lumbar spine specimens from mCIA mice. (G) A schematic diagram illustrating the theory in the current study.

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FRI0150 MTOR BLOCKADE BY RAPAMYCIN DECREASES ARTHRITIS AND SPONDYLITIS DEVELOPMENT AND SEVERITY IN HLA-B27 TRANSGENIC RATS

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Background: HLA-B27 misfolding is thought to play an important role in the pathogenesis of spondyloarthritis (SpA), possibly through triggering of ER stress and the unfolded protein response. One of the mechanisms that regulates the unfolded protein response is autophagy. Autophagy is a process that degrades proteins, cytoplasmic particles and organelles in lysosomes and is regulated by protein kinases, mechanistic target of rapamycin (mTOR) and AMP activated protein kinase.

Objectives: To study whether blockade of mTOR will affect spondyloarthritis development and/or severity in the *Mycobacterium tuberculosis* (*M. tub*) induced disease HLA-B27 tg rat model.

Methods: 6 weeks old, female or orchiectomized male HLA-B27/Huβ2m transgenic rats were immunised with 60–90 µg heat-inactivated *M. tub* in IFA. Rats were prophylactically or therapeutically treated three times a week intra-peritoneally with 1.5 mg/kg rapamycin or vehicle. Clinical measurements included weight, clinical scores for spondylitis and arthritis, and hind paw swelling measured by plethysmometry. After 5 weeks of treatment rats were sacrificed; axial and peripheral joints were isolated for histology and metacarpophalangeal joints, spleen and lymph nodes were isolated for RNA isolation.

Results: In the prophylactic experiment 72.7% (8/11) and 18.2% (2/11) rapamycin treated rats developed arthritis and spondylitis compared to respectively 100% (13/13; p=0.0225) and 92.3% (12/13; p<0.0001) control animals. Also severity of arthritis and spondylitis was significantly decreased in rapamycin treated animals compared to control treated animals; mean arthritis severity of diseased rats was respectively 0.45 versus 7.15 on a scale from 0–12 (p<0.0001) and mean spondylitis severity was respectively 0.18 versus 2.07 on a scale from 0–3 (p<0.0001). Clinical findings were confirmed by histology with a significant decrease of inflammation (p<0.0001), bone- and cartilage destruction (p=0.0021) and new bone formation (p=0.0010) in peripheral joints of rapamycin treated rats compared to vehicle treated rats and a similar trend was observed in spinal joints. Also in a therapeutic setting rapamycin treatment decreased arthritis severity (mean score of 6 compared to 8.8 in controls; p=0.0317) and spondylitis severity (mean score of 1.23 compared to 2.8 in controls; p=0.0159). Histology for the therapeutic experiment is currently being performed as well as RNA analyses for autophagy genes and pro-inflammatory cytokines, like IL-17A and TNF.

Conclusions: mTOR blockade significantly suppressed arthritis and spondylitis in the *M. tub* induced disease HLA-B27 transgenic rat model of SpA.

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FRI0151 PROTEIN INHIBITOR OF ACTIVATED STAT3 PREVENTS PERIPHERAL ARTHRITIS AND GUT INFLAMMATION BY REGULATING TH17/TREG CELL IMBALANCE VIA STAT3 SIGNALLING IN MICE MODEL OF SPONDYLOARTHRITIS

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Background: Spondyloarthritis (SpA) is inflammatory arthritis, and interleukin (IL) –17 is crucial on pathogenesis of SpA. Type 17 helper T cell (Th17) is one of major IL-17 secreting cells. Signal transducer and activator of transcription (STAT)–3 signalling induces Th17 cell differentiation. Present study investigated the effect of protein inhibitor of activated STAT3 (PIAS3) on SpA pathogenesis.

Methods: Curdlan was injected to SKG ZAP-70^{W163C} mice for SpA induction. Then PIAS3 or Mock vector was inserted to mice for 10 weeks. Clinical score and histologic scores of paw, spine, and gut were evaluated. Expressions of IL-17, tumour necrosis factor-α (TNF-α), STAT3, bone morphogenic protein (BMP) were

measured. Confocal stain and flow cytometry were used to assess helper T cell differentiation.

Results: PIAS3 significantly diminished the histological scores of paw and gut. PIAS3 group displayed lesser expression of IL-17, TNF-α, and STAT3 in the paw, spine, and gut. BMP-2/4 expressions were lower in spine of PIAS3 group. Helper T cell differentiation was polarised toward upregulation of regulatory T cell (Treg) and downregulation of Th17 in the PIAS3 mice.

