and rtPCR. PsA FLS bioenergetics was assessed using an XF24 analyser, which simultaneously quantifies two energetic pathways- glycolysis (ECAR) and Oxidative phosphorylation (OCR). **Results:** OSM-induced Migration and Invasion was suppressed by all JAKi with Peficitinib, Filgotinib and Baricitinib showing the greatest effect. Analysis by ELISA and rtPCR showed reduction in MCP-1 and IL-6 expression in response to JAKi, in contrast, an increase in IL-8 was observed. These functional effects were accompanied by a change in the cellular bioenergetic profile of PsA FLS, where OSM significantly increased the ECAR:OCR ratio in favour of glycolysis where PsA FLS displayed a hypermetabolic phenotype. This effect was reversed in the presence of JAKi, which specifically targeted the glycolytic pathway with PsA FLS returning to a more quiescent phenotype.

Conclusion: This study demonstrates that JAK/STAT signalling mediates the complex interplay between inflammation and cellular metabolism in PsA pathogenesis, inhibition of which shows effective suppression of the pathogenic phenotype of PsA FLS that drives joint destruction.

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POS0412 CD21-LOW B-CELLS ARE INCREASED IN PATIENTS WITH ANKYLOSING SPONDYLITIS

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Background: Ankylosing spondylitis (AS) is a chronic immune mediated inflammatory rheumatic disease, in which primarily the sacroiliac joints and the spine are affected. Extra-skeletal manifestations (ESM) include uveitis, psoriasis, inflammatory bowel disease and peripheral arthritis. In studies into the pathogenesis of AS, B-cells have received little attention most likely due to the lack of auto-antibodies¹. A B-cell subset that has been particularly associated with auto-reactivity is characterized by low expression of CD21. These CD21^{low} B-cells are increased in systemic autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus and primary Sjögren syndrome (pSS)². At least part of the CD21^{low} B-cells are considered to represent anergic, autoreactive B-cells, that fail to become activated through conventional B-cell receptor and CD40 signaling².

Objectives: To phenotypically study the peripheral B-cell compartment in the blood of AS patients compared to pSS patients, a typical B-cell-associated autoimmune disease, and healthy controls (HC). Special emphasis was given to the CD21^{low} compartment.

Methods: The proportions and phenotype of peripheral B-cells were assessed in cryopreserved peripheral blood mononuclear cells of 45 AS patients (62% male, mean age 49.2±13.2 years, mean ASDAS 2.5±1.0), 20 age-matched patients with pSS (20% male, mean age 50.6±12.0, median (IQR) ESSDAI 3±6.25) and 30 age- and sex-matched HCs, using 15-color flow-cytometry analysis. Differences between groups were tested using the Independent Samples t-test or Mann-Whitney U test depending on the distribution of variables. Associations between CD21^{low} B-cells and clinical parameters were explored using the Pearson or Spearman correlation coefficient.

Results: The percentage of total B-cells in AS patients did not differ from pSS patients and HCs. In AS patients, percentages of CD27⁺ memory B-cells and CD27⁺IgD⁺ naïve B-cells were also similar to HCs, whereas CD27⁺IgD⁺ memory B-cells were significantly reduced in pSS patients, as expected. The proportions of CD27⁺CD38^{low}CD21^{low} B-cells among total B-cells were significantly increased in both AS (median 6.4%, $p<0.0001$) and pSS patients (median 7.8%, $p<0.0001$) compared to HCs (median 4.9%). Interestingly, only in AS patients, expression of chemokine receptors CXCR3 and CXCR5 was significantly elevated on CD27⁺CD38^{low}CD21^{low} B-cells compared to HCs ($p<0.001$ and $p<0.01$, respectively). In comparison to HCs the expression of the immune markers T-bet and CD11c by CD27⁺CD38^{low}CD21^{low} B-cells was significantly lower in AS patients ($p<0.01$ and $p<0.01$, respectively). The distribution of IgM and IgD expression within the CD27⁺CD38^{low}CD21^{low} B-cell population was similar between all three study groups. Regarding the association between CD27⁺CD38^{low}CD21^{low} B-cells and clinical parameters in AS patients, we observed a positive correlation with age ($r=0.347$, $p=0.02$) and erythrocyte sedimentation rate ($p=0.386$, $p=0.01$). Furthermore, AS patients with ESM showed increased proportions of CD27⁺CD38^{low}CD21^{low} B-cells compared to patients without ESM ($p<0.05$).

Conclusion: In this cross-sectional study, we observed an increased proportion of circulating CD27⁺CD38^{low}CD21^{low} B-cells in AS patients, similar as in patients