Synovial Tissue Heterogeneity in Rheumatoid Arthritis and Changes With Biologic and Targeted Synthetic Therapies to Inform Stratified Therapy

Lylia Ouboussad 1†, Agata N. Burska 1†, Andrew Melville 1 and Maya H. Buch 1,2*

1 Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, United Kingdom, 2 NIHR Leeds Biomedical Research Centre, Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom

The treatment of rheumatoid arthritis (RA) has been transformed with the introduction of biologic disease modifying anti-rheumatic drugs (bDMARD) and more recently, targeted synthetic DMARD (tsDMARD) therapies in the form of janus-kinase inhibitors. Nevertheless, response to these agents varies such that a trial and error approach is adopted; leading to poor patient quality of life, and long-term outcomes. There is thus an urgent need to identify effective biomarkers to guide treatment selection. A wealth of research has been invested in this field but with minimal progress. Increasingly recognized is the importance of evaluating synovial tissue, the primary site of RA, as opposed to peripheral blood-based investigation. In this mini-review, we summarize the literature supporting synovial tissue heterogeneity, the conceptual basis for stratified therapy. This includes recognition of distinct synovial pathobiological subtypes and associated molecular pathways. We also review synovial tissue studies that have been conducted to evaluate the effect of individual bDMARD and tsDMARD on the cellular and molecular characteristics, with a view to identifying tissue predictors of response. Initial observations are being brought into the clinical trial landscape with stratified biopsy trials to validate toward implementation. Furthermore, development of tissue based omics technology holds still more promise in advancing our understanding of disease processes and guiding future drug selection.

Keywords: rheumatoid arthritis, biologics, JAK inhibitors, synovial tissue, histology, cytokine, gene expression, pathotypes

INTRODUCTION

Rheumatoid arthritis (RA) is a complex, genetically and biologically heterogeneous autoimmune disease. It is characterized by a systemic inflammatory arthritis. The treatment of patients with RA has evolved considerably in recent years owing to the successful development and widespread use of biologic disease modifying anti-rheumatic drug (bDMARD) therapy, with more recent introduction of targeted synthetic DMARDs (tsDMARD) in the form of small molecules inhibitors.
However, up to 40% patients in clinical trials fail to respond, also reflected in real-world practice; and a sizeable proportion fail to achieve the target of therapy, mainly clinical remission where appropriate or low disease activity (1, 2). Personalized medicine, i.e., tailoring therapy to individual patient (or, put simply, “choosing the right drug for the right patient”), has the potential to improve response rates, but has proven challenging to implement. If it is to be successful, the identification of reliable biomarkers will be of prime importance.

In this mini-review, we summarize the evidence for synovial tissue heterogeneity, and tissue studies that have evaluated change in cellular and molecular markers following currently available bDMARD and tsDMARD specifically that could aid treatment selection.

THE SYNOVIIUM, PRINCIPAL TARGET OF INFLAMMATION

The synovium is the principal target of inflammation in RA, undergoing marked pathological changes compared to healthy tissue. The study of RA synovial tissue has offered insights at a cellular level into multiple aspects of the disease, from identifying pathogenic processes and pathways (3, 4); to explaining clinical manifestations. Furthermore, changes in synovial tissue following successful treatment allow better understanding of mechanism of drug action (5–7).

Synovial tissue samples can be obtained via arthroscopic or ultrasound (US)-guided biopsies. The US-guided approach has been shown to be safe, with reproducible tissue quality/RNA yield (8), and has the advantage of enabling joint assessment for synovial thickness (gray-scale score) and vascularity (Power Doppler-PD), associated with active synovial inflammation (9).

HEALTHY SYNOVIUM

In health, the synovial membrane contains relatively few cells, consisting of an intimal lining layer of 1–2 cell thickness and a distinct synovial sublining layer (10). The intima comprises fibroblast-like synoviocytes (FLS, also known as synovial fibroblasts or type B synoviocytes) intercalated with macrophage-like synoviocytes (MLS, also called type A synoviocytes) (11). The sub-lining layer is a well-vascularized connective tissue, containing collagen fibers and evenly dispersed FLS and MLS (11).

The synovial membrane is key to the structure and function of the healthy synovial joint. The synovial membrane controls transport to and from the synovial cavity, thus maintaining the composition of synovial fluid as well as overall joint homeostasis and integrity. The intimal lining is particularly important, as its lack of tight junctions or a true basement membrane allows the ingress and egress of various cells and proteins (12). Intimal FLS orchestrate proceedings, controlling the synovial fluid volume, secreting hyaluronan for lubrication, clearing intra-articular debris, regulating various immunological processes, and maintaining the extracellular matrix (ECM) of the sublining (13).

RA SYNOVIUM

In RA, the synovial tissue becomes markedly expanded, with a striking increase in cellular infiltration. This leads to hallmark “pannus” formation at cartilage-bone interfaces; pannus can be composed of macrophages, FLS, leucocytes, plasma cells, and mast cells (14), and behaves like a locally invasive tumor, mediating damage and erosion formation in later disease (15). The intimal lining can expand to 10–20 cells in thickness, partly due to an increase in FLS, but mostly due to infiltration by bone marrow-derived MLS recruited from the circulation (15). Highly activated macrophages send pro-inflammatory signals to intimal FLS, inducing invasiveness, and to B cells, which in turn produce various pro-inflammatory mediators. Paracrine and autocrine signaling networks develop in this way, further propagating synovitis (16). Sub-lining MLS have been associated with disease activity (17) and synovial inflammation measured on magnetic resonance imaging (MRI) (18), and therefore appear of paramount importance to the inflammatory joint reaction (19). Proliferation of FLS are a prime cause of synovial hyperplasia, and major mediators of damage to cartilage and bone, via both direct and indirect interactions, including production of inflammatory mediators, adhesion molecules, proteolytic enzymes and pro-osteoclastogenic factors (13). T cells are able to establish important crosstalk with antibody-producing plasma cells (15, 20, 21). When present, CD3+ T cells in the RA synovium are mostly found in deeper sub-lining layers, where they may be homogeneously or randomly distributed, or clustered in follicle-like structures (19). Similarly, B cells, when present, are mostly organized in follicular structures, which can act as pro-inflammatory, immunological niches (19).

