JAK inhibitors alleviate metabolic dysregulation, inflammation and fibrosis in osteoarthritis: insights from human joint cells and synovium

Geneviève Paulissen[1]*, Olivier Malaise[1]*, Céline Deroyer[1], Edith Charlier[1], Sophie Neuville[1], Zelda Plener[1], Thierry Thirion[2], Christophe Daniel[2], Clio Ribbens[1], Dominique de Seny[1]

[1] Laboratory of Rheumatology, GIGA Institute, CHU Liège, University of Liège, Liège, Belgium

[2] Orthopedic Surgery Department, CHU Liège, Liège, Belgium

Statement of equal contribution: Paulissen G and Malaise O contributed equally to this study.

Corresponding author details:

Geneviève Paulissen

Laboratory of Rheumatology, GIGA Institute, CHU Liège, University of Liège, Liège, Belgium

Avenue de l'Hôpital 1

4000 Liège, Belgium

genevieve.paulissen@chuliege.be

Abstract

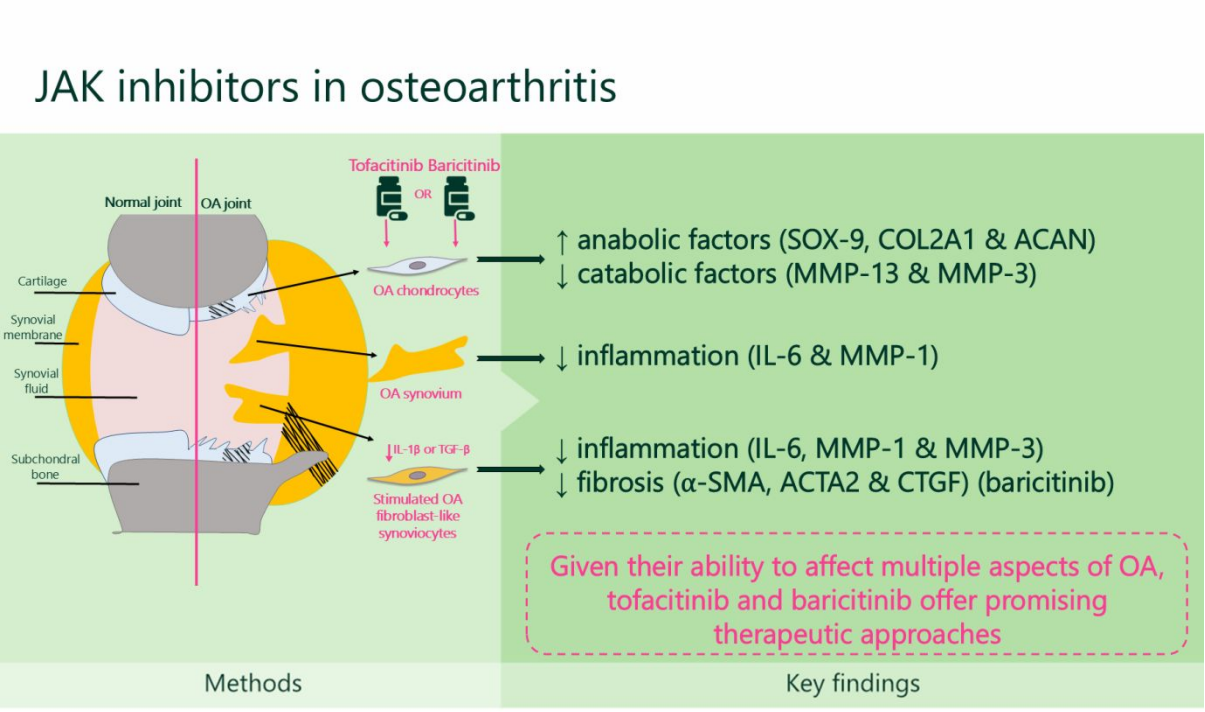
Objectives: Osteoarthritis (OA) presents a significant clinical challenge due to its heterogeneous nature, characterized by cartilage degradation, inflammation, and fibrosis. Current treatments offer limited efficacy, highlighting the need for novel therapeutic approaches. Our study aimed to investigate the effects of two JAK inhibitors, tofacitinib and baricitinib, on various hallmarks of OA in human joint cells and synovium.

Methods: Human OA fibroblast-like synoviocytes (FLS), OA chondrocytes, and synovial explants were cultured with tofacitinib or baricitinib, with or without additional stimulation (IL-1β or TGF-β). The levels of p-STAT1, p-STAT3, SOX9, and α-SMA were assessed by Western blot whereas *SOX9*, *COL2A1*, *ACAN*, *ACTA2*, *CTGF* and *COL3A1* gene expression was examined by RT-qPCR. Secreted IL-6, MMP-1, MMP-3, MMP-13 were measured in supernatants by ELISA.

Results: Tofacitinib or baricitinib increased the expression of anabolic factors *SOX9*, *COL2A1*, and *ACAN* while decreasing MMP-13 and MMP-3 levels in OA chondrocytes. Secreted levels of IL-6 and MMP-1 were significantly reduced in IL-1β-stimulated OA FLS and in OA synovial explants treated with tofacitinib or baricitinib. Finally, baricitinib decreased some fibrotic markers: α-SMA expression, *ACTA2* gene expression, and *CTGF* levels in TGF-β-stimulated OA FLS.

Conclusion: Tofacitinib and baricitinib modulate some features of OA pathophysiology by promoting anabolic processes in OA cartilage, reducing inflammation in OA synovium, and attenuating some fibrotic factors in OA FLS. These findings demonstrate the potential use of tofacitinib and baricitinib as therapeutic options for managing OA, and highlight pathogenic pathways to target for further research and development of new OA treatment strategies.

Graphical Abstract



RHEUMATOLOGY Paulissen et al. JAK inhibitors alleviate metabolic dysregulation, inflammation and fibrosis in osteoarthritis: insights from human joint cells and synovium. *Rheumatology.*

Key words:

tofacitinib, baricitinib, osteoarthritis, joint cells, synovium, fibrosis, inflammation, anabolism, cartilage

Key messages:

- Tofacitinib and baricitinib promote anabolic processes in OA chondrocytes
- Tofacitinib and baricitinib decrease inflammation in IL-1β-stimulated OA synoviocytes and *ex vivo* synovial explants
- Baricitinib reduces fibrotic factors in OA synoviocytes

Introduction

Osteoarthritis (OA) is a significant public health issue, affecting more than 200 million people worldwide; and its prevalence is increasing as a result of the aging population. OA manifests as a heterogeneous whole-joint disease characterized by cartilage degradation, osteophytosis, subchondral bone remodeling [1], synovial hypertrophy and/or joint effusion [2,3]. The condition arises from an imbalance between the catabolic and anabolic functions of chondrocytes in the articular cartilage, resulting in extracellular matrix (ECM) degradation [4,5]. While OA was previously viewed as a "wear and tear" disease affecting cartilage, it is now widely acknowledged that synovial inflammation is another key characteristic [6,7]. Immune cells infiltrate the OA synovial membrane inducing the secretion of pro-inflammatory cytokines such as IL-1β, IL-6 and matrix metalloproteinases (MMPs) like MMP-1, which further drive cartilage degradation and inflammation [8]. OA also exhibits profibrotic features, as evidenced by the presence of fibrotic markers such as α-smooth muscle actin (α-SMA) in the synovium and cartilage of OA patients [9,10]. These structural alterations collectively contribute to the pathophysiology of OA.

The main symptoms experienced by OA patients include joint pain, joint deformity, morning stiffness, swelling, reduced range of motion, and moderate to severe disability, all of which significantly diminish the quality of life. Unfortunately, to date, there are no curative treatments available for OA. Pharmacological treatments only provide pain relief. In more advanced cases, glucocorticoid injections or surgical procedures to implant hip or knee prostheses are necessary. Symptomatic slow-acting drug for OA (SYSADOA) like glucosamine sulphate or chondroitin sulphate lack evidence of a robust structural effect in human. Additionally, biological treatments such as anti-IL-1β or anti-TNF-α therapies have limited efficacy [11].

