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# Unveiling the macrophage dynamics in osteoarthritic joints: From inflammation to therapeutic strategies

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Osteoarthritis (OA) is an incurable, painful, and debilitating joint disease affecting over 500 million people worldwide. The OA joint tissues are infiltrated by various immune cells, particularly macrophages, which are able to induce or perpetuate inflammation. Notably, synovitis and its macrophage component represent a target of interest for developing treatments. In this review, we describe the latest advances in understanding the heterogeneity of macrophage origins, phenotypes, and functions in the OA joint and the effect of current symptomatic therapies on these cells. We then highlight the therapeutic potential of anticytokines/chemokines, nano- and microdrug delivery, and future strategies to modulate macrophage functions in OA.

**Keywords:** macrophages; immune cells; synovitis; inflammation; osteoarthritis; joint; personalized therapy

## Introduction

Osteoarthritis (OA) was long considered as a degenerative disease affecting the cartilage but is now increasingly recognized as a heterogeneous, multifaceted joint disease with multitissue involvement of varying severity. OA is primarily characterized by the degradation of articular cartilage, varying degrees of synovial inflammation, and osteophyte formation, which lead to



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**Anaïs Cardon** obtained her PhD in immunology in 2022 at Nantes University, France. She investigated the role of autoreactive CD4<sup>+</sup> T cells in autoimmune hepatitis at the Center for Research in Transplantation and Translational Immunology. Since 2023, she has been working as a postdoctoral research fellow at RMeS, and her project aims to develop innovative tools to target synovial immune cells for the development of novel personalized therapies.



**Marie-Astrid Boutet** is a head of the 'Immune cells and osteoarthritis personalized therapies -StratOA-' at RMeS in Nantes, France, and a visiting fellow at the Centre for Experimental Medicine and Rheumatology, Queen Mary University of London, UK. Her current research projects aim to understand how synovial tissue and its immune cell infiltration drive joint inflammation in the context of arthritic diseases to improve disease understanding and patient care and to set the basis for clinically relevant personalized therapeutic approaches.

the loss of joint function. Patients with OA were initially categorized into three major clinical phenotypes related to the most important risk factors of the disease: age-, trauma-, and obesity-associated. However, recent studies have highlighted a greater heterogeneity of these phenotypes, including metabolic syndrome- and inflammatory-driven ones, highlighting that OA is a phenotypically heterogeneous disease driven by a variety

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of etiological and pathological factors.<sup>(p1),(p2)</sup> Despite a considerable number of clinical trials testing various therapeutic strategies for patients with OA, to date, there is still no curative treatment able to reduce or halt the progression of the disease. In this context, the treatments are limited to symptomatic care, including the administration of analgesics and anti-inflammatory drugs, and, in the late stages, joint replacement is often inevitable. Consequently, given its constantly increasing prevalence, OA imposes a major and growing individual-level and socioeconomic burden.<sup>(p3)</sup>

Over the course of OA, the synovial tissue undergoes major changes even before the onset of cartilage degradation.<sup>(p4)</sup> Synovitis, and in particular its macrophage infiltrate, correlates with osteophyte formation, OA-related pain, disease severity, and loss of joint function,<sup>(p4),(p5)</sup> thus representing a target of interest for the development of treatments. In addition, resident and infiltrating macrophages in other joint tissues, such as the infrapatellar Hoffa's fat pad (IFP) in the knee, might also influence joint homeostasis by affecting intercompartmental cross-talk, notably through the production of secreted mediators. In summary, each joint tissue exerts multidirectional and multimodal effects on the surrounding ones, thereby establishing a network of finely tuned interactions that can be disturbed in the onset and development of chronic inflammatory joint diseases, including OA.

Here, we will highlight the latest evidence related to joint macrophage diversity in OA, considering their various locations, origins, and phenotypes. We will then focus on the beneficial and deleterious functions of macrophages in maintaining joint homeostasis, with particular emphasis on the roles played by these cells in orchestrating the communication between synovial tissue, cartilage, and IFP, notably through the production of soluble mediators released into the synovial fluid. We will then report the current knowledge about the influence of OA symptomatic treatments on macrophage biology and provide an updated view of the development of macrophage-targeted therapeutic strategies in the context of OA.

## Synovial macrophages in OA: a very heterogeneous group of cells

### *Distribution of macrophages within the OA synovial tissue*

The synovial tissue delimitates the joint capsule and is composed of two layers: the lining layer or intima that is directly in contact with the joint cavity and the synovial fluid, and the sublining layer or subintima. As presented in [Figure 1](#), in homeostatic conditions, the intima is composed of one or two layers of macrophages and fibroblast-like synoviocytes (FLSs) (previously called type A and type B synoviocytes, respectively), whereas the subintima harbors a variety of resident cells such as macrophages, fibroblasts, endothelial cells, and adipocytes. Beyond its key role in regulating joint homeostasis and inflammation, as further developed in the next sections, one of the significant functions of synovial tissue is the production of the main components of the synovial fluid [e.g. hyaluronic acid (HA), lubricin] by FLSs to lubricate the joint and provide nutrients to chondrocytes.<sup>(p6)</sup>

The synovial tissue might present with major changes during chronic joint diseases, such as OA. Indeed, the OA synovium

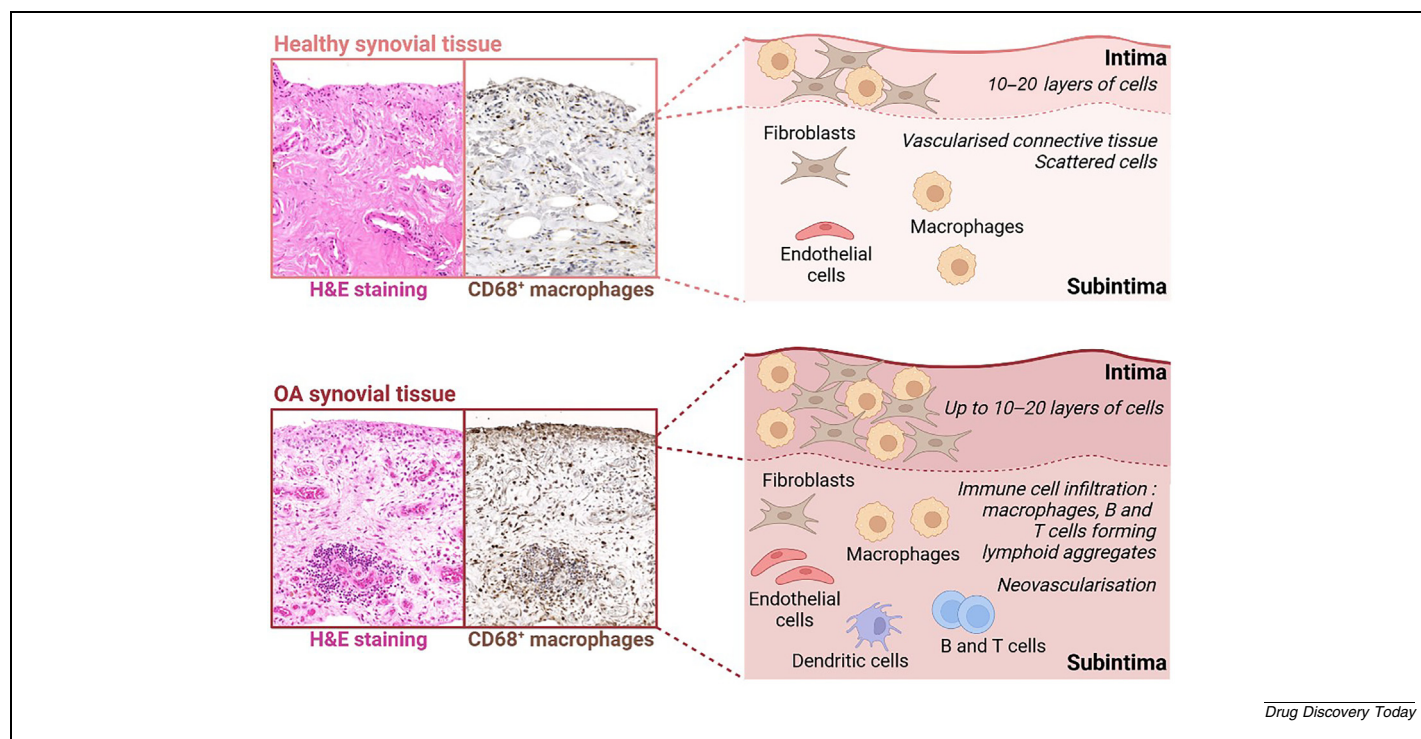
often presents with a thickening of the intima and an infiltration of the subintima by distinct innate and adaptive immune cell types (e.g. inflammatory macrophages, B cells, or T cells) in variable amounts. These changes are summarized in [Figure 1](#). Currently, the histological characterization of OA synovial tissue is mainly performed using the Krenn's score,<sup>(p7)</sup> which is based on the assessment of three reproducible histopathological criteria among all types of arthropathies, including OA and rheumatoid arthritis (RA): (1) the thickness of the synovial intimal layer, (2) the cellular density of the stroma, and (3) the presence of a lymphocytic infiltrate. In 2019, we contributed to the improvement of this score by defining the IMMunologic SYnovitis sCore (IMSYC), which quantifies the cellular infiltrates of macrophages, T/B cells, endothelial cells, and proliferative cells.<sup>(p8)</sup>

In the context of RA, a prototypic synovitis-driven disease, the presence of three synovial pathotypes based on the quantity and distribution of synovial immune cells has been described. Specifically, the lympho–myeloid pathotype is characterized by the presence of B and T cells forming highly organized ectopic lymphoid structures (ELSs), plasma cells, and macrophages in the subintima; the diffuse-myeloid pathotype is marked by a predominant infiltration of CD68<sup>+</sup> synovial cells (monocytes, macrophages) and diffuse lymphocytes without distinctly organized follicular structures; and the pauci-immune is defined by a scant immune cell infiltrate and a fibroblast-rich stroma. In RA, these pathotypes are associated with disease severity, predict the radiographic progression of the disease, and enable stratification of the clinical response to therapy.<sup>(p9),(p10),(p11)</sup> Importantly, our recent work demonstrates that these pathotypes are also observed in the OA synovium and could contribute to the stratification of patients with OA, as they reflect different levels of peripheral inflammation.<sup>(p2)</sup>

