Figure 1: FTS therapy reduces the levels of pathogenic autoantibody and alters glycosylation of autoantibodies.

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SAT0045

A NOVEL SMALL MOLECULE, MBS2133, MODULATES OSTEOCLAST PRE-CURSOR METABOLISM TO INHIBIT OSTEOCLAST DIFFERENTIATION: AN ALTERNATIVE THERAPY FOR OSTEOLYTIC PATHOLOGY IN RA

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with substantial local and systemic bone loss. Despite the availability of several treatment options many patients do not reach low disease activity. Furthermore, current therapeutics generally target inflammation rather than erosive pathology. Thus, there remains a need for new therapies that can target both aspects of the disease. Prior studies have shown that biphenylcarboxylic acid small molecule derivatives not only inhibit murine osteoclastogenesis but also attenuate inflammation and bone destruction in murine models of RA^{1,2}.

Objectives: To evaluate a novel small molecule derivative, MBS2133, on human osteoblastogenesis, osteoclastogenesis and cellular function, and to investigate the *in vitro* mechanism-of-action.

Methods: Osteoblasts were derived from human mesenchymal stem cells. Cells were differentiated in the presence or absence of MBS2133 and mineralization assessed by Alizarin Red staining. Human CD14⁺ blood monocytes were differentiated into osteoclasts (OCs) with M-CSF and RANK-L, in the presence or absence of MBS2133, and/or metabolites. Mature OCs were stained with tartrate-resistant acid phosphatase (TRAP) and quantified by light microscopy. Osteolytic activity was assessed on mineral-coated surfaces. Western blot analysis was used to assess downstream signalling pathways. Changes in the metabolic profile of pre-osteoclasts following 4h exposure to MBS2133 was carried out by liquid chromatography mass spectrometry.

Results: MBS2133 had no effect on the differentiation and function of primary human osteoblasts. In comparison, exposure of RANK-L stimulated CD14⁺ monocytes to MBS2133 significantly reduced OC differentiation and osteolytic activity of mature OCs. Notably, exposure of pre-OCs to MBS2133 for 2h at initiation of osteoclastogenesis, was sufficient to significantly reduce subsequent OC differentiation. Evaluation of treated pre-osteoclasts revealed that RANKL-mediated phosphorylation of p38 was reduced. Metabolomic analysis of pre-osteoclasts revealed that MBS2133 induced a substantial reduction in a range of metabolites associated with glycolysis, oxidative phosphorylation and fatty acid oxidation pathway. Notably, L-carnitine, which facilitates the transportation of fatty acids to the mitochondrial matrix and enables processing and entry into tricarboxylic acid (TCA) cycle for further energy production, was significantly reduced. *In vitro* supplementation of L-carnitine inhibited the ability of the compound to switch off OC differentiation and osteolytic activity.

Conclusion: The results of this study demonstrate that MBS2133 specifically modulates the metabolome of myeloid cells, which has a substantial impact on their ability to differentiate into mature osteoclasts. These findings highlight the importance of modulating the glycolysis/oxidative phosphorylation axis in osteoclastogenesis and suggest that targeting the metabolic state of pre-osteoclasts could offer a new therapeutic approach to treat bone resorption in rheumatic diseases.

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SAT0046

ARE SENSE OF SOCIAL SUPPORT AND LOW DECISION LATITUDE AT WORK LINKED TO RISK OF RHEUMATOID ARTHRITIS, AND IF SO, HOW DO THEY RELATE TO OTHER RISK FACTORS? RESULTS FROM THE SWEDISH EIRA STUDY

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Background: The role of psycho-social factors in the development of rheumatoid arthritis (RA) is debated.

Objectives: We investigated whether psychosocial stress measured as low sense of social support, and low decision latitude at work, were linked to risk of RA, and whether they related to known lifestyle risk factors for RA.

Methods: The Swedish population-based EIRA study included incident RA cases (N=3724) and controls (N=5937), matched for age, sex and residential area. Responders filled in questionnaires regarding self-reported social support, decision latitude at work and life-style-factors.

The distribution of answers among controls were used to define exposure, thus for social support, those in the lowest quartile of social support were considered exposed to low social support and similarly for decision latitude, those in the lowest quartile were considered exposed to low decision latitude at work.

Using logistic regression, we first evaluated whether exposures associated with RA risk, considering potential confounding of established risk factors. Then, we investigated whether the frequency of those factors differed between individuals reporting low social support or low decision latitude at work or not, among cases and controls.