HETEROGENEITY OF RA SYNOVITIS

RA synovitis is highly heterogeneous, with diverse cellular and molecular signatures (22, 23). In recent years distinct patterns have been recognized, primarily according to the composition, organization and localization of cellular infiltrates. Studies have revealed RA synovial ’pathotypes’ (7, 24), namely, lymphoid, myeloid, pauci-immune, and fibroid variants (other patterns, such as granulomatous synovitis, have also been described). The lymphoid pathotype is characterized by lymphoid infiltrates, which may be diffuse (small, loosely arranged lymphocyte clusters) or follicular (large aggregates of lymphocytes organized in ectopic lymphoid structures). The latter may develop germinal centers containing T follicular helper (Tfh) cells highly expressing of programmed cell-death (PD-1), C-X-C chemokine receptor 5 (CXCR5), B-cell lymphoma (Bcl6), and Inducible T cell costimulator (ICOS) (7, 24, 25). Cellular composition of tissue defined as myeloid pathotype shows a less abundant B and T cells aggregates compared to the lymphoid subgroup, and presence of sublining macrophages. By contrast, the ’pauci-immune’ (7) (or ‘low inflammatory’) pathotype shows minimal infiltrating immune cells (24). The fibroid pathotype has complete absence of aggregates and little immune infiltration comprising hyperplastic tissues.
SYNOVIAL TISSUE GENE EXPRESSION PROFILES

Early gene expression of RA synovial tissue studies identified distinct profiles and revealed the presence of multiple activated signaling pathways (29–31). Perhaps unsurprisingly given its clinical heterogeneity, expression of molecular signatures in RA is likewise heterogeneous. Gene expression profiles can be modulated by disease activity and the burden of inflammation in synovial tissue (32). Gene expression in RA synovial (intimal) lining cells specifically has been analyzed using a laser mediated micro-dissection (LIMM) approach (33). Data analysis using clustering revealed two distinct RA subgroups associated with increased expression levels of inflammation-related genes [compared with osteoarthritis (OA) control tissue] involved in the tumor necrosis factor TNF-activated interferon regulatory factor 1 (IRF1)- interferon (IFN)- signal transducer and activator of transcription 1 (STAT1)- pathway (34). Three molecularly distinct forms of RA tissues have also been identified by the same group; the first characterized by genes involved in inflammation and the adaptive immune response [matrix metalloproteinase (MMP) 1 and 3 genes, STAT-encoding and -induced genes and antigen-presenting-cell–related genes], the second characterized by genes involved in extracellular matrix remodeling (genes involved in inflammation-driven joint remodeling in RA, characterized by uncoupling of destructive and reparative processes (37). A number of transcription factor families, such as nuclear factor κB (NF-κB) and the activator protein 1 (AP-1), were established early on as chief regulators of gene expression in the inflamed synovium (38). Gene expression analysis of FLS indicates the presence of 2 subtypes, with high-inflammatory FLS expressing transforming growth factor (TGF)-β/activin A–inducible genes and FLS from low inflammatory synovial tissue predominantly expressing growth factor genes (39). Distinct molecular signatures indicating pathways relating to T cell-mediated immunity and major histocompatibility complex (MHC) class II mediated immunity (amongst others) upregulated in early RA, and pathways relating to the cell cycle upregulated in later disease (40) have been reported. Similarly differential gene expression between high and low inflammatory subsets of RA patients in relation to disease duration has been observed (29).
defined by enrichment of transforming growth factor β pathways, glycoprotein synthesis, and cell adhesion genes (45). Distinct myeloid and lymphoid synovial histological subtypes were not identified, in contrast to previous studies (24), but the high inflammatory subtype displayed elevated expression of genes previously attributed to these in the literature.

SYNOVIAL TISSUE STUDIES TO PREDICT RESPONSE TO BIOLOGIC AND TARGETED THERAPIES

General Synovial Tissue Biomarkers of Response To Therapy

CD68 Macrophage
Effective treatment can modify synovial histology, cytokine and gene expression, with ineffective treatment having little impact, thus providing a means to assess for pathological response (46). Synovial sublining (CD68) macrophage numbers and macrophage expressed cytokines have been shown to correlate with disease activity, and change in sublining macrophage to be the optimal indicator of effective therapy, thus providing a potential early predictive biomarker of drug response (6, 47, 48). A recent study demonstrated that the transcriptional profile of isolated RA synovial macrophages highlighted different subpopulations of patients and identified 6 novel transcriptional modules that were associated with disease activity and therapy (49). The authors suggest that transcriptional signatures in macrophages regardless of location (sublining vs. synovial lining) predict responsiveness to specific non-biologic and/or biologic therapies.

Synovial Pathotypes and Response
A study by Dennis et al. suggested myeloid and lymphoid pathotypes may predict therapeutic success with TNF inhibitors (TNFi) and IL-6-targeted tocilizumab, respectively (24). Analysis of serum chemokines further suggested these two pathotypes correlate with raised serum soluble intercellular adhesion molecule 1 (sICAM) and CXCL13 (sICAM/CXCL13) compared to high CXCL13/sICAM, respectively. These initial observations however have not been validated in other cohorts using the serum correlates (50) indicating the need for additional such synovial tissue studies. Nevertheless, stratifying patients by synovial pathotype may inform choice of targeted therapy.

Multiple types of therapies will be discussed in detail below, these are summarized in Table 1 together with key findings which indicate response to biologic and synthetic targeted DMARDs.

