western blot analysis. ROS levels were analyzed with MitoSOX red mitochondrial superoxide indicator using flow cytometry analysis. Cell migratory ability was examined using transwell migration assay and invaded cells were stained with crystal violet. For quantification, the crystal violet dye was eluted with 0.1% sodium dodecyl sulfate (SDS). A769662 was treated as a specific activator of AMPK (AMP-activated protein kinase)-mediated signaling.

**Results:** When RA FLS were stimulated with recombinant interleukin 17 (IL-17; 10 ng/ml) and tumor necrosis factor alpha (TNF-α; 10 ng/ml), SLC7A11 expression was markedly decreased. Following LKB1 knock-down for 24h, the protein level of SLC7A11 was also decreased in RA FLS. To determine whether SLC7A11 directly regulates FLS migration, SLC7A11 siRNA was transfected and then analyzed in intracellular ROS level and performed transwell assay. SLC7A11 inhibition induced ROS expression (1.74-fold) and migration ability (1.74-fold) compared to control in RA FLS. Next, cells were treated with A769662 for AMPK activation, which is directly phosphorylated by LKB1 stimulation. LKB1 knockdown reduced SLC7A11 level and that expression was increased by LKB1 siRNA, and that increase was suppressed by A769662 treatment. When LKB1 inhibition enhanced cell migration about 1.81-fold, and those increases were also reduced in 0.92-fold by AMPK activation compared to control.

**Conclusion:** These findings suggest that LKB1 regulate ROS production and SLC7A11 expression and, eventually lead to decrease migration, thereby further contributing to alleviate inflammation of RA FLS.

**REFERENCES:**


**Acknowledgements:** NIL.

**Disclosure of Interests:** None Declared.

**DOI:** 10.1136/annrheumdis-2023-eular.1175
CD4+ T cells through histological and cellular and molecular biology techniques, and propose possible molecular regulatory pathways. **Methods:** Patients with RA from December 2019 to January 2022, and gender and age-matched different controls (OA) were enrolled. Peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cells (SFMC) were collected and CD4+ T cells were sorted by magnetic beads. The expression levels of different phenotypes of CD4+ T cells and important cytokines were analyzed by flow cytometry; the secretion levels of IL-10 and IFN-γ were detected by ELISA technology. In the intervention experiment, the CD4+ T cells were incubated with siRNA-CH25H-mediated decrease in IL-10+CD4+ T cells was reversed and NKp46+ ILC3-like cells infiltration in the joints, higher and bone destruction. Adoptive transfer of NKp46+ ILC3-like cells before disease onset resulted in increased NKp46+ ILC3-like cells infiltration in the joints, higher and bone destruction. Adoptive transfer of NKp46+ ILC3-like cells before disease onset resulted in increased NKp46+ ILC3-like cells infiltration in the joints, higher and bone destruction. Adoptive transfer of NKp46+ ILC3-like cells before disease onset resulted in increased NKp46+ ILC3-like cells infiltration in the joints, higher

between ERA and reFrA, using unsupervised clustering and linear mixed-effects models, which adjusted for age, sex, seropositivity and batch, a false discovery rate of 0.05 was applied. All analyses were performed using R v4.0.2. **Results:** The cohort had a higher proportion of females (73.3% ERA and 87% reFrA) and a median age of 61 years. The majority were seropositive (86.7% ERA, 91.3% reFrA). Among the reFrA patients, median number of failed b/tsDMARDs was 3 (range 2-7). Overall, we identified 17 phenotypically distinct subsets from the immune cell population. ERA and reFrA had similar abundance of immune cell sub-populations. However, functional marker differences in these cell subpopulations were observed between reFrA and ERA higher TNFR expression, monocytes, dendritic and FcεRI+ cells, CD80 (FcεRI+ monocytes), CD127 (NK cells), CD38 (basophils), NKp46 (CD8+ naïve T cells) and PD1 (CD8+ naïve T cells). **Conclusion:** We report alteration in the functional profile across multiple immune cell types between reFrA and ERA. These findings provide new insights into the immunological profiles of reFrA and potential targets for therapy.

**REFERENCES:**

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Euras, 77(12), 1705.

