Conclusion: This study showed the apoptosis of lining cells derived from macrophages resulted in the formation of the discoid fibrosis. These findings indicated TNFi might induce apoptosis of macrophage leading to the suppression of RA synovitis.

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Fig.1 a Discoid fibrosis in sublining layers. **b** TUNEL stain positive cells are around Discoid fibrosis

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SAT0020

MOMELOTINIB, A JANUS KINASE 1/2 AND ACTIVIN RECEPTOR 1 INHIBITOR, AMELIORATES JOINT INFLAMMATION, SYSTEMIC TH17 DIFFERENTIATION AND ARTHRITIS-LINKED ANEMIA IN PRE-CLINICAL AUTOIMMUNE RA

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Background: Janus kinases (JAKs) serve as signaling hubs orchestrating inflammation, innate and adaptive immunity and erythropoiesis. Unfortunately, some of these agents cause suppression of JAK-dependent erythropoiesis, thereby exacerbating inflammation-associated anemia, leading to potential under-dosing and reduced therapeutic benefit. We previously showed that the JAKi momelotinib (MMB) can correct anemia in a rat model of RA, an effect that has been clinically reproduced in myelofibrosis patients treated with MMB. Subsequently, the molecular basis for MMB's anemia benefit was determined to be a consequence of its potent inhibition of Activin Receptor Type 1 (ACVR1), resulting in decreased hepcidin and, as a consequence, increased systemic iron availability and improved erythropoiesis.

Objectives: The goal of the current study was to investigate the effects of MMB on arthritis in pre-clinical RA models.

Methods: The anti-arthritis activity of daily administration of MMB was assessed in Streptococcus cell wall-induced arthritis in Lewis rats (PG-PS model) and in collagen antibody-induced arthritis (CAIA) in DBA/1 mice. Consecutive assessment of arthritis was performed by joint thickness measurements and paw scoring. Following 3 weeks of treatment, synovial immune cell infiltration and T cell subset differentiation was quantified. Cytokine gene expression was profiled by quantitative rt-PCR. Anemia was assessed by determination of blood hemoglobin and serum, spleen and liver iron levels.

Results: MMB reduced inflammatory granulocyte and macrophage infiltration in synovial tissue by more than 60% at all tested doses as compared to vehicle treatment in PG-PS animals. Importantly, MMB treatment effectively decreased arthritogenic Th17 cell differentiation and overall CD4+ T cells in the synovia beginning at the lowest tested dose and coincided with complete remission of joint swelling at 25 mg/kg. Anti-arthritis activity of MMB was confirmed with significant reductions in arthritis scoring, which demonstrated non-inferiority versus the TNF- α inhibitor, etanercept, in the CAIA model. Consistent with its inhibitory

activity on the ACVR1-hepcidin axis, MMB reduced circulating hepcidin levels and mobilized systemic iron, resulting in substantial improvement of the RA-associated anemia in rats.

Conclusion: MMB is a highly efficacious anti-arthritis agent that ameliorates local joint inflammation and reduces the systemic differentiation of major arthritogenic effector cell population, Th17 lymphocytes. In accord with our previous report, MMB is distinct from other JAKi due to its ability to inhibit ACVR1 signaling leading to decreased plasma hepcidin, improved iron homeostasis and increased erythropoiesis. The dual anti-inflammatory and anemia-improving pharmacologic activities of MMB position it as a promising and differentiated therapeutic agent for the treatment of RA and other inflammatory diseases with an anemia component.

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SAT0021

STRUCTURAL EFFECTS OF LOCAL CRYOTHERAPY IN RHEUMATOID ARTHRITIS: A STUDY IN ADJUVANT-INDUCED ARTHRITIS

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Background: The control of joint destruction caused by rheumatoid arthritis (RA) is a key issue in the treatment of this disease. Recent evidence showed that radiographic progression of joint damage occur despite a sharp decrease in disease activity and the use of aggressive Disease Modifying Anti-Rheumatic Drug (DMARD) therapies [1]. Whether alternative treatments such as cryotherapy may have beneficial effects on joint destruction at the early stages of the disease remains to be demonstrated, but such strategy would be of interest as it would not interfere with DMARDs treatment.