Anti-cytokine Therapies

Tumor-Necrosis Factor-Inhibitors
Synovial studies have offered useful insights into the mechanism of action of TNFi. TNFi have been shown to regulate chemokine and leukocyte trafficking (69) likely explaining the reduction in the synovial cellular infiltrate observed; with reductions in synovial tissue expression of IL-6, IL-8, granulocyte macrophage colony stimulating factor (GM-CSF), macrophage chemoattractant protein-1 (MCP-1), IL-1β, TNF, and vascular endothelial growth factor (VEGF) (70).

Several studies have sought to identify predictors of response to TNF blockade through examination of synovial tissue cytokine expression. Baseline synovial TNF levels (intimal and sub-lining) predicted response to infliximab in one study (54), although another similar study did not reproduce this finding (53). Decreased sub-lining TNF expression was, however, seen in responders. A prospective study of 86 patients found higher proportions of synovial lymphoid aggregates in poor responders to treatment, despite higher rates of TNFi use. Baseline lymphoid aggregates were an independent predictor of poor response in multivariate analysis, and reversal of these histological changes was seen in over half of treatment responders (57). Addition of lymphocyte aggregates to sub-lining TNF expression (54) improved infliximab response prediction, but still only accounting for 29% variance (71); thus insufficient for clinical application. An early RA synovial gene expression study found that mRNA levels pertaining to several inflammatory pathways were associated with response to TNFi therapy, suggesting a role for synovial gene expression profiles as response predictors (72). Another study identified a number of negative predictors of response to adalimumab, another TNFi biologic, including baseline synovial expression of IL-7 receptor alpha chain (IL-7R), CXCL11, IL-18, IL-18 receptor accessory (IL-18rap), and MBL67 (63). However, a larger gene expression study using whole synovial tissue samples pre- and post-infliximab did not identify any predictors, perhaps because of the confounding presence of lymphoid aggregates (55).

Tocilizumab
Tocilizumab is a clinically effective humanized anti–IL-6R monoclonal antibody that inhibits membrane IL-6R– and soluble IL-6R (sIL-6R)–mediated signaling. The aforementioned study by Dennis et al. (24), suggested lymphoid pathotype as predictive of response. In another study, paired synovial tissue biopsies taken at baseline and post-treatment with tocilizumab showed a significant decrease in the expression of various chemokines and T-cell activation genes (51). When compared with gene expression data following other treatments, results showed strong correlation with methotrexate and B-cell depleting agent rituximab, but notable differences with adalimumab (51). A further study of synovial histology post-tocilizumab demonstrated a complete block of synovial IL-6 and a significant reduction of B-cells, CD29 and phospho-JNK. ERK was increased in the tocilizumab group compared to a methotrexate-treated control group, whilst TNF, MMP-3, and CD68 were similarly expressed in both groups. Therefore, inhibition of IL-6/CD20/CD29 may be differentially involved in tocilizumab efficacy compared with methotrexate (52). A more recent study in 33 early RA patients suggested higher expression of TNF-induced transcripts in early RA synovitis was associated with higher disease activity, and predicted poor response to first-line therapy (that comprised either methotrexate, tocilizumab or rituximab therapy) (65). Finally, an exploratory study by Das et al. suggested persistent synovial IL-6 mRNA expression
| Drug                  | RA Population                                                                 | Analysis type                        | Key findings                                                                                                                                      | References |
|----------------------|-------------------------------------------------------------------------------|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| ANTI CYTOKINE THERAPY |                                                                                |                                      |                                                                                                                                                  |            |
| IL-6 blockade        | TCZ 30 early RA (disease duration < 1 year); treatment-naive                  | Gene expression microarrays, IHC     | Significant decrease in the expression of various chemokines and T-cell activation genes.                                                        | (51)       |
|                      | TCZ 10 bDMARD treated RA patients 10 controls: RA patients on no bDMARDs      | IHC                                  | Complete blockade of IL-6. Inhibition of CD20, CD29, and JNK in MAPK implicates TCZ efficacy compared with MTX.                                    | (52)       |
|                      | IFX 32 RA patients                                                            | IHC                                  | Reduction in synovial TNF expression in IFX responders and non-responders. Unchanged TNF in extreme non-responders                               | (53)       |
|                      | IFX 143 active RA patients                                                     | IHC                                  | Higher intimal and sub-lining TNF expression in IFX responders vs. non-responders.                                                               | (54)       |
|                      | IFX 62 RA patients                                                            | IHC and gene expression arrays       | Baseline whole synovial biopsy microarray unable to identify TNFi non-responders.                                                                | (55)       |
|                      | ADA 25 RA patients                                                            | Global gene expression profiles arrays at T0 and T16, IHC | Poor response to ADA associated with: - Upregulation of genes from cell division and immune responses pathways in poor responders.  
- High baseline synovial expression of IL-7R, CXCL11, IL-18, IL-18ra), and MKI67. | (56)       |
|                      | Several TNFi 86 RA patients                                                   | IHC                                  | High synovial lymphoid neogenesis, with B and T cell aggregates, correlated with poorer clinical outcomes. Reversal of these aggregates associated with good response. | (57)       |
| CELL-MEDIATED THERAPY |                                                                                |                                      |                                                                                                                                                  |            |
| B-Cell depletion     | RTX 13 RA patients                                                            | IHC, digital image analysis, gene expression | Significant decrease synovial B cells post-RTX but not completely depleted compared to peripheral B cells.  
No strong correlation with clinical response.  
Responders have higher expression of macrophage and T cell genes. Non-responders showed higher expression of interferon-α and signaling genes. | (58)       |
|                      | RTX 20 RA patients                                                            | qPCR                                 |                                                                                                                                                  | (59)       |
|                      | RTX 24 RA patients                                                            | IHC, flow cytometry                  | Significant lower infiltration of CD79+/CD20+ plasma cells in the synovium associated with the reduction in peripheral blood B-cell repopulation. | (60)       |
|                      | RTX 24 RA patients                                                            | IHC                                  | Clinical response predicted by changes in cell types other than B cells, mainly number of synovial plasma cells.                                  | (61)       |
|                      | RTX 17 RA patients                                                            | IHC                                  | RTX treatment associated with rapid decrease in synovial B cell numbers.                                                                       | (62)       |
| T-CELL CO-STIMULATION BLOCKADE |                                                                                |                                      |                                                                                                                                                  |            |
|                      | ABT 16 RA patients                                                            | IHC                                  | Significant downregulation of pro inflammatory genes, notably IFNγ. Only specific reduction in synovial CD20+ B cells, in responders.           | (63)       |
|                      | ABT 20 RA patients (10 ABA and 10 MTX)                                        | IHC                                  | Increase in CD29 and ERK in MAP kinases.                                                                                                         | (64)       |
| MIXED BDMARD COHORT  | NSAIDs and DMAPRs with/without bDMARD (ADA, ETN, IFX, ANK, RTX)              | GeneChip Human Genome U133 Plus 2.0 Arrays (Affymetrix, Inc.) ELISA, IHC | A myeloid phenotype (high serum sICAM1/low CXCL13) prevalent in responders to TNFi therapy  
A lymphoid pathotype (high serum CXCL13/low sICAM1) prevalent in responders to TCZ. | (24)       |
(following rituximab inefficacy) associated with subsequent tocilizumab response (73).

