metabolite ensuring linked choreography between fibroblast and macrophage movement in the synovium which may become uncoupled in disease. We propose that dysfunctional crosstalk between these two cell types due to high lactate levels, promotes inflammation and the establishment of persistent disease in RA. Targeting lactate/MCTs pathway may provide a novel therapeutic strategy, to restore cellular crosstalk and to reduce inflammation in RA patients.

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**Disclosure of Interests:** Valentina Pucino: None declared, Meriam Nefla: None declared, Vincent Gauthier: None declared, Sally A Clayton: None declared, Andrew Filer Consultant of: Abbvie, Roche, Janssen, Grant/research support from: Roche, UCB, Nascient, Mestag, GSK, Janssen, Andy R Clark: None declared, Karim Raza: None declared, Christopher D Buckley Consultant of: GSK, Astra-Zenica, Roche, Pfizer, Lilly, Janssen Mestag, Grant/research support from: GSK, Roche, Pfizer, Janssen Mestag.
**DOI:** 10.1136/annrheumdis-2022-eular.2323

## OP0076 L-ARGININE REPROGRAMS OSTEOCLAST PURINE METABOLISM AMELIORATING BONE LOSS IN RHEUMATOID ARTHRITIS

S. Cao[1,2], R. Song[1,2], X. Meng[1], K. Kachler[1], M. Fuchs[3], X. Meng[2], Y. Li[2], V. Taudte[4], M. Kunz[3], U. Schloetzer-Schrehardt[5], U. Schleicher[6], X. Chen[2], G. Schett[1], A. Bozec[1]. [1]*Universitätsklinikum Erlangen, Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg, Department of Internal Medicine 3–Rheumatology and Immunology, Erlangen, Germany*; [2]*Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Department of Rheumatology, Shanghai, China*; [3]*Friedrich-Alexander University of Erlangen-Nürnberg, Chair of Medical Informatics, Erlangen, Germany*; [4]*Friedrich-Alexander University of Erlangen-Nürnberg, Institute of Experimental and Clinical Pharmacology and Toxicology, Erlangen, Germany*; [5]*Universitätsklinikum Erlangen, Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg, Department of Ophthalmology, Erlangen, Germany*; [6]*Universitätsklinikum Erlangen, Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg, Mikrobiologisches Institut – Klinische Mikrobiologie, Immunologie und Hygiene, Erlangen, Germany*

**Background:** Bone erosion is a clinical feature of rheumatoid arthritis related to disease severity and poor functional prognosis. Excessive osteoclast differentiation and insufficient osteoblast function are the main reasons for the erosive process in RA. Our previous investigation indicated that L-arginine supplementation not only diminished arthritic inflammation in the serum-induced arthritis (K/BxN) model but also decreased inflammatory joints osteoclast numbers (1).
**Objectives:** In the present study, we aim to investigate the metabolic action of L-arginine supplementation in RA, especially on periarticular bone erosion and systemic bone loss. We plan to depict the metabolic features of TNFα induced inflammatory osteoclasts after *in vitro* L-arginine supplementation.
**Methods:** Three murine arthritis models (serum-induced arthritis (K/BxN) model, collagen-induced arthritis model, and hTNFtg mice model) were analysed in this study. L-arginine was supplemented within the drinking water after the onset of arthritis. Bone parameters for axial skeleton (spine) and peripheral skeleton (tibia) from the respective group were quantified by µCT. HE and TRAP staining were performed to address further the erosion area and osteoclast numbers in periarticular sites. *In vitro* osteoclast differentiation was conducted with or without L-arginine treatment, in the presence or not of TNFα activation. Seahorse and SCENITH analyses were adopted to delineate the metabolic features. JC-1 staining and transmission electron microscopy (TEM) were used to depict the mitochondria metabolism. RNA-seq and mass spectrometry (MS) were performed to investigate the underlying molecular mechanism.
**Results:** Inflammation was diminished in all three arthritis models after L-arginine supplementation with a significant reduction in arthritic score. Moreover, an amelioration of periarticular bone erosion, systemic bone loss, and decreased osteoclast numbers in periarticular sites were observed in arthritic mice after L-arginine treatment. L-arginine also inhibited osteoclastogenesis *in vitro*, particularly under TNFα activation. Seahorse and SCENITH analyses indicated TNFα promoted glycolysis while blocking mitochondria-driven oxidative phosphorylations (OXPHOS) in pre-osteoclasts. Meanwhile, JC-1 staining and TEM images also showed that TNFα decreased mitochondria membrane potential and prompted damage of mitochondria. Surprisingly, L-arginine rescued the TNFα inhibition of OXPHOS while promoting ATP production. RNA-seq and MS data confirmed the boost of OXPHOS after L-arginine treatment under TNFα activation. To interfere with OXPHOS, L-arginine inhibited cJun thus altered arginase-1 and arginase-2 expression. Moreover, the increased ATP in L-arginine treated cells facilitated purine metabolism, especially the production of inosine and hypoxanthine, contributing to the inhibition of osteoclastogenesis. Increasing Adenosine deaminase (ADA) is essential for the production of inosine and hypoxanthine due to the decreased inhibitory regulation of the transcription factor c-Jun.
**Conclusion:** These data strongly demonstrated that L-arginine ameliorates bone erosion in RA through metabolic reprogramming and perturbation of purine metabolism in osteoclasts. L-arginine might therefore benefit RA therapy by reducing joint inflammation and also ameliorating bone destruction.

