AUTOINFLAMMATORY DISEASES

Somatic mutations cause VEXAS syndrome

> myeloid lineage-restricted somatic mutations in *UBA1* [cause] diverse clinical manifestations

Using a genotype-driven, phenotype-neutral approach to discover a genetic cause of inflammatory disease, researchers have identified a new disorder arising from somatic mutations in *UBA1*, an X-chromosome gene encoding ubiquitin-like modifier-activating enzyme 1 (UBA1). The disorder, which the researchers named VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome, was identified in a total of 25 men with seemingly unrelated late-onset inflammatory diseases.

"Discovery of new disease entities usually begins with clinical recognition of a syndromic condition. By employing a genotype-first approach, we were able to discover a disease that would have been challenging to recognize clinically," explains Peter Grayson, co-corresponding author of the report in *The New England Journal of Medicine*. The first three participants with missense mutations affecting codon 41 in *UBA1* were identified by analysis of genetic data from 2,560 individuals in the NIH's Periodic Fever Database and Undiagnosed Diseases Program. They had all developed severe inflammatory syndromes associated with progressive haematologic abnormalities. On the basis of overlapping clinical similarities, the researchers identified another 15 men in NIH observational cohorts, and seven more were identified in UK study populations.

Credit: Brilly/DigitalVision Vectors

The 25 patients, who had a median age at disease onset of 64 years, each had one of three somatic variants in *UBA1* at p.Met41. Extensive clinical assessment revealed common clinical features including fever, skin involvement, pulmonary infiltrate, ear and nose chondritis, venous thromboembolism, macrocytic anaemia and bone marrow vacuoles. Most of the men met diagnostic or classification criteria for various inflammatory syndromes and/or haematologic conditions, including relapsing polychondritis, myelodysplastic syndrome, polyarteritis nodosa and giant cell arteritis.

Sequencing of isolated cell populations revealed that *UBA1* variants were found in more than half of progenitor cells and myeloid lineages but were absent in T cells, B cells and fibroblasts. Gene expression patterns in peripheral blood were consistent with activation of multiple innate immune pathways; consistent with these findings, men with VEXAS syndrome had elevated serum concentrations of C-reactive protein and pro-inflammatory cytokines including IFNγ and IL-8.

The researchers determined that p.Met41 variants lead to loss of cytoplasmic UBA1 function by generating the novel, catalytically deficient UBA1c isoform of UBA1. Monocytes from the study participants, most of which carried mutated *UBA1* variants, had decreased levels of the catalytically proficient UBA1b isoform and detectable levels of UBA1c; by contrast, UBA1 isoforms in T cells (most of which did not have mutated UBA1) were similar to those in T cells from unaffected individuals. Monocytes carrying *UBA1* variants had decreased ubiquitylation activity.

As zebrafish *uba1* and human *UBA1* are highly homologous, the researchers established CRISPR-Cas9–edited zebrafish models to assess *UBA1* gene function in vivo. In this model, knockout of the UBA11b isoform homologue led to upregulation of genes encoding pro-inflammatory cytokines including TNF, IL-8 and IL-6, supporting the idea that disruption of cytoplasmic UBA1 leads to systemic inflammation.

Together, the results implicate myeloid lineage-restricted somatic mutations in *UBA1* as the common underlying cause of a syndrome with diverse clinical manifestations and highlight the importance of somatic mutations in adult-onset inflammatory conditions. The researchers also suggest that VEXAS syndrome might explain the co-occurrence of myelodysplasia with conditions such as relapsing polychondritis, polyarteritis nodosa and giant cell arteritis.

Further study of VEXAS syndrome could also shed new light on the role of UBA1 and ubiquitylation in human diseases, as well as identify potential treatments for affected patients. "The VEXAS syndrome is a severe, progressive disease. To date, effective therapies other than glucocorticoids have not been identified," highlights Grayson. "We hope to leverage our understanding of the molecular basis of the disease towards discovery of novel treatment paradigms, which could include gene-editing therapies and bone marrow transplantation."

Sarah Onuora

ORIGINAL ARTICLE Beck, D. B. et al. Somatic mutations in *UBA1* and severe adult-onset autoinflammatory disease. *N. Engl. J. Med.* https://doi.org/10.1056/NEJMoa2026834 (2020)

IN BRIEF

RHEUMATOID ARTHRITIS

RA remission maintained after MTX withdrawal

For patients with rheumatoid arthritis (RA) who achieved sustained (24-week) remission with the combination of etanercept plus methotrexate (MTX) in the SEAM-RA study ($n = 253$), the proportion of patients with remission without disease worsening was higher among those who switched to etanercept monotherapy (that is, after MTX withdrawal) than to MTX monotherapy (49.5% versus 28.7%; $P = 0.004$), and was similar to that in the group who remained on combination therapy (52.9%), after a further 48 weeks. Among patients who received rescue therapy upon disease worsening, 70–80% in each monotherapy arm recaptured remission.

ORIGINAL ARTICLE Curtis, J. R. et al. Etanercept or methotrexate withdrawal in rheumatoid arthritis patients in sustained remission on combination therapy: a randomized, double-blind trial. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41589 (2020)

PAIN

Fasinumab effective for chronic low back pain

In a phase II–III trial, subcutaneous treatment with the nerve growth factor inhibitor fasinumab at doses of 9 mg every 4 weeks or every 8 weeks, but not 6 mg every 4 weeks, improved pain and function in patients with chronic low back pain at 16 weeks. Rates of treatment-emergent adverse events were similar across the fasinumab and placebo groups (65.6% and 67.1%, respectively). Arthropathies, most frequently rapidly progressive osteoarthritis (OA), occurred in 20 joints in 17 patients, 16 of whom were in the fasinumab groups; of the 20 joints with arthopathies, all but one were in patients who had peripheral OA at baseline.

ORIGINAL ARTICLE Dakin, P. et al. Efficacy and safety of fasinumab in patients with chronic low back pain: a phase II/III randomised clinical trial. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2020-217259 (2020)

OSTEOARTHRITIS

Intensive electroacupuncture reduces OA pain

In a multicentre randomized trial, patients with knee osteoarthritis (OA) who received intensive (thrice weekly) electroacupuncture simultaneously achieved minimal clinically important improvement in pain and function at week 8 more frequently than those who underwent sham acupuncture, with a between-group difference in response rate of 13.0% (97.5% CI 0.2–25.9; $P = 0.0234$); the effects persisted at week 26. The response rate with manual acupuncture did not differ from that with sham acupuncture at week 8 (between-group difference of 11.3%; 97.5% CI -1.6–24.4; $P = 0.0507$), although benefits of manual acupuncture were apparent at week 26.

ORIGINAL ARTICLE Tu, J.-F. et al. Efficacy of intensive acupuncture versus sham acupuncture in knee osteoarthritis: a randomized controlled trial. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41584 (2020)

SPONDYLOARTHRITIS

Long-term fatigue relief with secukinumab for AS

In both the MEASURE 1 and MEASURE 2 placebo-controlled randomized controlled trials, treatment with the anti-IL-17A antibody secukinumab provided rapid improvements in fatigue that were sustained for up to 3 years in patients with active ankylosing spondylitis (AS). Improvements were particularly prominent in patients who had not previously been treated with a TNF inhibitor.

ORIGINAL ARTICLE Kvien, T. K. et al. Secukinumab provides sustained reduction in fatigue in patients with ankylosing spondylitis: long-term results of two phase III randomized controlled trials. *Arthritis Care Res.* https://doi.org/10.1002/acr.24517 (2020)

RHEUMATOID ARTHRITIS

Induced T_{reg} cells stay on course

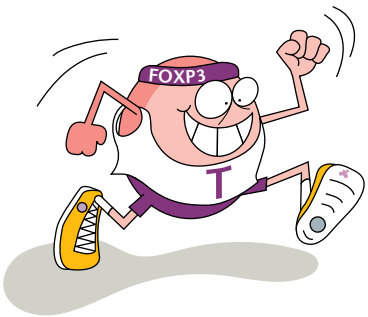
Regulatory T (T_{reg}) cells are important for preventing autoimmunity and are typically divided into two subgroups: natural T_{reg} (nT_{reg}) cells and induced T_{reg} (iT_{reg}) cells. New findings published in *Science Advances* suggest that iT_{reg} cells, but not nT_{reg} cells, maintain their regulatory function after exposure to arthritic conditions, which could have implications for T_{reg} cell-based therapies in autoimmune conditions such as rheumatoid arthritis (RA).

nT_{reg} cells develop in the thymus whereas iT_{reg} cells arise in the periphery and can be generated in vitro from naive T cells. Emerging data suggest that the two subgroups have overlapping and differing features. Previous findings had shown that adoptively transferred nT_{reg} cells lose the expression of the transcription factor FOXP3 (a master regulator of these cells) and transdifferentiate into pathogenic T helper 17 (T_H17) cells in mice with collagen-induced arthritis (CIA).

The authors on this latest study decided to take these investigations further by comparing the responses of nT_{reg} cells and iT_{reg} cells under similar conditions. In co-cultures with synovial fibroblasts from mice with CIA (inflamed synovial fibroblasts), iT_{reg} cells, but not nT_{reg} cells, retained their FOXP3 expression and suppressive capacity, whereas some nT_{reg} cells transdifferentiated into T_H17 cells, concurring with previous data.

In both a mouse model of colitis and in mice with CIA, adoptively transferred iT_{reg} cells or nT_{reg} cells could suppress the development of disease. However, this suppressive capacity was lost for nT_{reg} cells, but not for iT_{reg} cells, if the cells were primed with inflamed synovial fibroblasts prior to infusion.

Given the important contribution of synovial fibroblasts to RA pathogenesis and the relatively unknown relationship between



Credit: N.Smith/Springer Nature Limited

T_{reg} cells and synovial fibroblasts, the authors next sought to investigate the effect of T_{reg} cells on synovial fibroblasts. In vitro, iT_{reg} cells, but not nT_{reg} cells, could inhibit the migration, proliferation and cytokine production of inflamed synovial fibroblasts. In mice with CIA, infusion of nT_{reg} cells had a reduced capacity to inhibit CIA development over time compared with infusion of iT_{reg} cells. Notably, 45–60 days after immunization, the data suggested that the infused iT_{reg} cells had a superior effect on inhibiting the inflammatory activities of synovial fibroblasts than the infused nT_{reg} cells.