**Cell Mediated Therapies**

**B-Cell Depletion: Rituximab**

Treatment with the anti-CD20 monoclonal antibody rituximab significantly decreases synovial B cells, but, unlike in the periphery, does not completely eradicate them. In addition, synovial B cell depletion does not correlate strongly with clinical response in RA, suggesting the effects of rituximab on synovial B cells may be necessary but not sufficient for inducing clinical efficacy (58). A separate study of RA synovial histology pre- and post-rituximab confirmed these findings, but also examined changes in other cell populations at 4 and 16 weeks. A reduction in short-lived CD138+ plasma cells, possibly generated locally within the synovial membrane, was found to predict clinical response, whilst delayed reductions in T cell, intimal macrophages and lymphoid aggregates were also seen, highlighting the role of B cells in sustaining inflammation and cell recruitment (74). Another study suggested that clinical response to rituximab is associated with higher residual levels of CD79+CD20− plasma cells in the synovium (together with persistence of circulating ACPA+IgM plasmablasts) (60). In addition, there is evidence that baseline synovial gene expression may be able to predict response to rituximab (and lack of response), as composite “gene scores” were found to correlate with changes in disease activity (DAS-28 score) in one study (59). Genes relating to macrophage and T cell function were activated in responders.

At a more fundamental level, B cells have shown to be central to T-cell mediated synovial inflammation. This was elegantly demonstrated by a study showing that synovial T-cell clones adoptively transferred into human leukocyte antigen (HLA)-DR-matched synovial tissues xenotransplanted into severe combined immunodeficient (SCID) mice are able to enhance local production of IFNγ, TNF, and IL-1β, but only when transplanted tissues contain B-cell follicles (75). Furthermore, treatment of synovial grafts with anti-CD20 depleting agents induces not only a decrease in B-cell density but also a disruption of the overall lymphoid architecture and reduction of cytokine expression, as well as a dramatic depletion of T cells and macrophages, in keeping with the existence of an active cell network supported by B cells.

**T-Cell Co-stimulation Blockade (Abatacept (CTLA4-Fc))**

Abatacept, a recombinant fusion protein approved for the treatment of RA, blocks T cell co-stimulation by competing with CD28 for CD80/86 on antigen presenting cells. Synovial studies of the effect of and mechanism of abatacept are relatively lacking. A study of 16 RA patients compared synovial tissue pre- and 16 weeks post-abatacept in terms of gene expression and immunohistochemistry. Amongst responders, there was notable downregulation of several pro-inflammatory mediators, particularly the T-cell-related cytokine IFNγ. However, only

| Drug | RA Population | Analysis type | Key findings | References |
|------|---------------|---------------|-------------|------------|
| TCZ, MTX, RTX | Early RA (mainly <1 year disease duration), pre- and post-3 months | GeneChip Human Genome U133 Plus 2.0., Affymetrix, IHC | Over-expressed baseline tissue GADD45B and PDE4D in first-line MTX and bDMARD non-responders | (65) |
| TOFA | 14 RA patients | ELISA, IHC, qPCR. | Reduced synovial mRNA expression of MMP1 and MP3 and IFN-regulated genes. Clinical improvement correlated with reductions in STAT1 and STAT3 phosphorylation. | (66) |
| TOFA | Varied/unclear | Synovial explants and tissue culture of primary RASFs, qPCR, WB, and ELISA | Decrease in metabolic functions (mitochondrial pathways, ROS production and glycolysis), indicating that the JAK-STAT signaling is a mediator between inflammation and cellular metabolism. | (67) |
| Baricitinib | 27 RA samples | Tissue culture experiments on FLS | Abrogation of IFNγ-stimulated FLS invasion by targeted inhibition of JAK. | (68) |

---

**SMALL INHIBITORS (JAKI)**

| Drug | RA Population | Analysis type | Key findings | References |
|------|---------------|---------------|-------------|------------|
| TOFA | Varied/unclear | GeneChip Human Genome U133 Plus 2.0., Affymetrix, IHC | Decrease in metabolic functions (mitochondrial pathways, ROS production and glycolysis), indicating that the JAK-STAT signaling is a mediator between inflammation and cellular metabolism. | (67) |
| Baricitinib | 27 RA samples | Tissue culture experiments on FLS | Abrogation of IFNγ-stimulated FLS invasion by targeted inhibition of JAK. | (68) |