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**Disclosure of Interests:** Shan Cao: None declared, Rui Song: None declared, Xianyi Meng: None declared, Katerina Kachler: None declared, Maximilian Fuchs: None declared, Xinyu Meng: None declared, Yixuan Li: None declared, Verena Taudte: None declared, Meik Kunz: None declared, Ursula Schloetzer-Schrehardt: None declared, Ulrike Schleicher: None declared, Xiaoxiang Chen Speakers bureau: AbbVie, Roche and Novartis, Georg Schett Speakers bureau: AbbVie, BMS, Celgene, Janssen, Eli Lilly, Novartis, Roche and UCB, Aline Bozec: None declared.
**DOI:** 10.1136/annrheumdis-2022-eular.4338

## OP0077 SYNOVIAL RNA-SEQ ANALYSIS OF THE R4RA TRIAL IDENTIFIES SIGNATURES OF TREATMENT RESISTANCE AND REFRACTORY STATE IN RHEUMATOID ARTHRITIS

A. Surace[1,2], E. Sciacca[1,2], K. Goldmann[1,2], F. Rivellese[1,2], C. Cubuk[1,2], G. Giorli[1,2], L. Fossati-Jimack[1], M. Ahmed[1], E. Prediletto[1], M. Bombardieri[1], M. Lewis[1], C. Pitzalis[1], on behalf of R4RA investigators. [1]*Queen Mary University of London, Centre for Experimental Medicine and Rheumatology, London, United Kingdom*; [2]*Queen Mary University of London, Centre for Translational Bioinformatics, London, United Kingdom*

**Background:** Although up to 5-20% of rheumatoid arthritis (RA) patients do not respond to all current medications including biologic therapies, relatively little is known about the underlying pathogenic mechanisms driving non-response. In the first biopsy-driven randomized clinical trial in RA (R4RA)[1], patients, in whom synthetic-DMARDs and at least one anti-TNF drug were not effective, were randomised 1:1 to rituximab (RTX) or tocilizumab (TOC) with a balanced stratification based on their synovial B-cell rich/poor signature, and response was assessed at 16 weeks. Non-responders were subsequently allowed to switch to the alternative drug with 48-week follow-up.
**Objectives:** Investigate mechanisms of response and non-response to RTX and TOC through deep molecular (RNA-Sequencing) profiling of synovial tissue.
**Methods:** RNA-Seq from baseline synovial tissue biopsies of patients who received RTX (n=88) or TOC (n=94) at any point in the trial was analysed for differentially expressed genes and associated modules between responders and non-responders. Response was defined as 50% improvement in clinical disease activity index (CDAI) score. Patients who had received both drugs during the trial were subdivided into RTX only responders (pro-RTX, n=9), TOC only responders (pro-TOC, n=12) and refractory patients (no response to both RTX & TOC, n=32) and analysed for differential gene expression and performed gene module analysis.
**Results:** 6625 genes were significantly differentially expressed between RTX responders compared to non-responders, with a predominance of antigen presentation as well as T- and B-cell genes being associated with response, while non-response was linked to fibroblast associated genes. Comparison between TOC responders and non-responders identified fewer (85) differentially expressed genes, however lymphocyte and immunoglobulin genes were also high in the synovial tissue of TOC responders similar to RTX responders, while non-responder genes and modules also included a fibroblast signature. The cross-over study design enabled comparison of rituximab-specific responders (pro-RTX), tocilizumab-specific responders (pro-TOC) and refractory patients (non-responders to both RTX & TOC, n=32) in a 3-way analysis (see Figure 1). This identified 1980 genes upregulated both in pro-RTX and pro-TOC patients, 175 genes exclusive to the pro-RTX group and 306 to the pro-TOC group, while 1277 genes were exclusive to the refractory group. While leukocyte modules and genes dominated RTX & TOC response, the refractory state was strongly associated with fibroblast genes and modules. We confirmed the observed expansion of fibroblasts from the RNA-Seq data by immunohistochemistry showing the presence of DKK3+ sublining fibroblasts in refractory rather than responder patients.