Further in vitro analysis suggested that, although cell-to-cell contact between the synovial fibroblasts and T_{reg} cells contributed to the loss of FOXP3 expression on nT_{reg} cells, the conversion of nT_{reg} cells to T_H17 cells mainly occurred as a result of IL-6 production by the synovial fibroblasts.

"This study advances efforts to use cell therapy in autoimmune disease with the use of in vitro expanded T_{reg} cells," says George Tsokos, an expert on T cells in autoimmune diseases who was not involved in the study. "The use of T_{reg} cell therapy in rheumatic diseases is plausible but there are a number of important considerations including the cost and the need for advanced facilities."

Jessica McHugh

ORIGINAL ARTICLE Yang, S. et al. Induced, but not natural, regulatory T cells retain phenotype and function following exposure to inflamed synovial fibroblasts. *Sci. Adv.* **6**, eabb0606 (2020)

GOUT

Febuxostat cardiovascular safety revisited

The xanthine oxidase inhibitors febuxostat and allopurinol are widely used as urate-lowering therapy, but the results of the CARES trial reported in 2018 raised concerns that febuxostat therapy was associated with an increased risk of death in patients with gout and cardiovascular disease. In the newly published results from the Febuxostat versus Allopurinol Streamlined Trial (FAST), however, the two treatments did not differ with respect to cardiovascular outcomes or mortality in patients with gout and cardiovascular risk factors. "The findings of FAST should reassure patients with gout and doctors treating patients with gout that febuxostat can be used as an alternative to allopurinol," highlights lead author Isla Mackenzie.

Credit: Bruce Rolff/Alamy Stock Photo

In FAST, which was conducted between 2011 and 2019, 6,128 patients were treated in a lead-in phase with allopurinol at dosages optimized to achieve a serum urate concentration of <6 mg/dl, then randomly assigned in a 1:1 ratio to receive treatment with febuxostat or allopurinol. Patients in the febuxostat group received a daily dose of 80 mg or 120 mg (mean daily dose 81 mg) and in the allopurinol group daily doses ranged from 100 mg to 900 mg (mean daily dose 279 mg).

At the end of the follow-up (median 1,467 days) in FAST, febuxostat was noninferior to allopurinol with respect to the primary outcome, which was a composite of non-fatal stroke, hospitalization for non-fatal myocardial infarction or biomarker-positive acute coronary syndrome, or death due to a cardiovascular event (adjusted HR 0.85, 95% CI 0.70–1.03; $P < 0.0001$).

> in FAST, febuxostat was noninferior to allopurinol with respect to the primary outcome

In the febuxostat group, 222 (7.2%) of 3,063 patients died and 1,720 (57.3%) had ≥1 serious adverse event; in the allopurinol group, 263 (8.6%) of 3,065 patients died and 1,812 (59.4%) had ≥1 serious adverse event. Rates of treatment discontinuation were 32.4% in the febuxostat group and 16.5% in the allopurinol group; 5.8% of patients in the trial withdrew from follow-up.

"The FAST study should lead to regulators reconsidering and updating their advice about the use of febuxostat that was issued following the results of CARES," says Mackenzie. "Not all patients with gout are able to tolerate allopurinol therapy, so it is useful to have an alternative treatment option."

Sarah Onuora

ORIGINAL ARTICLE Mackenzie, I. S. et al. Long-term cardiovascular safety of febuxostat compared with allopurinol in patients with gout (FAST): a multicentre, prospective, randomised, open-label, non-inferiority trial. *Lancet* https://doi.org/10.1016/S0140-6736(20)32234-0 (2020)

OSTEOARTHRITIS

IL-33 is a potential new target in OA

Osteoarthritis (OA) is the most common form of arthritis worldwide, yet effective treatments are lacking, leading researchers to constantly search for plausible new treatment targets. One such target is IL-33, a member of the IL-1 cytokine family. According to the results of a new study, blockade of IL-33 has the potential to reduce both pain and joint damage in experimental OA.

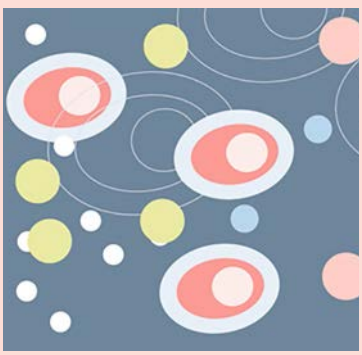
"The role of the immune system has been downplayed in OA," says corresponding author Pradeep Kumar Sacitharan. "Decades of research showed the classical cytokines (IL-1 and TNF) do not have disease relevance in pre-clinical models of OA. Moreover, IL-1 and TNF blockade have not shown any clinical efficacy for OA. However, immune cells and other cytokines are detectable in human OA joints. Hence, our lab's approach was to go back to the drawing board and investigate these other cytokines and rebuild the immunological puzzle in OA."

Sacitharan and colleagues first examined tissue from patients with OA and found an upregulation of IL-33 in the synovial fluid, and of both IL-33 and its receptor ST2 in chondrocytes. Exogenous IL-33 caused human chondrocytes to produce cartilage-degrading proteases in vitro and exacerbated disease in mice with experimental OA induced by destabilization of the medial meniscus (DMM).

> IL-33 knockout in chondrocytes reduced joint damage, pain and total IL-33 concentrations

Next, the researchers investigated the origin of IL-33 in mice with DMM-induced OA using tissue-specific conditional knockouts. Interestingly, although synovial fibroblast-specific knockout of IL-33 reduced synovitis, it had no effect on total IL-33 concentrations, joint damage or pain. By contrast, IL-33 knockout in chondrocytes reduced joint damage, pain and total IL-33 concentrations.

Pharmacological blockade of either IL-33 or ST2 with monoclonal antibodies was able to reduce pain and joint damage in mice with DMM-induced OA, suggesting that IL-33 signalling could be a potential future therapeutic target for OA.



Credit: S.Harris/Springer Nature Limited

"At this stage, we need to further investigate if targeting IL-33 and ST2 can be plausible in human OA," suggests Sacitharan. "We also need to elucidate methods of precision medicine to be able to target the right cytokine at the right time in a complex joint with multiple tissues."

Joanna Clarke

ORIGINAL ARTICLE He, Z. et al. Blockade of IL-33 signalling attenuates osteoarthritis. *Clin. Transl Immunol.* **9**, e1185 (2020)
RELATED ARTICLE Dinarello, C. A. The IL-1 family of cytokines and receptors in rheumatic diseases. *Nat. Rev. Rheumatol.* **15**, 612–632 (2019)

PAEDIATRIC RHEUMATOLOGY

New genetic risk loci found for JIA

Juvenile idiopathic arthritis (JIA) is a common form of childhood arthritis, but its low prevalence and extensive clinical heterogeneity hampers our understanding of this condition. To reduce clinical heterogeneity, past genetic studies have focused on specific clinical subtypes of JIA, resulting in the identification of 17 susceptibility loci. Taking a different approach, a group of researchers have jointly analysed all JIA subtypes in the largest genome-wide association study of JIA to date.

"We implemented a novel multinomial approach to systematically explore the sharing and specificity of genetic factors across the clinical subtypes and chose to analyse all available case samples, regardless of clinical subtype, to increase the success rate in shared locus discovery," explains corresponding author John Bowes. "We found that the effects of the majority of JIA genetic risk factors are shared across the various clinical subgroups and this combined analysis led to the discovery of five novel risk factors for susceptibility to JIA, bringing the total to 22."

To link these variants to target genes, the researchers used a combination of approaches including fine-mapping, transcriptomic analysis and chromatin interaction maps in relevant cell types. This integrative approach led to the prioritization of causal genes at six loci.

Importantly, this approach implicated *IL6ST*, and not the classically reported gene *ANKRD55*, as the target gene of the single nucleotide polymorphism rs7731626. This gene encodes a signal transducer involved in the IL-6 pathway and is the target of the biologic drug satralizumab, supporting future investigations of this drug in the treatment of JIA.

The enrichment analysis also identified two transcription factors — RELA and EBFI (involved in B cell and regulatory T cell development,

> "The enrichment analysis … identified two transcription factors … as key contributors to disease risk"

Credit: bortonia/DigitalVision Vectors

respectively) — as key contributors to disease risk.

"Although our results show that most risk factors are shared, there is strong evidence to also support clinical subtype-specific risk factors," says Elena López-Isac, first author of the study. "Large, international collaboration will facilitate further investigation of subtype-specific risk factors, which in turn will provide information on potential therapeutic targets."

Jessica McHugh

ORIGINAL ARTICLE López-Isac, E. et al. Combined genetic analysis of juvenile idiopathic arthritis clinical subtypes identifies novel risk loci, target genes and key regulatory mechanisms. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2020-218481 (2020)

RHEUMATOID ARTHRITIS

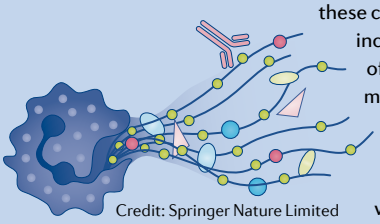
NETs revealed as source of carbamylated proteins in RA

Post-translational protein modification has an important role in the pathogenesis of rheumatoid arthritis (RA); however, although clear pathogenic pathways have been established for citrullination, less is known about the role of carbamylation.

"Around 50% of patients with RA develop autoantibodies to carbamylated proteins (CarP). The presence of these autoantibodies is associated with a higher prevalence of radiographic bone erosions and with increased morbidity and mortality in RA," explains Carmelo Carmona-Rivera, co-corresponding author of a new study published in *Science Advances* that describes the role of carbamylation in RA.