**Table 1** | Continued

| Drug | RA Population | Analysis type | Key findings | References |
|------|---------------|---------------|-------------|------------|
| TCZ, MTX, RTX | Early RA (mainly <1 year disease duration), pre- and post-3 months | GeneChip Human Genome U133 Plus 2.0., Affymetrix, IHC | Over-expressed baseline tissue GADD45B and PDE4D in first-line MTX and bDMARD non-responders | (65) |
| TOFA | 14 RA patients | ELISA, IHC, qPCR. | Reduced synovial mRNA expression of MMP1 and MP3 and IFN-regulated genes. Clinical improvement correlated with reductions in STAT1 and STAT3 phosphorylation. | (66) |
| TOFA | Varied/unclear | Synovial explants and tissue culture of primary RASFs, qPCR, WB, and ELISA | Decrease in metabolic functions (mitochondrial pathways, ROS production and glycolysis), indicating that the JAK-STAT signaling is a mediator between inflammation and cellular metabolism. | (67) |
| Baricitinib | 27 RA samples | Tissue culture experiments on FLS | Abrogation of IFNγ-stimulated FLS invasion by targeted inhibition of JAK. | (68) |

**ABT, abatacept; ADA, adalimumab; ANK, anakinra; bDMARD, biologic disease modifying anti-rheumatic drug; ELISA, enzyme-linked immunosorbent assay; ERK, Extracellular signal-Regulated Kinase; ETN, etanercept; FLS, fibroblast-like synoviocytes; GADD45B, Growth Arrest And DNA Damage Inducible Beta; IHC, immunohistochemistry; IFN, interferon; IFX, infliximab; JAKI, janus kinase inhibitor; MAPK, mitogen activated protein kinase; MMP, matrix metalloproteinase; MTX, methotrexate; PDE4D, Phosphodiesterase 4D; qPCR, quantitative polymerase chain reaction; RA, rheumatoid arthritis; RTX, rituximab; SF, synovial fibroblast; STAT, signal transducers and activators of transcription; TCZ, tocilizumab; TNF, tumor necrosis factor.**
a specific reduction in synovial CD20+ B cells without significant disruption in other cell populations was observed (contrasting with the observations following anti-cytokine therapies, perhaps in keeping with the more immunomodulatory role of CTLA4) (63). Whilst effects on tertiary lymphoid structures were not analyzed, these observations suggest that disruption of T-/B-cell interactions may be critical to abatacept’s mode of action. In contrast to this study, a smaller study on 5 patients treated with abatacept indicated inhibition of cell proliferation, with decreases in the expression of MMP-3, CD68, CD4, CD8, CD20, CD80, and CD86 in the synovium (64).

**Small Molecule Janus-Kinase (JAK) Inhibitors**

Multiple inflammatory cytokines signal via JAK-STAT pathway. Thus, JAK/STAT signaling plays a key role in several immune mediated inflammatory diseases, including RA (76). As small molecules with intracellular targets (i.e., JAK family members), JAK inhibitors represent a novel targeted therapeutic approach in RA (77).

Tofacitinib is an oral JAK inhibitor effective for the treatment of RA (78). It is a pan-selective JAKi, blocking signaling mediated via JAK1, JAK3 and, to a lesser extent, JAK2 (79). A comparison of RA synovial tissue at pre- and 4 weeks post-treatment with tofacitinib showed no change in an overall inflammation score or levels of T cells, B cells or macrophages, but reduced expression of MMPs (MMP1 and MMP3) and interferon-regulated genes, notably CXCL10. Furthermore, clinical improvement at 4 months was found to correlate with reductions in STAT1 and STAT3 phosphorylation, indicating the importance of IFNγ and IL-6 inhibition, respectively (66). In addition, a recent metabolomics study showed that adding tofacitinib to RA synovial explants and synovial fibroblasts in vitro led to decreased mitochondrial pathway activity, reactive oxygen species (ROS) production and glycolysis, suggesting modulation of cellular metabolism may contribute to its therapeutic effect (67).

Baricitinib, a JAK inhibitor targeting JAK1/JAK2, is another licensed treatment for RA (80). A study specifically examining FLS activity in RA showed that baricitinib abrogates IFNγ-induced invasiveness of FLS (68), which is of importance given their key contribution to pannus formation (aggressive cell masses that destroy articular cartilage and bone), one of the hallmarks of RA synovial pathobiology (81).

**CONCLUSION**

It is well-accepted that the considerable advances in the treatment of RA need to be accompanied by a stratified approach that mitigates against the current trial and error approach of treatment decision-making, and the associated individual patient and health-economic consequences. Significant investment in biomarker studies has failed to deliver clinically meaningful tools, with the vast majority focusing on peripheral blood-based evaluation. The emphasis on synovial tissue, the primary site of RA is intuitive, from which tissue and thus disease subtypes are emerging.

The need to pull through benchside investigation of tissue biomarkers to the bedside demands more refined and innovative stratified trial design (82). We will soon see the outcomes of such initiatives [including STRAP—Stratification of Biologic Therapies for RA by Pathobiology (ISRCTN10618686) and R4-RA—A Randomized, open labeled study in anti-TNFα inadequate responders to investigate the mechanisms for Response—Resistance to Rituximab vs. Tocilizumab in RA (ISRCTN97443826)] that will inform future tissue driven trial design. These trials and other tissue-based programmes such as the recently established NIH Accelerating Medicines Partnership (AMP) RA/SLE network will also exploit high-dimensional analyses including mass cytometry, RNA-seq of selected cell populations, and single cell RNA-seq (83). Whilst the sheer volume of data in itself presents massive challenges in the clinically meaningful interpretation, the richness of data matched with improved sophisticated analytical techniques holds the promise of being able to join the field of personalized RA targeted therapy use.

**AUTHOR CONTRIBUTIONS**

LO, AB, and AM: literature Search, write up, final approval of the manuscript; MB: conception and design of the work, write up, final approval of the manuscript.

**FUNDING**

MB research grants Pfizer Ltd., Roche, UCB. Consulting fees, Abbvie, Lilly Merck-Serono, Pfizer Ltd., Sandoz, Sanofi.