Currently, there is a new therapeutic class emerging in rheumatology, referred to as Janus kinase inhibitors (JAKi). These molecules are commonly used for the treatment of chronic inflammatory diseases such as atopic dermatitis, alopecia areata, Crohn's disease, spondyloarthritis and rheumatoid arthritis (RA). Tofacitinib and baricitinib are the first molecules of the JAKi family to have received approval from both the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for RA treatment. They function by blocking the activation of STATs (Signal Transducer and Activator of Transcription) through the inhibition of their phosphorylation. The JAK/STAT signaling pathway is involved in many processes including cell proliferation, inflammation, immune

regulation, differentiation, ECM degradation and apoptosis [12]. JAKi demonstrate a reduction in inflammatory responses [13], along with a protective effect on joint damage in RA [14]. Furthermore, they play a role in fibrosis process in scleroderma-associated lung disease [15]. In animal models of OA, several authors have described the effects of JAKi. Tofacitinib has been studied in a surgically induced rat model of OA [16,17], demonstrating reduced cartilage ECM degradation, increased chondrocyte autophagy, and inhibition of synovitis and osteoclast formation. Nevertheless, there is limited information available in the literature regarding the efficacy of JAKi for treating OA in humans. Hence, we hypothesize that tofacitinib and baricitinib could represent a novel therapeutic approach in OA owing to their pleiotropic effects. To address this issue, we studied the impact of tofacitinib and baricitinib on various pathophysiological parameters of OA using human OA cells and tissue, such as fibroblast-like synoviocytes (FLS), chondrocytes, and synovial explants *ex vivo,* aiming to demonstrate the potential benefit of these JAKi in OA.

Methods

Reagents

Tofacitinib (Tofa) (CP-690550) and baricitinib (Bari) (INCB028050) (Selleckchem, Houston, Texas, USA) [18] were used at 0.1, 1 or 10 μM. Control cells were treated with 0.1% DMSO. Recombinant human Il-1β (R&D systems, Minnesota, USA) and TGF-β protein (Thermo Fisher Scientific, Waltham, Massachusetts, USA) were used at 10 ng/ml. See Supplementary Data S1 for further information.

Primary cell culture

Synovial membranes and cartilage were obtained from OA patients undergoing total knee arthroplasty (TKA) or total hip replacement (THR) surgery in collaboration with the orthopedic surgery department of CHU of Liege. Patients were included only if the prosthetic indication was OA. Exclusion criteria were joint replacement for fracture or active inflammatory disease. This study was approved by the ethics committee of the CHU of Liege (2023/4) and the biobank research committee (BB190058). All experiments were performed in accordance with the relevant guidelines and regulations. Patients were recruited using an opt-out methodology. This study includes biological samples provided from 59 patients (36F/23M) with median age of 66 (47-80) years old and median BMI of 29.7 (21.4-44.9). Synovial membranes and cartilage were digested as previously described [19,20]. Further information can be found in Supplementary Data S2.

Ex vivo synovial explant culture

Upon receiving synovial tissue from an osteoarthritic knee in the operating room, the synovium was directly dissected into small, equally sized pieces. These fragments were then transferred into a 24-well plate containing cell medium, and were stimulated with DMSO 0.1% for the control condition or with tofacitinib or baricitinib, in a humidified incubator at 37°C with 5% CO_2. Seventy-two hours later, supernatants were harvested and stored at -20°C for ELISA. To ensure that tissue morphology remains intact at the end of the protocol, H&E coloration was performed (see Supplementary Figure S1).

Western blot analysis

Proteins were separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). Proteins were then blocked prior to

incubation with a specific primary antibody. Enhanced chemiluminescence detection reagent (ECL kit, Thermo Fisher Scientific, Waltham, Massachusetts, USA) was applied for bands detection. Densitometric analysis was performed using the Image Studio Lite Software version 5.2 (Li-Cor Biosciences, Linkolin, Nebraska, USA). Further description can be found in Supplementary Data S3.

ELISA

Supernatants were recovered and used to determine the concentration of secreted IL-6, MMP-1, MMP-3 and MMP-13 by commercially available Duoset kits (R&D systems, Minneapolis, MN, USA) according to manufacturer's instructions. Absorbance was read on a spectrophotometric ELISA plate reader SpectraMax 384Plus with SoftMax Pro Software version 7.0.2 (Molecular Devices, San Jose, USA).

RT-qPCR

Total RNAs were reverse-transcribed into cDNA using RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Scientific, Pittsburgh, Pennsylvania, USA). Thereafter, a real-time reverse transcription quantitative PCR (RT-qPCR) allowed to amplify the cDNA products using the KAPA SYBR FACT detection system (Sopachem, Nazareth, Belgium) and specific primers for gene of interest. Further information was described in Supplementary Data S4. Primers used were listed in Table 1.

Data analysis

All the results were reported as mean ± SEM and the graphs were carried out using Graphpad Prism software (version 9.3.1, La Jolla, CA, USA). The samples are paired, and the comparisons involve more than two groups. A normality test was conducted, followed by either a Friedman test with Dunn's *post-hoc* test or a one-way ANOVA with Sidak *post hoc* test based on the results of the normality test.

For all the tests, p-values < 0.05 were considered as significant: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$.

Results

Tofacitinib and baricitinib inhibit STAT1 and STAT3 phosphorylation

In order to ensure the efficacy of tofacitinib and baricitinib treatments, the expression of p-STAT1 and p-STAT3 was investigated in OA FLS and OA chondrocytes (Figure 1). Cells were stimulated with tofacitinib or baricitinib at concentrations of 0.1, 1 or 10 μM for 2 hours. The expression of p-STAT1 significantly decreased in OA FLS after stimulation with tofacitinib (1 μM, p=0.002 and 10 μM, p=0.0001) or baricitinib (1 μM, p=0.0013 and 10 μM, p<0.0001) compared to the control condition (stimulated with DMSO) (Figure 1A). Similarly, the expression of p-STAT3 was significantly reduced after stimulation with tofacitinib or baricitinib at 1 μM and 10 μM (p<0.0001) compared to the control condition (Figure 1B). The expression of p-STAT1 was also significantly diminished in OA chondrocytes after stimulation with tofacitinib or baricitinib 10 μM (p=0.0434 and p=0.0082, respectively) (Figure 1C); and the expression of p-STAT3 was significantly reduced with tofacitinib (1 μM, p=0.008 and 10 μM, p<0.0001) or baricitinib (1 μM and 10 μM, p<0.0001) compared to the control condition (Figure 1D).

Tofacitinib and baricitinib promote anabolic process in OA chondrocytes

To assess the treatment's effect on some anabolic and catabolic factors, OA chondrocytes were treated with either tofacitinib or baricitinib at concentrations of 1 and 10 μM for 24 hours. Regarding anabolic factors, levels of *SOX9* gene expression significantly increased after stimulation with tofacitinib (10 μM, p=0.0001) or baricitinib (10 μM, p<0.0001) compared to the control condition (DMSO) (Figure 2A). Levels of SOX9 protein expression were also significantly higher after stimulation with tofacitinib (10 μM, p=0.0139) or baricitinib (10 μM, p=0.0010) (Figure 2B). Additionally, levels of *COL2A1* were notably elevated after stimulation with tofacitinib (10 μM, p<0.0001) or baricitinib (1 μM, p=0.0115 and 10 μM p=0.0002) compared to the control condition (Figure 2C). Moreover, levels of *ACAN* gene expression significantly increased after stimulation with tofacitinib (10 μM, p=0.0063) or baricitinib (10 μM, p<0.0001) compared to the control condition (Figure 2D). Conversely, regarding catabolic factors, the expression level of secreted MMP-13 was significantly decreased after stimulation with tofacitinib (1 μM, p=0.0119 and 10 μM, p=0.0046) or baricitinib (1 μM, p=0.0119; 10 μM, p=0.0016) (Figure 2E). The expression level of secreted MMP-3 protein was significantly

decreased only at the highest dose of tofacitinib (10 μM, p=0.0016) compared to the control condition (Figure 2F). Together, these data demonstrate that tofacitinib and baricitinib tend to promote some anabolic processes over catabolic ones in OA chondrocytes.

Tofacitinib and baricitinib decrease some markers of inflammation in OA synovium

To find out whether tofacitinib and baricitinib exert an anti-inflammatory effect in OA joint cells, the secretion levels of IL-6, MMP-1, and MMP-3 were assessed in OA FLS supernatants by ELISA. FLS were treated with tofacitinib or baricitinib for 24 hours without additional stimulation. Under spontaneous conditions, a decreasing trend in IL-6 secretion was observed in a dose-dependent manner with tofacitinib (Figure 3A), along with lower MMP-1 secretion with tofacitinib (Figure 3B). No modulation of MMP-3 secretion was observed with either tofacitinib or baricitinib treatment (Figure 3C). By contrast, under pro-inflammatory condition (*i.e.* IL-1β stimulation), tofacitinib and baricitinib displayed significant anti-inflammatory effects. As expected, the expression level of secreted IL-6, MMP-1, and MMP-3 significantly increased after IL-1β stimulation compared to the control condition: p<0.0001 (IL-6), p=0.0469 (MMP-1), p=0.0029 (MMP-3) (Figure 3D-F). In IL-1β-stimulated cells, levels of IL-6 significantly decreased with tofacitinib (1 μM, p=0.0289 and 10 μM, p=0.0080) or with baricitinib (1 μM, p=0.0335 and 10 μM, p=0.0196) (Figure 3D). Levels of MMP-1 were significantly reduced with both drugs, tofacitinib (1 μM, p=0.0469 and 10 μM, p=0.0209) and baricitinib (1 μM, p=0.0136) (Figure 3E). Moreover, MMP-3 secretion was significantly decreased with tofacitinib (10 μM, p=0.0294) (Figure 3F) but not with baricitinib.