The researchers discovered that carbamylated proteins (particularly histones) are present on neutrophil extracellular traps (NETs) produced by neutrophils in patients with RA and that these carbamylated NETs (cNETs) can be recognized by anti-CarP antibodies in RA sera. Immunization of HLA-DRB1*04:01 transgenic mice with cNETs also led to the generation of anti-CarP antibodies in these animals.

> "carbamylated NETs … can be recognized by anti-CarP antibodies in RA sera"



Credit: Springer Nature Limited

Incubation of RA fibroblast-like synoviocytes with cNETs induced a pro-inflammatory phenotype in these cells that included increased production of osteoclastogenic mediators. Interestingly, the presence of anti-carbamylated histone antibodies was associated with the severity of bone erosion in patients with RA, and immune complexes of anti-carbamylated histone antibodies stimulated osteoclast formation in vitro.

"These results may provide a mechanistic explanation for the association between anti-CarP antibodies and increased bone erosions and worse prognosis in RA, and further support the rationale of NET inhibition as a putative therapeutic strategy in this disease," says co-corresponding author Mariana Kaplan.

"In the future, we plan to better understand the kinetics of these autoantibody responses and their association with genetic variants that predispose to RA but also promote anti-CarP responses and the generation of carbamylated autoantigens," states Carmona-Rivera. Such investigations will include characterization of the pathways that trigger carbamylation.

Joanna Clarke

ORIGINAL ARTICLE O'Neil, L. J. et al. Neutrophil-mediated carbamylation promotes articular damage in rheumatoid arthritis. *Sci. Adv.* **6**, eabd2688 (2020)

Credit: Yuichiro Chino/Moment

DATA ANALYSIS

Machine learning in precision medicine: lessons to learn

Darren Plant and Anne Barton

The ability to predict how a patient might respond to a medication would shift treatment decisions away from trial and error and reduce disease-associated health and financial burdens. Machine learning approaches applied to genomic datasets offer great promise to deliver personalized medicine but their application must first be optimized.

Refers to Tao, W. et al. Multi-omics and machine learning accurately predicts clinical response to adalimumab and etanercept therapy in patients with rheumatoid arthritis. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41516 (2020).

The arguments for precision medicine to optimize the benefits of treatment for patients are well-rehearsed; if robust biomarkers could be identified, fewer patients would be treated with drugs that are unlikely to be effective, which would accelerate the pathway to better outcomes, thereby improving the patient's experience and reducing the risk of unwanted effects. Machine learning approaches offer great promise in the identification of patterns, particularly in datasets that contain large numbers of different types of biomarkers that might not be discoverable using traditional statistical techniques. However, the use of machine learning to inform precision medicine is a field that is still in its infancy.

A new study published by Tao et al.[1] represents an important step in this particular learning curve. In this study, the authors developed machine learning models that were based on genome-wide gene expression data and DNA methylation signatures (measured in whole blood cells) to predict treatment response to adalimumab and etanercept in patients with rheumatoid arthritis (RA). Using this approach, the authors report being able to predict responses to adalimumab or etanercept with an accuracy of 86% and 79%,

> “the use of machine learning to inform precision medicine is a field that is still in its infancy”

respectively, using gene expression signatures; and with an accuracy of 85% and 88%, respectively, using models based on DNA methylation. The study by Tao et al. has a number of strengths, including the use of real-world data, the selection of samples to mitigate baseline differences in patient characteristics, the analysis of peripheral blood mononuclear cells (PBMCs), $CD4^+$ T cells and monocytes from the same individuals and an attempt to validate the findings. The datasets have also been published to enable further independent analyses[2]. However, it is now vital that the field adopts these good practices and also recognizes where study design could be improved in the future.

One important issue faced by researchers carrying out prediction modelling using 'omics' data is dimensionality; as the number of features (the potential predictive variants, which could be clinical, genetic, transcriptomic or other omics factors) grows larger, so does the number of samples that are required to create useful models[3]. A large number of samples is required because high-dimensional data will contain many features that are either redundant or irrelevant, and keeping those features in the dataset can make it more difficult to identify patterns that group patients effectively, impeding model performance, generalizability and interpretation. In the real world, it is often difficult to achieve large sample sizes, particularly if omics datasets are prohibitively expensive to generate or if the condition being studied is uncommon. Indeed, the total cohort size available to Tao and colleagues was only 80 individuals, 40 adalimumab-treated patients and 40 etanercept-treated patients[1]. Approximately 50% of these patients did not respond well to their medication, meaning that drug-specific analysis was performed on only ~20 individuals with a good response and ~20 individuals with a poor response from each treatment group. Now, if we consider that over 700,000 features were tested in the DNA methylation data alone, and that more than one cell type was assessed for gene expression levels, the number of redundant or irrelevant

> we are still learning the strengths and limitations of machine learning as an approach

features tested is likely to be high, and the chance of false positive and false negative findings will also be high for this dataset.

One way to alleviate the problem of small sample sizes is to only focus on relevant features and to exclude those that are not important for the analysis. Feature reduction can be achieved by generating many models using different subsets of the available features and then selecting those features that result in the best performing model, or by applying methods during the data pre-processing to evaluate the relationship (or correlation) between each feature and the outcome being investigated, and then only including those that show some correlation at this first stage of analysis[4]. In the study by Tao et al.[1], the features used to build the prediction model were pre-selected from the complete dataset using regression-based methods. However, this approach risks overestimating how well the model predicts response (known as overfitting), as every sample in the dataset is included in the step to identify relevant features, including the samples used for subsequent model evaluation. This methodological problem is referred to as double dipping[5]. Learning from the study by Tao et al., the authors of future studies should be encouraged to consider sample size carefully and ensure that their training data are used exclusively for feature selection and a completely separate dataset is used to test the performance of the model.

Tao and colleagues also attempted validation in nine samples from four patients treated with adalimumab and five treated with etanercept[1]; however, these patient samples were selected from the same dataset in which the original feature selection was performed, meaning that they were not truly independent. So, although the models reported by the authors seem to be moderately predictive of response in the nine samples tested, the results must be interpreted with caution until they can be replicated in truly independent samples; yet another lesson that we, as a field, must take on board for future studies.

The dataset analysed by Tao and colleagues is a hugely important resource[2] containing gene expression and DNA methylation data from PBMCs, $CD4^+$ T cells and monocytes, all from the same individuals. The differences in gene expression and DNA methylation signatures that occur between those cell types could reveal important mechanistic insights into RA. Similarly, a multi-omics approach that combines transcriptomics, proteomics and cell-count-based models was used in a 2018 study to identify a molecular signature for long-term clinical remission following treatment in patients with RA[6]. In the future, it will be important to integrate the data generated using different omics techniques in samples from the same individuals to produce a combined model. Methods exist to integrate data across omics platforms (described elsewhere[7]), and it will be important to see if such integration results in an improved ability to classify those who do and do not respond to medication, as has been shown for prognostic assessment in human cancers[8].

Overall, although advances in computational processing and programming have increased the accessibility and enthusiasm for applying state-of-the-art machine learning methods to new fields, such as precision medicine, the corollary is that we are still learning the strengths and limitations of machine learning as an approach. Lessons from studies such as that by Tao et al.[1] need to be applied if we are to identify robust, reproducible biomarkers of treatment response. We recognize that large sample sizes are not always readily available for treatment response studies; however, other areas of study design could instead be optimized, including the use of standardized machine learning approaches to improve feature selection, standardized reporting of model performance (including model accuracy, area under the curve values and model calibration[3]) and, most importantly, performing independent replication. If these lessons are not learnt, in the future it is likely that the scientific literature will be filled with papers claiming to have identified treatment response biomarkers that are unlikely to be generalizable to independent patient samples.

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Competing interests
The authors declare no competing interests.

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SYSTEMIC LUPUS ERYTHEMATOSUS

How well do the new classification criteria for SLE perform?

Guillermo J. Pons-Estel and Graciela S. Alarcón

The new 2019 EULAR–ACR classification criteria for systemic lupus erythematosus (SLE) performed well in the initial derivative and validation cohorts. But do these criteria outperform previous classification criteria across sexes, disease durations or ethnicities?

Refers to Johnson, S. R. et al. Performance of the 2019 EULAR/ACR classification criteria for systemic lupus erythematosus in early disease, across sexes and ethnicities. *Ann. Rheum. Dis.* **79**, 1333–1339 (2020).

Systemic lupus erythematosus (SLE) is a complex, heterogeneous, multisystemic disease with variable clinical expression among patients from different ethnic or racial groups; these differences are related to both the genetic characteristics (ancestral and non-ancestral genes) and non-genetic characteristics (such as socioeconomic factors) of these groups and account for the variable course of this disease, including the frequency and severity of flares, the extent of damage accrual and the frequency of remission[1]. Thus, the diagnosis of SLE is a challenge and puts the talent of the clinician to the test to identify disease from a broad set of symptoms, signs and laboratory tests without the help of a true 'gold standard' assay; this caveat is why no SLE diagnostic criteria truly exist. Alternatively, classification criteria are useful in identifying well-defined, relatively homogeneous patient groups for clinical and research purposes across the world; these criteria also have teaching value in the clinical setting[2]. However, the performance of classification criteria can vary across different cohorts and patient subsets. In a new study, Johnson et al.[3] attempt to evaluate the performance of the 2019 EULAR–ACR SLE classification criteria[4] across different subgroups, including across sexes, disease durations and ethnicities.

Previously developed SLE classification criteria (the 1982/1997 ACR criteria and the 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria) perform overall better in patients with longstanding disease than in patients with new-onset SLE; however, there is an increasing recognition and demand for the inclusion of patients with early SLE in clinical studies and trials. In 2019, EULAR and the ACR joined forces and developed a new set of criteria with the objective to achieve a better sensitivity than the 1982/1997 ACR criteria and specificity than the 2012 SLICC criteria[4]. The 2019 EULAR–ACR criteria present some unique characteristics that include the use of antinuclear antibodies (ANAs) as an entry criterion; the requirement that patients must accumulate ≥10 points; the stipulation that the occurrence of a criterion at least once is sufficient, that criteria need not occur simultaneously and that within each of the seven clinical and three immunological domains, only the highest weighted criterion is counted towards the total score; and finally, the inclusion that a clinical or laboratory feature is not counted if there is a more likely explanation than SLE for its occurrence. In view of these unique characteristics, the new classification criteria examined in the derivation and validation cohorts have shown outstanding sensitivity and specificity: 96–98% and 93–96%, respectively[4].