**REFERENCES**

1. Nam J, Winthrop K, van Vollenhoven RF, Pavelka K, Valesini G, Hensor E, et al. Current evidence for the management of rheumatoid arthritis with biological disease-modifying antirheumatic drugs: a systematic literature review informing the EULAR recommendations for the management of RA. *Ann Rheum Dis.* (2010) 69:976–86. doi: 10.1136/ard.2009.126573

2. Sokka T, Hetland ML, Mäkinen H, Kautiainen H, Horslev-Petersen K, Luukkainen RK, et al. Remission and rheumatoid arthritis: data on patients receiving usual care in twenty-four countries. *Arthritis Rheumat.* (2008) 58:2642–51. doi: 10.1002/art.23794

3. Devauchelle V, Marion S, Cagnard N, Mistou S, Falgarone G, Breban M, et al. DNA microarray allows molecular profiling of rheumatoid arthritis and identification of pathophysiological targets. *Genes Immun.* (2004) 5:597–608. doi: 10.1038/sj.gene.6364132

4. Gerlag DM, Tak PP. How to perform and analyse synovial biopsies. *Best Pract Res Clin Rheumatol.* (2009) 23:221–32. doi: 10.1016/j.berh.2013.03.006

5. Tak P. Lessons learnt from the synovial tissue response to anti-rheumatic treatment. *Rheumatology.* (2000) 39:817–20. doi: 10.1093/rheumatology/39.8.817

6. Tak PP, Smets Tj, Daha MR, Kluin PM, Meijers KA, Brand R, et al. Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue
in relation to local disease activity. *Arthritis Rheumat.* (1997) 40:217–25. doi: 10.1002/1.1780400206

7. Pitzalis C, Kelly S, Humby F. New learnings on the pathophysiology of RA from synovial biopsies. *Carr Opin Rheumatol.* (2013) 25:334–44. doi: 10.1097/BOR.0b013e32835dd4eb

8. Kelly S, Humby F, Filer A, Ng N, Di Cicco M, Hands R, et al. Ultrasound-guided synovial biopsy: a safe, well-tolerated and reliable technique for obtaining high-quality synovial tissue from both large and small joints in early arthritis patients. *Ann Rheuma Dis.* (2015) 74:611–7. doi: 10.1136/annrheumdis-2013-204603

9. Koski JM, Saarakkala S, Helle M, Hakulinen U, Heikkinen JO, Hermunen H, Power Doppler ultrasonography and synovitis: correlating ultrason imaging with histopathological findings and evaluating the performance of ultrasound equipment. *Ann Rheuma Dis.* (2006) 65:1590–5. doi: 10.1136/ard.2005.051235

10. Smith MD. The normal synovium. *Open Rheumat J.* (2011) 5:100–6. doi: 10.2174/187439210105010100

11. Lindblad S, Hedfors E. The synovial membrane of healthy individuals—immuno-histological overlap with synovitis. *Clin Exp Immunol.* (1987) 69:41–7.

12. Steenvoorden MMC, Tolboom TCA, van der Pluijm G, Löwik C, Verser CPJ, DeGroot J, et al. Transition of healthy to diseased synovial tissue in rheumatoid arthritis is associated with gain of mesenchymal/fibrotic characteristics. *Arthritis Res Ther.* (2006) 8:R165. doi: 10.1186/ar1427

13. Bottini N, Firestein GS. Duality of fibroblast-like synoviocytes in RA: passive responders and imprinted aggressors. *Nat Rev Rheumatol.* (2013) 9:24. doi: 10.1038/nrrheum.2012.190

14. Bromley M, Woolley DE. Histopathology of the rheumatoid lesion. *Arthritis Rheumat.* (1984) 27:857–63. doi: 10.1002/art.1780270804

15. Firestein GS. Etiology and Pathogenesis of Rheumatoid Arthritis. Kelley’s Textbook of Rheumatology. Philadelphia, PA: Elsevier (2012), 921–66.

16. Tak PP, Bresnihan B. The pathogenesis and prevention of joint damage in rheumatoid arthritis advances from synovial biopsy and tissue analysis. *Arthritis Rheumat.* (2000) 43:2619–33. doi: 10.1002/1529-0131(200012)43:12<2619::AID-ANR1>3.0.CO;2-V

17. Vordenbäumen S, Schleich C, Miese F, Schleich C, Bleck E, et al. Inflammation and vascularisation markers of arthoscopically-guided finger joint synovial biopsies reflect global disease activity in rheumatoid arthritis. *Clin Exp Rheumatol.* (2014) 32:117–20.

19. Mucke J, Hoyer A, Brinks R, Bleck E, Pauly T, et al. Dynamic contrast-enhanced magnetic resonance imaging of the synovial tissue: perspectives for biomarker development in rheumatoid arthritis. *Arthritis Res Ther.* (2014) 16:2073.

20. Buckley CD, McGettrick HM. Leukocyte trafficking between stromal compartments: lessons from rheumatoid arthritis. *Nat Rev Rheumat.* (2018) 14:476–87. doi: 10.1038/s41584-018-0042-4

21. Fonseca J, Canhao H, Resende C, Saraiva F, da Costa JT, Pimentão JB, et al. Histology of the synovial tissue: value of semiquantitative analysis for the prediction of joint erosions in rheumatoid arthritis. *Clin Exp Rheumatol.* (2000) 18:559–64.

22. Klimiuk PA, Goronyz JY. Tissue cytokine patterns distinguish variants of rheumatoid synovitis. *Am J Pathol.* (1997) 151:1311–9.