To emphasize these anti-inflammatory properties in OA synovium, *ex vivo* synovial explant cultures were also used. The explants were treated with JAKi at a concentration of 1 μM for 72 hours. Under spontaneous conditions, IL-6 secretion was significantly decreased with both drugs, tofacitinib (1 μM, p=0.0028) and baricitinib (1 μM, p=0.0089) compared to the control condition (Figure 4A). Similarly, MMP-1 secretion was also significantly reduced with tofacitinib (1 μM, p=0.0075) and baricitinib (1 μM, p=0.0051) (Figure 4B). A non-significant decrease of MMP-3 secretion was observed with tofacitinib or baricitinib (Figure 4C). Taken together, these data suggest that tofacitinib and baricitinib exert an anti-inflammatory effect in OA synovium.

Baricitinib decreases some fibrotic markers in OA FLS

Fibrosis markers such as α-SMA, CTGF and COL3 expressions have been reported in OA tissues and cells [10,21]. We assessed their spontaneous and TGF-β-induced expression in OA FLS. Next, we challenged the potential anti-fibrotic effect of tofacitinib and baricitinib. As expected, under TGF-β stimulation, a significant increase of α-SMA protein (Figure 5A, p=0.0004) and gene (*ACTA2*, Figure 5B, p=0.008) expression was observed compared to the control OA FLS. Similarly, *CTGF* (p=0.0004) and *COL3A1* (p<0.0001) gene expression were also increased upon TGF-β stimulated OA FLS when compared to the control cells (Figure 5C-D).

After baricitinib stimulation, levels of α-SMA were significantly decreased, both at protein (Figure 5A, p=0.0129) and mRNA (*ACTA2*, Figure 5B, p=0.0216) level. This was not the case after tofacitinib stimulation (Figure 5A-B). *CTGF* gene expression was also significantly reduced (p=0.0452) after baricitinib stimulation but not with tofacitinib (Figure 5C). However, regarding *COL3A1* gene, baricitinib could not significantly decrease its expression level (Figure 5D). Moreover, there was a significant (p=0.0025) increase of *COL3A1* gene in TGF-β-stimulated cells under tofacitinib treatment (Figure 5D). Together, these findings suggest that baricitinib exhibits a partial anti-fibrotic effect in OA FLS.

Discussion

Our study demonstrates interesting effects of tofacitinib and baricitinib on primary OA cells (FLS and chondrocytes) as well as on *ex vivo* synovial explants isolated from human OA joints. In our study, tofacitinib and baricitinib promote anabolic molecules in OA chondrocytes, but also reduce the levels of pro-inflammatory mediators, and modulate some fibrotic factors in FLS, suggesting that they may play a promising role in the treatment of OA.

Our results confirm that tofacitinib and baricitinib significantly decrease STAT1 and STAT3 phosphorylation in OA FLS and OA chondrocytes. These results are consistent with other studies using inflammatory joints provided from inflamed synovial tissue from patients with RA or psoriatic arthritis [22,23]. In the study by Gao *et al*, tofacitinib at 1 μM reduced p-STAT1 and p-STAT3 in psoriatic arthritis synovial fibroblasts [22]. In our study, both drugs at 1 μM and 10 μM significantly decreased p-STAT1 and p-STAT3 expression, confirming their efficacy in inhibiting the JAK/STAT pathway.

Tofacitinib and baricitinib promoted the expression of anabolic molecules in OA chondrocytes. Type II collagen and aggrecan, encoded by the *COL2A1* and *ACAN* genes respectively, are the major components of the extracellular matrix of cartilage. SOX9, a transcription factor associated with cartilage formation, is involved in embryonic chondrogenesis [24,25] and in the regulation of the *COL2A1* gene [26]. During OA, chondrocytes lose their ability to produce these molecules, both *in situ* in cartilage and also when cultured *in vitro* [27]. In this study, we show that SOX9*, COL2A1* and *ACAN* expressions were increased in OA chondrocytes after stimulation with tofacitinib or baricitinib at 10 μM, which is in line with two other studies suggesting a potential chondroprotective effect of tofacitinib [28,29]. Furthermore, tofacitinib and baricitinib attenuated the secretion of some catabolic factors. Catabolic factors are numerous and include MMPs, ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs), and cytokines. MMPs degrade the extracellular matrix of cartilage by cleaving structural proteins such as collagen and aggrecan [30]. Cytokines such as IL-1β and TNF-α are inflammatory mediators that can induce the production of MMPs and ADAMTS, thereby promoting cartilage degradation. In this study, we focused on the secretion of MMP-13 and MMP-3, both of which were decreased after stimulation of OA chondrocytes with tofacitinib or baricitinib. These findings corroborate the results by Machida *et al* [28] who observed a decrease in the expression of MMP-13 induced by mechanical stress in chondrocytes after treatment with tofacitinib. It is also in line with

Thorpe *et al*., who demonstrated that tofacitinib decreased MMP-3 and MMP-13 expression in the human chondrocyte C28/I2 cell line stimulated with rhIL-6 [31].

The protective role of tofacitinib and baricitinib on inflammation is well described in other rheumatic diseases, but remains understudied in OA, particularly in synovial fibroblasts. In this study, we highlight their anti-inflammatory properties in the synovium (FLS and *ex vivo* synovial explants) of OA patients. We showed that tofacitinib and baricitinib decreased the secretion of the pro-inflammatory mediators IL-6 and MMP-1 in synovial explant cultures. *Ex vivo* synovial explants add supplementary value by mimicking the synovial cell network in OA patients. McGarry *et al* observed a decrease in IL-6 secretion in ex vivo synovial explants from RA patients after stimulation with tofacitinib [32]. We also showed that tofacitinib and baricitinib decreased the secretion of IL-6 and MMP-1 in supernatants of IL-1β stimulated OA FLS while a decreasing trend could be observed under spontaneous inflammation in OA FLS. Similarly, in RA synovial fibroblasts, McGarry *et al* did not observe any IL-6 secretion with tofacitinib 1 μM under spontaneous conditions [32]. Of particular interest, a prospective clinical study has already showed that baricitinib may be a promising treatment for inflammatory knee OA patients because some inflammatory markers (such as ESR or hsCRP) and the WOMAC score of OA severity were decreased [33]. In this study, OA patients were enrolled only if they had clinical signs of inflammation and elevated sedimentation rate or C-reactive protein levels.

Interestingly, in RA synovial fibroblasts, tofacitinib and baricitinib, at concentrations of 1-5μM, were also able to decrease IL-6 in response to other pro-inflammatory stimulators like oncostatin M (OSM) [34]. By contrast, no IL-6 decrease was observed either with tofacitinib or baricitinib in RA synovial fibroblasts stimulated with IL-1β [34,35]. This difference compared to our data might be explained by the different pathophysiology of RA *vs* OA. Indeed, in RA, oncostatin plays a predominant role, whereas in OA, it is rather IL-1β that is the key player in IL-6 generation.

Finally, OA is associated with fibrosis, characterized by the excessive deposition of connective tissue due to dysregulated fibroblast activation [36,37]. The tissues affected are cartilage and synovium, the latter being affected at the onset and during the progression of OA, contributing to the joint pain and stiffness. Here, we show that baricitinib decreases some fibrotic markers in OA FLS. A decrease of α-SMA and its associated gene *ACTA2*, as well as a decrease in *CTGF* expression, was observed in OA FLS treated with baricitinib at 10 μM.

Limited data are available on the potential effect of baricitinib on fibrosis. Liu *et al* demonstrated that baricitinib reduced fibrosis in lung tissue, especially by reducing p-STAT3 expression in a mouse model of RA-associated interstitial lung disease [38]. In another *in vivo* study, baricitinib decreased α-SMA, type I collagen and fibronectin levels in lung tissue from fibrotic mice [39]. Several studies have reported that JAK 1/2 inhibition can help alleviate fibrosis. For example, the combination of baricitinib and nintedanib has shown a synergistic effect in the treatment of idiopathic pulmonary fibrosis [40], and baricitinib reduces myocardial fibrosis [41]. In our study, tofacitinib did not reduce fibrotic markers, possibly because it targets JAK1 and JAK3, whereas baricitinib inhibits JAK1 and JAK2[42]. The JAK2 pathway is more prominent in renal fibrosis and myelofibrosis, where JAK2 inhibitors are used in myeloproliferative diseases [43]. Although tofacitinib did not reduce fibrotic markers here, it has shown efficacy in scleroderma mouse models [44,45]. Further studies are needed to investigate JAK signaling in fibrosis.