Results: There were 898 cases with low social support and 285 cases with low decision latitude at work (latter only available in first part of EIRA).

Low social support was not associated with RA risk in unadjusted analyses (OR= 1.05, 95%CI=0.95-1.15). Low decision latitude at work did associate with a higher RA risk in the unadjusted analyses (OR=1.52, 95% CI=1.20-1.94), but this association was no longer significant after further adjustment for smoking, obesity and university degree (adjusted OR=1.24, 95% CI=0.93-1.63). Associations between those life-style risk factors and RA were confirmed (no university degree, OR=1.50; smoking OR=1.71; obesity OR=1.15).

Next, we evaluated whether low social support or low decision latitude at work differed by previously established risk factors. Cases with RA reporting low sense of social support were more often men (OR=1.60, 95% CI=1.40-1.83), current smokers (OR=1.46, 95%CI=1.26-1.70), obese (OR=1.29, 95%CI=1.09-1.54), physically inactive (OR=2.78, 95%CI=1.98-3.90) and without a university-degree (OR=2.04, 95%CI=1.77-2.36); with similar pattern among the controls. For working-conditions, cases reporting low decision latitude at work were also more often current smokers (OR=2.05, 95%CI=1.33-3.16) and with no university degree (OR=8.23, 95%CI=5.13-13.22), but less often male (OR = 0.40, 95%CI=0.26-0.60). Again, the pattern was similar among controls. RF/ACPA-positivity did not associate with low social support or low decision latitude.

Conclusion: Neither low social support nor low decision latitude at work were associated with an increased risk of RA after adjustment for other known lifestyle risk factors for RA. An initial crude association between

low decision latitude at work and risk for RA was explained by differences in smoking and educational level. However, both low social support and low decision latitude at work associate strongly with known, and here validated, risk factors for RA (smoking, obesity, no university degree) with similar pattern among both cases and controls.

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SAT0047

PRIMARY LYMPHOID TISSUE FROM RHEUMATOID ARTHRITIS PATIENTS HARBOUR CITRULLINE SPECIFIC PLASMA CELLS

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Background: Anti-citrullinated protein antibodies (ACPA) are specific markers with pathological effects in rheumatoid arthritis (RA). ACPA-specific B-cells have been identified in synovial joint fluid and peripheral blood. An increased concentration of ACPA is found in synovial fluid and lung tissue compared to blood suggest local production, partly confirmed by detection ACPA synovial plasma blasts, but primary lymphoid tissue have not previously been examined.

Objectives: In this study we investigated the plasma cell repertoire in the bone marrow and occurrence of autoantibody producing plasma cells.

Methods: For this study we developed a method to collected and process bone marrow samples from proximal femur in RA patients undergoing hip joint replacement. Bone marrow samples were processed and mononuclear cells were obtained by Ficoll separation. CD138+ plasma cells were single cell sorted by flow cytometry. Paired heavy and light chains were PCR amplified, sequenced, and analyzed by V-Quest and IgBLAST towards the IMGT database to annotate variable gene usage. To enrich for ACPA, sequences were selected based on high somatic hypermutations number and Fab N-glycosylation sites. Selected sequences were cloned and expressed as IgG in Expi293 cells. Monoclonal antibody reactivity to citrullinated and arginine form of vimentin, enolase, fibrinogen, histone, tenascin C peptides; carbamylated and malondialdehyde-acetaldehyde bovine serum albumin, and tetanus toxoid antigens were examined by ELISA. Included patients were clinical examined and donating peripheral blood samples.

Results: After method development and quality analysis we were able to include five bone marrow samples in this study. The five included RA patients, median age 74 (range 66-82) years, had in median 20 (range 4-44) years of destructive disease, all were RF positive, four were females and anti-CCP2-positive, and all except one were treated with anti-rheumatic drugs.

Overall, from the processed bone marrow, 465 paired heavy-light IgG plasma cell sequences were obtained, in total 368 (range 20-194) from ACPA positive patients and 97 from the ACPA negative patient. We observed statistically significant changes in heavy chain variable gene usage with lower VH-1 and higher VH-3 frequency in sequences from ACPA positive RA patients compared to sequences from the ACPA negative RA patient. We also found statistically significant increase in VH N-glycosylation in sequences from the ACPA positive RA patients (22.6% ACPA positive vs 12.4% ACPA negative; $p=0.03$) but no difference in mutation numbers. From obtained sequences, 34 interesting clones were selected for monoclonal antibody-expression. Among the 34 clones we found two citrulline specific reactive clones (reactivity towards CCP2, citrullinated vimentin and fibrinogen peptides) and one malondialdehyde-acetaldehyde (MAA) reactive clone, originating from three different APCA positive patients with the most longstanding disease. None of these

clones had reactivity towards control antigens such as arginine versions of peptides.