Within the OA synovium, macrophages and CD4<sup>+</sup> T cells represent the main immune cell populations.<sup>(p12),(p13)</sup> Recently, we further highlighted that the level of synovial immune cell infiltration in the pauci-immune and diffuse-myeloid pathotypes appears similar between OA and RA. However, the T cells and subintimal macrophages are present in higher proportions in RA tissues presenting with a lympho–myeloid pathotype but not in OA displaying the same pathotype.<sup>(p2)</sup> Notably, the spatial distribution of macrophages is not homogeneous within the synovial tissue, allowing us to differentiate OA from RA.<sup>(p14)</sup> These differences in cell distribution might have pathophysiological implications in the development and perpetuation of chronic joint diseases, as well as the response to treatment, and future high-throughput studies assessing the spatial diversity of synovial macrophages will help address this burning question.<sup>(p15)</sup>

### *Origin of synovial macrophages*

Macrophages are innate immune cells that play a major role in the initiation, perpetuation, and resolution of inflammation. In every organ, including the synovium, macrophages with different origins can be distinguished. Tissue-resident macrophages, such as osteoclasts, microglial cells, and Kupffer cells, mostly derive from progenitors present in the tissues since the early stages of embryonic development, marked by successive hematopoietic waves involving different embryonic precursors.<sup>(p16),(p17)</sup> Tissue-resident macrophages differ from infiltrating



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**FIGURE 1**

Histological and schematic representation of the cellular distribution in healthy and osteoarthritic (OA) synovial tissue. In the left panel, sections of either healthy or OA synovial tissues were stained with hematoxylin and eosin (H&E) and CD68 (monocyte/macrophage) antibodies. Synovial tissue is composed of two main layers: the intima and the subintima. The main changes occurring in the synovium during OA (e.g. thickening of the intima, immune cell infiltration, neovascularization) are represented in the right panel.

macrophages that derive from circulating monocytes, whose precursor cells are hematopoietic stem cells in the bone marrow.<sup>(p16),(p18)</sup> It is now clear that macrophages represent a highly diverse group of cells, exhibiting various phenotypes and functions and characterized by significant plasticity, which are involved in maintaining homeostasis as well as in chronic inflammation or disease development.<sup>(p19)</sup>

Currently, although most of our knowledge about synovial macrophage origins comes from fate-mapping studies performed in the context of rodent RA models, it is likely that a similar cellular dynamism exists in OA. Recently, using cell lineage-tracing approaches in a serum transfer-induced arthritis model, Culemann and colleagues highlighted the existence of a dynamic continuum in the differentiation of resident macrophages.<sup>(p20)</sup> In particular, the authors revealed that a pool of proliferative macrophages, expressing a high level of major histocompatibility complex class II (MHC-II) molecules, is present in the synovial tissue independently of the migration of infiltrating macrophages towards the inflamed tissue. These cells can differentiate into interstitial and intimal macrophages displaying proresolving and anti-inflammatory properties (e.g. efferocytosis and the production of anti-inflammatory mediators, respectively).

In addition to tissue-resident macrophages, circulating monocytes travel within the bloodstream and infiltrate inflamed tissues, where they differentiate into macrophages that can adopt various phenotypes under the control of growth factors,

cytokines, and other molecules secreted in the microenvironment. Three main populations of monocytes have been distinguished based on their expression of the markers cluster of differentiation (CD)14 and CD16: classical CD14<sup>+</sup> CD16<sup>-</sup> monocytes (Ly6C<sup>high</sup> in mice), nonclassical CD14<sup>dim</sup> CD16<sup>+</sup> monocytes (Ly6C<sup>low</sup> in mice), and intermediate CD14<sup>+</sup> CD16<sup>+</sup> monocytes (Ly6C<sup>+</sup> TremL4<sup>+</sup> in mice). Each population has a different role in homeostasis and disease.<sup>(p21),(p22)</sup> Nonclassical Ly6C<sup>-</sup> monocytes, known as ‘patroller monocytes’, are involved in the repair process and production of vascular endothelial growth factors (VEGFs), and have been shown to be specifically responsible for the development of inflammatory arthritis in mice by migrating to the synovium and differentiating into proinflammatory macrophages.<sup>(p23)</sup> Macrophages might then be capable of adapting their phenotype *in situ* and participating in the resolution of the disease. In addition, in a collagenase-induced mouse model of OA, increased migration of Ly6C<sup>+</sup> monocytes to the synovium has been observed under the influence of S100A8 and S100A9 alarmins released as a consequence of cartilage degradation.<sup>(p24)</sup>

Interestingly, in OA, *in vitro* experiments have demonstrated amplified migration of classical CD14<sup>+</sup> CD16<sup>-</sup> monocytes, as compared with intermediate CD14<sup>+</sup> CD16<sup>+</sup> monocytes and nonclassical CD14<sup>dim</sup> CD16<sup>+</sup>, subjected to conditioning medium obtained from the OA synovium in a Transwell migration assay.<sup>(p25)</sup>



### Diversity of macrophage identities in joint tissues

Macrophages have long been divided into two subcategories based on the T helper (Th)1/Th2 dichotomic classification: M1 (classically activated macrophages known to exert proinflammatory functions) and M2 (alternatively activated macrophages that broadly act to maintain or restore tissue homeostasis).<sup>(p26)</sup> M1 macrophages adopt their phenotype in response to bacterial lipopolysaccharides (LPSs) or Th1 cytokines, such as tumor necrosis factor (TNF)- $\alpha$  and interferon (IFN). They in turn produce numerous proinflammatory mediators, such as interleukin (IL)-1 $\beta$ , IL-6, IL-12, IL-23, and cyclooxygenase (COX), which ultimately participate in recruiting and activating other immune cells. M2 macrophages are polarized by Th2-type cytokines such as IL-4 and IL-13, and produce anti-inflammatory cytokines such as transforming growth factor (TGF)- $\beta$  and IL-10, thus playing a role in maintaining tissue homeostasis.<sup>(p27)</sup> M1 and M2 macrophages are *in vitro*-differentiated subtypes often obtained under nonphysiological conditions that, although useful for addressing several mechanistic questions, represent only the two extremes of a larger spectrum of possible phenotypes. Although the M1/M2-restricting paradigm once provided an instructive framework, this simplistic classification has notably been challenged in the context of human diseases, including OA. Thanks to the development of high-throughput transcriptomic and proteomic analyses, a broader synovial macrophage repertoire including multiple phenotypes and a wide diversity of functions has recently been revealed, especially in RA.<sup>(p18)</sup> The use of standardized protocols for tissue collection by minimally invasive ultrasound-guided biopsies, digestion, and analysis pipelines<sup>(p28),(p29)</sup> has contributed to the deeper characterization of this complex synovial state.<sup>(p30)</sup> For instance, Alivernini and colleagues have described nine synovial macrophage subpopulations,<sup>(p31),(p32)</sup> including five tissue-resident and four tissue-infiltrating macrophages. In the OA synovium, similar high-throughput techniques have identified 12 distinct cell types with significant phenotypic diversity among macrophages.<sup>(p33),(p34)</sup> Interestingly, Zhang and collaborators recently identified 15 synovial myeloid populations, including 4 resident macrophage populations, 6 infiltrating monocyte populations, and 5 dendritic cell populations, present in patients with OA and RA using a multiomic single-cell cellular indexing of transcriptomes and epitopes (CITE)-seq approach.<sup>(p35)</sup> Among these populations, three tissue-resident macrophage populations expressing the phagocytic markers CD206, myeloid-epithelial-reproductive tyrosine kinase (MERTK), and CD163 were abundant in OA tissues, although they were less represented compared with in healthy tissues.<sup>(p31)</sup> Some similarities observed between OA and RA myeloid populations suggest that their participation in disease progression might be more synovitis-driven rather than disease specific, but further comparative studies are required. In a murine post-traumatic OA model, nine subtypes of macrophages with specific transcriptomic signatures, very similar to the ones reported in human arthritic synovium,<sup>(p31)</sup> have been described, emphasizing the strong correlation between human and mouse macrophage populations.<sup>(p36)</sup>

Beyond the variety of phenotypes displayed by macrophages, these cells also present with significant plasticity throughout

their lifetime, which is enhanced in chronic inflammation.<sup>(p19)</sup> Several epigenetic mechanisms, such as histone modification, DNA methylation, and microRNA (miRNA) expression, participate in regulating the identity and functions of macrophages.<sup>(p19),(p37)</sup> Infiltrating macrophages have an increased plasticity compared with resident macrophages, influenced by both the surrounding inflammation and the macrophage niche within the tissue.<sup>(p38)</sup> A recent preprint from Knights and colleagues highlighted that synovial macrophage phenotypes vary over time, mimicking early and late stages of the disease in a post-traumatic mouse model of OA.<sup>(p39)</sup> This underscores the need for longitudinal studies of changes in OA synovial immune cells and macrophages across disease stages.