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<td>Cell type</td>
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</tr>
<tr>
<td>Increased expression</td>
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<td>Monocytes</td>
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<td>NKp46+ ILC3</td>
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<td>Reduced expression</td>
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<tr>
<td>Monocytes</td>
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<td>NKp46+ ILC3</td>
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Functional makers differences (reFrA vs ERA): Table 1 shows functional markers expression across immune cell clusters in reFrA compared to ERA, using linear mixed modelling (FDR <0.05).

Acknowledgements: This study was supported by a UCB Pharma PhD stu-
dentship (TB).

Disclosure of Interests: None Declared.

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**POS0014** SINGLE-CELL PERIPHERAL BLOOD ANALYSIS IDENTIFIES KEY IMMUNE SIGNATURES ASSOCIATED WITH REFRACTORY RHEUMATOID ARTHRITIS

Keywords: Rheumatoid arthritis, Biomarkers, -omics

Y. Tan1,2, T. Bhatt3, M. Sutcliffe1, J. Fitton3, P. Emery3,4, A. Aslam5, D. Plant1,2, M. H. Buch1,2,3, Y. Liu1, F. J. T. Godson1, K. G. et al (2021). EULAR definition of difficult-to-treat rheumatoid arthri-

**Background:** Refractory rheumatoid arthritis (reFrA) persists in the targeted therapy era with up to 20% of patients failing multiple classes of biologic (b) and/or targeted synthetic DMARDs (2). Currently, there is limited understanding of the immune composition of reFrA.

**Objectives:** To characterise the peripheral immune landscape of patients with reFrA as compared to early RA.

**Methods:** In the Leeds observational cohort study, peripheral blood mononuc-
lear cells were collected from 30 treatment naïve early rheumatoid arthritis (ERA) and 23 refractory rheumatoid arthritis (reFrA) patients. ERA patients have >12 months symptoms duration and reflect least moderate-
disease) RA despite ≥2 classes of b and/or DMARDs. Using a panel of 36 antibodies, we conducted mass cytometry by time of flight (CyTOF) analysis to identify alterations in peripheral immune cell subsets and functional markers expression of CH25H and LXR in CD4+ T cells of RA synovial tissue increased disease) RA despite ≥2 classes of b and/or DMARDs. Using a panel of 36 antibodies, we conducted mass cytometry by time of flight (CyTOF) analysis to identify alterations in peripheral immune cell subsets and functional markers

Y. Tan1,2, T. Bhatt3, M. Sutcliffe1, J. Fitton3, P. Emery3,4, A. Aslam5, D. Plant1,2, M. H. Buch1,2,3, Y. Liu1, F. J. T. Godson1, K. G. et al (2021). EULAR definition of difficult-to-treat rheumatoid arthri-

**Disclosure of Interests:** None Declared.

DOI: 10.1136/annrheumdis-2023-eular.2622

**POS0015** EXPANSION OF NKp46+ INNATE LYMPHOID CELL IN THE JOINTS EXACERBATES COLLAGEN-INDUCED ARTHRITIS

Keywords: Rheumatoid arthritis

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**Background:** NK cells, a subset of innate lymphoid cells (ILCs), possessing both cytotoxicity and cytokine-producing properties, participate in many immune activi-
ties, e.g., control of viral infections, antitumor immunity, and autoimmune diseases. The role of innate Lymphoid cell (ILC) in rheumatoid arthritis remains controversial.

**Objectives:** This study investigates the alteration in proportion of IL-10+ ILC3-like cells in CIA mice over disease development in different tissues and possible association with the severity of arthritis. We further examined the role of NKp46+ ILC3-like cells cells via cell transfer and the receptor NKp46 via NKp46 knockout mice.

**Methods:** The percentage of NKp46+ ILC3-like cells in the peripheral blood, spleen, lymph nodes and inflamed paws from collagen-induced arthritis mice were examined through the disease progression. Correlation between the proportion of NKp46+ ILC3-like cells and subsets with arthritis score, histopathological changes, and bone destruction were evaluated. Adoptive cell transfer was performed to determine the effect of NKp46+ ILC3-like cells on arthritis development, and the role of receptor NKp46 was explored with NKp46 knockout mice.

**Results:** The percentage of NKp46+ ILC3-like cells in peripheral blood decreased at the late stage of the disease and negatively correlated with arthritis score. NKp46+ ILC3-like cells increased in the inflamed paws during arthritis development and were positively associated with arthritis score, histopathological change, and bone destruction. Adoptive transfer of NKp46+ ILC3-like cells before disease onset resulted in increased NKp46+ ILC3-like cells infiltration in the joints, higher