Conclusions: PIAS3 showed preventive effects in mice with SpA by suppressing peripheral arthritis and gut inflammation. Proinflammatory cytokines and Th17/Treg differentiation were controlled by PIAS3. Additionally BMPs were decreased in spine of PIAS3 mice. These findings suggest that PIAS3 could be a potential therapeutic choice of SpA treatment.

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FRI0152 INFLAMMASOMES ACTIVATION OCCURS IN THE INFLAMED TISSUES OF AS PATIENTS AND DRIVES IL-23 EXPRESSION

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Background: A growing body of evidences indicate that the aberrant activation of innate immune systems, occurring in genetically predisposed patients, drives inflammatory processes in Ankylosing Spondylitis (AS).¹

Objectives: Aim of this study was to evaluate the activation and the functional relevance of inflammasome pathways in patients with AS.

Methods: Intestinal, synovial and bone marrow expression of inflammasome pathways, pyroptosis and IL-1b and IL-18 was evaluated in AS patients. Organic acid extraction was performed on ileal samples as previously described on. The expression of the metabolite-sensing receptors GPR43 and GPR109A involved in the regulation of the intestinal inflammasome was also assessed. The role of intestinal dysbiosis in modulating inflammasome activation was also studied in AS patients and HLA-B27 transgenic rats. Inflammasome activation was evaluated in isolated peripheral AS monocytes. The role of LPS, PGE2 and nicotine in inducing monocyte inflammasome activation and the role of inflammasome in modulating IL-23 production was also evaluated.

Results: Activation of inflammasomes was observed in the inflamed gut, synovial and bone marrow samples of AS patients and associated with an increased expression of caspase-1, IL-1b and IL-18. In AS, AIM2 expression was observed in the context of tuft cells and of adherent ileal bacteria. Inflammasome activation in AS gut, was associated with the occurrence of dysbiosis and increased pyroptosis as demonstrated by the membrane localization of Gasdermin D. Isolated intestinal bacteria from AS ileal samples, significantly modulated inflammasome activation in isolated monocytes. Reduced Short-chain fatty acids concentrations and increased expression of GPR43 and GPR109 were demonstrated in the AS ileal samples. Inflammasome activation was also observed in the inflamed gut of HLA-B27 TG rats and suppressed by antibiotics treatment. Increased expression of NLRP3, NLRC4 and AIM2 was confirmed in AS isolated peripheral monocytes. Serum levels of IL-1b and IL-18 were increased in AS patients, especially in smoker patients, and directly correlated with the ASDAS-CRP. In *in vitro* studies, LPS and nicotine strongly activated NLRP3, NLRC4 and AIM2 pathways in AS monocytes. The CC genotype of PTGER4 SNP rs6896969 was associated with a significantly increased activation of inflammasome in AS. Finally, inflammasome activation in AS monocytes was required for the induction of IL-23p19 expression in an IL-1b-dependent way.

Conclusions: Inflammasome activation occurs in AS patients being modulated by a plethora of different stimuli. Inflammasome drives IL-23 production in an IL-

1b-dependent mechanism in AS patients supporting the auto-inflammatory nature of the disease and possibly representing a future therapeutic target in AS.

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FRI0153 ROLE OF ROR GAMMA T INHIBITION IN THE IL-23 MINICIRCLE MODEL AND SPA PATIENT TISSUE CULTURES

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Background: Retinoic acid receptor related Orphan Receptor gamma t (RORγt) is a nuclear hormone receptor expressed in a subset of pathogenic T cells and innate lymphoid cells. RORγt regulates the transcription of key pro inflammatory cytokines such as IL-17 and IL-22 in response to multiple activation signals including cytokines and T cell receptor engagement. Antagonism of RORγt is hypothesised to block transcription of these pathogenic cytokines resulting in reduced tissue inflammation and aberrant joint remodelling.

Spondyloarthropathy (SpA) is characterised by a peripheral oligoarthritis and enthesitis. A key feature of SpA is the imbalance between bone resorption and formation leading to aberrant bone formation and ankylosis. The contribution of the IL-23/IL-17 axis to the pathogenesis of SpA is supported by several lines of evidence. However, the specific role of RORγt in regulating the cellular and molecular pathways contributing to enthesal and synovial inflammation remains incompletely understood.

Objectives: The goal of this study was to determine the role of RORγt antagonism in both an IL-23 minicircle *in vivo* model and *ex vivo* tissue culture model.

Methods: Model 1: Mice were administered an IL-23 minicircle via hydrodynamic injection to induce systemic IL-23 expression on day 1. Starting on day 2, mice were dosed p.o. for 28 days. Mice were monitored daily for signs of arthritis. Upon sacrifice tissue was collected for mRNA biomarker analysis and histologic assessment.