Although similarities between mouse and human synovial macrophage phenotypes have been noted,<sup>(p20)</sup> the significant disparities observed in other contexts must be considered when translating *in vivo* findings to clinical application.<sup>(p40)</sup>

### Role of macrophages in maintaining, restoring, and disturbing synovial and joint homeostasis

#### Roles of synovial macrophages in the steady state and OA

Given their significant phenotypic diversity, the roles played by macrophages within the synovial tissue are numerous.<sup>(p18)</sup> Under physiological conditions, resident macrophages are the main macrophage population in the synovial intima and subintima, participating in the maintenance of tissue homeostasis primarily by sensing their microenvironment.<sup>(p41)</sup> Their protective role is notably illustrated by their phagocytic activity, which clears potential cartilage debris and apoptotic cells<sup>(p42)</sup> through the expression of markers such as T-cell immunoglobulin and mucin domain containing 4 (TIMD-4) or MERTK. They also secrete immunoregulatory cytokines such as IL-4, IL-13, TGF- $\beta$ , IL-10, and IL-1 receptor antagonist (IL-1Ra).<sup>(p43)</sup> Culemann and colleagues demonstrated the role of intimal-resident CX3C motif chemokine receptor 1 (CX3CR1)<sup>+</sup> macrophages in the maintenance of an immune barrier separating the synovium from the synovial cavity in mice.<sup>(p20)</sup> Consequently, given their distribution throughout the tissue and their complementarity, macrophages, together with FLSS, play a central and crucial protective role in maintaining and restoring joint health.<sup>(p44)</sup> However, in contrast to their protective functions, macrophage populations can have deleterious effects within the OA synovium.<sup>(p5)</sup> Macrophages, especially infiltrating macrophages, are major producers of proinflammatory mediators such as cytokines (e.g. TNF- $\alpha$ , IL-1 $\beta$ , IFN- $\gamma$ ), chemokines [e.g. C-X-C motif chemokine ligand 8 (CXCL8)], and alarmins (e.g. S100A12), which promote the recruitment of immune cells (e.g. T and B cells) and contribute to the thickening of the synovial tissue. Importantly, the activation of inflammatory pathways in macrophages also actively promotes the restoration of homeostasis by fostering the subsequent production of proresolving or anti-inflammatory mediators,<sup>(p45)</sup> as recently reviewed in the context of OA.<sup>(p46)</sup>

Macrophages also produce metalloproteinases and reactive oxygen species (ROS) involved in cartilage and extracellular matrix (ECM) degradation,<sup>(p33)</sup> which contribute to overall joint inflammation, as further described in Section 3.2. During OA,

activated macrophages might also express MHC-II molecules, highlighting their potential role in antigen presentation and T cell-mediated immune responses, which could contribute to the chronicity of synovial inflammation.<sup>(p18),(p34)</sup> Although OA is not recognized as an autoimmune disease, understanding the interplay between innate and immune cells in its pathophysiology requires further investigation. Indeed, the presence of autoantibodies against methionine adenosyltransferase 2 beta (MAT2B)<sup>(p47)</sup> or post-translationally modified proteins<sup>(p48)</sup> in the sera of patients with OA suggests a possible contribution of self-reactivity mechanisms to its pathogenesis, and further studies investigating the role of macrophages in this process need to be conducted.

Macrophages have also been shown to be key players in the resolution of inflammation in various contexts, not only by dampening inflammation but also by actively clearing dead cells through efferocytosis and by promoting tissue repair.<sup>(p49)</sup> In an obesity-associated OA model, the intra-articular injection of growth arrest-specific gene 6 (Gas6) can prevent disease progression by restoring the phagocytic capacity of macrophages.<sup>(p50)</sup> Moreover, proresolving mediators produced by macrophages, such as resolvin D1, can reduce the severity of synovitis and cartilage degradation *in vivo*.<sup>(p51)</sup>

Altogether, the multiple functions of synovial macrophages influence the physiological and pathophysiological processes happening in the OA joint.

#### *Role of macrophages from distinct joint structures in OA development*

In addition to their major role within the synovial tissue, macrophages are also present in other joint compartments, such as the IFP in the knee and the synovial fluid, and can strongly influence the phenotype of many other cells through complex mechanisms.<sup>(p52)</sup> Indeed, intertissue communication plays a role in maintaining homeostasis. As summarized in [Figure 2](#) and [Table 1](#) and further described in the following sections, in the context of OA, synovial macrophages interact with the other main cell types of the joint, thereby contributing to disease progression.

#### **Role of synovial macrophages in cartilage breakdown in OA**

Bidirectional communication between cartilage and synovium, notably through chondrocytes and macrophages, has been extensively studied in recent years.<sup>(p5)</sup> The ECM plays a key role in the joint by absorbing and distributing mechanical stress and ensuring joint homeostasis,<sup>(p53),(p54)</sup> and macrophages play a key role in the maintenance of ECM integrity ([Table 1](#)). In particular, three-dimensional cocultures of chondrocytes or cartilage explants and macrophages highlight a direct influence of macrophage phenotypes/secretory function on chondrocytes, and vice versa.<sup>(p55)</sup> Of note, the involvement of matrix metalloproteinases (MMPs) and structural proteins, such as flightless I, has been recognized in driving these autocrine/paracrine effects through the activation of specific intracellular signaling pathways (e.g. mitogen-activated protein kinases (MAPKs)/extracellular signal-regulated kinase (ERK) 1/2).<sup>(p54),(p56)</sup>

In 2020, using single-cell RNA sequencing of paired cartilage and synovium samples from human patients with OA, Chou and colleagues revealed the presence of 12 and 7 distinct cell populations in these tissues, respectively.<sup>(p34)</sup> The authors

highlighted that the largest variety of cytokines and growth factors predicted to be associated with the development of OA are expressed by synovial cells, particularly macrophages, when compared with chondrocytes. Their work used single-cell analyses to establish a predictive model of intercellular communication between cartilage and synovial cells through the synovial fluid, and provided crucial information for the development of novel therapeutic strategies for patients with OA through the targeting of specific pathogenic cell populations (e.g. macrophages, dendritic cells) and their associated surface proteins [e.g. Toll-like receptor (TLR)] or mediators (e.g. IL-6, TNF).

#### **Involvement of IFP macrophages in the development of knee OA**

The IFP is an intracapsular but extrasynovial structure localized in the knee joint that stabilizes and protects the joints from mechanical damage.<sup>(p57)</sup> Richly vascularized and innervated, the IFP also exerts a key endocrine function through the expression of adipokines (e.g. adiponectin, leptin) and is capable of secreting various mediators, such as cytokines and growth factors, as summarized in a recent review ([Table 1](#)).<sup>(p58)</sup> The IFP is therefore involved in the development of several joint diseases.<sup>(p59)</sup> Notably, its size has been shown to increase with age, and it is associated with the development of OA.<sup>(p60)</sup> Moreover, as the synovial tissue and fat pad form the same anatomofunctional unit,<sup>(p61)</sup> the influence of soluble molecules produced by synovial tissue on IFP, and vice-versa, should be considered.

Paired analysis of synovium and fat pad samples from patients with OA by flow cytometry revealed similar percentages of macrophages, mast cells, and T cells within both tissues.<sup>(p12)</sup> Notably, the immune cells infiltrating the IFP present a specific inflammatory phenotype distinguishing them from those infiltrating other types of adipose tissue.<sup>(p62)</sup> Indeed, the IFP contains more macrophages when compared with subcutaneous adipose tissue samples. These macrophages express the surface marker CD206, which typically identifies macrophages with a repair phenotype, and conditioned medium prepared with OA IFP is able to inhibit the catabolic process observed in cartilage explant.<sup>(p63)</sup> Recent evidence highlights a crucial detrimental role for apolipoprotein E (APOE) signaling in synovial and IFP fibroblasts and macrophages in cartilage catabolism.<sup>(p64)</sup>

The precise functions and origins of IFP macrophages, as well as the communication between IFP immune cells and the surrounding tissues affected by OA, remain poorly understood and should be addressed in future studies.

#### **Pivotal role of the synovial fluid in the OA joint**

The synovial fluid lubricates the joint, helps to overcome mechanical constraints, and notably provides the nutrients necessary for the survival of chondrocytes.<sup>(p65),(p66)</sup> Diverse immune cells can also be found in the synovial fluid, and their numbers vary during joint inflammation.<sup>(p67),(p68)</sup> Monocytes, macrophages, and neutrophils are the most abundant leukocytes in the synovial fluid. In particular, compared with peripheral blood, OA synovial fluid is enriched in proinflammatory CD14<sup>+</sup>CD16<sup>+</sup> cells.<sup>(p68)</sup> In the same study, the authors reported that all macrophages found in the synovial fluid express a significant level of major histocompatibility complex (MHC)-II molecules, reflecting an activated phenotype.<sup>(p68)</sup>

Recently, four distinct subgroups of patients with OA have been described based on the proportions and features of immune

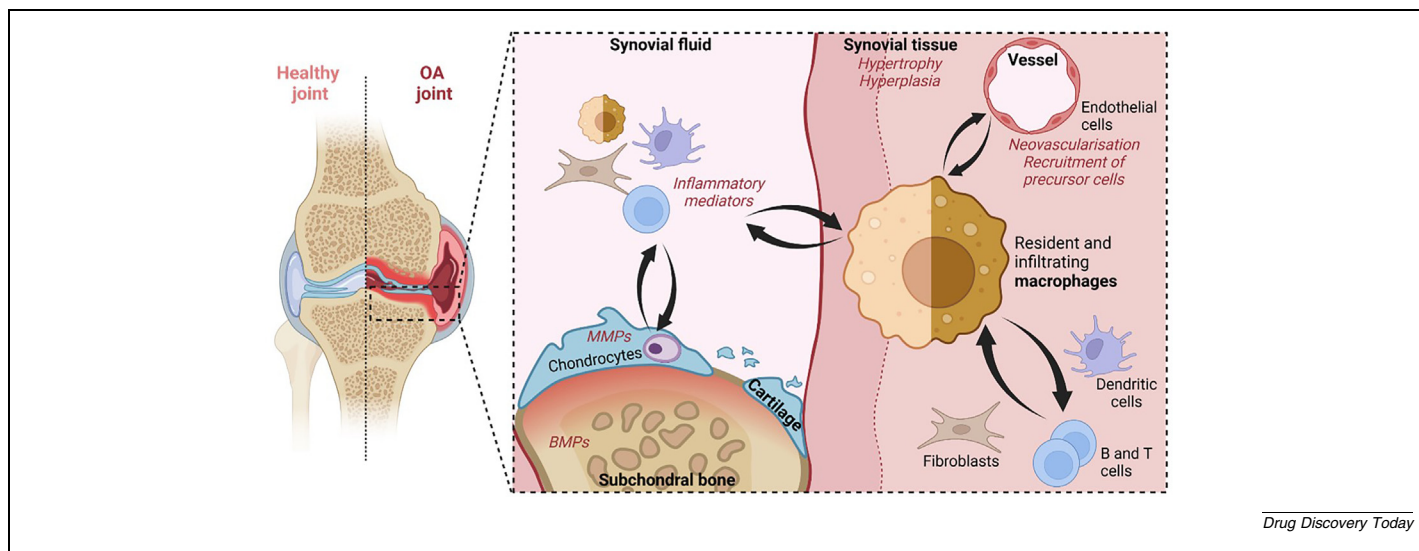


FIGURE 2

Central role of synovial macrophages in osteoarthritis (OA) pathophysiology. Resident and infiltrating macrophages within the synovial tissue can interact with different cells within the same compartment (e.g. fibroblasts, dendritic cells, B and T cells) or within other compartments of the joint (e.g. synovial fluid, cartilage, subchondral bone, the infrapatellar Hoffa's fat pad – not represented). Macrophages participate in the recruitment of other immune cells, enhancing synovial hypertrophy and hyperplasia, and contribute to the production of inflammatory mediators such as cytokines or matrix metalloproteinases (MMPs) and bone morphogenic proteins (BMPs).