Our study has several limitations. It addresses OA features at an advanced stage of the disease, since tissues were obtained from patients undergoing surgery, and results cannot be extrapolated to early OA. It constitutes an initial investigation of the effect of tofacitinib and baricitinib on certain human OA parameters, before delving deeper into the effects of the two molecules. The use of an *in vivo* model, such as the CIOA or DMM mouse models, could provide valuable insights into the impact of JAKi on OA progression. Two studies using *in vivo* OA models found that intra-articular tofacitinib reduced arthritis scores, bone degradation [16], and cartilage degeneration by preserving ECM and enhancing chondrocyte autophagy [17]. In our study, we used tofacitinib and baricitinib, that inhibit multiple isoforms of JAKs, but there are other JAKi selectively inhibiting only one isoform. Therefore, a comparative analysis between a selective JAK inhibitor and a non-selective one could be performed in future research studies.

OA is a complex disease, involving numerous pathophysiological pathways. Current treatments targeting inflammation remain controversial and SYSADOA agents show limited effects in humans. Therefore, tofacitinib and baricitinib, given their ability to affect multiple aspects of OA, offer promising therapeutic approaches. Specifically, tofacitinib and baricitinib promote anabolic processes in osteoarthritic cartilage, decrease the levels of pro-inflammatory mediators in OA FLS and synovial explants, and attenuate some fibrotic factors in OA FLS. Furthermore, the short half-life and easy production of JAKi as synthetic molecules provide additional advantages. However, considering that these small molecules have only emerged in

the last decade, it is worth bearing in mind that there is limited data on their long-term side effects. Tofacitinib and baricitinib have recently received FDA and EMA warnings about cardiovascular events, blood clots, cancer, and serious infections, mainly based on the ORAL Surveillance study [46]. However, recent data [47–49] suggest these risks are mostly seen in high-risk populations. Despite this, JAK inhibitors are used in conditions with high pro-thrombotic risk, such as thrombocythemia vera, systemic lupus erythematosus, and giant cell arteritis. Moreover, OA patients frequently use NSAIDs [50] and have limited mobility—both strongly associated with cardiovascular disease. An effective OA treatment, as suggested by our *in vitro* data, could help mitigate these risks. Nevertheless, tofacitinib and baricitinib offer promising therapeutic approaches in the treatment of OA, particularly given their ability to affect multiple aspects of the disease. In addition, in our study, baricitinib appears to be more promising than tofacitinib because it has an effect on some fibrotic factors such as α-SMA and *CTGF*.

In conclusion, although further studies are needed to confirm and extend these observations, our findings identify multiple beneficial effects of a single therapeutic drug on different pathophysiological pathways involved in OA and raise hope on new therapeutic modalities in this widespread disease.

Acknowledgements:

We thank the GIGA-Immunohistology platform for their help.

Group authorship list:

Paulissen G: Conceptualization, Formal analysis, Methodology, Validation, Writing - Original Draft. Malaise O: Conceptualization, Funding acquisition, Investigation. Deroyer C & Charlier E: Conceptualization. Neuville S & Plener Z: Data Curation. Thirion T: Resources, Daniel C: Resources. Ribbens C: Conceptualization, Supervision. de Seny D: Conceptualization, Project administration, Supervision, Validation.

All the authors reviewed critically and approved the final version to be published.

Funding:

This work was supported by “Fonds d’Investissement pour la Recherche Scientifique” [FIRS, N° 981487200], CHU Liège, Belgium.

Conflict of interest statement:

The authors declare no conflict of interest related to this study, which was financed only by an academic grant (FIRS) from University Hospital of Liège. Malaise O. et Ribbens C. received, for their clinical activities, educational grants, payments for lectures and support for attending meetings from all compagnies commercializing JAK inhibitors in rheumatic diseases.

Data availability statement:

The data underlying this article will be shared on reasonable request to the corresponding author.

References

1. Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. *Lancet* 2019;393:1745–59.

2. D'Agostino MA. EULAR report on the use of ultrasonography in painful knee osteoarthritis. Part 1: Prevalence of inflammation in osteoarthritis. *Ann Rheum Dis* 2005;64:1703–9.

3. Atukorala I, Kwoh CK, Guermazi A, Roemer FW, Boudreau RM, Hannon MJ, et al. Synovitis in knee osteoarthritis: a precursor of disease? *Ann Rheum Dis* 2016;75:390–5.

4. Zheng L, Zhang Z, Sheng P, Mobasheri A. The role of metabolism in chondrocyte dysfunction and the progression of osteoarthritis. *Ageing Res Rev* 2021;66:101249.

5. Mueller MB, Tuan RS. Anabolic/Catabolic balance in pathogenesis of osteoarthritis: identifying molecular targets. *PM & R* 2011;3:S3-11.

6. Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). *Osteoarthritis Cartilage* 2013;21:16–21.

7. Goldring MB, Otero M. Inflammation in osteoarthritis. *Curr Opin Rheumatol* 2011;23:471–8.

8. Chou C-H, Jain V, Gibson J, Attarian DE, Haraden CA, Yohn CB, et al. Synovial cell cross-talk with cartilage plays a major role in the pathogenesis of osteoarthritis. *Sci Rep.* 2020;10:10868.

9. Deroyer C, Charlier E, Neuville S, Malaise O, Gillet P, Kurth W, et al. CEMIP (KIAA1199) induces a fibrosis-like process in osteoarthritic chondrocytes. *Cell Death Dis*. 2019;10.

10. Deroyer C, Poulet C, Paulissen G, Ciregia F, Malaise O, Plener Z, et al. CEMIP (KIAA1199) regulates inflammation, hyperplasia and fibrosis in osteoarthritis synovial membrane. *Cellular and Molecular Life Sciences*. 2022;79.

11. Meng F, Li H, Feng H, Long H, Yang Z, Li J, et al. Efficacy and safety of biologic agents for the treatment of osteoarthritis: a meta-analysis of randomized placebo-controlled trials. *Ther Adv Musculoskelet Dis*. 2022;14:1759720X2210803.

12. Xin P, Xu X, Deng C, Liu S, Wang Y, Zhou X, et al. The role of JAK/STAT signaling pathway and its inhibitors in diseases. *Int Immunopharmacol* 2020;80:106210.

13. Dowty ME, Lin TH, Jesson MI, Hegen M, Martin DA, Katkade V, et al. Janus kinase inhibitors for the treatment of rheumatoid arthritis demonstrate similar profiles of in vitro cytokine receptor inhibition. *Pharmacol Res Perspect* 2019;7.

14. Emery P, Durez P, Hueber AJ, de la Torre I, Larsson E, Holzkämper T, et al. Baricitinib inhibits structural joint damage progression in patients with rheumatoid arthritis—a comprehensive review. *Arthritis Res Ther* 2021;23:3.

15. Lescoat A, Lelong M, Jeljeli M, Piquet-Pellorce C, Morzadec C, Ballerie A, et al. Combined anti-fibrotic and anti-inflammatory properties of JAK-inhibitors on macrophages in vitro and in vivo: Perspectives for scleroderma-associated interstitial lung disease. *Biochem Pharmacol* 2020;178:114103.

16. Chiu YS, Bamodu OA, Fong IH, Lee WH, Lin CC, Lu CH, et al. The JAK inhibitor Tofacitinib inhibits structural damage in osteoarthritis by modulating JAK1/TNF-alpha/IL-6 signaling through Mir-149-5p. *Bone* 2021;151.

17. Zhang P, Xiao J, Luo C, Liu X, Li C, Zhao J, et al. The Effect of JAK Inhibitor Tofacitinib on Chondrocyte Autophagy. *Inflammation* 2023;46:1764–76.

18. Migita K, Izumi Y, Torigoshi T, Satomura K, Izumi M, Nishino Y, et al. Inhibition of Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway in rheumatoid synovial fibroblasts using small molecule compounds. *Clin Exp Immunol* 2013;174:356–63.

19. Relic B, Zeddou M, Desoroux A, Beguin Y, de Seny D, Malaise MG. Genistein induces adipogenesis but inhibits leptin induction in human synovial fibroblasts. *Laboratory Investigation* 2009;89:811–22.

20. Relić B, Bentires-Alj M, Ribbens C, Franchimont N, Guerne PA, Benoît V, et al. TNF-α protects human primary articular chondrocytes from nitric oxide-induced apoptosis via nuclear factor-κB. *Laboratory Investigation* 2002;82:1661–72.