> “the performance of classification criteria can vary across different cohorts and patient subsets”

Differences in SLE disease expression affect the performance of classification criteria in different patient groups; furthermore, the criteria need to also perform well in early disease. To tackle these unmet needs, Johnson et al.[3] conducted a post-hoc analysis of the same multiethnic cohort of 1,270 patients (in which the patients self-reported as Asian (9%), Black (5%), Hispanic (Latin American heritage; 10%) and white (74%)) that was used to validate the 2019 EULAR–ACR criteria. The authors showed that these criteria have strong operating characteristics in terms of sensitivity and specificity across subsets of patients with SLE, including those patients with early disease (<3 years of disease duration) (sensitivity 97%, specificity 96%) and among patients with 3 to <5 years of disease duration (sensitivity 96%, specificity 99%). Likewise, good performance was observed in men (sensitivity 93%, specificity 96%) and women (sensitivity 97%, specificity 94%) and in white (sensitivity 95%, specificity 94%), Black (sensitivity 98%, specificity 100%), Hispanic (sensitivity 100%, specificity 96%) and Asian patients (sensitivity 97%, specificity 91%). But these excellent operating characteristics have some limitations. Only 18.1% (230 of 1,270 patients) had incident disease, defined as <3 years from the date of the physician diagnosis to the date of data submission. Thus, the 2019 EULAR–ACR criteria have excellent sensitivity, but classification might be missed or delayed, especially at very early disease stages.

In another study in an early SLE cohort of 690 patients[5], who had a median disease duration of 48 months, 79 of the patients could not be classified using the 2019 EULAR–ACR criteria; this group had a high incidence (55.7%) of moderate and severe disease as measured by the physician global assessment, and 40.5% of them had organ damage as measured by the SLICC damage index ≥1. In short, there was high disease burden in patients who could not be classified by the 2019 EULAR–ACR criteria. Only one quarter of the patients included in the study by Johnson et al.[3] was non-white; in fact, Black patients made up only 5.4% of the total cohort. As Black patients tend to have a more frequent and aggressive disease than white patients (for example, as shown in this study of African American and white patients[6]), a proportional participation of these patients in SLE and control groups is needed to refine the precision of the estimates of the operating characteristics of the new criteria. Thus, future collaborative studies by EULAR and ACR should consider recruiting referral centers from Africa, Asia Pacific and Latin America to avoid similar limitations.

Performance evaluation of the new 2019 criteria have been carried out in two separate multiethnic cohorts[7,8]; in these studies, the investigators examined whether patients from uncontrolled real-life clinical settings can be classified earlier using the 2019 EULAR–ACR criteria compared with using either the 1982/1997 ACR or the 2012 SLICC criteria. In the GLADEL cohort, the new set of criteria enabled an earlier classification of SLE in 7.4% (mean 0.67 years) and 0.6% (mean 1.47 years) of patients compared with the 1982/1997 ACR and the 2012 SLICC criteria,

> future collaborative studies … should consider recruiting referral centers from Africa, Asia Pacific and Latin America

respectively[7], whereas in the LUMINA cohort, the new criteria enabled an earlier classification in 13.3% (mean 0.66 years) and 15.3% (mean 0.63 years) of patients compared with the 1982/1997 ACR and the 2012 SLICC criteria, respectively[8]. However, in both cohorts, between 12.8% and 34.4% of patients fulfilled the new criteria at a later stage than with the 1982/1997 ACR or the 2012 SLICC criteria, respectively. In conclusion, both cohorts found that the EULAR–ACR criteria achieved the goal of classifying patients earlier only in a small proportion of the patients, particularly in a subset of patients with more severe disease. In addition, in a large multinational, multiethnic cohort study that compared all three sets of criteria (as well as a weighted SLICC criteria)[9], the investigators concluded that there are no differences between these criteria sets in terms of their performance, and suggested that the unweighted SLICC criteria might be preferred by clinicians and researchers because of their simplicity. However, the performance of any classification criteria will depend on the population from which patients and controls are drawn and whether specificity or sensitivity is prioritized.

Despite the points discussed, the findings from Johnson et al.[3] and the other studies described (TABLE 1) suggest that the 2019 EULAR–ACR criteria perform satisfactorily in terms of sensitivity and specificity in general, including across sexes, in different ethnic groups and in patients with early disease. We hope that the ACR and EULAR working group will keep track of the limitations mentioned and, in the future, will collaborate with the international rheumatology community to improve these criteria to enable their worldwide use.

Table 1 | **Performance of the SLE classification criteria across different groups**

Cohort or patient subset	Sensitivity %			Specificity %		
	ACR 1982/1997	SLICC 2012	EULAR–ACR 2019	ACR 1982/1997	SLICC 2012	EULAR–ACR 2019
Cohorts						
EULAR–ACR derivation cohort[4]	85	97	98	95	90	96
EULAR–ACR validation cohort[4]	83	97	96	93	84	93
Adamichou et al. cohort[5]	86	91	89	93	94	97
Petri et al. cohort[8]	83	97	91	96	84	89
Dahlström et al. cohort[10]	83	100	93	82	75	73
Subpopulations[3]						
Women	83	97	97	93	82	94
Men	78	94	93	94	90	96
<1 year disease duration	56	89	89	92	92	92
1 to <3 years disease duration	81	98	97	95	88	96
3 to <5 years disease duration	81	91	96	94	89	99
≥5 years disease duration	84	97	96	93	81	93
White	83	96	95	93	83	94
Black	82	98	98	100	92	100
Hispanic	86	100	100	96	78	96
Asian	77	99	97	93	91	91

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Competing interests
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PAEDIATRIC RHEUMATOLOGY

How to close the gap between paediatric and adult care

Kirsten Minden

For young people with rheumatic diseases, the transition from paediatric to adult rheumatology care is a vulnerable time, and delays or disruption in their care can lead to adverse outcomes. Research into the factors associated with gaps in transitional care could improve the identification and targeting of vulnerable groups.

Refers to Bitencourt, N. et al. Time to completed visit and healthcare utilization among young adults transferring from pediatric to adult rheumatologic care in a safety-net hospital. *Arthritis Care Res.* https://doi.org/10.1002/acr.24409 (2020).

Continuity of care is crucial for the successful transition of young people from paediatric to adult care, but has hardly been evaluated in rheumatology. A retrospective study by Bitencourt and colleagues on 141 patients with various rheumatic diseases transferring from paediatric to adult rheumatology care provides new information in this regard[1]. By quantifying the time between the last paediatric and the first adult rheumatology visit and evaluating post-transfer outcomes, the results highlight indicators of continuity of care that still need to be investigated — decades after the basic groundwork for the transition of adolescents with chronic conditions to adult care was laid.

At two conferences held in the 1980s, on "Youth with Disability: The Transition Years" and "Growing Up and Getting Medical Care: Youth with Special Health Care Needs", it was acknowledged that the issue of transition has not been adequately addressed by the health-care system and presents a substantial barrier to adolescents and young adults as they attempt to obtain developmentally appropriate medical care[2]. Today, despite numerous policy statements, expert panels, transition guidelines and numerous publications on rheumatological transitional care, considerable gaps in the care of young people with juvenile-onset rheumatic diseases remain. At present, up to half of these young people do not make a successful transfer to adult rheumatology services and are therefore particularly at risk of unfavorable outcomes[3,4]. Those who fall through the care gaps may face morbidity, damage accrual and even mortality.

Indicators of continuity of care include engagement and retention in adult care; the few available studies in rheumatology identified deficits in this regard. According to these studies, young people completed the first visit to an adult rheumatologist on average only 7–9 months after their last paediatric visit[5,6]. The study by Bitencourt et al. corroborates these findings[1]. In this study, the authors found that an average of 221 days

> "Those who fall through the care gaps may face morbidity, damage accrual and even mortality"

elapsed between the last paediatric visit and the first completed adult rheumatology visit. This observation implies that a substantial proportion of patients did not establish care with an adult rheumatologist within 6 months after the last paediatric rheumatology visit, which is considered by paediatric rheumatologists to be one of the most important indicators of a successful transition, along with patient survival and maintenance of insurance coverage[7].

Bitencourt and colleagues revealed that referral by a paediatric rheumatologist (versus any other physician) shortened the length of time between a paediatric and completed adult visit (mean 144 versus 529 days; $P<0.0001$)[1]. This finding underlines the importance of paediatric rheumatological care for young people with inflammatory rheumatic diseases. Surveys from North America and Europe show that paediatric rheumatologists consider a transition policy as being necessary for good clinical practice, with the majority of them pursuing at least informal transitional care[8]. Other identified determinants of continuity of care include communication between paediatric and adult rheumatologists and an overlapping visit in which a patient is seen by an adult rheumatologist before discharge from paediatric care[1]. Cross-sectoral collaboration and good communication between paediatric and adult rheumatologists are undoubtedly necessary to share critical patient-specific information by or before the transfer in order to provide appropriate care. The need for direct communication between paediatric and adult caregivers is also emphasized in the recommendations for transition issued by

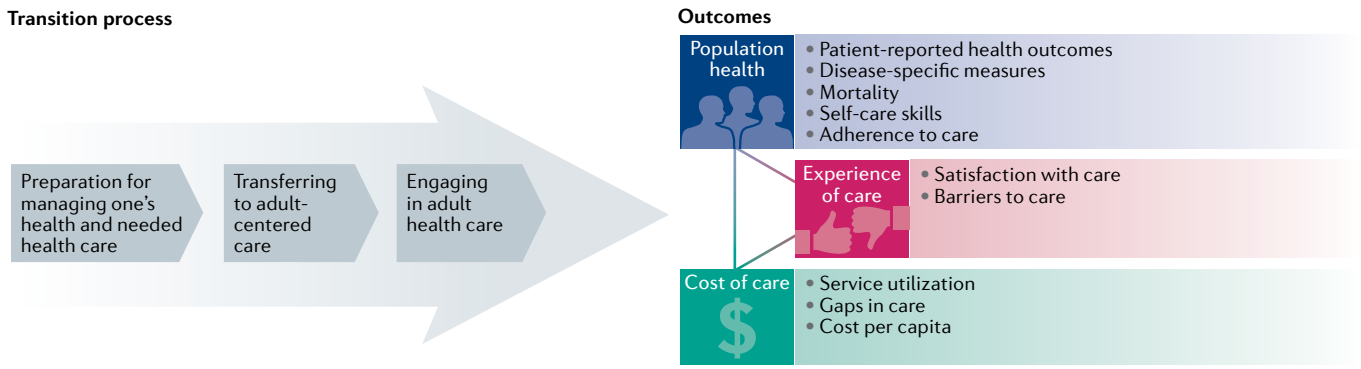

Fig. 1 | **Phases and outcome domains of transition from paediatric to adult care, categorized according to the Triple Aim framework.** Continuity of care is considered within the 'cost of care' domain, with gaps in care measured by the time frame between attendance at paediatric and adult clinics, the rate of loss to follow-up or the rate of missed appointments.