TABLE 1

#### Summary of the main roles of macrophages in the joint environment described in recent studies

Macrophage location	Target tissues	Roles	Mediators and related pathways	Refs
Synovium	Cartilage	Promotion of ECM degradation and chondrocytes hypertrophy	TNF, MMP9, and MMP14 via ATF2, STAT3, and NF-κB pathways	(p54)
IFP	Cartilage	Inhibition of catabolic processes	Flightless I and TLR4 via MAPK, ERK1, and ERK2 pathways	(p56)
IFP	Cartilage	Inhibition of cartilage anabolism and promotion of cartilage catabolism	NO, MMP1	(p63)
IFP	All joint tissues	Secretion of proinflammatory mediators and adipokines	APOE through modulation of COL2A1, ACAN, SOX9, MMP, and ADAMTS expression	(p64)
IFP	All joint tissues	Secretion of proinflammatory mediators and adipokines	IL-6, adipin, adiponectin, visfatin	(p62)
Synovial fluid	Cartilage, synovium	Secretion of proinflammatory mediators	VCAM-1, ICAM1, MMP3, TIMP-1, VEGF, MCP-1	(p70)

Abbreviations, ACAN, aggrecan; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; APOE, apolipoprotein E; ATF2, activating transcription factor 2; ERK, extracellular signal-regulated kinase; ICAM1, intercellular adhesion molecule-1; IL, interleukin; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa B; NO, nitric oxide; SOX, SRY-box transcription factor; STAT, signal transducer and activator of transcription; TIMP-1, tissue inhibitor matrix metalloproteinase-1; TLR, Toll-like receptor; TNF, tumor necrosis factor; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor.

cells present in their synovial fluid.<sup>(p69)</sup> Interestingly, these immune phenotypes, also described as 'iPhens', were related to the clinical trajectory at 3–6 months postsampling. In particular, low expression of C-X-C motif chemokine receptor 4 (CXCR4) and C-C chemokine receptor type 7 (CCR7) in synovial fluid macrophages correlates with clinical improvement of OA, considering the evolution of pain and joint effusion.<sup>(p69)</sup> In addition, several synovial fluid biomarkers, described in Table 1, have recently been associated with synovial inflammation and radiographic severity (Kellgren–Lawrence grading) in OA.<sup>(p70)</sup> Of note, these biomarkers significantly correlate with the synovial fluid levels of soluble CD14 and CD163 shed by activated macro-

phages. Another study, investigating cell phenotypes from matched samples of synovial tissue and fluid, highlighted that, although macrophages were predominant in the OA synovial tissue, neutrophils constituted the predominant cell type in the synovial fluid. These cells produce mediators specifically associated with radiographic OA severity and have been recognized as being capable of identifying patients with a higher risk of disease progression (Table 1).<sup>(p67)</sup>

Altogether, the results presented in the above section highlight the importance of considering phenotypic changes in all joint compartments, including the synovial fluid, synovial tissue, and the IFP, to design novel and effective personalized therapies for OA.

## Targeting macrophages in OA: current and future perspectives

### Impact of current symptomatic treatments on joint macrophages

Thanks to their great plasticity, macrophages rapidly react to various stimuli arising from their environment. Among these stimuli, the effects of therapeutic agents used in the clinic to reduce joint symptoms in OA have been reported.

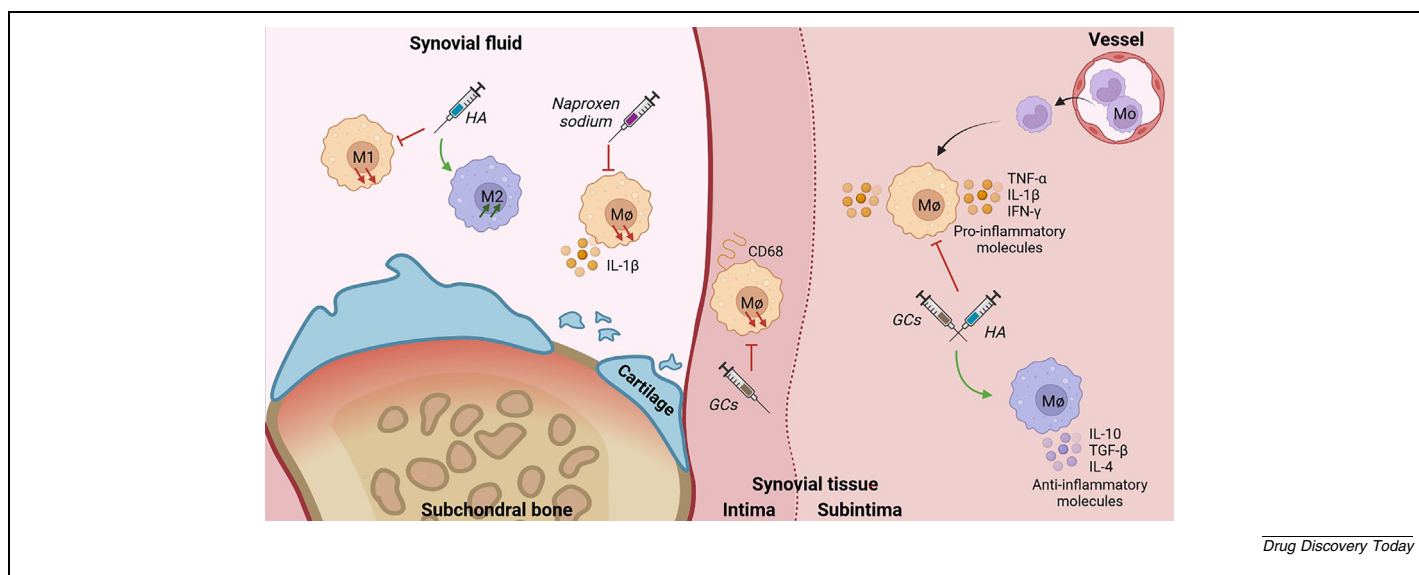
#### Nonsteroidal anti-inflammatory drugs (NSAIDs)

Although there is currently no treatment available to slow down or stop the progression of OA, symptomatic therapeutic strategies are offered to patients to reduce pain and joint discomfort. First-line therapies consist of the administration of oral analgesics (e.g. acetaminophen) and oral or topical NSAIDs. Several studies have identified a role for NSAIDs in modulating macrophage phenotypes in the context of OA. Using a cell type-specific depletion strategy, a study using a rat model of OA identified macrophages as major players in the persistence of pain in animals resistant to COX inhibitors.<sup>(p71)</sup> Two other studies also showed that chronic COX inhibition could promote macrophage differentiation into a proinflammatory phenotype.<sup>(p72),(p73)</sup> Despite the effect of NSAIDs on macrophages and monocytes, these molecules have only a moderate impact on pain relief, as patients are affected by a persistent pain regardless of treatment monitoring, and they might even increase the risk of adverse events like gastrointestinal toxicity or cardiovascular, renal, and hepatic disorders.<sup>(p74)</sup>

#### Corticosteroids and HA

Second-line therapies are conditionally recommended by clinicians, mostly for patients with moderate-to-severe OA, and consist of intra-articular injections of corticosteroids or HA (also

called hyaluronan).<sup>(p75)</sup> In patients with OA, glucocorticoid (GC) injections have been shown to impact the number of synovial macrophages and their secretion of proinflammatory and procatabolic molecules when compared with placebo injections.<sup>(p76)</sup> However, in that study, the authors demonstrated that the number of intimal macrophages was reduced by only 30% following intra-articular injection of GCs. *In vitro*, dexamethasone, a synthetic GC, has also been shown to exert an anti-inflammatory effect on OA synovial explants through modulation of the balance between the expression of pro- and anti-inflammatory cytokines by macrophages.<sup>(p77)</sup> However, despite their beneficial effect on inflammation, intra-articular injections of GCs fail to induce long-lasting pain relief, with an average of 4 weeks of pain alleviation seen after administration.<sup>(p78)</sup> Among the treatments proposed for patients with OA to reduce joint discomfort, intra-articular HA injections are often performed.<sup>(p79)</sup> HA is a natural component of the synovial fluid and cartilage, and it contributes to the viscoelastic properties of the synovial fluid.<sup>(p80)</sup> *In vitro*, HA impedes the proinflammatory response of macrophages through its binding to the CD44 receptor and inhibition of nuclear factor kappa B (NF- $\kappa$ B) signaling, leading to decreased production of proinflammatory cytokines and increased production of anti-inflammatory cytokines.<sup>(p81),(p82)</sup> Following intra-articular injections of HA in patients with OA, IL-6 expression is downregulated in the synovial fluid, potentially due to decreased secretion by macrophages.<sup>(p81),(p83),(p84)</sup> Nonetheless, because of potentially underestimated placebo effects, the efficacy of HA injections specifically for the treatment of OA is still controversial.<sup>(p85)</sup> The Osteoarthritis Research Society International (OARSI) 2019 guidelines only recommend HA injections in very specific conditions, highlighting that they



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**FIGURE 3**

Influence of current symptomatic treatments on macrophages in the osteoarthritic (OA) joint. Schematic representation summarizing the currently limited *in vitro*, *ex vivo*, and *in vivo* data available in the literature about the effects of nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and hyaluronic acid (HA) on macrophages in OA. Overall, these drugs act as anti-inflammatory therapies, dampening proinflammatory M1 macrophages and favoring proresolving M2 macrophages. Abbreviations: GCs, glucocorticoids; HA, hyaluronic acid; Mø, macrophages; Mo, monocytes. ↗↗, increased number; ↘↘, decreased number; →, stimulation; —|, inhibition.



might have beneficial effects on pain at 12 weeks post-treatment.<sup>(p75)</sup> Overall, although NSAIDs, corticosteroids, and HA injections exhibit relative effectiveness in OA pain alleviation, to our knowledge, there is no extensive study recapitulating the precise consequences of these treatments on macrophages. Considering the difficulty of precisely studying local and systemic molecular mechanisms triggered by these therapeutics in macrophages, most studies are conducted in *in vitro* settings; however, given the plasticity of macrophages, which is dependent on their complex *in vivo* microenvironment, these results should be interpreted very carefully. Figure 3 presents a summary of the effects of NSAIDs, GCs, and HA on macrophage phenotypes within the joint. Although there is currently no efficient and targeted therapy for the treatment of OA, these studies highlight the broad effects of current symptomatic drugs on joint macrophages. Considering the recognized pathogenic role of macrophages in the OA joint, it is now important to develop relevant cell-specific therapeutic strategies for the treatment of this disease. These strategies should not only induce anti-inflammatory mechanisms but also exert effects in proresolutive and proanabolic pathways.