21. Vaamonde-Garcia C, Malaise O, Charlier E, Deroyer C, Neuville S, Gillet P, et al. 15-Deoxy-Δ-12, 14-prostaglandin J2 acts cooperatively with prednisolone to reduce TGF-β-induced pro-fibrotic pathways in human osteoarthritis fibroblasts. *Biochem Pharmacol* 2019;165:66–78.

22. Gao W, McGarry T, Orr C, McCormick J, Veale DJ, Fearon U. Tofacitinib regulates synovial inflammation in psoriatic arthritis, inhibiting STAT activation and induction of negative feedback inhibitors. *Ann Rheum Dis* 2016;75:311–5.

23. Amrhein J, Drynda S, Schlatt L, Karst U, Lohmann CH, Ciarimboli G, et al. Tofacitinib and baricitinib are taken up by different uptake mechanisms determining the efficacy of both drugs in RA. *Int J Mol Sci.* 2020;21:1–13.

24. Dy P, Wang W, Bhattaram P, Wang Q, Wang L, Ballock RT, et al. Sox9 Directs Hypertrophic Maturation and Blocks Osteoblast Differentiation of Growth Plate Chondrocytes. *Dev Cell* 2012;22:597–609.

25. Haseeb A, Kc R, Angelozzi M, de Charleroy C, Rux D, Tower RJ, et al. SOX9 keeps growth plates and articular cartilage healthy by inhibiting chondrocyte dedifferentiation/osteoblastic redifferentiation. Proceedings of the National Academy of Sciences 2021;118.

26. Bell DM, Leung KKH, Wheatley SC, Ng LJ, Zhou S, Wing Ling K, et al. SOX9 directly regulates the type-ll collagen gene. *Nat Genet* 1997;16:174–8.

27. Anderson HC, Chacko S, Abbott J, Holtzer H. The loss of phenotypic traits by differentiated cells in vitro. VII. Effects of 5-bromodeoxyuridine and prolonged culturing on fine structure of chondrocytes. *Am J Pathol* 1970;60:289–312.

28. Machida T, Nishida K, Nasu Y, Nakahara R, Ozawa M, Harada R, et al. Inhibitory effect of JAK inhibitor on mechanical stress-induced protease expression by human articular chondrocytes. *Inflammation Research* 2017;66:999–1009.

29. Kjelgaard-Petersen CF, Sharma N, Kayed A, Karsdal MA, Mobasheri A, Hägglund P, et al. Tofacitinib and TPCA-1 exert chondroprotective effects on extracellular matrix turnover in bovine articular cartilage ex vivo. *Biochem Pharmacol* 2019;165:91–8.

30. Wang M, Sampson ER, Jin H, Li J, Ke QH, Im H-J, et al. MMP13 is a critical target gene during the progression of osteoarthritis. *Arthritis Res Ther* 2013;15:R5.

31. Thorpe JR, Wilson RA, Mesiano S, Malemud CJ. Tofacitinib Inhibits STAT Phosphorylation and Matrix Metalloproteinase-3, -9 and -13 Production by C28/I2 Human Juvenile Chondrocytes. *Open Access Rheumatol*. 2022;14:195–209.

32. McGarry T, Orr C, Wade S, Biniecka M, Wade S, Gallagher L, et al. JAK/STAT Blockade Alters Synovial Bioenergetics, Mitochondrial Function, and Proinflammatory Mediators in Rheumatoid Arthritis. *Arthritis & Rheumatology* 2018;70:1959–70.

33. Wang Q, Chen Z, Dai S-M. Baricitinib is a potential treatment in inflammatory osteoarthritis: a proof of concept study. *Rheumatology* 2022;61:e213–5.

34. Diller M, Hasseli R, Hülser ML, Aykara I, Frommer K, Rehart S, et al. Targeting activated synovial fibroblasts in rheumatoid arthritis by peficitinib. *Front Immunol* 2019;10.

35. Weston S, Macdonald JL, Williams LM, Roussou E, Kang N V, Kiriakidis S, et al. The JAK inhibitor baricitinib inhibits oncostatin M induction of proinflammatory mediators in ex-vivo synovial derived cells *Clin Exp Rheumatol* 2022;40:1620-1628.

36. Bolia IK, Mertz K, Faye E, Sheppard J, Telang S, Bogdanov J, et al. Cross-Communication Between Knee Osteoarthritis and Fibrosis: Molecular Pathways and Key Molecules. *Open Access J Sports Med* 2022;13:1–15.

37. Rim YA, Ju JH. The Role of Fibrosis in Osteoarthritis Progression. *Life* 2020;11:3.

38. Liu H, Yang Y, Zhang J, Li X. Baricitinib improves pulmonary fibrosis in mice with rheumatoid arthritis-associated interstitial lung disease by inhibiting the Jak2/Stat3 signaling pathway. Advances in Rheumatology 2023;63:45.

39. Gu S, Liang J, Zhang J, Liu Z, Miao Y, Wei Y, et al. Baricitinib Attenuates Bleomycin-Induced Pulmonary Fibrosis in Mice by Inhibiting TGF-β1 Signaling Pathway. *Molecules* 2023;28:2195.

40. Wan Q, Li D, Shang S, Wu H, Chen F, Li Q. Novel synergistic therapeutic approach in idiopathic pulmonary fibrosis: Combining the antifibrotic nintedanib with the anti-inflammatory baricitinib. *Pulm Pharmacol Ther* 2025;89.

41. Feng R, Liu H, Chen Y. Baricitinib represses the myocardial fibrosis via blocking JAK/STAT and TGF-β1 pathways in vivo and in vitro. *BMC Cardiovasc Disord* 2025;25.

42. Traves PG, Murray B, Campigotto F, Galien R, Meng A, Di Paolo JA. JAK selectivity and the implications for clinical inhibition of pharmacodynamic cytokine signalling by filgotinib, upadacitinib, tofacitinib and baricitinib. *Ann Rheum Dis* 2021;80:865–75.

43. Liu J, Wang F, Luo F. The Role of JAK/STAT Pathway in Fibrotic Diseases: Molecular and Cellular Mechanisms. *Biomolecules* MDPI; 2023.

44. Aung WW, Wang C, Xibei J, Horii M, Mizumaki K, Kano M, et al. Immunomodulating role of the JAKs inhibitor tofacitinib in a mouse model of bleomycin-induced scleroderma. *J Dermatol Sci* 2021;101:174–84.

45. Karatas A, Oz B, Celik C, Akar ZA, Akkoc RF, Etem EO, et al. Tofacitinib and metformin reduce the dermal thickness and fibrosis in mouse model of systemic sclerosis. *Sci Rep* 2022;12.

46. Ytterberg SR, Bhatt DL, Mikuls TR, Koch GG, Fleischmann R, Rivas JL, et al. Cardiovascular and Cancer Risk with Tofacitinib in Rheumatoid Arthritis. *New England Journal of Medicine* 2022;386:316–26.

47. Kristensen LE, Danese S, Yndestad A, Wang C, Nagy E, Modesto I, et al. Identification of two tofacitinib subpopulations with different relative risk versus TNF inhibitors: An analysis of the open label, randomised controlled study ORAL Surveillance. *Ann Rheum Dis* 2023;82:901–10.

48. Charles-Schoeman C, Buch MH, Dougados M, Bhatt DL, Giles JT, Ytterberg SR, et al. Risk of major adverse cardiovascular events with tofacitinib versus tumour necrosis factor inhibitors in patients with rheumatoid arthritis with or without a history of atherosclerotic cardiovascular disease: a post hoc analysis from ORAL Surveillance. *Ann Rheum Dis* 2023;82:119–29.

49. Curtis JR, Yamaoka K, Chen Y-H, Bhatt DL, Gunay LM, Sugiyama N, et al. Malignancy risk with tofacitinib versus TNF inhibitors in rheumatoid arthritis: results from the open-label, randomised controlled ORAL Surveillance trial. *Ann Rheum Dis* 2023;82:331–43.

50. Neogi T, Dell'Isola A, Englund M, Turkiewicz A. Frequent use of prescription NSAIDs among people with knee or hip osteoarthritis despite contraindications to or precautions with NSAIDs. *Osteoarthritis Cartilage* 2024;32:1628–35.

Figures

Figure 1 Inhibition of STAT1 and STAT3 phosphorylation by tofacitinib and baricitinib in OA FLS and chondrocytes

OA FLS (A-B) and OA chondrocytes (C-D) were incubated with tofacitinib or baricitinib at 0.1 µM, 1 µM or 10 µM for 2 hours. (A-C) p-STAT1, STAT1 and GAPDH protein expression level. (B-D) p-STAT3, STAT3 and GAPDH protein expression level. For each panel, the upper picture shows a representative Western blot for one patient and the lower graph is the protein quantification for all patients reported on GAPDH expression level (loading control). Numbers of patients included: n=8 (A); n=10 (B); n=6 (C); n=11 (D). Values are expressed as mean ± SEM; * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. %

Alt text: Western blots showing STAT 1 and 3 phosphorylation in osteoarthritic fibroblast-like-synoviocytes and chondrocytes after 2 hours treatment with tofacitinib or baricitinib at increasing concentrations with subfigures labelled from A to D, illustrating representative picture and statistical analyses.