Conclusion: Plasma cells producing ACPA are present in primary lymphoid tissue from RA patients. Further studies of difference in characteristics, IgG gene usage and N-glycosylation, between ACPA-positive and ACPA-negative patients are needed.

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SAT0048

ANTI-FRACTALKINE MONOCLONAL ANTIBODY AMELIORATE JOINT DESTRUCTION AND SYNOVIAL THROUGH SUPPRESSION OF OSTEOCLAST PRECURSOR MIGRATION AND INDUCTION OF SYNOVIAL CELL DEATH IN COLLAGEN-INDUCED ARTHRITIS MODEL

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Background: In the Phase 1/2 clinical study, E6011, a novel humanized anti-fractalkine (FKN) mAb demonstrated a promising efficacy in active RA patients who were inadequately controlled by MTX and/or TNF- α inhibitors (NCT 02196558)¹. In RA joint tissue, increased expression of fractalkine (FKN) and abundant infiltration of CX3CR1-positive cells were observed². FKN is expressed on endothelial cells and fibroblast-like synovocytes in synovium and also expressed on osteoblasts. CX3CR1 is expressed on monocytes/macrophages and osteoclast precursors (OCPs). Therefore, FKN-CX3CR1 interaction could play pivotal roles in migration, differentiation and activation of those cells. However, the precise mechanism(s) of FKN-CX3CR1 axis in RA, especially on joint destruction remains to be elucidated.

Objectives: To elucidate the roles of FKN-CX3CR1 axis in cartilage destruction, bone damage and proliferated synovial cells by using anti-mouse FKN mAb (anti-mFKN mAb).

Methods: For the induction of CIA, mice were immunized with bovine type II collagen. Anti-mFKN mAb was injected twice a week. The clinical arthritis score was monitored, and joint destruction was evaluated by soft x-ray and histology. The mRNA expression levels were assessed by quantitative RT-PCR. Blood parameters were measured using ELISA. In vitro, effect of immobilized FKN on RANK ligand (RANKL)-induced osteoclast differentiation was examined. In vivo, bone marrow-derived OCPs were fluorescein-labeled and transferred to CIA mice to evaluate the migration of OCPs into synovium. Inhibitory effect of anti-mFKN mAb, etanercept or tofacitinib against OCP migration was assessed. To examine the effect of anti-mFKN mAb against proliferated synovial cells, propidium iodide (PI) was injected to anti-mFKN mAb-treated CIA mice to detect the synovial cell death.

Results: Anti-mFKN mAb significantly reduced the arthritis and soft x-ray scores in both prophylactic and therapeutic treatment. Anti-mFKN mAb histologically improved synovitis, cartilage destruction and bone damage, with marked reduction of osteoclast numbers. Plasma levels of COMP and MMP-3 were also decreased. Interestingly, anti-mFKN mAb significantly suppressed Tnf and Il6 mRNA expression in the affected joints. In vitro, RANKL-induced osteoclast differentiation was enhanced by immobilized FKN, and anti-mFKN mAb suppressed FKN-dependent osteoclast formation. In vivo, anti-mFKN mAb strongly inhibited the migration of OCPs into the proliferated synovial cells of mice with CIA, whereas etanercept or tofacitinib had no significantly effect. Importantly, synovial cell death was abundantly found in proliferated synovial cells of mice with CIA after the anti-mFKN mAb treatment³.

Conclusion: Anti-mFKN mAb remarkably ameliorated the joint destruction with the marked reduction of osteoclasts by the inhibition of both OCP survival and OCP migration in inflamed joint tissues. In addition, anti-mFKN mAb immediately induced synovial cell death in the proliferated synovial cells, suggesting the direct inhibitory effect in the synovitis. These results strongly indicate that inhibition of FKN-CX3CR1 axis by a humanized anti-FKN mAb, E6011, is an attractive and affected joints-selective therapeutic strategy for the treatment of both inflammatory synovitis, cartilage destruction and bone damage in RA patients.

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