Objectives: The aim of this study was to evaluate the effect of a 14-days-treatment of local cryotherapy on radiological outcomes in rat adjuvant induced arthritis.

Methods: Adjuvant-induced arthritis (AIA) was induced in 6-weeks old male Lewis rats by injection of *Mycobacterium butyricum* in Freund's incomplete adjuvant at the basis of the tail. A control group received saline. At the onset of arthritis, AIA rats were treated or not by application of cryotherapy on paws using either a cold spray or ice, twice a day for 14 days. Arthritis score and paws skin temperature was daily monitored. At the end of treatment, radiological exam of hind paws was performed and a score taking into account (swelling, osteoporosis, cartilage destruction, bone erosion, bone destruction and new bone formation) was assigned, according to Ackerman *et al* [2]. Circulating levels of cytokines (IL-6, IL-1 β , TNF- α) was measured by Magpix Luminex kit.

Results: Compared to untreated AIA, local cryotherapy significantly reduced the progression of arthritis score, whatever the modality ($p < 0.05$), and to the same extent (reduction of arthritis score at day 24 post-immunization: -38% with cold spray, $p < 0.01$, -37% with ice, $p < 0.01$). Radiological score was significantly reduced by both treatments with no difference between the two treatments (-33% with cold spray, $p < 0.01$, -44% with ice, $p < 0.01$). All the items of the radiological score were equally reduced by ice and cold spray except swelling that was significantly reduced only by ice. Interestingly, the use of the cold spray induced a greater decrease in the skin temperature than the ice treatment (18.32 ± 0.07 °C vs 20.46 ± 0.08 °C, $p < 0.001$). Conversely, cryotherapy did not significantly change the level of cytokines. No correlation was found between radiological score and arthritis score or cytokine levels.

Conclusion: These data demonstrated that local cryotherapy had positive effects on structural damage in adjuvant-induced arthritis. The mechanisms involved remain now to be determined. These results suggest that local cryotherapy would be an interesting complement to conventional DMARDs in early RA.

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SAT0022

CXCL7 PROMOTES OSTEOCLASTOGENESIS IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by bone destruction[1]. Chemokine signaling by skeletal cells or by other cells of the bone marrow niche regulates bone formation and resorption[2]. Recent studies have found that CXCL7 enhanced the osteoclast formation in mouse bone marrow cells[3, 4]. Whether CXCL7 plays a role in human osteoclastogenesis especially in RA patients remains unclear.

Objectives: To examine the functional role of CXCL7 in the induction of osteoclastogenesis in RA.

Methods: The level of CXCL7 in CD14⁺ monocyte supernatant was assessed via enzyme-linked immunosorbent assay. Osteoclastogenesis of CD14⁺ monocyte from RA patients and healthy donors were evaluated by TRAP staining and F-actin ring immunofluorescence. Bone resorption pit was observed by scanning electron microscopy. We performed quantitative reverse transcription polymerase chain reaction (RT-PCR) to detect changes in osteoclast markers. RAW264.7 macrophages were also used to investigate specific signaling pathway by which CXCL7 stimulated during osteoclast formation.

Results: CXCL7 level in CD14⁺ monocyte supernatant from RA patients (5690 ± 627.05 pg/ml) was significantly higher than that in healthy controls (2301 ± 535.52 pg/ml) (n=5, P<0.001). In the presence of M-CSF and RANKL, CXCL7 promoted osteoclast formation (Figure 1A and B) and increased bone resorption area (Figure 1C) of CD14⁺ monocyte from healthy donors in the low concentration (10ng/ml) group (n=3, p < 0.05). While in high concentration of CXCL7 (50ng/ml) group, there were no significant changes in the number of osteoclasts. Transcription level of the osteoclast markers RANK, cathepsin K, and MMP-9 was significantly increased in the CXCL7 (10ng/mL) group after 3 days in the presence of M-CSF and sRANKL (n=3, p < 0.05). When using CD14⁺ monocyte from RA patients, the optimal concentration of CXCL7 was 50ng/ml, which significantly increased the number of osteoclasts (Figure 2A and B) and bone resorption area (Figure 2C) (n=3, p < 0.01). Flow cytometry analysis revealed that a higher proportion of CD14⁺ monocytes expressed CXCR2 from healthy donors than those from RA patients (n=6, p < 0.01). Consistent with the results obtained in CD14⁺ monocytes, the effects of exogenous CXCL7 on osteoclast formation were also observed in RAW264.7 cells (p < 0.01). The addition of CXCL7 dramatically promoted phosphorylation ERK1/2 in RAW264.7 cells, but it did not affect the phosphorylation of P65.