Figure 2 Impact of tofacitinib and baricitinib on metabolism balance in OA chondrocytes

OA chondrocytes were treated with tofacitinib or baricitinib at 1 and 10 µM for 24 hours. (A,C,D) SOX9, COL2A1, ACAN relative gene expression was assessed by RT-qPCR.(B) SOX9 and HSP90 protein expression level. The upper picture shows a representative Western blot for one patient and the lower graph is the protein quantification for all patients reported on GAPDH expression level. (E-F) Secreted MMP-13 and MMP-3 levels were measured by ELISA. Numbers of patients: n=9 (A; C); n=6 (B); n=8 (D); n=10 (E; F). Data are shown as mean ± SEM; * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Alt text: Bar graphs illustrate how tofacitinib and baricitinib modulate anabolic gene expression and the secretion of catabolic factors in osteoarthritic chondrocytes after 24 hours of treatment, with subfigures A to F displaying the statistical analysis. Panel B also includes a representative western blot for one anabolic marker.

Figure 3 Effect of tofacitinib and baricitinib on inflammatory mediators in spontaneous or IL-1β-stimulated OA FLS

In the spontaneous model (A,B,C), OA FLS were treated with tofacitinib or baricitinib at 0.1 µM, 1 µM or 10 µM for 24 hours. In the IL-1β-stimulated model (D,E,F), OA FLS were pre-incubated for 2 hours with tofacitinib or baricitinib at 0.1 µM, 1 µM or 10 µM, then stimulated with IL-1β for 24 hours (10 ng/ml). Secreted IL-6 (A-D), MMP-1 (B-E) or MMP-3 (C-F) levels were measured in supernatants in OA FLS by ELISA. Numbers of patients included: n=6 (A; C; D); n=5 (B; E; F). Data are represented as mean ± SEM; * $p<0.05$, ** $p<0.01$, **** $p<0.0001$.

Alt text: Bar graphs illustrate a reduction in the secretion of inflammatory markers by tofacitinib and baricitinib in osteoarthritic fibroblast-like synoviocytes, under both spontaneous and interleukin-1 beta-stimulated conditions, with subfigures A to F displaying the statistical analyses.

Figure 4 Effect of tofacitinib and baricitinib on inflammatory mediators in OA synovial explants

OA synovium explants were incubated with tofacitinib or baricitinib at 1 µM for 72 hours. Secreted IL-6 (A), MMP-1 (B) and MMP-3 (C) levels were measured in supernatants in OA synovial explants by ELISA. Numbers of patients: n=6 (A); n=4 (B); n=5 (C). Data are expressed as mean ± SEM; ** $p<0.01$.

Alt text: Bar graphs on secretion of inflammatory markers in osteoarthritic synovial explants after 72 hours with tofacitinib or baricitinib, with subfigures labelled from A to C illustrating statistical analyses.

Figure 5 Impact of tofacitinib and baricitinib on fibrotic markers in OA FLS

OA FLS were stimulated with TGF-β (10 ng/ml) for 72 hours. At 48 hours, tofacitinib or baricitinib (10 µM) was added for 24 hours. (A) α-SMA and HSP90 protein expression level. The upper picture shows a representative Western blot for one patient and the lower graph is the protein quantification for all patients reported on GAPDH expression level (loading control). (B-D) ACTA2, CTGF and COL3A1 relative gene expression in OA FLS by RT-qPCR. Numbers of patients: n=10 (A); n=12 (B); n=13 (C; D). Data are shown as mean ± SEM; * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Alt text: Graphs illustrate the expression of transforming growth factor-beta-induced fibrotic markers in osteoarthritic fibroblast-like synoviocytes after treatment with tofacitinib or baricitinib. Subfigures A to D present the statistical analyses, with panel A also showing a representative western blot for one fibrotic marker.

Tables/Figures

Table

Table 1

Primer sequences used for RT-qPCR

Gene	Forward sequence	Reverse Sequence
human *SOX9*	5'-ACAACCCGTCTACACACAGC-3'	5'-ACGATTCTCCATCATCCTCC-3'
human *COL2A1*	5'-TTTTCCAGCTTCACCATCATC-3'	5'-CCTCAAGGATTTCAAGGCAAT-3'
human *ACAN*	5'-AGATTCACAGAACTCCAGTGC-3'	5'-ACCTACGATGTCTACTGCTTTG-3'
human *ACTA2*	5'-CGTGTTGCCCCTGAAGAGCAT-3'	5'-ACCGCCTGGATAGCCACATACA-3'
human *CTGF*	5'-TTGGCAGGCTGATTTCTAGG-3'	5'-GGTGCAAACATGTAACTTTTGG-3'
human *COL3A1*	5'-GCGGTTTTGCCCCGTATTAT-3'	5'-TGCAGTTTCTAGCGGGGTTT-3'
human *GAPDH*	5'-TGTAGTTGAGGTCAATGAAGGG-3'	5'-ACATCGCTCAGACACCATG-3'

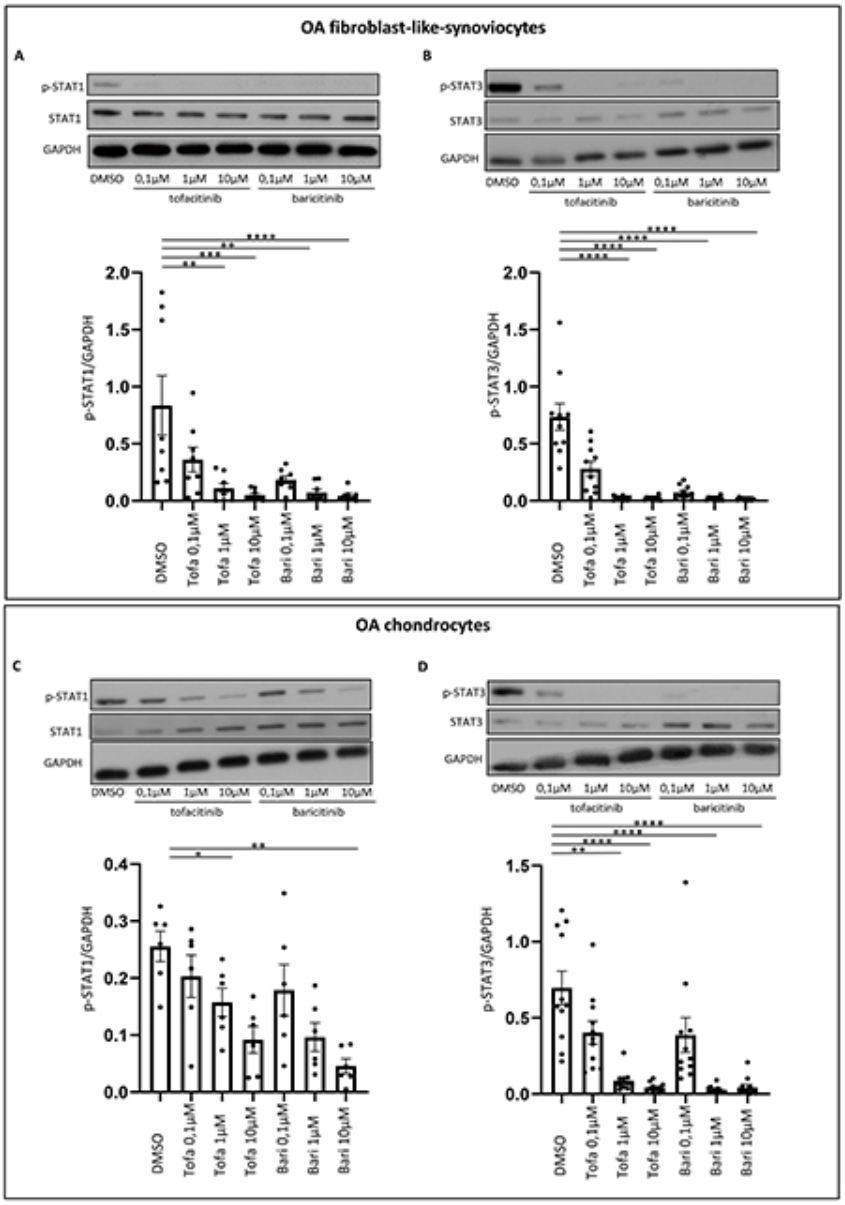


Figure 1 **Inhibition of STAT1 and STAT3 phosphorylation by tofacitinib and baricitinib in OA FLS and chondrocytes** OA FLS (A-B) and OA chondrocytes (C-D) were incubated with tofacitinib or baricitinib at 0.1 µM, 1 µM or 10 µM for 2 hours. (A-C) p-STAT1, STAT1 and GAPDH protein expression level. (B-D) p-STAT3, STAT3 and GAPDH protein expression level. For each panel, the upper picture shows a representative Western blot for one patient and the lower graph is the protein quantification for all patients reported on GAPDH expression level (loading control). Numbers of patients included: n=8 (A); n=10 (B); n=6 (C); n=11 (D). Values are expressed as mean ± SEM; * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. %"

