spondyloarthritis, a prototype chronic inflammatory disease. Furthermore, it attenuated inflammation and improved disease activity, function, and quality of life.

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Disclosure of Interest None declared.

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PEFICITINIB TARGETS SYNOVIAL FIBROBLASTS IN RHEUMATOID ARTHRITIS

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Career situation of first and presenting author Post-doctoral fellow.

Introduction Tofacitinib and baricitinib are the first approved Janus kinase inhibitors (JAKi) in Europe for treatment of rheumatoid arthritis (RA). Other JAKi differing in the inhibition profile of the four isoforms JAK1, JAK2, JAK3 and TYK2 are currently examined in clinical trials. Especially filgotinib, inhibiting mainly JAK1/2, and the panJAKi peficitinib are well tolerated up to doses causing C_{max} values higher than 1 μ M. However, less is known about the effect of JAKi in RA on synovial fibroblasts (RASf) activated by IL-1 β . RASf contribute to the growing pannus and develop an aggressive cartilage-destructive phenotype.

Objectives The aim of the study was to examine the effect of JAKi on activated RASf and further characterize the most effective inhibitor.

Methods Isolated human RASf were pretreated with JAKi for 2 hour and then stimulated with IL-1 β and JAKi with/without soluble IL-6 receptor (sIL-6R). IL-6 and MMP-3 were measured in supernatants by ELISA. Stimulation with oncostatin M served as control. The proliferation of RASf was detected using a BrdU-incorporation assay. The effect of peficitinib on RASf migration was investigated by cell culture inserts using a FCS gradient. For adhesion assays, RASf were treated with JAKi, detached and seeded in culture plates. Then, plates were extensively shaken and adherent RASf quantified by counting crystal violet stained cells. Cell viability, cytotoxicity and apoptosis were measured using commercially available assays.

Results Addition of the sIL-6R caused an additional release of IL-6 in RASf treated with IL-1 β compared to IL-1 β alone. This release was fully blocked by tofacitinib at 0.5 and 1 μ M (both $p < 0.01$). However, tofacitinib was not able to reduce the IL-6 release of IL-1 β activated RASf without sIL-6R. In contrast, peficitinib attenuated the IL-6 levels by 62% at 5 μ M ($p < 0.001$, $n = 7$) and by 24% at 1 μ M. Filgotinib resulted in a reduction of 30% at 5 μ M only ($p < 0.05$). The MMP-3 release was reduced by peficitinib (88%, $p < 0.001$) at 5 μ M. Peficitinib was the only JAKi able to reduce the proliferation of activated RASf by 23% at 1 μ M ($p < 0.05$).

Furthermore, peficitinib at 1 and 5 μ M decreased the migration of RASf by 38% and 92% ($p < 0.001$, $n = 4$). Peficitinib up to 5 μ M did not act cytotoxic on RASf even after 48 hour.

Conclusions JAKi suppress the inflammatory response induced by transsignaling of IL-6 in RASf. Peficitinib appears to be superior to other JAKi in suppressing the IL-1 β induced response and the proliferation of activated RASf. Thus, patients suffering specifically from severe synovial proliferation could benefit from treatment with peficitinib.

Disclosure of Interest None declared.

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TARGETING ACTIVATED SYNOVIAL FIBROBLASTS USING PHOTODYNAMIC THERAPY IN HUMAN RHEUMATOID ARTHRITIS SYNOVIAL TISSUE

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Career situation of first and presenting author Student for a master or a PhD.

Introduction Activated synovial fibroblasts (ASF) play an important role in the pathogenesis of rheumatoid arthritis (RA). They contribute to the pro-inflammatory environment in the joint and degradation of cartilage and bone. Depleting ASF could ameliorate both these hallmarks of RA. ASF are characterized by the expression of fibroblast activation protein (FAP).

Objectives Here, we investigated the potential of FAP-targeted photodynamic therapy (tPDT) using the photosensitizer IRDye700DX conjugated to the anti-FAP antibody 28 H1 (28H1–700DX) to selectively kill these cells.

Methods To demonstrate the proof-of-concept of FAP-based tPDT in *ex vivo* human tissue, RA synovial tissue obtained during joint replacement surgery was trypsin digested and fibroblasts were cultured for at least 5 passages. Cells were incubated with or without 1 μ g/well 28H1–700DX for 4 hour, washed with PBS and either exposed to 690 nm light or not exposed. A luminescent cell viability assay (CellTiter-Glo) was used to measure cell viability. In parallel, 6 mm biopsies of synovial tissue were taken and used for FAP-based tPDT ($n = 8$ patients). They were subjected to tPDT as described above and formalin fixed after 1 hour. To measure cell death, 5 μ m paraffin slices were stained for FAP, gamma-H2AX and caspase-3 expression for activated fibroblasts, DNA double-strand breaks and early apoptosis, respectively.