> … transition interventions result in fewer lapses in care

the Paediatric Rheumatology European Society (PReS) and EULAR[8]. However, the translation of this guidance into routine clinical practice remains a challenge. Even at an institution with an established transitional care programme, a retrospective case note review revealed that referral letters and health summaries were not sent by the paediatric team to the adult team in 24% of cases or to patients themselves in 59% of cases[3].

In response to the need to implement best practices for transfer of care, various point-of-care resources and tools have been developed, most notably those of the Got Transition initiative.

To what extent these and other transition measures improve the outcome of transfer remains to be determined. The US Institute for Healthcare Improvement's Triple Aim framework has been proposed to evaluate transition interventions[9] (FIG. 1). This framework is organized around three goals, namely improving the individual experience of care, improving the health of populations and reducing the per capita cost of care, and is based on the assumption that high-value health care can only be achieved if these three interdependent goals are pursued. Using this framework, a systematic review of 43 paediatric-to-adult transition studies found that structured transition interventions led to positive outcomes in the majority of studies. Seven of the 43 studies involved young people with rheumatic diseases and all of these studies had positive outcomes, including regarding continuity of care and informational continuity[10].

The study by Bitencourt et al. also provides evidence that transition interventions result in fewer lapses in care. Moreover, the authors assessed transfer outcomes in terms of cost of care and population health. A diagnosis of connective tissue disease, gaps in insurance coverage and race or ethnicity (for Black patients in particular) were associated with (unnecessary) service use, which was assessed on the basis of the proportion of patients who had unscheduled hospitalization or emergency department visits within 1 year of their final paediatric rheumatology visit. These parameters, as well as referral from a physician other than a paediatric rheumatologist, were also associated with serious consequences such as end-stage renal disease (ESRD) or even death, which occurred in 9% of patients[1]. A risk group can be defined according to these parameters for which a successful transition is less likely and which must be prioritized in the preparation for transfer to adult care. Bitencourt and colleagues could not clarify whether the poor outcomes in their study were related to a poor transition or to disease severity. However, studies of patients with other diseases (for example, type 1 diabetes mellitus or sickle cell disease) have also shown an increase in worse acute and chronic complications following transfer[1].

Yet despite the need, increasing evidence and efforts, the provision of uninterrupted, high quality and developmentally appropriate health-care services for patients moving from adolescence to adulthood remains challenging. Among the main obstacles to transition are the lack of secure funding for dedicated resources to provide uninterrupted clinical care and transition services and the lack of accountability for health outcomes when young people change providers or move to new insurance plans. Further action is needed.

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Competing interests
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How COVID-19 is changing rheumatology clinical practice

Eloisa Bonfá, Laure Gossec, David A. Isenberg, Zhanguo Li and Soumya Raychaudhuri

Abstract | The emergence of COVID-19 in early 2020 led to unprecedented changes to rheumatology clinical practice worldwide, including the closure of research laboratories, the restructuring of hospitals and the rapid transition to virtual care. As governments sought to slow and contain the spread of the disease, rheumatologists were presented with the difficult task of managing risks, to their patients as well as to themselves, while learning and implementing new systems for remote health care. Consequently, the COVID-19 pandemic led to a transformation in health infrastructures and telemedicine that could become powerful tools for rheumatologists, despite having some limitations. In this Viewpoint, five experts from different regions discuss their experiences of the pandemic, including the most challenging aspects of this unexpected transition, the advantages and limitations of virtual visits, and potential opportunities going forward.

Q Since the COVID-19 pandemic began, what have been the biggest challenges to managing patients with non-COVID-19 conditions?

Soumya Raychaudhuri. In mid-March 2020, Massachusetts, USA, had about 100 reported cases of coronavirus disease 19 (COVID-19), most emerging from an outbreak from a meeting of pharmaceutical company executives at a Boston hotel in late February[1]. Simultaneously, my colleagues and I were confronted with rapidly emerging data about the asymptomatic spread of this virus[2]. By 13 March, Boston and the surrounding public schools were shutting down, and our research laboratories were directed to work from home except for the most essential functions. At the same time, our outpatient clinic shifted to limit capacity to all but those patients most in need of care. Brigham and Women's Hospital (BWH) made the remarkable switch to making virtual visits available to our patients. Although virtual care worked well for some of our patients, it did mean that we faced unprecedented challenges in taking care of our newest and most active patients. Many of our patients are on immunomodulatory therapies and were appropriately reluctant to travel into health-care facilities, including for diagnostic tests and clinical laboratory monitoring or even infusions. The result was that with the COVID-19 pandemic, many of the tools that we commonly wielded became unwieldy.

This issue was particularly problematic for patients seeing us for a first visit, for patients who were failing to respond to therapies, for patients who needed to be seen urgently for concerning new symptoms or for patients who needed a referral to another specialist for evaluation and work-up of related independent diagnoses. In many instances, we used inadequate temporizing measures rather than a durable solution. For example, some patients with newly diagnosed inflammatory arthritic diseases were prescribed courses of prednisone until an in-person visit became possible. COVID-19 has also taken an emotional toll on our patients, as, like many, they struggled to balance their personal lives as our society shifted towards a lockdown and with the anxiety of a pandemic.

Eloisa Bonfá. On 23 March, 1 week after the first death from COVID-19 was reported in the city of São Paulo, Brazil, a major and difficult decision was taken by the largest tertiary public hospital in Latin America, consisting of 2,400 beds and eight specialized Institutes (Heart and Lung, Orthopaedic, Psychiatry, Children, Cancer, Central, Rehabilitation and Radiology Institutes). As the clinical director of the hospital and one of the coordinators of the COVID-19 crisis Committee, I was involved in the decision to isolate the Central Institute (containing 900 beds) solely for patients with COVID-19 (REF.[3]). This decision meant that the other seven Institutes remained at low exposure for COVID-19. All non-COVID patients from our General Tertiary Emergency Unit and from more than 30 specialized ward Units allocated in the Central Institute, including the rheumatology unit, were transferred to these COVID-cold Institutes. Patients from the rheumatology unit were transferred to the Orthopaedic Institute, along with patients from almost all specialized clinical wards. Each specialized ward was allocated to one Unit that had approximately 50% as many beds as were previously allocated to that ward. Overall, the pandemic resulted in delays in non-emergency hospitalizations.

One main challenge during this period was to divide the team between those who would work in the non-COVID-19 area and those who were recruited to exclusively care for patients with COVID-19 in the isolated COVID-19 Institute. A safe hospitalization flow for inpatients and employee safety was quickly established and upon suspicion of COVID-19, the patient was rapidly transferred to the transition area of the isolated COVID-19 Institute. Another challenge was to increase the number of intensive care unit (ICU) beds available in this Central Institute from 100 to 300 in 2 months. To achieve this goal, we had to convert 34 surgery rooms into 76 ICU beds. During the first 4 months of the pandemic (April–July) in São Paulo, >4,000 patients with severe COVID-19 were hospitalized in the isolated institute, and ICU beds accounted for more than half of these patients. In terms of patients with rheumatic diseases, the number of hospitalizations decreased by ~40% compared with the same period in the previous year and the number of patients in our Rheumatology outpatient clinics decreased by ~34%,

reducing from a mean of ~1,730 patients per month to ~1,148 patients per month. The Rheumatology Biological Center, a separate Unit dedicated exclusively to patients under biologic therapy, remained opened during the pandemic, and the number of appointments reduced by only ~16% compared with the same period in the previous year.

Zhanguo Li. As a rheumatologist practicing at Peking University People's Hospital, Beijing, the biggest challenge during the COVID-19 pandemic has been how to manage patients with rheumatic diseases remotely using online systems, social media platforms (such as WeChat) or telephone calls, because the patients simply could not physically attend the hospital. This alternative access to care was unprecedented and was previously even prohibited by our medical systems and insurance policies. The situation was extremely challenging for rheumatologists and patients for quite a few months, as rheumatologists had no existing online, regulated system for prescribing treatments. Consequently, the ceasing of medication or inappropriate self-management occurred in many patients across the country, resulting in flares of disease in some patients.

David Isenberg. Managing patients with serious autoimmune rheumatic diseases (who are often on steroids, immunosuppressives and/or biologics) who you cannot see and examine and do blood tests on has been a huge challenge. It is clear that many patients who have been carefully shielding have not wanted to come to hospital (at the University College Hospital, situated in the centre of London) and some have clearly tried hard to deny (to themselves as well as to their physicians) the fact that their underlying disease was getting worse. We had a particularly troubling time 2 months into the pandemic when, in a period of about 1 week in April, we had to admit six patients with systemic lupus erythematosus (SLE) who were experiencing acute flares — three of whom went straight into the ICU and two of whom died.

