Table 1. Tcell subsets in Rheumatoid Arthritis and Osteoarthritis.

CD4 subtype	RA		OA	
	PB	SF	PB	SF
	N=80	N=30	N=30	N=30
Th1	26.65 ± 5.59	34.99 ± 1.30	25.97 ± 7.24	26.45 ± 1.87*
Th2	5.19 ± 1.9	16.36 ± 1.73	5.24 ± 2.15	1.44 ± 0.10*
Th17	14.05 ± 3.29	21.18 ± 2.04	10.81 ± 2.78*	2.45 ± 0.23*
Treg	10.68 ± 2.47	12.53 ± 2.10	11.162.95	13.04 ± 2.57

*P<0.05

Conclusion: Tregs in RA may be converted to Th1 and Th17 phenotype on exposure to inflammatory cytokine in the synovial fluid, thus losing their regulatory functions. Understanding factors influencing stability of Treg cells may help improve future therapeutics.

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Fig1: A. Comparison of plasma cytokine levels between RA and OA patients; B. Comparison of synovial fluid cytokine levels between RA and OA patients; C. Surface characterization of in-vitro cultured Peripheral blood Treg cells of RA patients. C1 & C2 CXCR3(Th1) and CXCR6(Th17) chemokines expressing Treg cells isolated from peripheral compartment of RA patients; C3 & C4 CXCR3(Th1) and CXCR6(Th17) chemokines expressing Treg cells cultured in autologous synovial fluid; D. Comparison of expression of transcription factors in peripheral blood Treg cells of RA and OA patients: cultured with autologous synovial fluid. Expression of (D1) T-bet, (D2) ROR-g, (D3,D4) Foxp3. Mann-Whitney U test was used to analyze cytokine data, unpaired student t-test was used to analyze Treg frequency data, Paired t-test was used to analyze gene expression data; * P Value < 0.05 considered significant

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AB0095

PRECLINICAL CHARACTERIZATION OF CJ-15314, A HIGHLY SELECTIVE JAK1 INHIBITOR, FOR THE TREATMENT OF AUTOIMMUNE DISEASES

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Background: Janus kinases (JAK1, JAK2, JAK3, and TYK2) play critical roles in mediating various cytokine signaling, and has been developed as a target for autoimmune diseases such as RA. Tofacitinib, oral Pan-JAK inhibitor, demonstrated efficacy in RA patients, but its widespread use is limited by safety issues. Baricitinib, JAK1/2 inhibitor, is also known to interfere with the hematopoiesis system, such as anemia and thrombocytopenia associated with suppression of JAK2 signals. Therefore, it is necessary to develop a new potent compound that selectively inhibits JAK1 over JAK2, 3

Objectives: To identify the pharmacological characteristic based on efficacy of CJ-15314 as potent and selective JAK1 inhibitor for treatment of autoimmune disease.

Methods: In vitro, cell-based, kinase panel, Kd value and human whole blood assay were performed to determine the inhibition potency and selectivity for JAK subfamily kinases. In vivo therapeutic potential was evaluated by RA model including rat Adjuvant-Induced Arthritis (AIA) and collagen-induced arthritic (CIA). To confirm the possibility of further expansion into the autoimmune disease, BioMAP® Diversity PLUS® Panel was performed by discoverX.

Results: In vitro assay, CJ-15314 inhibited JAK kinase family in a concentration-dependent manner with IC50 values of 3.8 nM against JAK1, Selectivity for

JAK1 over JAK2, 3 was approximately 18, 83 fold greater for CJ-15314. In 1mM ATP condition, CJ-15314 has been confirmed to have the highest JAK1 selectivity over competing drugs, under 1mM ATP condition that reflects the physiological environment in the body. Similarly, Kd values has also confirmed the selectivity of JAK1, which is 10 fold higher than JAK2, 3. Accordingly, in human whole blood assays, CJ-15314 is 11 fold more potent against IL-6 induced pSTAT1 inhibition through JAK1 (IC50 value: 70 nM) than GM-CSF-induced pSTAT5 inhibition (JAK2) whereas baricitinib and filgotinib exhibited only 2 fold and 7 fold respectively.

In vivo efficacy model, CJ-15314 inhibited disease severity scores in a dose dependent manner. In the rat AIA model, CJ-15314 at 30mg/kg dose showed 95.3% decrease in arthritis activity score, 51.2% in filgotinib at 30 mg/kg, 97.7% showed baricitinib at 10 mg/kg. CJ-15314 showed superior anti-arthritis efficacy than filgotinib. CJ-15314 also minimally affected anemia-related parameters but not bicitinib end of the 2-week treatment. In the rat CIA model, like 10 mg/kg of bicitinib, 30 mg/kg of CJ-15314 also has a similar effect, with a significant reduction in histopathological scores.