23. Lauwereys BR, Hernández-Lobato D, Gramme P, Ducreux J, Dessy A, Focant I, et al. Heterogeneity of synovial molecular patterns in patients with arthritis. *PLoS ONE.* (2015) 10:e0122104. doi: 10.1371/journal.pone.0122104

24. Dennis G, Holweg CT, Kummerfeld SK, Choy DE, Setiadi AF, Hackney JA, et al. Synovial phenotypes in rheumatoid arthritis correlate with response to biologic therapeutics. *Arthritis Res Ther.* (2014) 16:R90. doi: 10.1186/147555

25. Orr C, Najm A, Biniecka M, McGarry T, Ng C, Young F, et al. Synovial immunophenotype and anti–citrullinated peptide antibodies in rheumatoid arthritis patients: relationship to treatment response and radiologic prognosis. *Arthritis Rheumat.* (2017) 69:2114–23. doi: 10.1002/art.40218
43. Srirangan S, Choy EH. The role of interleukin 6 in the pathophysiology of rheumatoid arthritis. *Therapeuic Adv Musculoskeletal Dis.* (2010) 2:247–56. doi: 10.1177/1759720X10378372

44. Klimiuk PA, Sierakowski S, Latosiewicz R, Skowronski J, Cylwik JP, Cylwik B, et al. Histological patterns of synovitis and serum chemokines in patients with rheumatoid arthritis. *J Rheumatol.* (2005) 32:1666–72.

45. Orange DE, Agius P, DiCarlo EF, Robine N, Geiger H, Szymonifka J, et al. Identification of three rheumatoid arthritis disease subtypes by machine learning integration of synovial histologic features and RNA sequencing data. *Arthritis Rheumatol.* (2018) 70:690–701. doi: 10.1002/art.40428

46. Baeten D, Houbiers J, Kruithof E, Vandooren B, Van den Bosch F, Roots AM, et al. Synovial inflammation does not change in the absence of effective treatment: implications for the use of synovial histopathology as biomarker in early phase clinical trials in rheumatoid arthritis. *Ann Rheum Dis.* (2006) 65:990–7. doi: 10.1136/ard.2005.047852

47. Bresnahan B, Tak PP, Emery P, Klaresgt L, Breedveld F. Synovial biopsy in arthritis research: five years of concerted European collaboration. *Ann Rheum Dis.* (2000) 59:506–11. doi: 10.1136/ard.59.7.506

48. Haringman JJ, Gerlag DM, Zwijnderman AH, Smeets TJ, Kraan MC, Baeten D, et al. Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis. *Ann Rheum Dis.* (2005) 64:834–8. doi: 10.1136/ard.2004.029751

49. Mandelin AM, Homan PJ, Shaffer AM, Cuda CM, Dominguez ST, Bacalao E, et al. Transcriptional profiling of synovial macrophages using minimally invasive ultrasound-guided synovial biopsies in rheumatoid arthritis. *Rheumatol. (2018)* 70:841–54. doi: 10.1002/art.40453

50. Sornasse T, Gabay C, Townsend M, Laubender R, Wang J, Tuckwell K. The value of synovial cytokine expression in predicting the clinical response to TNF antagonist therapy (infliximab). *Rheumatology.* (2008) 47:1469–75. doi: 10.1093/rheumatology/ken261

51. Buch MH, Boyle DL, Rosengren S, Saleem B, Reece RJ, Rhodes LA, et al. Mode of action of abatacept in rheumatoid arthritis patients having failed tumour necrosis factor blockade: a histological, gene expression and dynamic magnetic resonance imaging pilot study. *Ann Rheum Dis.* (2009) 68:1220–7. doi: 10.1136/ard.2008.091876

52. Kanbe K, Oh K, Chiba J, Inoue Y, Taguchi M, Yabuki A. Analysis of mitogen-activated protein kinases in bone and cartilage of patients with rheumatoid arthritis treated with abatacept. *Clin Med Insights Arthritis Musculoskelet. Disord.* (2016) 9:51–6. doi: 10.4137/CMAMD.S34424

53. Du Toit A, Dureex J, Hambly F, Nzesseu Toukap A, Badot V, Pitralis C, et al. Higher expression of TNFα-induced genes in the synovium of patients with early rheumatoid arthritis correlates with disease activity, and predicts absence of response to fist line therapy. *Arthritis Res Ther.* (2016) 18:19. doi: 10.1186/s13075-016-0919-9

54. Boyle D, Soma K, Hodge J, Kavanaugh A, Mandel D, Mease P, et al. The JAK inhibitor tofacitinib suppresses synovial JAK1-STAT signalling in rheumatoid arthritis. *Ann Rheum Dis.* (2015) 74:1311–6. doi: 10.1136/annrheumdis-2014-206028

55. McGarry T, Orr C, Wade S, Biniecka M, Wade S, Gallagher L, et al. JAK-STAT blockade alters synovial bioenergetics, mitochondrial function and pro-inflammatory mediators in rheumatoid arthritis. *Arthritis Rheumatol.* (2018) 70:1959–70. doi: 10.1002/art.40569

56. Karonitsch T, Beckmann D, Dalwijk K, Niederreiter B, Studenici D, et al. Assessment of rituximab’s immunomodulatory synovial effects (ARISE study). *Ann Rheumat Dis.* (2009) 68:563–8. doi: 10.1136/ard.2007.074229

57. Boonen A, Janssens AC, Cudworth AE, van Handel E, Verweij CL, et al. Responsiveness to anti-tumour necrosis factor α therapy in rheumatoid arthritis down-regulates synovial tumour necrosis factor α synthesis. *Arthritis Rheumatol.* (2000) 43:2391–6. doi: 10.1002/1529-0131(200011)43:11<2391::AID-ANR3-3.0.CO;2-F

58. van den Breek R, Thurlings RM, Wijbrandts CA, van Schaardenburg D, Gerlag DM, et al. The relationship between synovial lymphocyte aggregates and the clinical response to infliximab in rheumatoid arthritis: a prospective study. *Arthritis Rheumatol.* (2009) 60:3217–24. doi: 10.1002/art.24913

