group, the proportion of Th/Tfr in CIA group was significantly higher than healthy control group. Compared with CIA model group, Th/Tfr in all intervention groups (Ox,Td,C4d,21d,IL-2) were significantly lower.

**Conclusions:** Early administration of low-dose IL-2 may help reduce the incidence of arthritis and disease activity in CIA by restoring Th/Tfr balance, but it has time heterogeneity.

**REFERENCES:**


**Figure 1.** Early intervention of CIA with low dose of IL-2 at different time points. (A) The percentage of mice that remained arthritis free over time. (B) Arthritis scores of each experimental group over time. (C) Comparison of Th (CD4+CXCR5+) cells percentage and (D) Thfr(CD4+CXCR5+CD225+Foxp3+) cells of CD4+T cells in different groups. (E) Th/Tfr ratio in different group.

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**Disclosure of Interests:** None Declared.

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**POS0104**

**INVESTIGATING THE SYNOVIAL PATHOLOGY RELATED TO TREATMENT RESISTANCE IN JAPANESE RHEUMATOID ARTHRITIS PATIENTS USING SINGLE-CELL ANALYSIS**

**Keywords:** Rheumatoid arthritis, Synovium


**Background:** Despite of recent developments in therapeutic agents for rheumatoid arthritis (RA) patients[1], it is reported that half of the patients are unable to achieve remission even with existing drugs[2]. Therefore, there is an urgent need to gain mechanistic insight into treatment resistance. Nowadays, single-cell RNA sequencing (scRNA-seq) technology have dramatically improved our understanding of the heterogeneity in synovial cells. However, it has not been fully elucidated how the cell clusters are related to treatment response, especially in Asian races[3].

**Objectives:** We intend to analyze the RA synovium from Japanese patients based on single-cell transcriptomics to explore the pathology of key players related to treatment resistance.

**Methods:** Synovial specimens were collected from 31 RA patients using an ultrasound-guided needle biopsy. The proportion of 5 immune cell subsets (CD4+ T cells, CD8+ T cells, B cells, NK cells, monocytes) and mesenchymal cells (synovial fibroblasts (SF), endothelial cells, mural cells) were analyzed by flow cytometry. CD45+ and CD45- live cells were isolated, and scRNA-seq libraries were prepared using the 10x chromium system.

**Results:** We classified the patients into the following three groups based on treatment status at the time of biopsy; treatment-naïve, inadequate response to conventional synthetic disease-modifying antirheumatic drugs (csDMARDs-IR), or biological DMARDs (bDMARDs-IR). The proportion of CD8+ T cells, especially GZMB+ GZMK+ CD8+ T cells, was significantly lower in csDMARDs-IR patients compared to treatment-naïve patients. This population was characterized by enhanced expression of IFNG and GZMK, the cooperative inducers of IL-6 production from SF. Meanwhile, an increased proportion of SF, especially THY1+ sublining and CD44+ sublining, was observed in csDMARDs-IR and bDMARDs-IR patients. Intriguingly, by integrating gene set variation analysis (GSVA) with transcriptomic data of cytokine-stimulated SF in vitro, THY1+ sublining was indicated to be activated independently of the effects of inflammatory cytokines (e.g., TNF-α, IL-1), IFNs (Figure 1). Collectively, this SF subpopulation was inferred to be less susceptible to cytokine-blocking agents such as IL-6 receptor or TNF-α inhibitors.

**Conclusion:** The synovial analysis has the potential to be useful in parsing the mechanism of treatment resistance in Japanese RA patients, and gaining insights into novel therapeutic targets.