#### *Development of novel therapeutic strategies to target macrophage subpopulations*

Because of (i) the absence of treatments able to sustainably restore normal joint function, (ii) the moderate efficacy of current symptomatic therapeutic strategies for OA, (iii) their short-lived effect, and (iv) the potential for adverse events, it is crucial to highlight the importance of developing targeted and personalized therapies. Over the last few years, different innovative therapeutic approaches targeting various aspects of OA pathophysiology, such as cartilage degradation, bone remodeling, or inflammation (e.g. MMP inhibitors, anticytokine antibodies, cell therapy), have been tested.<sup>(p86)</sup>

#### **Cytokines and chemokine targeting**

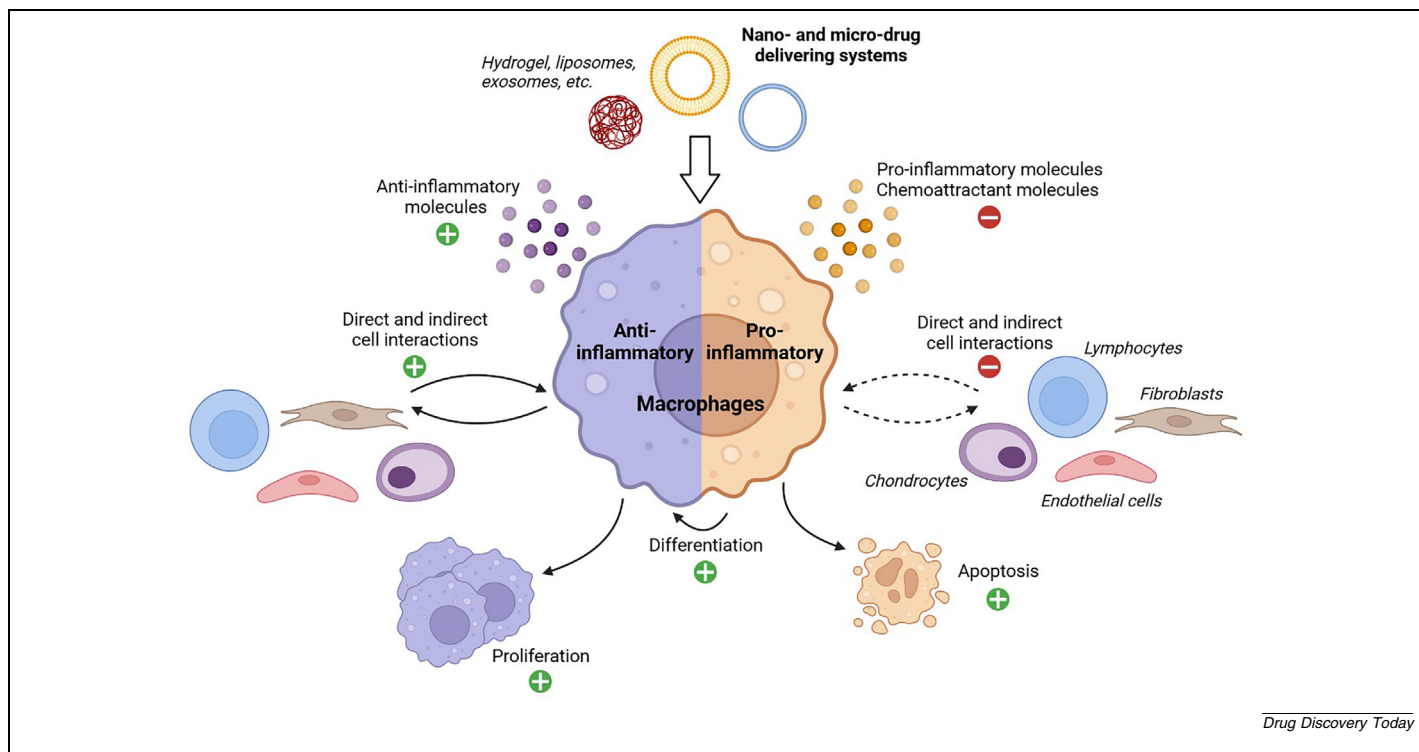
Synovial macrophages are able to produce proinflammatory cytokines (e.g. IL-1 $\beta$ , IL-6, TNF- $\alpha$ ) that contribute to cartilage degradation and bone alterations.<sup>(p87)</sup> These cytokines can be specifically targeted using neutralizing antibodies that are routinely and efficiently used in the clinic for the treatment of autoimmune or inflammatory diseases. However, anti-IL-1 antibodies demonstrate minimal-to-no effects with respect to OA pain relief, with limited clinical benefit.<sup>(p88),(p89),(p90)</sup> Indeed, a nonsignificant decrease in the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score in lutikizumab (anti-IL-1 $\alpha/\beta$ )-treated patients with OA, comparable with the placebo group, has been observed and is characterized by an absence of synovitis improvement.<sup>(p89)</sup> Similarly, strategies targeting the IL-6 signaling pathway have not proven their efficacy in patients with OA, as shown in a recent clinical trial using tocilizumab, an anti-IL-6R antibody, in patients with hand OA.<sup>(p91),(p92)</sup> Additionally, anti-TNF- $\alpha$  therapies do not significantly impact pain relief or OA symptoms, including synovitis.<sup>(p93),(p94)</sup> However, interestingly, a subgroup of etanercept-treated patients with OA, characterized by a symptomatic erosive inflammatory disease and persistent pain despite NSAID treatment, presented with fewer bone marrow lesions on magnetic resonance imaging (MRI) and more radiographic remodeling, as well as better pain

alleviation, compared with the placebo group after 1 year of follow-up, calling for further investigations to confirm these observations.<sup>(p94)</sup> This suggests that subgroups of patients could respond differently to anticytokine therapies and underlines that primary outcomes should be carefully defined according to the timing of interim and final analyses when designing clinical trials in the context of OA. Finally, a recent clinical trial explored the efficacy of otilimab, an antigranulocyte-macrophage colony-stimulating factor (GM-CSF) antibody, for the treatment of hand OA. They observed a nonsignificant reduction in pain and functional impairment compared with the placebo group.<sup>(p94)</sup> Therefore despite the demonstrated involvement of cytokine-mediated inflammatory process in OA pathogenesis, and encouraging preclinical data, these approaches have limited effects with respect to pain relief and no significant effects on OA manifestations, such as synovitis and bone remodeling. The failure of specific cytokine-targeting strategies highlights that these cytokines are not solely responsible for OA pathophysiology but take part in larger pathological mechanisms involving various players, such as cytokines, degradation products, or cells. Of note, although these biological drugs target the expression of specific cytokines, they broadly modulate their expression without cell or tissue-specific actions, whereas OA perturbations are mainly local. Given the dual and sometimes divergent effects of the same cytokine in different cell types or distinct niches, these results overall underline the importance of a better understanding of OA's pathophysiological mechanisms to develop targeted and personalized treatments.

#### **Nano- and microdrug delivery strategies**

The use of nano- and microdrug delivery strategies (e.g. nanoparticles, liposomes, exosomes) has already proven effective in pre-clinical models for the treatment of arthritis.<sup>(p18),(p95)</sup> Notably, several studies demonstrate that using such delivery strategies could improve the efficacy of NSAIDs<sup>(p96)</sup> and GCs<sup>(p97)</sup> and induce the inhibition of OA progression and the restoration of cartilage integrity in preclinical OA models. Recently, the US Food and Drug Administration approved intra-articular injections of Zilretta, i.e. triamcinolone acetonide (TCA) combined with poly(lactic-co-glycolic acid) (PLGA) microspheres, for the management of knee OA. This drug demonstrated the ability to improve the management of OA-related pain and stiffness compared with TCA alone or placebo.<sup>(p98)</sup>

Nano- and microdrug delivery strategies have also been used to demonstrate the relevance of novel therapeutic targets, favoring a proresolving phenotype in macrophages. Nanoliposomes loaded with resolvin D1, a proresolution mediator,<sup>(p99)</sup> were able to enhance the recruitment of M2-like macrophages at the site of inflammation in a surgically induced OA mouse model and reduced the proinflammatory activity of synovial macrophages.<sup>(p100)</sup> Likewise, the use of zeolitic imidazolate framework-8 (ZIF-8)-modified nanoparticles with anti-CD16/32 antibody, targeting M1 macrophages, promoted a beneficial proinflammatory/proresolving macrophage balance in an anterior cruciate ligament transection (ACLT)-induced OA mouse model.<sup>(p101)</sup> Another study, using immunoglobulin G-opsonized nanoparticles coupled with the anti-inflammatory agent berberine, showed preferential internalization by M1 macrophages *in vitro*, leading to polarization towards an M2

**FIGURE 4**

Potential macrophage-specific targeting strategies for the development of personalized osteoarthritis (OA) treatments. This figure presents the pathways and mechanisms that could be targeted in specific macrophage subpopulations using nano- and microdrug delivery systems. +, indicates stimulation; –, inhibition.

phenotype. *In vivo*, the same nanoparticles were used in an ACLT-induced OA rat model and induced cartilage repair.<sup>(p102)</sup> Interestingly, lipoplex-mediated targeting of macrophages demonstrated successful silencing of the expression of several genes in the context of inflammatory arthritis,<sup>(p103),(p104)</sup> leading to reduced inflammation and the prevention of joint destruction. Considering the presence of proinflammatory infiltrating macrophages in the OA synovium, such strategies could also be tested in the context of OA.

Although encouraging, the results presented above highlight some limitations that might limit the clinical translation of these strategies in OA. First, proinflammatory cytokine inhibitors are clinically relevant as they have previously been tested and approved for the treatment of other joint diseases, but they lack the capacity for local and specific targeting of macrophages, increasing associated adverse events and reducing the specificity of their actions, respectively. Therapeutic strategies aiming to improve the tissue targeting of free drug therapies rely on already-tested and short-lasting treatments for OA (e.g., NSAIDs). These strategies have been shown to enhance the delivery and effects of these therapies. However, so far, they have only been tested in early-stage OA animal models following prophylactic administration. Moreover, although being locally injected, they require repeated injections over several weeks to be efficient with respect to OA-related pain and symptoms, which could represent an impediment for clinical translation, and no data are available

on their long-lasting effect after withdrawal. These strategies often rely on the intrinsic capacity of macrophages for phagocytosis, and improvement of delivery systems should allow more precise targeting of macrophage subpopulations in the future.