158x225mm (72 x 72 DPI)

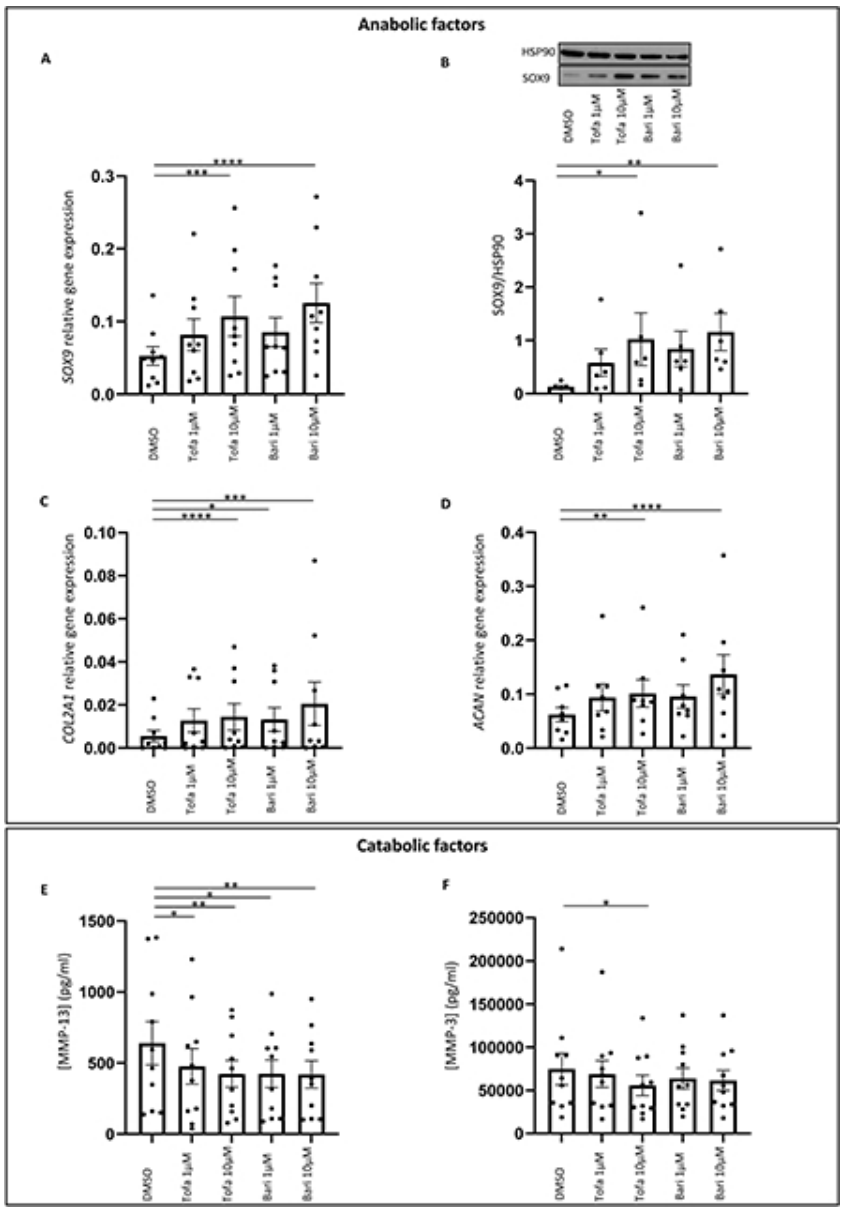


Figure 2 **Impact of tofacitinib and baricitinib on metabolism balance in OA chondrocytes** OA chondrocytes were treated with tofacitinib or baricitinib at 1 and 10 µM for 24 hours. (A,C,D) SOX9, COL2A1, ACAN relative gene expression was assessed by RT-qPCR.(B) SOX9 and HSP90 protein expression level. The upper picture shows a representative Western blot for one patient and the lower graph is the protein quantification for all patients reported on GAPDH expression level. (E-F) Secreted MMP-13 and MMP-3 levels were measured by ELISA. Numbers of patients: n=9 (A; C); n=6 (B); n=8 (D); n=10 (E; F). Data are shown as mean ± SEM; * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001."

157x225mm (72 x 72 DPI)

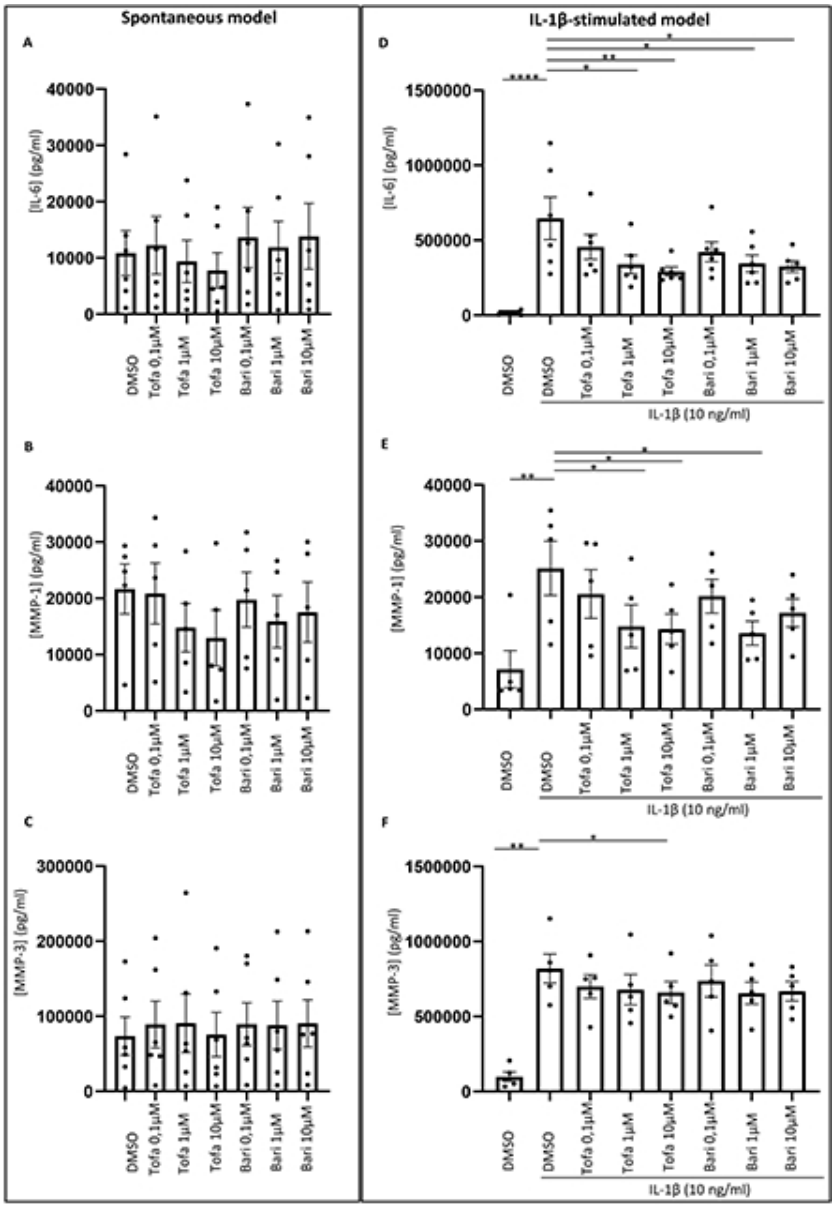


Figure 3**Effect of tofacitinib and baricitinib on inflammatory mediators in spontaneous or IL-1β-stimulated OA FLS** In the spontaneous model (A,B,C), OA FLS were treated with tofacitinib or baricitinib at 0.1 µM, 1 µM or 10 µM for 24 hours. In the IL-1β-stimulated model (D,E,F), OA FLS were pre-incubated for 2 hours with tofacitinib or baricitinib at 0.1 µM, 1 µM or 10 µM, then stimulated with IL-1β for 24 hours (10 ng/ml). Secreted IL-6 (A-D), MMP-1 (B-E) or MMP-3 (C-F) levels were measured in supernatants in OA FLS by ELISA. Numbers of patients included: n=6 (A; C; D); n=5 (B; E; F). Data are represented as mean ± SEM; * p<0.05, ** p<0.01, **** p<0.0001.