**REFERENCES:**


**Figure 1.** THY1+ sublining is a cluster scarcely affected by inflammatory cytokine.

**Acknowledgements:** NIL.

**Disclosure of Interests:** None Declared.

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**POS0105**

**A NOVEL DRUG COMBINATION OF IGuratimod AND Tofacitinib ALleviates RHEUMATOID ARTHRITIS AND SECONDARY OSTEOPOROSIS**

**Keywords:** Osteoporosis, Rheumatoid arthritis, Animal models

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**Background:** At present, specific immune targeted therapeutics including biologics and kinase inhibitors has made significant progress in Rheumatoid arthritis (RA). However, the response to targeted therapies in some refractory RA patients suggests more attention should be paid on cell death pathways, such as pyroptosis. TNF-α is a prionlarily pyroptotic cytokine that plays a fundamental role in the pathogenesis of RA, which could induce pyroptosis in monocytes and macrophages and cause the destruction of bone and cartilage. Igruratimod (IGU) has been confirmed as a highly efficacious and safe conventional synthetic disease-modifying cytokine.
anti-rheumatic drug for RA in Asia. Whether the combination of IGU and Tofacitinib (TOF), the Janus kinase inhibitor, would be better partner need to be elucidated.

**Objectives:** To evaluate the therapeutic effect of IGU and TOF on active RA and secondary osteoporosis in collagen-induced arthritis (CIA)+TNF model.

**Methods:** In this study, hematoxylin and eosin (HE) staining were used to evaluate the pathological changes in ankle joints of CIA+TNF model. Immunohistochemistry (IHC) were used to evaluate the level of pyroptosis related proteins in synovial tissue. We performed Micro-computed tomography (Micro-CT), HE staining and IHC to analyze the trabecular bone changes in distal femoral metaphyses to investigate the destruction of knee joint.

**Results:** After 6 weeks treatment of IGU and/or TOF, the diameter of ankle joint and the level of interleukin (IL)-18, IL-1 of CIA+TNF model. Immunohistochemistry (IHC) were used to evaluate the level of pyroptosis related proteins in synovial tissue. We performed M group. HE staining showed that only a small amount of inflammatory cell infiltration and less pannus were seen in synovial tissue of both monotherapy and combination group, when compared with the CIA+TNF group. Of importance, the pyroptosis related proteins, such as gasdermin D (GSDMD), nucleotide-binding domain (NOD)-like receptor protein 3 (NLRP3), caspase-1, and IL-1β were significantly less expression in synovial tissue of combination group compared with CIA+TNF group. Both the osteoblast bone formation and osteoclast bone absorption were sharply ruined in CIA+TNF model. However, the bone destruction was significantly alleviated and bone turnover rate was remarkably increased in combination group, detected by Micro-CT, HE staining and IHC.

**Conclusion:** The TOF-IGU combination synergistically alleviated the disease severity of the CIA model, including relieving joint inflammation and bone erosion, with suppressing the activation of the NLRP3 inflammasome and reducing GSDMD-related proteins in synovial tissue; (D) Micro-CT analysis in distal femurs; (E) HE staining in synovial tissue. We performed Micro-computed tomography (Micro-CT), HE staining and IHC to analyze the trabecular bone changes in distal femoral metaphyses to investigate the destruction of knee joint.

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**Disclosure of Interests:** None Declared.

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**POST016**

**ANALYSIS OF GUT MICROBIOTA IN RHEUMATOID ARTHRITIS-ASSOCIATED INTERSTITIAL LUNG DISEASE**

**Keywords:** Rheumatoid arthritis

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**Background:** The gut microbiota has been related to rheumatoid arthritis (RA), inflammation, and its severity. Interstitial lung disease (ILD) causes high morbidity and mortality in RA patients. However, the association between gut microbiota and RA- associated ILD (RA-ILD) is still unknown.

**Objectives:** To analyze the gut microbiota and gut permeability in RA-ILD patients.

**Methods:** Nested case-cohort study of 2 prospective cohorts of patients with RA with and without ILD. The cohorts were matched for age, sex, and time of RA evolution. All patients systematically underwent high-resolution computed tomography (HRCT) and pulmonary function testing (PFT) on the diagnosis of ILD. The ILD was defined according to the lung biopsy or HRCT based on the standard criteria of the American Thoracic Society/European Respiratory Society. The progression was defined as the worsening of the FVC > 10% or DLCO > 15%. The gut microbiota was measured by the 16S rRNA gene and the sequences were processed using the Quantitative Insights Into Microbial Ecology (QIME2). Serum lipopolysaccharide-binding protein (LBP) and lipopolysaccharide (LPS) were measured as markers of gut permeability. Demographic, clinical, laboratory, and treatment-related data were recorded. The disease activity was measured by Disease Activity Score-28 with Erythrocyte Sedimentation Rate (DAS28-ESR), and the function using the Health Assessment Questionnaire (HAQ). We performed a descriptive analysis and Cox regression analysis to identify prognostic factors for the time to progression of RA-ILD.