Conclusion: CXCL7 level in CD14⁺ monocyte supernatant was higher in RA patients than that of healthy donors. CXCL7 promoted osteoclastogenesis in CD14⁺ monocyte both from RA patients and healthy donors. CXCL7 could be a potential therapeutic target for bone destruction in RA.

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Figure1. CXCL7 induced osteoclast differentiation and bone resorption in healthy donors. CD14⁺ monocytes isolated from healthy donors were cultured in the presence of M-CSF (50 ng/ml) and RANKL (100 ng/ml) with CXCL7 (1, 10, 50ng/ml) during the whole period of osteoclastogenesis.

Figure2. CXCL7 induced osteoclast differentiation and bone resorption in RA patients. CD14⁺ monocytes isolated from RA patients were cultured in the presence of M-CSF (50 ng/ml) and RANKL (100 ng/ml) with CXCL7 (10, 50, 100ng/ml) during the whole period of osteoclastogenesis.

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SAT0023

THE ROLE OF ADAM12 UPREGULATED PROLIFERATION OF SYNOVIAL MEMBRANE IN RHEUMATOID ARTHRITIS

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Background: ADAM12 is a member of a disintegrin and metalloproteinase family and has been reported to participate in the development of a variety of tumors by degrading ECM and shed precursors, thus promoting cell proliferation, invasion, and metastasis¹. Additionally, ADAM12 is involved in chondrocyte differentiation from osteoarthritis (OA) patients by regulation of TGFβ1-induced IGF-1 and RUNX-2 expressions². However, there is no report on the role of ADAM12 for rheumatoid arthritis (RA).

Objectives: To investigate the expression and role of ADAM12 in the synovial tissue of RA.

Methods: (1) The expression of ADAM12 in synovial tissues from RA (18 cases), OA (5 cases) and healthy control (HC) (3 cases) was examined by immunohistochemistry. The synovial tissues of HC were obtained during surgery of hemiarthroplasty for bone tumors. Three researchers evaluated the positive cell ratio. The samples were scored according to the percentage of positive staining: 0 points (weak positive, positive expression was less than 5%), 1 point (moderate positive, positive expression was between 5% and 50%) and 2 points (strong positive, positive expression was greater than 50%). In addition, the samples were scored according to the staining intensity: 0 points (weak intensity), 1 point (moderate intensity) and 2 points (high intensity). (2) The cultured synovial fibroblasts obtained from RA patients at the surgery (RASf) were stimulated by TNFα (1, 5, 10 ng/mL), TGFβ1 (1, 5, 10 ng/mL), PDGF-BB (1, 5, 10 ng/mL) and TNFα+TGFβ1+PDGF-BB (all 10 ng/mL), and the expression levels of ADAM12 relative mRNA was examined by real-time PCR. (3) siADAM12 was transfected in RASf, and the proliferation was examined by WST-1 assay, and the expression of ADAM12 protein was examined by western blotting.

Results: (1) ADAM12 positive cells were found in synovial lining cells, plasma cells, and vascular endothelial cells. ADAM12 was highly expressed in RA synovial tissues. The immunostaining scores of RA, OA, and HC were 3.9±0.01, 1.9±0.27, and 0.8±0.18, respectively. (2) Stimulation by TNFα, TGFβ1, and PDGF-BB resulted in the upregulation of the expression of ADAM12 relative mRNA in RASf, and TGFβ1 stimulation notably tended to increase the expression by about 5 to 6 times. (3) siADAM12 successfully suppressed the expression of ADAM12 protein and simultaneously suppressed the proliferation of RASf.

Conclusion: ADAM12 might be involved in the pathogenesis of RA, promoting the cell proliferation of RASf.