Laure Gossec. An overall and overarching challenge to my practice as an academic full-time rheumatologist at Sorbonne Université and Pitié-Salpêtrière Hospital, Paris, France, was my inner turmoil. When I was young, I spent a few months doing volunteer medical work in a developing country, but for me, this role led to less personal risk than the current pandemic, especially as personal protection equipment was scarce or lacking, and I feared bringing COVID-19 back home. This situation challenged my conviction that my job as a rheumatologist is the best in the world!

For me, the second biggest challenge to managing my (non-COVID) patients over the past months has been my fear of putting them at risk through my prescriptions. I mainly see patients with inflammatory arthritis, most of whom are treated with biologics or other targeted therapies. Initially, we had no information as to the potential risk associated with such treatments, in terms of increasing the risk of or severity of COVID-19. Thus, whereas I have always prescribed such treatments with the conviction of helping my patients, the challenge here is a profound rethinking of the benefit-to-risk balance of my prescriptions.

Q *How have your clinical and research activities changed? What adaptations have you put in place?*

Eloisa Bonfá. For the first time, the Rheumatology Outpatient Clinics of our Hospital provided virtual care over the phone to define which patients could have their visit postponed, which patients needed a change in prescription or which patients had to come to the clinic for an appointment. Postponing all previously scheduled rheumatology outpatient appointments was a challenging task owing to the large number of patient appointments per week (approximately 400), and it required a team of staff fully dedicated to this assignment. Those health-care workers who were at a high risk of severe illness from COVID-19 were selected for this job. This procedure required several adaptations for the medical staff and patients due to the lack of previous experience with virtual care, as telehealth was only endorsed by the Federal Council of Medicine during the pandemic[4]. Several measures of care and risk assessment were established for patients who were required to come into the clinic for an appointment, such as screening for COVID-19 symptoms at entry and at the reception as part of the routine clinical assessment. Patients were recommended not to attend a face-to-face appointment if they had any symptoms of COVID-19. Other adaptations included reviewing appointment scheduling, physical distancing in waiting rooms, hand hygiene care and appropriate personal protective equipment. Mask wearing is still mandatory in Brazil for any outside activity during the pandemic[5] and is also compulsory for patients during appointments.

The contributors

Eloisa Bonfá is a full professor of rheumatology and the clinical director of the largest tertiary public hospital of Latin America. Her main clinical and research interests are systemic lupus erythematosus and autoimmunity, with relevant contributions in the fields of autoantibodies, vaccines and drug monitoring in autoimmune diseases. She graduated at the University of São Paulo Medical School, Brazil, and undertook specialist training in rheumatology in the same university followed by a 4-year rheumatology research fellowship at the Hospital for Special Surgery, New York.

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Zhanguo Li. To adapt to the totally unexpected changes to clinical practice, one option in my department of the People's Hospital was to set up a consultant team consisting of 26 rheumatologists to provide medical service free to patients with rheumatic diseases, supported technically by an internet company. It was the first rheumatologist team to provide such support to patients in the country. Many patients nationwide were helped by this group over a 2-month period, from early February to late March 2020.

In addition, we used a previously developed smartphone application (smart system of disease management (SSDM)) as a patient self-care instrument to evaluate disease activity and remind patients to contact rheumatologists. The SSDM system was designed for a research project[6], and the clinical value was also clearly shown in the patients who used this SSDM system during the initial months of the COVID-19 pandemic.

David Isenberg. My practice has changed completely. During the first 3 months of the pandemic, no routine appointments were offered (although an emergency clinic once a week was available) so that all outpatient consultations took place over the phone or occasionally by video conferencing. For patients with longstanding, well-established disease and on low or moderate doses of steroids and immunosuppressives, I was reasonably content to miss seeing the patients at routine follow-up appointments, but increasingly I have become concerned about the inadequacies of what can be done when not seeing patients face-to-face.

Among the pleasures and responsibilities of running clinics in an academically inclined institution are doing research and educating both undergraduate and postgraduate students. The introduction of more remote patient assessment has had, and will always have, a detrimental effect on both. It will be harder to recruit patients to trials. We cannot, for example, perform ACR20, ACR50 or ACR70 assessments of our patients with rheumatoid arthritis or British Isles Lupus Activity Group (BILAG) assessments of our patients with SLE, to help determine their eligibility for a clinical trial. The patient cannot agree to have their blood taken remotely for a project. Likewise, teaching opportunities are restricted if we cannot, for example, demonstrate the use of the cross fluctuation test to show fluid in the knee of a patient, identify an enlarged liver or spleen or identify an extensor plantar response. These problems will obviously be detrimental for patient care too.

Laure Gossec. My professional life has profoundly changed since February. My research activities usually involve very frequent travels to other countries, which have completely stopped since February. My academic work as a professor of rheumatology involves face-to-face interactions with students, which likewise have disappeared completely and have been replaced (partly) by online courses, which are by essence much less interactive.

As regards my clinical work, my practice has changed because the hospital has become a place of dread and doom. My patients with inflammatory arthritis do not want to come to the hospital anymore, and I myself feel reluctant to ask them to come. For this reason, for 3 months, all of my patient clinics were switched to teleconsultation, where no physical examination is possible and where the quality of care is lower. In the hospital, instead of accommodating patients with severe rheumatic diseases, our beds were taken over for patients with non-rheumatic diseases, for whom my added value and competency is much lower.

One of the fun and interesting parts of my work is interactions within the medical and non-medical team as well as with colleagues outside of rheumatology (such as through staff meetings). Most of this social interaction has now disappeared, replaced somewhat by e-mail exchanges.

Soumya Raychaudhuri. I spend most of my time running a research lab in an academic setting. That part of my life has completely changed. Like many workplaces, we have moved almost entirely to virtual work environments. Hence, research and education has become much less interactive and we have had to shift our culture to accommodate this major change.

My clinical practice is within the BWH Arthritis Center, which is a large clinic that hosts 30,000 patient visits per year. My practice specifically has shifted to include more virtual visits and fewer in-person visits. From March to July, my practice was almost entirely virtual.

For in-person visits, to reduce the risk of infection for our staff and our patients, the BWH Arthritis Center has made dramatic changes in the way we interact with each other and with our patients, the flow of patients in and out of the clinic and the clinic rooms, how clinic rooms are turned over and many other components. The changes have been well executed and have affected every aspect of our clinical experience. The result is that I feel confident to encourage my patients to come in and see me, especially when so many of them are worried about COVID-19 and the personal risk to themselves. Although these changes are essential to our ability to see patients in person, they do make the experience of being a doctor somewhat less personal. Implementing social distancing has meant that I see fewer colleagues and staff. It also means that many of the spouses and family members that often accompanied my patients are no longer present. I no longer greet my patients in a crowded waiting room, rather they are brought in from an empty waiting room. Masks are essential to protect our patients, especially those on immunomodulatory therapies, but they do make non-verbal cues harder to glean. Overall, in-person visits continue to be essential, but they do not feel quite as warm or friendly.

On the other hand, virtual visits have been much more effective than I might have anticipated. Our clinical infrastructure has enabled video visits, which have proven to be far more productive than a simple phone call. The video visits are very practical and effective for my longstanding patients who are doing well on established therapeutics. Previously, some patients who live further away might have taken a day off to drive into Boston — in some instances from out of state — for a physical visit. For some of these patients, the ability to do a visit virtually has saved them valuable time. The virtual visit is often more efficient as visits can be easily started and ended, and the next visit can be started immediately. But the virtual visit has definite limitations. Most obviously, the inability to do an in-person physical examination and joint examinations cannot be reproduced via video. The exam is essential for assessing our patient's disease activities or making diagnoses, and taking care of new patients or patients with active disease can hence be really challenging. Video visits expose the digital divide of our society, and some of our patients are unable to fully take advantage of our infrastructure, especially those who are of fewer means, have poorer internet access or are older and less comfortable with technology.

Q *Will COVID-19 change your clinical and research activities for good, or will you return to business as usual once the situation is back to 'normal'?*

David Isenberg. Although apocryphal, there is a story that the then Chinese Premier Zhou Enlai, when asked by Henry Kissinger, Richard Nixon's secretary of state,

for his opinion on the effects of the French Revolution, replied "too early to say". I think the same is true for assessing the long-term effects of COVID-19. The pandemic has highlighted the value (at least in the short term) of fully electronic record systems, which makes it possible to see patient records, including letters, imaging and blood test results, remotely. I can certainly envisage that some routine follow-up appointments can be undertaken remotely and safely (provided local blood tests can be done), which may well reduce the numbers of patients attending specialist clinics.

Eloisa Bonfá. Engaging back to 'normal' activities will take time and it will probably have to wait for a vaccine. Until then, all adaptations and risk assessments will remain. But one of the major gains the COVID-19 pandemic will bring is the consolidation of telemedicine and televisits in the care of patients. Taking into account that many patients with rheumatic diseases have mobility difficulties, telehealth will provide an alternative approach to the care of these patients, when possible. Furthermore, in a large city such as São Paulo, with chaotic traffic and long distances, the possibility of avoiding public transportation, not only to prevent the spread of COVID-19 but also to avoid other issues beyond the pandemic, will be more convenient for the patient.

Zhanguo Li. COVID-19 has certainly changed rheumatology practice. Although the patient volume has now returned to normal in China, the demographics of patients attending outpatient clinics have altered in terms of disease severity and distance of travel. Patients with mild diseases who live in remote areas now tend to see their local doctors, rather than come to rheumatology centres.

Laure Gossec. At this stage, I do not really foresee the situation ever fully getting back to normal. It seems to me that social distances will be increased for a long time. In France, we usually hug and kiss a lot, which I do not think will go back to normal anytime soon. As for my professional life, I do not foresee going back to my previous rate of travel related to my research activities. I also think that medical teaching will be profoundly modified now with much more online resource use and much less face-to-face teaching. From that point of view, we were quite late in France in adopting these teaching methods, and this pandemic might well be an opportune moment for this change. As for my patients, I do think the situation will mostly go back to normal, as my clinics require the use of physical examinations and ultrasonography. I am planning to keep around 10% of consultations online for patients in the long term.