156x225mm (72 x 72 DPI)

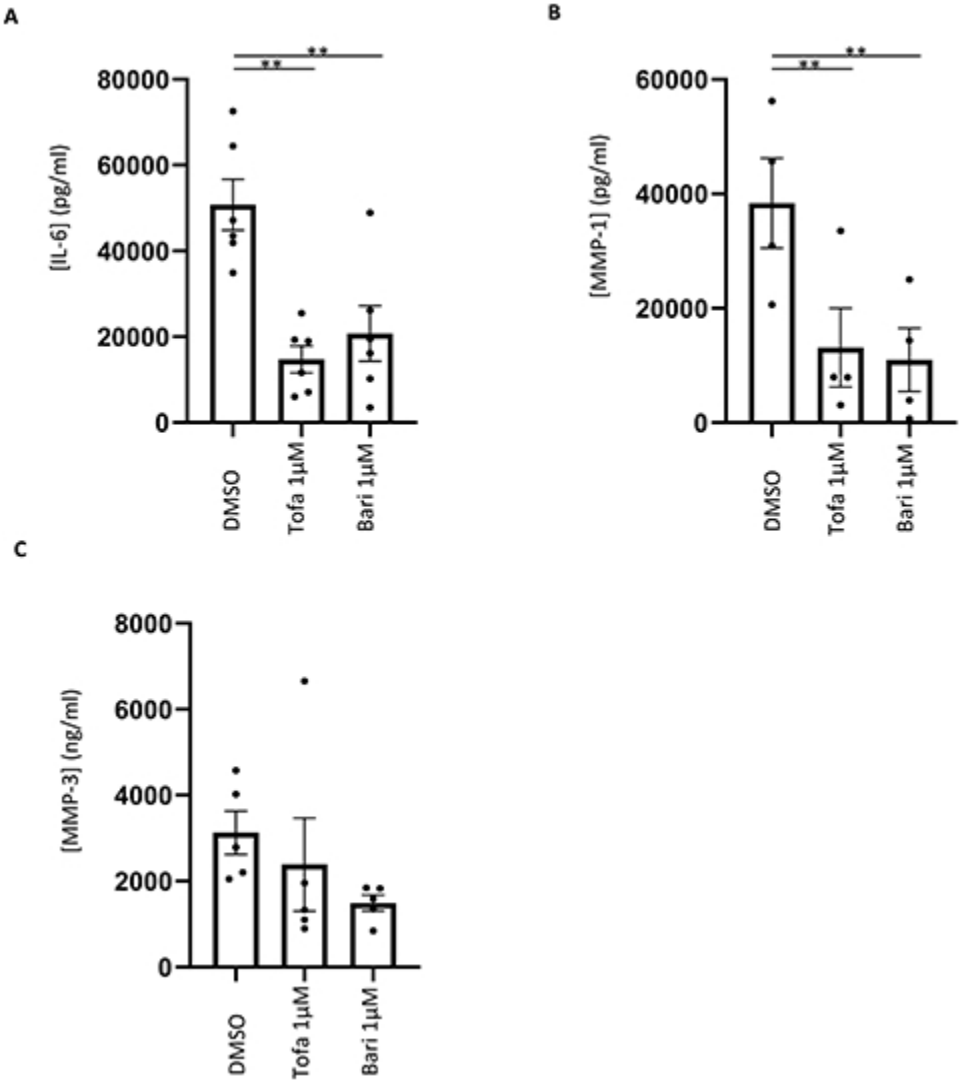


Figure 4**Effect of tofacitinib and baricitinib on inflammatory mediators in OA synovial explants** OA synovium explants were incubated with tofacitinib or baricitinib at 1 µM for 72 hours. Secreted IL-6 (A), MMP-1 (B) and MMP-3 (C) levels were measured in supernatants in OA synovial explants by ELISA. Numbers of patients: n=6 (A); n=4 (B); n=5 (C). Data are expressed as mean ± SEM; ** p<0.01.

169x190mm (72 x 72 DPI)

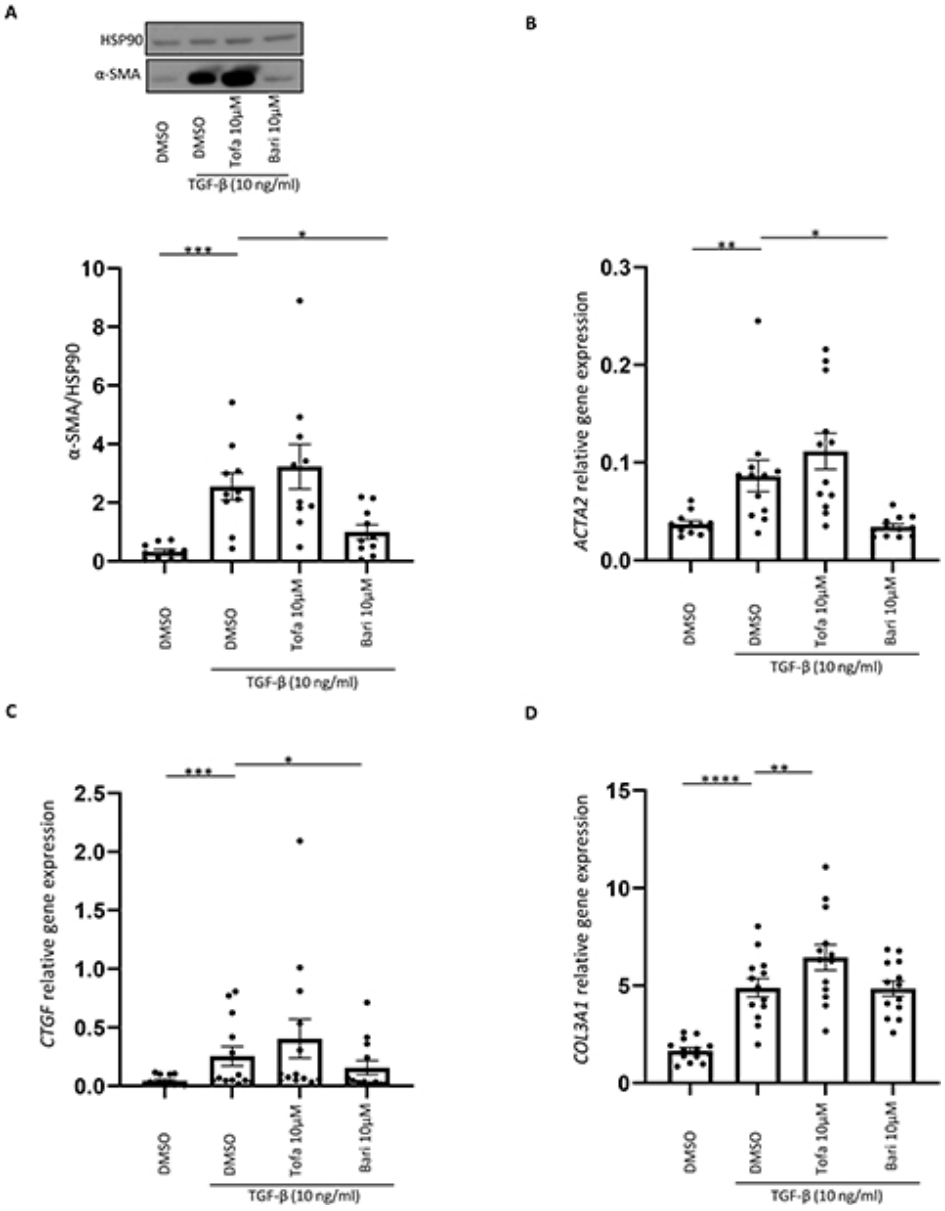


Figure 5**Impact of tofacitinib and baricitinib on fibrotic markers in OA FLS**OA FLS were stimulated with TGF-β (10 ng/ml) for 72 hours. At 48 hours, tofacitinib or baricitinib (10 μM) was added for 24 hours. (A) α-SMA and HSP90 protein expression level. The upper picture shows a representative Western blot for one patient and the lower graph is the protein quantification for all patients reported on GAPDH expression level (loading control). (B-D) ACTA2, CTGF and COL3A1 relative gene expression in OA FLS by RT-qPCR. Numbers of patients: n=10 (A); n=12 (B); n=13 (C; D). Data are shown as mean ± SEM; * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

169x216mm (72 x 72 DPI)