Results FAP-tPDT performed on the cultured fibroblasts showed a light dose dependent increase in cell death when incubated with 28H1–700DX for 4 hour. Cell viability was not affected when cells were incubated with 28H1–700DX without illumination ($101.37\% \pm 3.9\%$ remaining compared to $100\% \pm 5.07\%$ in the normalized control). Radiant exposure of 17.6 J/cm^2 did not significantly decrease cell viability ($6.4\% \pm 9.8\%$ decrease, ns). Radiant exposures of 52.8, 105.6 and 158.4 J/cm^2 significantly decreased cell viability ($38.6\% \pm 6.9\%$, $67.5\% \pm 10.9\%$ and $80.6\% \pm 5.8\%$ respectively, $p < 0.001$ for all). After FAP-tPDT, the human synovial biopsies showed a significantly increased staining of the caspase-3 marker, but not of gamma-H2AX (Friedman's ANOVA, $p = 0.007$ and $p = 0.810$ respectively). Pairwise comparison

showed that caspase-3 scores were significantly higher in biopsies treated with tPDT compared to those incubated with buffer ($p=0.009$).

Conclusions FAP-tPDT induces cell death of FAP-positive activated fibroblasts in synovial tissue from RA patients. This is a first indication that FAP-targeted PDT can be a feasible new treatment strategy in RA.

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EFFECTS OF RESVERATROL AND A NOVEL RESVERATROL-SALICYLATE HYBRID MOLECULE ON ACTIVATION OF HUMAN CD4⁺ T-CELLS

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Introduction T-cells are assumed to be crucially involved in the pathogenesis of systemic autoimmune diseases such as rheumatoid arthritis (RA). Consequently, substances modulating T-cell activation may have therapeutic benefit in RA and related rheumatic diseases. Resveratrol is a natural occurring polyphenol mainly produced in plants. The beneficial effects of resveratrol are due to its anti-inflammatory, anti-carcinogenic and anti-oxidant activities.

Objectives To compare the effects of resveratrol and a novel resveratrol-salicylate hybrid molecule termed C-10 (Aldawsari FS *et al*, 2016) on human CD4⁺ T-cells.

Methods CD4⁺ T-cells were isolated from healthy donors and pre-incubated with different concentrations of resveratrol or C-10 before being stimulated with anti-CD3/anti-CD28 antibodies. After 24 hour and 72 hour, respectively, cell culture supernatants were harvested and IL-2, IFN- γ and TNF- α release was quantified by ELISA. Proliferation rate was measured by thymidine incorporation. In addition, the up-regulation of the early activation markers CD25, CD69, CD71 and CD98 was analyzed and phosphorylation of signal transduction molecules were determined by western blot and/or flow cytometry.

Results Inhibition of IL-2, IFN- γ and particularly TNF- α release was significantly more effective when the cells were treated with C-10 as compared to resveratrol. Moreover, the proliferation rate was significantly more decreased in the presence of C-10. The expression of CD25, CD69, CD71 and CD98hc was reduced to a similar degree by both compounds. Furthermore, phosphorylation of Akt and STAT-5 was substantially attenuated by C-10 and to a lesser degree also by resveratrol. All T cell subsets investigated (Th1, Th2, Th17) were affected at a similar degree but the most pronounced effect was seen in naïve T cells.

Conclusions Our data demonstrate that C-10 suppressed cytokine secretion and proliferation more effectively than resveratrol. Both compounds influence the phosphorylation of important signalling molecules. The effect exerted on STAT-5 activation may be the key mechanism for inhibition of T cell activation. Thus, the resveratrol-salicylate hybrid molecule C-10 might be considered a candidate drug for treatment of RA and other T-cell driven autoimmune diseases.

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Disclosure of Interest None declared.

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CLINICAL AND IMMUNOLOGICAL EFFECTS OF TOFACITINIB THERAPY IN RHEUMATOID ARTHRITIS

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Career situation of first and presenting author Student for a master or a PhD.

Introduction During the treatment of rheumatoid arthritis (RA) we intend to achieve remission or low disease activity. In this regard, the most effective way is targeted therapy. Oral JAK inhibitor, tofacitinib appeared as a new therapeutic option, beside biological therapies, which has already proven its safety and effectiveness in RA.

Objectives The aim of this study was to assess the clinical and immunological effects of one-year tofacitinib therapy in patients with RA.

Methods Altogether 30 RA patients with active disease were recruited and treated with tofacitinib in this 12 months follow-up study. Mean duration of rheumatoid arthritis were 7.7 $\pm$ 5.0 years. Half of the patients haven't received biological treatment prior tofacitinib therapy, other half of the patients switched to tofacitinib therapy after completing washout. 15 patients received 2 $\times$ 5 mg and 15 patients received 2 $\times$ 10 mg tofacitinib daily for 12 months. Assessments were performed at baseline, month 6 and 12. Levels of CRP and IgM rheumatoid factor (RF) antibodies were measured by quantitative nephelometry and levels of anti-CCP assessed by ELISA. Lymphocyte subsets (CD3⁺, CD4⁺, CD8⁺ T cells, CD19⁺ B cells and CD56⁺/CD3⁻ NK cells) were assessed by cytofluorimetry. In addition, disease activity (DAS28), age and disease duration were also measured.

Results Tofacitinib therapy was clinically effective, significant improvements in physical function were observed in 26 patients. 4 patients (two from both arms) quit the study due to inefficacy. There were significant decrease in levels of DAS28 ($p<0.001$), CRP ($p<0.001$) and HAQ value ($p<0.05$) in 12 months. There were no changes in levels of RF and anti-CCP. Numbers and ratio of CD3⁺ and CD4⁺ T cells were significantly decreased ($p<0.05$), however significant increase was seen in the numbers and ratio of CD19⁺ B cells after 12 months ($p<0.05$). There were no significant changes in numbers and ratio of NK cells. There was significant correlation between absolute number of CD8⁺ T cells and disease