In biomap diversity panel, CJ-15314 inhibited the expression of genes such as MCP-1, VCAM-1, IP-10, IL-8, IL-1, sTNF-α and HLA-DR confirming the possibility of expansion into other diseases beyond arthritis.

Conclusion: CJ-15314 is a highly selective JAK1 inhibitor, demonstrates robust efficacy in RA animal model and is good candidate for further development for inflammatory diseases.

* CJ-15314 is currently conducting a phase I trial in south Korea.

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AB0096

FCER1G GENE METHYLATION AND MIR-106/MIR-17 AS A NEW POTENTIAL EPIGENETIC MARKERS IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease that leads to joints destruction. One of the most important cytokine responsible for this process is interleukin 6 (IL-6). Fc receptor gamma chain (FcRγ), encoded by *FCER1G* gene, is responsible for neutrophils activation, phagocytosis, cell surface signaling pathway as well as IL-4, IL-6, IL-10 and tumor necrosis factor production. Epigenetic factors, including DNA methylation and micro-RNAs (miRs) expression regulate the genes expression on transcriptional and post-transcriptional mechanisms. There are miRs responsible for cytokines production, for example GU rich miRs, miR-106b and miR-155 were reported as associated with IL-6 overproduction.

Objectives: The aim of our study was to evaluate *FCER1G* gene methylation and miR-17 family members as epigenetic markers associated with RA, disease activity and IL-6 expression.

Methods: Bioinformatics analysis were applied to select the miRs with a possible target sites in a promoter region of *FCER1G* gene. The MiR-17 family members, including miR-17, miR-93 and miR-106b were selected for investigation. A total of 74 individuals, 50 RA patients, 84% female, aged 53.7±12.3 years (mean±SD) and 24 healthy controls (HC), 87.5% female, aged 53±8.49 years were enrolled. RA patients were selected based on DAS-28 scoring. RA patients with high disease activity (DAS28 >5.1; 58%) and remission (≤2.6; 42%) were included in the analysis. DNA was extracted from a whole blood and miRs were extracted from plasma. Quantitative real-time PCR was used for analyze both methylation and expression levels. In a randomly selected samples (16 from high

disease activity group; 9 from remission and 19 from HC) the level of IL-6 in serum was evaluated.

Results: Patients with RA in comparison to HC have had a lower *FCER1G* methylation (0.98 [0.73-1.46] vs 1.96 [1.44-3], $p < 0.00001$; median [interquartile range]) and miR-106b (0.79 [0.49-1.68] vs 1.54 [0.88-2.51], $p = 0.008$) and miR-17 (1.26 [0.41-2.04] vs 2.44 [2.09-3.47], $p = 0.0001$) expressions. No difference in methylation between high and remission RA groups was found. MiR-106b and miR-17 expressions were different between RA patients with high disease activity and remission ($p = 0.009$ and $p = 0.003$, respectively), however a high disease activity group was not different to HC ($p = 0.82$ and $p = 0.12$, respectively). Detailed results are presented in Table 1. A strong correlation between IL-6 levels and *FCER1G* methylation ($r_s = -0.46$) was found.

Table 1. Methylation and expression between patients divided by disease activity in compare to controls.

	High disease activity, n=29	Remission, n=21	HC, n=24
<i>FCER1G</i> methylation	1.11 [0.83-1.52]	0.96 [0.61-1.18]	1.96 [1.44-3]
miR-106b expression	1.36 [0.63-1.76]	0.54 [0.19-1.19]	1.54 [0.88-2.51]
miR-93 expression	0.63 [0.49-1.21]	0.59 [0.15-1.5]	1.03 [0.65-1.38]
miR-17 expression	1.46 [1.05-2.54]	0.34 [0.11-1.26]	2.44 [2.09-3.47]

Data are given by median [interquartile range]. *FCER1G*, Fc receptor gamma chain gene; HC, healthy controls; miR, micro-RNA; RA, rheumatoid arthritis patients.

Conclusion: *FCER1G* methylation was found as a new epigenetic marker of RA, which is independent of disease activity and may be associated with IL-6 production. Plasma miR-17 and miR-106b can be considered as a novel molecular biomarkers of disease severity in RA.