Model 2: Patients undergoing total hip replacement were recruited into 2 groups: SpA (either psoriatic arthritis or ankylosing spondylitis) or OA (osteoarthritis). Enthesal and synovial tissues were collected and cultured ±RORγt inhibitor and cytokine stimulation (hTNFα/hIL23) for 24 and 48 hours and then analysed for gene expression.

Results: Model 1: Overexpression of IL-23 induced SpA like phenotype including enthesitis, synovitis and aberrant bone formation. RORγt inhibition resulted in significant reduction of inflammation and arthritic score, histologic parameters of bone remodelling, and tissue biomarkers associated with the IL-23 axis (IL17A, IL17F, IL22).

Model 2: Both enthesal and synovial cultures from SpA patients expressed increased Th17 axis genes relative to OA after cultures. RORγt inhibition reduced the expression of these Th17 axis genes (IL17A, IL17F, IL22) as well as reducing tissue remodelling related genes (FN1, MMP1/3, BMP5), in alignment with the results seen in model 1.

Conclusions: IL-23 induced Th17 axis gene expression induced a SpA-like phenotype in mice, including arthritis and aberrant bone formation, that was reversed upon RORγt inhibition. Similarly, SpA patient explants showed increased expression of Th17 axis cytokines and tissue remodelling related genes that were decreased with RORγt inhibition. Together, these complementary approaches support the hypothesis that RORγt treatment could block inflammation as well the underlying pathologies associated with SpA.

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FRI0154

ADDED VALUE OF BIOMARKERS COMPARED TO ROUTINE CLINICAL PARAMETERS FOR THE PREDICTION OF RADIOGRAPHIC SPINAL PROGRESSION IN AXIAL SPONDYLOARTHRITIS

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Background: Structural damage in the spine determines the functional status and spinal mobility in axial spondyloarthritis (axSpA). Already present syndesmophytes, elevated C-reactive protein, cigarette smoking, and to a lesser extent male sex are routine clinical parameters predicting radiographic spinal progression. In the last years, several biomarkers with a predictive value for radiographic spinal progression were identified. It is, however, not known, if biomarkers have a meaningful added value over clinical parameters in prediction of radiographic spinal progression in axSpA.

Objectives: The objective of the study was to examine whether adding biomarkers to the routine clinical parameters would improve prediction of radiographic spinal progression in axSpA.

Methods: Altogether 117 patients with ankylosing spondylitis who completed a 2 year clinical and radiographic follow-up in the ENRADAS trial were included. Radiographic spinal progression was defined as a worsening of the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) by ≥ 2 points after 2 years. A clinical prediction model for radiographic spinal progression was constructed out of already present syndesmophytes, elevated CRP, cigarette smoking, and male sex. Serum biomarkers measured at baseline included: matrix metalloproteinase-3, vascular endothelial growth factor (VEGF), calprotectin, leptin, high molecular weight adiponectin (HMW-APN), osteoprotegerin, sclerostin, N-terminal telopeptide, procollagen type II N-terminal propeptide, and serum amyloid A.

Results: Repeated cross-validation analyses revealed the following biomarker combination with added predictive value for radiographic progression compared to the clinical model (syndesmophytes, smoking, elevated CRP, and sex): Leptin + HMW-APN + VEGF. A combination of these biomarkers resulted in an Area Under the Curve (AUC) of $AUC_{\text{Biomarkers}} = 0.731$ (95%CI 0.614–0.848), thus numerically superior to the clinical model ($AUC_{\text{Clinical}} = 0.665$ (95%CI 0.553–0.776)). A combination of both models resulted in improvement of the predictive value reflected by the $AUC_{\text{Clinical+Biomarkers}} = 0.768$ (95%CI 0.666–0.871); though this improvement was not statistically significant compared to the clinical model in the permutation test ($p = 0.051$). However, when only considering the part of receiver operating characteristic (ROC) curves with a specificity of $\geq 75\%$, the improvement becomes statistically significant (partial $AUC_{\text{Clinical+Biomarkers}} = 0.119$ versus partial $AUC_{\text{Clinical}} = 0.053$; $p = 0.010$).

Abstract FRI0154 – Figure 1. ROC curve analyses of biomarker and clinical model alone versus the combined model for the prediction of radiographic spinal progression (mSASSS worsening ≥ 2 after 2 years) in ankylosing spondylitis. Area under the curves (AUC) and respective 95% confidence intervals (CI) shown. *Leptin + HMW-APN + VEGF.