#### **Future directions for macrophages targeting**

As described above, OA synovial macrophages present a multitude of phenotypes and functions, and their specific targeting could help reduce tissue inflammation and prevent joint damage, as well as favoring proresolving mechanisms. As summarized in Figure 4, such strategies could either induce apoptosis or inhibit the action of deleterious macrophage populations, specifically interfering with their secretion of proinflammatory and/or chemoattractant cytokines and chemokines, or conversely stimulate one or several beneficial macrophage populations by promoting their production of anti-inflammatory molecules or their proliferation or differentiation.<sup>(p5)</sup>

Future therapies should also consider the stage of the disease. Indeed, macrophage infiltration and phenotype might change according to the dynamics of the disease (e.g. disease stage, flare). Although a significant link between radiographic OA severity and macrophage activation is recognized,<sup>(p105)</sup> their temporal phenotype changes have never been investigated in human patients with OA across disease stages. In rat and murine OA models, rapid activation of macrophages is observed in the early stage of the disease and is sustained over time.<sup>(p106),(p107)</sup> Interestingly, the proportion of macrophages changes throughout dis-

ease development and, specifically, the proportion of proresolutive CD206<sup>+</sup> macrophages was significantly increased in the synovium at a late stage in a post-traumatic OA model.<sup>(p108)</sup> As detailed throughout this review, macrophage populations are heterogeneous and can present various and distinct phenotypes and associated functions in OA. Further studies are therefore needed to better understand their dynamics across OA stages and would benefit the development of novel personalized therapies to be administered at the most appropriate time point of the disease course.

Overall, given the interplay between and within joint tissues in the physiological and inflammatory conditions presented herein, the effect of targeting specific macrophage subpopulations in OA should be carefully assessed in preclinical studies using appropriate models. Moreover, according to the potential difference in epigenetic, pathophysiological, or anatomical changes between knee and hip OA development, and the current lack of knowledge about joint-specific macrophage phenotypes, further studies are needed to address this pressing question.

Defining relevant subgroups of patients, and associated preclinical models, for a precise stratification of cellular and molecular pathway involvement would be greatly beneficial for the design of targeted therapeutic strategies for OA.

## Concluding remarks

In the context of OA, synovial macrophages have been associated with disease severity and pain. Considerable advances have recently been made in the field of rheumatic diseases, highlighting the diversity in macrophage phenotypes, origins, and functions. Moreover, recent improvements in nano- and microdrug delivery systems have contributed to increased efficacy of pre-existing OA symptomatic treatments in preclinical models. However, further research is needed to better stratify patients with OA according to the diverse pathophysiological mechanisms underlying the disease's various symptomatic presentations, with a focus on macrophage subsets within and between the different joint tissues. This will facilitate the design of relevant targeted

and personalized therapeutic strategies for OA, helping to assign 'the right drug to the right patient' for efficient management of this complex disease.

## Authors' contributions

All authors have made substantial contribution to the work and approved it for publication. **N.G.**, **A.C.**, and **M.A.B.** wrote the manuscript and created the figures; **B.L.G.** and **J.G.** critically revised the whole manuscript.

## Data availability

No data was used for the research described in the article.

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## Declarations of interest

The authors declare that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## References

1. Deveza LA, Nelson AE, Loeser RF. Phenotypes of osteoarthritis: current state and future implications. *Clin Exp Rheumatol*. 2019;37(Suppl 120):64–72.
2. Boutet MA et al. Comparative analysis of late-stage rheumatoid arthritis and osteoarthritis reveals shared histopathological features. *Osteoarthritis Cartilage*. 2024;32:166–176. <https://doi.org/10.1016/j.joca.2023.10.009>.
3. Hunter DJ, March L, Chew M. Osteoarthritis in 2020 and beyond: a Lancet Commission. *Lancet*. 2020;396:1711–1712. [https://doi.org/10.1016/S0140-6736\(20\)32230-3](https://doi.org/10.1016/S0140-6736(20)32230-3).
4. Mathiessen A, Conaghan PG. Synovitis in osteoarthritis: current understanding with therapeutic implications. *Arthritis Res Ther*. 2017;19:18. <https://doi.org/10.1186/s13075-017-1229-9>.
5. Zhang H, Cai D, Bai X. Macrophages regulate the progression of osteoarthritis. *Osteoarthritis Cartilage*. 2020;28:555–561. <https://doi.org/10.1016/j.joca.2020.01.007>.
6. Bustamante MF, Garcia-Carbonell R, Whisenant KD, Guma M. Fibroblast-like synoviocyte metabolism in the pathogenesis of rheumatoid arthritis. *Arthritis Res Ther*. 2017;19:110. <https://doi.org/10.1186/s13075-017-1303-3>.
7. Krenn V et al. Synovitis score: discrimination between chronic low-grade and high-grade synovitis. *Histopathology*. 2006;49:358–364. <https://doi.org/10.1111/j.1365-2559.2006.02508.x>.
8. Najm A et al. IMSYC immunologic synovitis score: a new score for synovial membrane characterization in inflammatory and non-inflammatory arthritis. *Joint Bone Spine*. 2019;86:77–81. <https://doi.org/10.1016/j.jbspin.2018.04.004>.
9. Humby F et al. Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. *Ann Rheum Dis*. 2019;78:761–772. <https://doi.org/10.1136/annrheumdis-2018-214539>.
10. Lliso-Ribera G et al. Synovial tissue signatures enhance clinical classification and prognostic/treatment response algorithms in early inflammatory arthritis and predict requirement for subsequent biological therapy: results from the pathobiology of early arthritis cohort (PEAC). *Ann Rheum Dis*. 2019;78:1642–1652. <https://doi.org/10.1136/annrheumdis-2019-215751>.
11. Lewis MJ et al. Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes. *Cell Rep*. 2019;28:2455–2470.e5. <https://doi.org/10.1016/j.celrep.2019.07.091>.
12. Klein-Wieringa IR et al. Inflammatory cells in patients with endstage knee osteoarthritis: a comparison between the synovium and the infrapatellar fat pad. *J Rheumatol*. 2016;43:771–778. <https://doi.org/10.3899/jrheum.151068>.
13. Xie J, Huang Z, Yu X, Zhou L, Pei F. Clinical implications of macrophage dysfunction in the development of osteoarthritis of the knee. *Cytokine Growth Factor Rev*. 2019;46:36–44. <https://doi.org/10.1016/j.cytogfr.2019.03.004>.