Soumya Raychaudhuri. I think that some of the changes will be here to stay. Boston is a challenging city for many of our patients to get in and out of, particularly those who are coming from far away, or for those for whom driving or navigating public transportation is hard. For these patients, especially for routine follow-up visits, a virtual visit can offer real advantages. There are patients all over New England who would benefit from access to a referral centre. I can imagine if our institution or others are able to build a great virtual care infrastructure, we could be in a position to expand the scope of patients who our physicians are connecting with and caring for.

Q *If temporary adaptations are to become permanent, what barriers need to be overcome?*

Laure Gossec. Barriers to online consultations include poor access to the internet for some patients, low-quality internet connection on either side, a lack of user-friendly medical files and also a psychological reluctance from patients regarding online consultations (most patients prefer to see me face-to-face).

The wearing of masks is also a barrier to my clinical practice. It hinders the interactions with my patients, which makes shared decision-making (probably the most rewarding part of my clinics) more difficult. Will it be that masks will push us back in time, to paternalistic prescriptions? Who can say?

Zhanguo Li. Current barriers are the lack of a 'telehealth' and medical support system for patient care, which can facilitate patients and doctors in terms of consultations, efficient follow-up and clinical studies. If a second wave of COVID-19 comes, we will face the same difficulty as we had a few months ago.

Eloisa Bonfá. The most important adaptation is consolidation of the regulatory framework for telemedicine in Brazil, including reimbursement for this activity. Another notable barrier that is expected is the serious economic crisis resulting from the COVID-19 pandemic that will limit investment resources in all areas including health. This limitation of resources will hinder the development and implementation of innovations. Hopefully, increased solidarity, a hallmark of this crisis, and regional cooperation will help to overcome the challenges we will have during reconstruction.

Soumya Raychaudhuri. I think telemedicine and virtual medical care could become really powerful tools for the right patient with the right infrastructure. I think that we need to make sure that our patients have access to a proper IT infrastructure to mitigate access issues. If language is a barrier, we need to have a means of enabling translation services during our virtual visits. To realize the full potential of virtual care, we need to be able to arrange services and testing for our patients within their communities. After the visit, having an integrated health-care system that enables seamless data transfer is essential. With such an integrated health-care system, arranging imaging, lab work, therapeutic infusions and other services near to home becomes possible without cumbersome administrative barriers. Currently, for my more distant patients, I often need to bring them into Boston for tests and services. In many cases, they have alternative facilities near to their home, but those facilities are not connected to our system, and arranging local testing and services is challenging without extensive administrative effort.

David Isenberg. I anticipate that it will be even more important to stress to patients, if their disease is worsening and they have not been seen by a physician (or nurse), that they must contact the hospital and arrange a face-to-face appointment as soon as possible. From the administrative point of view, there will need to be greater flexibility about determining whether patients are to be seen face-to-face or via a telephone consultation. Closer links with general practices will also be necessary as, in my experience, some general practitioners have been reluctant to take on routine monitoring of patients on immunosuppressive medication.

Q *What other opportunities lie ahead for transforming rheumatology practice?*

Zhanguo Li. Many opportunities lie ahead, as long as we focus on the needs of patients and rheumatologists. Undoubtedly, more patient-associated and doctor-associated activities will be held online, providing opportunities for patient education and

virtual conferences, although patients with severe or difficult-to-treat disease will still need face-to-face appointments with their rheumatologist.

Laure Gossec. Improving access to best care, through online consultations but also by improving the patient trail (that is, the way in which patients first see their general practitioner before being referred to a rheumatologist) and decreasing the delay before a consultation, is a priority. Better use of online resources and maybe of rheumatology nurses, if they are allowed to play a bigger role in France, are options to move forward, which may be facilitated by the COVID-19 pandemic.

Soumya Raychaudhuri. The implementation of effective virtual visits will be really powerful for rheumatology. The need for an in-person visit will always be there, especially for patients with very active disease or for new patients with uncertain diagnoses. But for patients who we know well, managing them to some extent virtually will have great value. I think in practice these are the patients we talk to on the phone informally and e-mail with. So, having a formal mechanism to take care of them will be beneficial to them and to us.

David Isenberg. By doing more telephone consultations and reducing the numbers of patients attending clinics face-to-face, it should be possible to reduce the waiting times for patients referred to rheumatologists. I am, though, becoming increasingly concerned about the 'downsides' of what has happened in the past 6 months, notably the missed occurrences of increased disease activity in patients, the loss of educational opportunities for physicians and the difficulties in undertaking translational research.

Eloisa Bonfá. Innovations associated with self-care, including smartphone apps and wearable technologies, consolidated during the pandemic, are interesting alternatives for the management of several chronic conditions and will certainly also be useful for patients with rheumatic diseases. Above all, there is no way back and the acceleration of digital transformation and the improvements in internet speed that occurred during the pandemic will continue and will transform our lives. This change will provide new opportunities for physicians to update their knowledge on the field and for continuing medical education online, without the need for physical travel. In addition, for organizations, a new way of dealing with administrative work took place with changes in workflows, including replacement of meetings with e-mails, increased working from home and accelerated automation that will forever change the way we work.

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Competing interests
L.G. receives research grants from Amgen, Galapagos, Janssen, Lilly, Pfizer, Sandoz and Sanofi, and receives consulting fees from AbbVie, Amgen, BMS, Celgene, Gilead, Janssen, Lilly, Novartis, Pfizer, Samsung Bioepis, Sanofi-Aventis and UCB, all unrelated to the present paper. S.R. is an employee of Brigham and Women's Hospital, and has recently served as a consultant for AbbVie, Biogen, Gilead, Merck and Pfizer. He is a founder of Mestag. He currently receives research funding from Biogen. E.B., D.A.I. and Z.L. declare no competing interests.

Persistent inflammatory and non-inflammatory mechanisms in refractory rheumatoid arthritis

Maya H. Buch[1,2,3]✉, *Stephen Eyre*[1,2] *and Dennis McGonagle*[3,4]

Abstract | Despite nearly three decades of advances in the management of rheumatoid arthritis (RA), a substantial minority of patients are exposed to multiple DMARDs without necessarily benefitting from them; a group of patients variously designated as having 'difficult to treat', 'treatment-resistant' or 'refractory' RA. This Review of refractory RA focuses on two types of patients: those for whom multiple targeted therapies lack efficacy and who have persistent inflammatory pathology, which we designate as persistent inflammatory refractory RA (PIRRA); and those with supposed refractory RA who have continued disease activity that is predominantly independent of objective evidence of inflammation, which we designate as non-inflammatory refractory RA (NIRRA). These two types of disease are not mutually exclusive, but identifying those individuals with predominant PIRRA or NIRRA is important, as it informs distinct treatment and management approaches. This Review outlines the clinical differences between PIRRA and NIRRA, the genetic and epigenetic mechanisms and immune pathways that might contribute to the immunopathogenesis of recalcitrant synovitis in PIRRA, and a possible basis for non-inflammatory symptomatology in NIRRA. Future approaches towards the definition of refractory RA and the application of single-cell and integrated omics technologies to the identification of refractory RA endotypes are also discussed.

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Despite advances in the management of rheumatoid arthritis (RA) that have been made over the past 30 years and the availability of effective targeted synthetic DMARDs (tsDMARDs) and biologic DMARDs (bDMARDs), treatment non-response remains an ongoing clinical challenge that can result in treatment-resistant RA[1]. The goal of treatment with existing conventional synthetic DMARDs, tsDMARDs and bDMARDs in RA is the complete abrogation of inflammation. The inability to achieve this goal leads to successive cycling of therapies (BOX 1). Increasingly, the term 'refractory' RA is being used to describe disease that is resistant to multiple DMARDs[2], yet what comprises refractory RA lacks consensus.

In this Review, we discuss the challenges of defining refractory RA and the current literature on the extent and burden of the condition. We posit that refractory RA consists of two overlapping subtypes on the basis of whether symptomatology persists in the presence or absence of inflammation, and that these subtypes have relevance for management strategies. Having defined these subtypes, we examine the heterogeneous overlapping innate and adaptive mechanisms of RA, as well as interrelated factors such as smoking, epigenetic factors and potential somatic mutations, that could contribute to the persistence or evolution of immune responses and ongoing inflammation in individuals with persistent synovitis. We also discuss how perturbations in the joint microenvironment and subtle emergent immune mechanisms linked to pain might contribute to ongoing symptomatology in individuals with little or no discernible inflammation. Finally, we provide some thoughts on how the challenges surrounding refractory RA might be addressed in the future.

Defining refractory RA

Refractory RA is often used interchangeably with 'difficult to treat' RA[3], a working definition of which was provided by a EULAR task force in 2020 (REF.[4]). As illustrated in the results of a survey published in 2018 (REF.[5]), the term difficult-to-treat RA can have a wide variety of interpretations; however, a common theme is the exposure of patients to (but not necessarily a lack of efficacy of) several advanced therapies. Typical reasons for exposing a patient to several DMARDs can include multidrug toxicity and concerns around the safety profiles of complex immunosuppressive therapies in patients with comorbidities (not necessarily directly related to RA).

Key points

- The term refractory rheumatoid arthritis (RA) implies treatment-resistant persistent joint and/or systemic inflammation; however, it is often used interchangeably with broader definitions such as 'difficult to treat' RA.
- Refractory RA could be stratified into two major categories; persistent inflammatory refractory RA (PIRRA), in which unabated inflammation is evident, and non-inflammatory refractory RA (NIRRA), which lacks discernible inflammation.
- Within the category of PIRRA, serological status and HLA associations can provide meaningful stratification that can inform potential therapeutic avenues.
- Epigenetic modifiers, including methylation, microRNAs and long non-coding RNAs, can influence the course of RA and could provide a basis for the emergence of refractory RA.
- NIRRA is typically mediated by ongoing pain and patient-reported outcomes; pain mechanisms might include autoimmune and neuroinflammatory pathways that are independent of joint synovitis.
- The classification of RA and other diseases along an innate-to-adaptive immunological axis can be applied to refractory RA to help discover targets that might be of therapeutic benefit.