**Results:** Thirty-five RA-ILD and 35 RA without ILD were included. Table 1 shows the baseline characteristics. After a median (SD) period of 66.1 (47.2) months, pulmonary progression criteria had observed in 13 patients (37.1%). Compared with controls, RA-ILD had greater values of DAS28-ESR (p=0.032) and higher HAQ scores (p=0.003). They also had higher levels of serum LPS (p = 0.007) and more abundance of Strep.

duction of Slackia genus (p = 0.090) and a lower abundance of genera (p = 0.082). The RA-ILD with progression had a higher abundance of Slackia genus (p = 0.090) and a lower abundance of Slackia genus. In Cox regression analysis, the moderate-high activity of DAS28-ESR (HR [IC 95%], 3.53 [120-6.98] = 0.017), LPS (HR [IC 95%], 1.12 [1.02-1.23] = 0.018) and the Slackia genus (HR [IC 95%], 0.98 [0.97-0.99] = 0.010) were associated with RA-ILD.

**Conclusion:** RA-ILD patients showed increased gut permeability and also displayed a different pattern of gut microbiota associated with ILD diagnosis and progression. These findings may enable the discovery of potential RA-ILD biomarkers.

**Table 1. Clinical and demographic characteristics**

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>RA-ILD</th>
<th>RA without ILD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, mean (SD)</td>
<td>69.7 (9.3)</td>
<td>66.6 (7.0)</td>
<td>0.130</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>20 (57.1)</td>
<td>20 (57.1)</td>
<td>1.000</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td>0.760</td>
</tr>
<tr>
<td>Never smoked, n (%)</td>
<td>17 (48.6)</td>
<td>18 (51.4)</td>
<td>0.690</td>
</tr>
<tr>
<td>Ex-smoker, n (%)</td>
<td>10 (28.6)</td>
<td>8 (22.9)</td>
<td>0.384</td>
</tr>
<tr>
<td>Active smoker, n (%)</td>
<td>8 (22.9)</td>
<td>9 (25.7)</td>
<td>0.384</td>
</tr>
<tr>
<td>Time since diagnosis RA, months, median (IQR)</td>
<td>149.8 (34.2-245.5)</td>
<td>133.7 (67.8-204.2)</td>
<td>0.384</td>
</tr>
<tr>
<td>ESR, mean (SD)</td>
<td>44.3 (34.3)</td>
<td>31 (88.6)</td>
<td>0.393</td>
</tr>
<tr>
<td>CRP, n (%)</td>
<td>32 (91.4)</td>
<td>31 (88.6)</td>
<td>0.690</td>
</tr>
<tr>
<td>Erosions, n (%)</td>
<td>21 (60.0)</td>
<td>19 (55.6)</td>
<td>0.705</td>
</tr>
<tr>
<td>DAS28-ESR, mean (SD)</td>
<td>3.1 (0.9)</td>
<td>2.6 (0.9)</td>
<td>0.032</td>
</tr>
<tr>
<td>HAQ, mean (SD)</td>
<td>12 (0.6)</td>
<td>0.8 (0.6)</td>
<td>0.003</td>
</tr>
<tr>
<td>FVC mean (SD)</td>
<td>60.0 (15.2)</td>
<td>65.9 (15.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>UIP, mean (SD)</td>
<td>29 (82.9)</td>
<td>0 (0.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NSIP, n (%)</td>
<td>6 (17.1)</td>
<td>0 (0.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Abbreviations:** ACPA: anti-citrullinated peptide antibody; DAS28: Disease activity score; DLO: diffusion capacity of the lung for carbon monoxide; ESR: erythrocyte sedimentation rate; FVC: forced vital capacity; HAQ: Health Assessment Questionnaire; IQR: interquartile range; NSIP: nonspecific interstitial pneumonia; RA: rheumatoid arthritis; RF: rheumatoid factor; SD: standard deviation; UIP: usual interstitial pneumonia.

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