14. Mucke J et al. Inhomogeneity of immune cell composition in the synovial sublining: linear mixed modelling indicates differences in distribution and spatial decline of CD68+ macrophages in osteoarthritis and rheumatoid arthritis. *Arthritis Res Ther*. 2016;18:170. <https://doi.org/10.1186/s13075-016-1057-3>.
15. De Lima J et al. Spatial mapping of rheumatoid arthritis synovial niches reveals specific macrophage networks associated with response to therapy. *bioRxiv*. 2023. <https://doi.org/10.1101/2023.10.20.563040>. Published online October 22, 2023.
16. Ginhoux F, Guillems M. Tissue-resident macrophage ontogeny and homeostasis. *Immunity*. 2016;44:439–449. <https://doi.org/10.1016/j.immuni.2016.02.024>.
17. Perdiguero EG, Geissmann F. The development and maintenance of resident macrophages. *Nat Immunol*. 2016;17:2–8. <https://doi.org/10.1038/ni.3341>.
18. Boutet MA et al. Novel insights into macrophage diversity in rheumatoid arthritis synovium. *Autoimmun Rev*. 2021;20, 102758. <https://doi.org/10.1016/j.autrev.2021.102758>.
19. Locati M, Curtale G, Mantovani A. Diversity, mechanisms, and significance of macrophage plasticity. *Annu Rev Pathol*. 2020;15:123–147. <https://doi.org/10.1146/annurev-pathmechdis-012418-012718>.
20. Culemann S et al. Locally renewing resident synovial macrophages provide a protective barrier for the joint. *Nature*. 2019;572:670–675. <https://doi.org/10.1038/s41586-019-1471-1>.
21. Kapellos TS et al. Human monocyte subsets and phenotypes in major chronic inflammatory diseases. *Front Immunol*. 2019;10:2035. <https://doi.org/10.3389/fimmu.2019.02035>.
22. Narasimhan PB, Marcovecchio P, Hamers AAJ, Hedrick CC. Nonclassical monocytes in health and disease. *Annu Rev Immunol*. 2019;37:439–456. <https://doi.org/10.1146/annurev-immunol-042617-053119>.
23. Misharin AV et al. Nonclassical Ly6C<sup>+</sup> monocytes drive the development of inflammatory arthritis in mice. *Cell Rep*. 2014;9:591–604. <https://doi.org/10.1016/j.celrep.2014.09.032>.
24. Cremers NAJ et al. S100A8/A9 increases the mobilization of pro-inflammatory Ly6Chigh monocytes to the synovium during experimental osteoarthritis. *Arthritis Res Ther*. 2017;19:217. <https://doi.org/10.1186/s13075-017-1426-6>.
25. Saffery NSI et al. Typical response of CD14+CD16<sup>+</sup> monocyte to knee synovial derived mediators as a key target to overcome the onset and progression of osteoarthritis. *Front Med (Lausanne)*. 2022;9, 904721. <https://doi.org/10.3389/fmed.2022.904721>.
26. Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. *J Pathol*. 2013;229:176–185. <https://doi.org/10.1002/path.4133>.
27. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest*. 2012;122:787–795. <https://doi.org/10.1172/JCI59643>.
28. Donlin LT et al. Methods for high-dimensional analysis of cells dissociated from cryopreserved synovial tissue. *Arthritis Res Ther*. 2018;20:139. <https://doi.org/10.1186/s13075-018-1631-y>.
29. Kelly S 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 Rheum Dis*. 2015;74:611–617. <https://doi.org/10.1136/annrheumdis-2013-204603>.
30. Zhang F et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat Immunol*. 2019;20:928–942. <https://doi.org/10.1038/s41590-019-0378-1>.
31. Alivernini S et al. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. *Nat Med*. 2020;26:1295–1306. <https://doi.org/10.1038/s41591-020-0939-8>.
32. Kurowska-Stolarska M, Alivernini S. Synovial tissue macrophages in joint homeostasis, rheumatoid arthritis and disease remission. *Nat Rev Rheumatol*. 2022;18:384–397. <https://doi.org/10.1038/s41584-022-00790-8>.
33. Wood MJ et al. Macrophage proliferation distinguishes 2 subgroups of knee osteoarthritis patients. *JCI Insight*. 2019;4, e125325. <https://doi.org/10.1172/jci.insight.125325>.
34. Chou CH et al. Synovial cell cross-talk with cartilage plays a major role in the pathogenesis of osteoarthritis. *Sci Rep*. 2020;10:10868. <https://doi.org/10.1038/s41598-020-67730-v>.
35. Zhang F et al. Deconstruction of rheumatoid arthritis synovium defines inflammatory subtypes. *Nature*. 2023;623:616–624. <https://doi.org/10.1038/s41586-023-06708-y>.
36. Sebastian A et al. Single-cell RNA-Seq reveals changes in immune landscape in post-traumatic osteoarthritis. *Front Immunol*. 2022;13, 938075. <https://doi.org/10.3389/fimmu.2022.938075>.
37. Daskalaki MG, Tsatsanis C, Kampranis SC. Histone methylation and acetylation in macrophages as a mechanism for regulation of inflammatory responses. *J Cell Physiol*. 2018;233:6495–6507. <https://doi.org/10.1002/jcp.26497>.
38. Guillems M, Svedberg FR. Does tissue imprinting restrict macrophage plasticity? *Nat Immunol*. 2021;22:118–127. <https://doi.org/10.1038/s41590-020-00849-2>.
39. Knights AJ, Farrell EC, Ellis OM, Song MJ, Appleton CT, Maerz T. Synovial macrophage diversity and activation of M-CSF signaling in post-traumatic osteoarthritis. *bioRxiv*. 2023. <https://doi.org/10.1101/2023.10.03.559514>. Published online October 5, 2023.
40. Human and mouse peritoneal macrophages and dendritic cells compared. *Nat Immunol* 2024;25:17–8. <https://doi.org/10.1038/s41590-023-01709-5>.
41. Mosser DM, Hamidzadeh K, Goncalves R. Macrophages and the maintenance of homeostasis. *Cell Mol Immunol*. 2021;18:579–587. <https://doi.org/10.1038/s41423-020-00541-3>.
42. Orlowsky EW, Kraus VB. The role of innate immunity in osteoarthritis: when our first line of defense goes on the offensive. *J Rheumatol*. 2015;42:363–371. <https://doi.org/10.3899/jrheum.140382>.
43. Mushenkova NV, Nikiforov NG, Shakhpazyan NK, Orekhova VA, Sadykhov AN, Orekhov AN. Phenotype diversity of macrophages in osteoarthritis: implications for development of macrophage modulating therapies. *Int J Mol Sci*. 2022;23:8381. <https://doi.org/10.3390/ijms23158381>.
44. Kemble S, Croft AP. Critical role of synovial tissue-resident macrophage and fibroblast subsets in the persistence of joint inflammation. *Front Immunol*. 2021;12, 715894. <https://doi.org/10.3389/fimmu.2021.715894>.
45. Saas P, Vetter M, Maraux M, Bonnefoy F, Perruche S. Resolution therapy: harnessing efferocytic macrophages to trigger the resolution of inflammation. *Front Immunol*. 2022;13:1021413. <https://doi.org/10.3389/fimmu.2022.1021413>.
46. Leite CBG, Merkely G, Charles JF, Lattermann C. From inflammation to resolution: specialized pro-resolving mediators in posttraumatic osteoarthritis. *Curr Osteoporos Rep*. 2023;21:758–770. <https://doi.org/10.1007/s11914-023-00817-3>.
47. Camacho-Encina M, Balboa-Barreiro V, Rego-Perez I, Picchi F, VanDuin J, Qiu J, et al. Discovery of an autoantibody signature for the early diagnosis of knee osteoarthritis: data from the osteoarthritis initiative. *Ann Rheum Dis*. 2019;78:1699–1705. <https://doi.org/10.1136/annrheumdis-2019-215325>.
48. Xie X et al. Auto-antibodies to post-translationally modified proteins in osteoarthritis. *Osteoarthritis Cartilage*. 2021;29:924–933. <https://doi.org/10.1016/j.joca.2021.03.008>.
49. Rodriguez-Morales P, Franklin RA. Macrophage phenotypes and functions: resolving inflammation and restoring homeostasis. *Trends Immunol*. 2023;44:986–998. <https://doi.org/10.1016/j.it.2023.10.004>.
50. Yao Z et al. Down-regulated GAS6 impairs synovial macrophage efferocytosis and promotes obesity-associated osteoarthritis. *eLife*. 2023;12, e83069. <https://doi.org/10.7554/eLife.83069>.
51. Sun AR et al. Pro-resolving lipid mediator ameliorates obesity induced osteoarthritis by regulating synovial macrophage polarisation. *Sci Rep*. 2019;9:426. <https://doi.org/10.1038/s41598-018-36909-9>.
52. Han S. Osteoarthritis year in review 2022: biology. *Osteoarthritis Cartilage*. 2022;30:1575–1582. <https://doi.org/10.1016/j.joca.2022.09.003>.
53. Vincent TL, McClurg O, Troeberg L. The extracellular matrix of articular cartilage controls the bioavailability of pericellular matrix-bound growth factors to drive tissue homeostasis and repair. *Int J Mol Sci*. 2022;23:6003. <https://doi.org/10.3390/ijms23116003>.
54. Hamasaki M et al. Transcriptional profiling of murine macrophages stimulated with cartilage fragments revealed a strategy for treatment of progressive osteoarthritis. *Sci Rep*. 2020;10(1):7558. <https://doi.org/10.1038/s41598-020-64515-1>.
55. Samavedi S, Diaz-Rodriguez P, Erndt-Marino JD, Hahn MS. A three-dimensional chondrocyte-macrophage coculture system to probe inflammation in experimental osteoarthritis. *Tissue Eng Part a*. 2017;23:101–114. <https://doi.org/10.1089/ten.tea.2016.0007>.
56. Ebata T et al. Flightless I is a catabolic factor of chondrocytes that promotes hypertrophy and cartilage degeneration in osteoarthritis. *iScience*. 2021;24, 102643. <https://doi.org/10.1016/j.isci.2021.102643>.
57. Macchi V et al. The infrapatellar adipose body: a histotopographic study. *Cells Tissues Organs*. 2016;201:220–231. <https://doi.org/10.1159/000442876>.
58. Braun S et al. The corpus adiposum infrapatellare (Hoffa's fat pad)—the role of the infrapatellar fat pad in osteoarthritis pathogenesis. *Biomedicines*. 2022;10:1071. <https://doi.org/10.3390/biomedicines10051071>.