FcRγ may plays a significant role in RA pathogenesis and *FCER1G* gene methylation was found as a new, epigenetic and promising marker of RA.

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AB0097

TISSUE CYTOKINES AS NEW DIAGNOSTIC BIOMARKER OF BONE METABOLISM DISORDERS IN RHEUMATOID ARTHRITIS

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Background: Bone mineral density and proteins/peptides determination in blood and urine as markers of bone resorption and formation are currently used to diagnose osteoporosis (OP) and metabolic bone diseases. Recent evidence suggests that in RA changes in the secretion of hormones of white adipose tissue can be revealed [1,2,3,4].

Objectives: To study the clinical and diagnostic value of serum fetuin A, nesfatin, hemerin, leptin, adiponectin, resistin, visfatin determination in RA patients complicated by OP.

Methods: We examined 88 women with documented diagnosis of RA and mean disease duration of 6.56±0.88 years. We used EULAR/ARA 2010 criteria to diagnose the patients. Female patients with II degree of disease activity (DAS28), Steinbrocker stage II (erosive), rheumatoid factor- and anti-cyclic-citrullinated peptide antibody-positive were prevalent. We excluded patients who had surgery or developed an infection within the last 8 weeks, pregnant and breast-feeding women, those with severe heart, liver or kidney disease, immune deficiency, leucopenia or chronic infection.

A control group of 45 healthy females aged of 25 and 59 years were included in the study. There were no reported findings of joint pain and RA symptoms in the group. The groups were adjusted for age ($p > 0.05$) and showed no statistically significant differences.

We measured serum fetuin A, nesfatin, hemerin, leptin, adiponectin, resistin, visfatin levels (μg/ml) using ELISA commercial test systems. We used spectrophotometer with wavelength of 450nm to detect the test results («Multiskan» immunoenzyme analyzer, Finland). We plotted a curve using computer software. We diagnosed OP using dual-energy X-ray absorptiometry with LUNAR DPX PRO (GE, USA).

Results: At the first stage, the level of pro-inflammatory cytokines was studied in a group of healthy individuals. Then, the reference values of these indicators were measured as $M \pm 26$. Patients with OP and RA had significantly higher levels of serum pro-inflammatory cytokines ($p < 0.001$). For example, mean serum Adiponectin levels in RA patients who had normal bone density and had no OP were 35.21±0.6 μg/ml. Mean serum Adiponectin levels in RA/OP patients with low bone mineral density were 52.42±0.69 μg/ml. Adiponectin levels of 44 μg/ml and higher were associated with osteoporosis. Adiponectin levels of 43.9 μg/ml and lower were associated with normal bone density. Other pro-inflammatory cytokines have demonstrated similar dynamics of level serum.

Conclusion: Thus, we revealed that fetuin A, nesfatin, hemerin, leptin, adiponectin, resistin, visfatin levels depend on osteoporosis presence in RA patients. The test may be used to reduce the risk of low-energy fractures and to improve the quality of life in RA.

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AB0098

THE EFFECT OF MALT1-DEFICIENCY ON THE EFFECTOR PHASE OF EXPERIMENTAL AUTOIMMUNE ARTHRITIS

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Background: The paracaspase Malt1 is a cysteine protease, which forms a complex leading to the activation of the in proinflammatory transcription factor NF-κB in lymphocytes with CARMA1 and Bcl10. Previously, we showed that the myeloid equivalent of CARMA1, Card9 is important in neutrophils in Fcγ receptor-mediated cytokine release together with Bcl10 and Malt1. In line with these findings, we observed a significant decrease in the severity of autoantibody-triggered arthritis in the absence of Card9 and Bcl10.

Objectives: Our aim was to directly investigate whether the genetic deficiency of Malt1, the third component of the complex altered the process of the K/BxN serum transfer arthritis (that resembles to the effector phase of rheumatoid arthritis).

Methods: We used wild type and Malt1^{-/-} mice for our experiments. Autoantibody-mediated arthritis was induced by a single intraperitoneal injection of K/BxN serum. Clinical signs of joint inflammation were scored on a scale based on the cardinal inflammatory clues for two weeks. Ankle thickness was measured by a spring-loaded caliper.

Results: Similar to the deficiency of the other two components of the complex, Malt1^{-/-} mice showed a partial, but significant decrease in the macroscopic joint inflammation compared to arthritic serum-treated wild type animals during the entire experimental process. In line with this phenomenon, Malt1^{-/-} animals had reduced autoantibody-triggered ankle thickening.