59. van der Pouw Kraan TC, Wijbrandts CA, van Baarsen LG, Rustenburg F, Baggen JM, Verweij CL, et al. Responsiveness to anti-tumour necrosis factor alpha therapy is related to pre-treatment tissue inflammation levels in rheumatoid arthritis patients. *Ann Rheum Dis.* (2008) 67:563–6. doi: 10.1136/ard.2007.081950

60. Teng YO, Levarht EN, Toes RE, Huizinga TW, van Laar JM. Residual inflammation after rituximab treatment is associated with sustained synovial plasma cell infiltration and enhanced B cell repopulation. *Ann Rheum Dis.* (2009) 68:1011–6. doi: 10.1136/ard.2008.092791

61. Thurlings RM, Vis K, Wijbrands CA, Zwijnderman AH, Gerlag DM, Tak PP. Synovial tissue response to rituximab: mechanism of action and identification of biomarkers of response. *Ann Rheum Dis.* (2008) 67:917–25. doi: 10.1136/ard.2007.078096

62. Vis K, Thurlings RM, Wijbrands CA, van Schaardenburg D, Gerlag DM, Tak PP. Early effects of rituximab on the synovial cell infiltrate in patients with rheumatoid arthritis. *Arthritis Rheumatol.* (2007) 56:772–8. doi: 10.1002/art.22400

63. Du Toit A, Dureex J, Hambly F, Nzesseu Toukap A, Badot V, Pitralis C, et al. Higher expression of TNFα-induced genes in the synovium of patients with early rheumatoid arthritis correlates with disease activity, and predicts absence of response to fist line therapy. *Arthritis Res Ther.* (2016) 18:19. doi: 10.1186/s13075-016-0919-9

64. Boyle D, Soma K, Hodge J, Kavanaugh A, Mandel D, Mease P, et al. The JAK inhibitor tofacitinib suppresses synovial JAK1-STAT signalling in rheumatoid arthritis. *Ann Rheum Dis.* (2015) 74:1311–6. doi: 10.1136/annrheumdis-2014-206028

65. McGarry T, Orr C, Wade S, Biniecka M, Wade S, Gallagher L, et al. JAK-STAT blockade alters synovial bioenergetics, mitochondrial function and pro-inflammatory mediators in rheumatoid arthritis. *Arthritis Rheumatol.* (2018) 70:1959–70. doi: 10.1002/art.40569

66. Karonitsch T, Beckmann D, Dalwijk K, Niederreiter B, Studenici D, et al. Assessment of rituximab’s immunomodulatory synovial effects (ARISE study). *Ann Rheumat Dis.* (2009) 68:563–8. doi: 10.1136/ard.2007.081950

67. Das S, Vital EM, Horton S, Bryer D, El-Sherbiny Y, Rawstron AC, et al. Abatacept or tocilizumab after rituximab in rheumatoid arthritis? An exploratory study suggests non-response to rituximab is associated with persistently high IL-6 and better clinical response to IL-6 blocking therapy. *Ann Rheum Dis.* (2014) 73:999–12. doi: 10.1136/annrheumdis-2013-204417

68. Thurlings RM, Wijbrands CA, Mebus RE, Cantaert T, Dinant HJ, van der Pouw-Kraan TC, et al. Synovial lymphoid neogenesis does not define a specific clinical rheumatoid arthritis phenotype. *Arthritis Rheumatol.* (2008) 58:1582–9. doi: 10.1002/art.23505
75. Takemura S, Klimiuk PA, Braun A, Goronzy JJ, Weyand CM. T cell activation in rheumatoid synovium is B cell dependent. *J Immunol*. (2001) 167:4710–8. doi: 10.4049/jimmunol.167.8.4710

76. O’shea JJ, Park H, Pesu M, Borie D, Changelian P. New strategies for immunosuppression: interfering with cytokines by targeting the Jak/Stat pathway. *Curr Opin Rheumatol*. (2005) 17:305–11. doi: 10.1097/01.bor.0000160781.07174.db

77. O’sullivan LA, Liongue C, Lewis RS, Stephenson SE, Ward AC. Cytokine receptor signaling through the Jak–Stat–Socs pathway in disease. *Mol Immunol*. (2007) 44:2497–506. doi: 10.1016/j.molimm.2006.11.025

78. Fleischmann R, Kremer J, Cush J, Schulze-Koops H, Connell CA, Bradley JD, et al. Placebo-controlled trial of tofacitinib monotherapy in rheumatoid arthritis. *N Engl J Med*. (2012) 367:495–507. doi: 10.1056/NEJMoa1109071

79. Furumoto Y, Gadina M. The arrival of JAK inhibitors: advancing the treatment of immune and hematologic disorders. *BioDrugs*. (2013) 27:431–8. doi: 10.1007/s40259-013-0040-7

80. Smolen JS, Genovese MC, Takeuchi T, Hyslop DL, Macias WL, Rooney T, et al. Safety profile of baricitinib in patients with active rheumatoid arthritis with over 2 years median time in treatment. *J Rheumatol*. (2018) 46(1):7-18. doi: 10.3899/jrheum.171361

81. Noss EH, Brenner MB. The role and therapeutic implications of fibroblast-like synoviocytes in inflammation and cartilage erosion in rheumatoid arthritis. *Immunol Rev*. (2008) 223:252–70. doi: 10.1111/j.1600-065X.2008.00648.x

82. Buch MH, Pavitt S, Parmar M, Emery P. Creative trial design in RA: optimizing patient outcomes. *Nat Rev Rheumatol*. (2013) 9:183–94. doi: 10.1038/nrrheum.2013.5

83. Donlin LT, Rao DA, Wei K, Slowikowski K, McGeehy MJ, Turner JD, et al. High dimensional analyses of cells dissociated from cryopreserved synovial tissue. *Arth Res Ther*. (2018) 20:139. doi: 10.1101/284844

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Ouboussad, Barska, Melville and Buch. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.