59. Wang MG, Seale P, Furman D. The infrapatellar fat pad in inflammaging, knee joint health, and osteoarthritis. *NPJ Aging*. 2024;10:34. <https://doi.org/10.1038/s41514-024-00159-z>.
60. Chuckpaiwong B, Charles HC, Kraus VB, Guilak F, Nunley JA. Age-associated increases in the size of the infrapatellar fat pad in knee osteoarthritis as measured by 3T MRI. *J Orthop Res*. 2010;28:1149–1154. <https://doi.org/10.1002/jor.21125>.
61. Macchi V et al. The infrapatellar fat pad and the synovial membrane: an anatomo-functional unit. *J Anat*. 2018;233:146–154. <https://doi.org/10.1111/joa.12820>.
62. Klein-Wieringa IR et al. The infrapatellar fat pad of patients with osteoarthritis has an inflammatory phenotype. *Ann Rheum Dis*. 2011;70:851–857. <https://doi.org/10.1136/ard.2010.140046>.
63. Bastiaansen-Jenniskens YM et al. Infrapatellar fat pad of patients with end-stage osteoarthritis inhibits catabolic mediators in cartilage. *Ann Rheum Dis*. 2012;71:288–294. <https://doi.org/10.1136/ard.2011.153858>.
64. Tang S et al. Single-cell atlas of human infrapatellar fat pad and synovium implicates APOE signaling in osteoarthritis pathology. *Sci Transl Med*. 2024;16:eadf4590. <https://doi.org/10.1126/scitranslmed.adf4590>.
65. Radin EL. Synovial fluid as a lubricant. *Arthritis Rheum*. 1968;11:693–695. <https://doi.org/10.1002/art.1780110513>.
66. Levick JR. Microvascular architecture and exchange in synovial joints. *Microcirculation*. 1995;2:217–233. <https://doi.org/10.3109/10739689509146768>.
67. Hsueh MF, Zhang X, Wellman SS, Bolognesi MP, Kraus VB. Synergistic roles of macrophages and neutrophils in osteoarthritis progression. *Arthritis Rheumatol*. 2021;73:89–99. <https://doi.org/10.1002/art.41486>.
68. Gómez-Arístizábal A, Gandhi R, Mahomed NN, Marshall KW, Viswanathan S. Synovial fluid monocyte/macrophage subsets and their correlation to patient-reported outcomes in osteoarthritic patients: a cohort study. *Arthritis Res Ther*. 2019;21(1):26. <https://doi.org/10.1186/s13075-018-1798-2>.
69. Trajerova M, Kriegova E, Mikulkova Z, Savara J, Kudelka M, Gallo J. Knee osteoarthritis phenotypes based on synovial fluid immune cells correlate with clinical outcome trajectories. *Osteoarthritis Cartilage*. 2022;30:1583–1592. <https://doi.org/10.1016/j.joca.2022.08.019>.
70. Haraden CA, Huebner JL, Hsueh MF, Li YJ, Kraus VB. Synovial fluid biomarkers associated with osteoarthritis severity reflect macrophage and neutrophil related inflammation. *Arthritis Res Ther*. 2019;21(1):146. <https://doi.org/10.1186/s13075-019-1923-x>.
71. Sakurai Y et al. Contribution of synovial macrophages to rat advanced osteoarthritis pain resistant to cyclooxygenase inhibitors. *Pain*. 2019;160:895–907. <https://doi.org/10.1097/j.pain.0000000000001466>.
72. Na YR, Yoon YN, Son D, Jung D, Gu GJ, Seok SH. Consistent inhibition of cyclooxygenase drives macrophages towards the inflammatory phenotype. *PLoS One*. 2015;10:e0118203.
73. Hull MA et al. Paracrine cyclooxygenase-2 activity by macrophages drives colorectal adenoma progression in the *Apc*<sup>Min/+</sup> mouse model of intestinal tumorigenesis. *Sci Rep*. 2017;7:6074. <https://doi.org/10.1038/s41598-017-06253-5>.
74. Wang Y et al. Relative safety and efficacy of topical and oral NSAIDs in the treatment of osteoarthritis: a systematic review and meta-analysis. *Medicine*. 2022;101:e30354.
75. Bannuru RR et al. OARSI guidelines for the non-surgical management of knee, hip, and polyarticular osteoarthritis. *Osteoarthritis Cartilage*. 2019;27:1578–1589. <https://doi.org/10.1016/j.joca.2019.06.011>.
76. Young L et al. Effects of intraarticular glucocorticoids on macrophage infiltration and mediators of joint damage in osteoarthritis synovial membranes: findings in a double-blind, placebo-controlled study. *Arthritis Rheum*. 2001;44:343–350. [https://doi.org/10.1002/1529-0131\(200102\)44:2<343::AID-ANR52>3.0.CO;2-O](https://doi.org/10.1002/1529-0131(200102)44:2<343::AID-ANR52>3.0.CO;2-O).
77. Utomo L, van Osch GJVM, Bayon Y, Verhaar JAN, Bastiaansen-Jenniskens YM. Guiding synovial inflammation by macrophage phenotype modulation: an in vitro study towards a therapy for osteoarthritis. *Osteoarthritis Cartilage*. 2016;24:1629–1638. <https://doi.org/10.1016/j.joca.2016.04.013>.
78. Savvidou O, Milonaki M, Goumenos S, Flevas D, Papagelopoulos P, Moutsatsou P. Glucocorticoid signaling and osteoarthritis. *Mol Cell Endocrinol*. 2019;480:153–166. <https://doi.org/10.1016/j.mce.2018.11.001>.
79. Pereira TV et al. Viscosupplementation for knee osteoarthritis: systematic review and meta-analysis. *Br Med J*. 2022;378, e069722. <https://doi.org/10.1136/bmj-2022-069722>.
80. Cai Z, Zhang H, Wei Y, Wu M, Fu A. Shear-thinning hyaluronan-based fluid hydrogels to modulate viscoelastic properties of osteoarthritis synovial fluids. *Biomater Sci*. 2019;7:3143–3157. <https://doi.org/10.1039/C9BM00298G>.
81. Lee BM, Park SJ, Noh I, Kim CH. The effects of the molecular weights of hyaluronic acid on the immune responses. *Biomater Res*. 2021;25:27. <https://doi.org/10.1186/s40824-021-00228-4>.
82. Qadri M, Almadani S, Jay GD, Elsaid KA. Role of CD44 in regulating TLR2 activation of human macrophages and downstream expression of proinflammatory cytokines. *J Immunol*. 2018;200:758–767. <https://doi.org/10.4049/jimmunol.1700713>.
83. Jin L, Xu K, Liang Y, Du P, Wan S, Jiang C. Effect of hyaluronic acid on cytokines and immune cells change in patients of knee osteoarthritis. *BMC Musculoskelet Disord*. 2022;23:812. <https://doi.org/10.1186/s12891-022-05767-y>.
84. Vincent HK, Percival SS, Conrad BP, Seay AN, Montero C, Vincent KR. Hyaluronic acid (HA) viscosupplementation on synovial fluid inflammation in knee osteoarthritis: a pilot study. *Open Orthop J*. 2013;7:378–384. <https://doi.org/10.2174/1874325001307010378>.
85. Bowman S, Awad ME, Hamrick MW, Hunter M, Fulzele S. Recent advances in hyaluronic acid based therapy for osteoarthritis. *Clin Transl Med*. 2018;7:6. <https://doi.org/10.1186/s40169-017-0180-3>.
86. Amirsaadat S, Amirzad H, Hashemihesar R, Zarghami N. An update on the effect of intra-articular intervention strategies using nanomaterials in osteoarthritis: possible clinical application. *Front Bioeng Biotechnol*. 2023;11, 1128856. <https://doi.org/10.3389/fbioe.2023.1128856>.
87. Thomson A, Hilkens CMU. Synovial macrophages in osteoarthritis: the key to understanding pathogenesis? *Front Immunol*. 2021;12, 678757. <https://doi.org/10.3389/fimmu.2021.678757>.
88. Cohen SB et al. A randomized, double-blind study of AMG 108 (a fully human monoclonal antibody to IL-1R1) in patients with osteoarthritis of the knee. *Arthritis Res Ther*. 2011;13:R125. <https://doi.org/10.1186/ar3430>.
89. Fleischmann RM et al. A phase II trial of lutikizumab, an anti-interleukin-1 $\alpha/\beta$  dual variable domain immunoglobulin, in knee osteoarthritis patients with synovitis. *Arthritis Rheumatol*. 2019;71:1056–1069. <https://doi.org/10.1002/art.40840>.
90. Yu L, Luo R, Qin G, Zhang Q, Liang W. Efficacy and safety of anti-interleukin-1 therapeutics in the treatment of knee osteoarthritis: a systematic review and meta-analysis of randomized controlled trials. *J Orthop Surg Res*. 2023;18:100. <https://doi.org/10.1186/s13018-023-03590-2>.
91. Richette P et al. Efficacy of tocilizumab in patients with hand osteoarthritis: double blind, randomised, placebo-controlled, multicentre trial. *Ann Rheum Dis*. 2021;80:349–355. <https://doi.org/10.1136/annrheumdis-2020-218547>.
92. Sanchez-Lopez E, Coras R, Torres A, Lane NE, Guma M. Synovial inflammation in osteoarthritis progression. *Nat Rev Rheumatol*. 2022;18:258. <https://doi.org/10.1038/s41584-022-00749-9>.
93. Aitken D et al. A randomised double-blind placebo-controlled crossover trial of HUMira (adalimumab) for erosive hand Osteoarthritis – the HUMOR trial. *Osteoarthritis Cartilage*. 2018;26:880–887. <https://doi.org/10.1016/j.joca.2018.02.899>.
94. Kloppenburg M et al. Etanercept in patients with inflammatory hand osteoarthritis (EHOA): a multicentre, randomised, double-blind, placebo-controlled trial. *Ann Rheum Dis*. 2018;77:1757–1764. <https://doi.org/10.1136/annrheumdis-2018-213202>.
95. Li S, Su J, Cai W, Liu JX. Nanomaterials manipulate macrophages for rheumatoid arthritis treatment. *Front Pharmacol*. 2021;12, 699245. <https://doi.org/10.3389/fphar.2021.699245>.
96. Kang ML, Kim JE, Im GI. Thermoresponsive nanospheres with independent dual drug release profiles for the treatment of osteoarthritis. *Acta Biomater*. 2016;39:65–78. <https://doi.org/10.1016/j.actbio.2016.05.005>.
97. Seo BB et al. Injectable polymeric nanoparticle hydrogel system for long-term anti-inflammatory effect to treat osteoarthritis. *Bioact Mater*. 2022;7:14–25. <https://doi.org/10.1016/j.bioactmat.2021.05.028>.
98. Paik J, Duggan ST, Keam SJ. Triamcinolone acetonide extended-release: a review in osteoarthritis pain of the knee. *Drugs*. 2019;79:455–462. <https://doi.org/10.1007/s40265-019-01083-3>.
99. Schmid M, Gemperle C, Rimann N, Hersberger M. Resolvin D1 Polarizes primary human macrophages toward a proresolution phenotype through GPR32. *J Immunol*. 2016;196:3429–3437. <https://doi.org/10.4049/jimmunol.1501701>.
100. Dravid AA, Dhanabalan KM, Agarwal S, Agarwal R. Resolvin D1-loaded nanoliposomes promote M2 macrophage polarization and are effective in the treatment of osteoarthritis. *Bioeng Transl Med*. 2022;7, e10281. <https://doi.org/10.1002/btm2.10281>.
101. Zhou F et al. Modified ZIF-8 nanoparticles attenuate osteoarthritis by reprogramming the metabolic pathway of synovial macrophages. *ACS Appl Mater Interfaces*. 2020;12:2009–2022. <https://doi.org/10.1021/acsami.9b16327>.

102. Kou L et al. Oposonized nanoparticles target and regulate macrophage polarization for osteoarthritis therapy: a trapping strategy. *J Control Release*. 2022;347:237–255. <https://doi.org/10.1016/j.jconrel.2022.04.037>.
103. Ammari M et al. Delivery of miR-146a to Ly6C<sup>high</sup> monocytes inhibits pathogenic bone erosion in inflammatory arthritis. *Theranostics*. 2018;8:5972–5985. <https://doi.org/10.7150/thno.29313>.
104. Pr sumey J et al. Nicotinamide phosphoribosyltransferase/visfatin expression by inflammatory monocytes mediates arthritis pathogenesis. *Ann Rheum Dis*. 2013;72:1717–1724. <https://doi.org/10.1136/annrheumdis-2012-202403>.
105. Kraus VB et al. Direct in vivo evidence of activated macrophages in human osteoarthritis. *Osteoarthritis Cartilage*. 2016;24:1613–1621. <https://doi.org/10.1016/j.joca.2016.04.010>.
106. Piscaer TM et al. Imaging of activated macrophages in experimental osteoarthritis using folate-targeted animal single-photon-emission computed tomography/computed tomography. *Arthritis Rheum*. 2011;63:1898–1907. <https://doi.org/10.1002/art.30363>.
107. Zhang H et al. Synovial macrophage M1 polarisation exacerbates experimental osteoarthritis partially through R-spondin-2. *Ann Rheum Dis*. 2018;77:1524–1534. <https://doi.org/10.1136/annrheumdis-2018-213450>.
108. Gowler PRW, Turnbull J, Shahtaheri M, Walsh DA, Barrett DA, Chapman V. Interplay between cellular changes in the knee joint, circulating lipids and pain behaviours in a slowly progressing murine model of osteoarthritis. *Eur J Pain*. 2022;26:2213–2226. <https://doi.org/10.1002/eip.2036>.