Patient compliance and adherence to therapies are also increasingly being recognized as contributors to the overall outcomes for patients.

By contrast, the term refractory RA indicates the inefficacy of multiple agents in conjunction with unabating joint and systemic inflammation — features at the core of the historically poor prognosis of RA[6]. In the real-world clinical setting, a state of genuine refractory RA is considered to exist when all potentially useful available therapeutic options have been exhausted (BOX 1). All of the currently licenced effective therapies for RA target joint inflammation mediated by integrated innate and adaptive immune system mechanisms in an effort to ablate inflammation and normalize inflammatory markers. Thus, implicit in our understanding of true refractory RA is the persistence of joint inflammation (which manifests as a state of high disease activity) and the need to explore alternative anti-inflammatory strategies to target disease. The need for the use of moderately high doses of glucocorticoids alongside DMARD therapy to achieve disease control in patients with RA also implies therapeutic inefficacy and, in the context of the cycling of multiple therapies, is consistent with a refractory disease state. What happens, however, if a patient has a high composite disease activity score that persistent inflammation does not necessarily contribute to? Some patients with RA cycle through multiple DMARDs in an attempt to bring their disease to an acceptable measured disease activity state (be it remission, low disease activity or equivalent) but have little objective evidence of ongoing inflammation. Does this type of disease also constitute refractory RA?

Observations on the effects of therapy, structural progression and patient-centred outcomes that extend back almost two decades can inform how we answer such a question. Many patients with RA who failed to meet the ACR composite outcome measures in a trial of infliximab plus methotrexate still had reductions in swollen joint counts, C-reactive protein (CRP) concentrations and radiographic progression[7], indicating meaningful suppression of inflammation in the face of apparent clinical non-response. A synovial tissue study drew similar conclusions, identifying CRP suppression and synovial tissue improvement in individuals who failed to meet ACR composite measures of response[8]. These findings could be interpreted as joint inflammation and damage being uncoupled and thereby representing two different processes. However, imaging studies have demonstrated that these two processes are coupled, with synovitis thought to precede joint erosion[9,10]. In addition, other studies have confirmed that clinical joint swelling and synovitis, but not tenderness and patient-reported outcome measures (PROMs), correlate with progressive joint destruction[11], and that seemingly controlled disease can be associated with persistent measured disease activity mediated by pain and PROMs[12–14]. Several studies have demonstrated this notion in the context of discordance between the 28-joint disease activity score (DAS28) and the risk of inappropriate escalation of immunosuppressive therapy[15], and also in promoting remission 'near misses'[16]. These data demonstrate that individuals with raised composite disease activity scores might not have underlying joint and/or systemic inflammation.

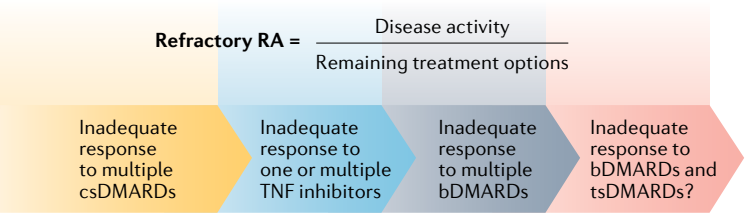
Applying this understanding to individuals who have already cycled through multiple DMARD therapies, we suggest that two types of disease state should be considered: persistent inflammatory refractory RA (PIRRA), in which an individual has unequivocal inflammatory joint synovitis that typically occurs, albeit not exclusively[17], in the presence of raised systemic markers of inflammation, despite the use of therapies with different mechanisms of action; and non-inflammatory refractory RA (NIRRA), in which symptomatology, typically pain, predominantly persists independently of discernible inflammation and is thus not directly amenable to DMARD therapy[2]. For some patients, although apparent joint swelling might suggest refractory synovitis, imaging studies can subsequently show no objective evidence of ongoing inflammation, consistent with the NIRRA subtype, and hence progressive erosion is unlikely[18]. Importantly, these two subtypes of refractory RA are unlikely to be mutually exclusive of one another, and both of these sit within the difficult-to-treat RA population (FIG. 1).

Although this subcategorization is not accepted or validated, and this approach is not without limitations (BOX 2), recognizing these two categories of refractory RA has fundamental conceptual implications for potential management strategies. Identifying PIRRA as the predominant basis for the cycling of multiple therapies will be of central importance for the emergent categorization, prognosis and testing of novel therapies for this condition. Similarly, although we acknowledge the potentially substantial functional incapacity in our suggested category of NIRRA, the prognosis for patients with this type of RA is likely to be very different from those with PIRRA.

Difficult-to-treat RA and the wide group of patients encompassed by this term can include refractory RA, but this concept is not discussed further in this Review. Nevertheless, it is worth acknowledging that a EULAR task force has recommended a threshold of a failure of at least two bDMARDs or tsDMARDs (with different mechanisms of action) as a definition of difficult-to-treat RA[4]. This recommendation is similar to that made in

Box 1 | **Treatment of RA and therapy cycling in refractory RA**

The therapeutic armamentarium for rheumatoid arthritis (RA) has rapidly expanded over the past 20 years with the introduction of biologic DMARDs (bDMARDs). The categorization of therapies in clinical practice and in clinical trials has often been dichotomous, with therapies described as either TNF inhibitors (the first bDMARDs to become available) or non-TNF inhibitor bDMARDs (including B cell-depleting therapies, T cell co-stimulation blockade and IL-6-targeted therapies). Refractory RA has traditionally been represented in the context of persistent disease activity following the exhaustion of all available treatment options (see figure). This concept has inevitably led to a continually expanding and changing definition of refractory RA as the number of available therapies has grown. Patients with refractory RA typically cycle from conventional synthetic DMARDs (csDMARDs), to bDMARDs, usually TNF inhibitors first, and then to bDMARDs with other mechanisms of action. This approach is pragmatic and can provide insights into meaningful pathways of disease. However, it remains to be seen whether the interruption of pan-cytokine signalling by the use of Janus kinase inhibitors, a type of targeted synthetic DMARD (tsDMARD), reduces the burden of refractory RA.



a 2018 viewpoint, which used the term refractory RA[2]; however, the term difficult-to-treat RA (as used by the EULAR task force) also includes the presence of more general signs, symptoms and clinical scenarios that can make the management of RA challenging. Going forwards in this Review, we use the term refractory RA to signify genuine disease that is resistant to treatment with multiple bDMARDs or tsDMARDs (as opposed to patients who are exposed to multiple DMARDs), and PIRRA and NIRRA when discussing data and concepts specific to these subcategories.

How common is refractory RA?

Only a handful of reports have described the prevalence of refractory RA, each of which used a different definition of the condition and none of which was designed to clearly identify PIRRA. A 2018 report from the British Society of Rheumatology Biologics Register for RA on individuals with refractory RA (defined as the failure of a minimum of two bDMARDs owing to toxicity and/or inefficacy) indicated a prevalence of approximately 6%, but the authors only examined patients who had used a TNF inhibitor as their first bDMARD[19]. A 2019 study in which refractory RA was defined as failure of a minimum of three DMARDs, including at least one bDMARD, and thus had a wider population of interest than the British Society of Rheumatology Biologics Register for RA study, reported that 17% of 412 patients had refractory RA[20]. Predictors of refractory RA included delayed initial treatment, being female and having high composite disease activity scores. The same group illustrated that treatment with a large number of prior DMARDs was associated with poor treatment response regardless of disease duration[21]. These results are consistent with the well-established observation that delayed treatment with a DMARD is associated with a poor response[17,22]. In other words, response rates are almost always higher when a drug is used as a first-line therapy than if the same drug is used as a second-line or third-line therapy later in the treatment pathway[1].

The differing definitions of refractory RA used in the studies published to date[19,20] make accurate estimation of the prevalence of this condition challenging. Such studies presume genuine persistent inflammation at each historic treatment failure. However, the difficulties associated with disentangling bona fide persistent inflammation from other factors that masquerade as refractory RA make it likely that the estimated 6–17% of individuals with refractory RA represents the overall proportion of individuals with refractory RA of any type, and that those with PIRRA constitute a smaller proportion of this group. Indeed, one of these studies reported no statistically significant difference in swollen joint counts, CRP concentration, erythrocyte sedimentation rate or radiographic damage between individuals with refractory RA and those with therapy-responsive disease[20], indicating that PIRRA is probably uncommon.

Characteristics of refractory RA

Persistent inflammatory refractory RA

Historically, severe and treatment-resistant (refractory) RA manifested as bulky synovitis, chronic progressive disability, complete incapacitation and loss of independence, accelerated atherosclerosis, tumorigenesis and early death[23,24]. Nowadays, refractory RA has a less aggressive phenotype and, in our experience, rarely emerges after years of sustained drug-induced remission. An individual can immediately and obviously fail to respond to successive treatments or can develop PIRRA more gradually, often as they partially respond to each new therapy before relapsing back into active disease (FIG. 2).

Patients with PIRRA can typically present with one of three clinical categories of refractory joint involvement (polyarthritis, oligoarthritis or monoarthritis), although this pattern of joint involvement has not been carefully studied. Individuals with unambiguously resistant disease generally have polyarthritis, which can become less extensive over time owing to the partial efficacy of targeted therapies. Some individuals can have an oligoarticular pattern of disease, which includes small-joint involvement of the hands and wrists and, very occasionally, patients can have a monoarticular large-joint pattern of disease with extensive synovitis. However, it is debatable whether a single refractory synovitic joint would meet any definition of refractory RA, which was historically a polyarticular disease. Notably, some individuals might have polysynovitis that is atypical and/or has a large-joint pattern but that nevertheless meets the criteria for RA; by designating such patients as having RA, the range of available treatment options is widened according to local eligibility criteria.

Distinct joint patterns in PIRRA might also be associated with autoantibody status. Involvement of large joints is associated with seronegative RA in what might be clinically termed a spondyloarthritis (SpA)-like pattern, whereas seropositive RA is typically associated with a small-joint symmetrical phenotype, although these patterns are not definitive[25]. Studies on the associations

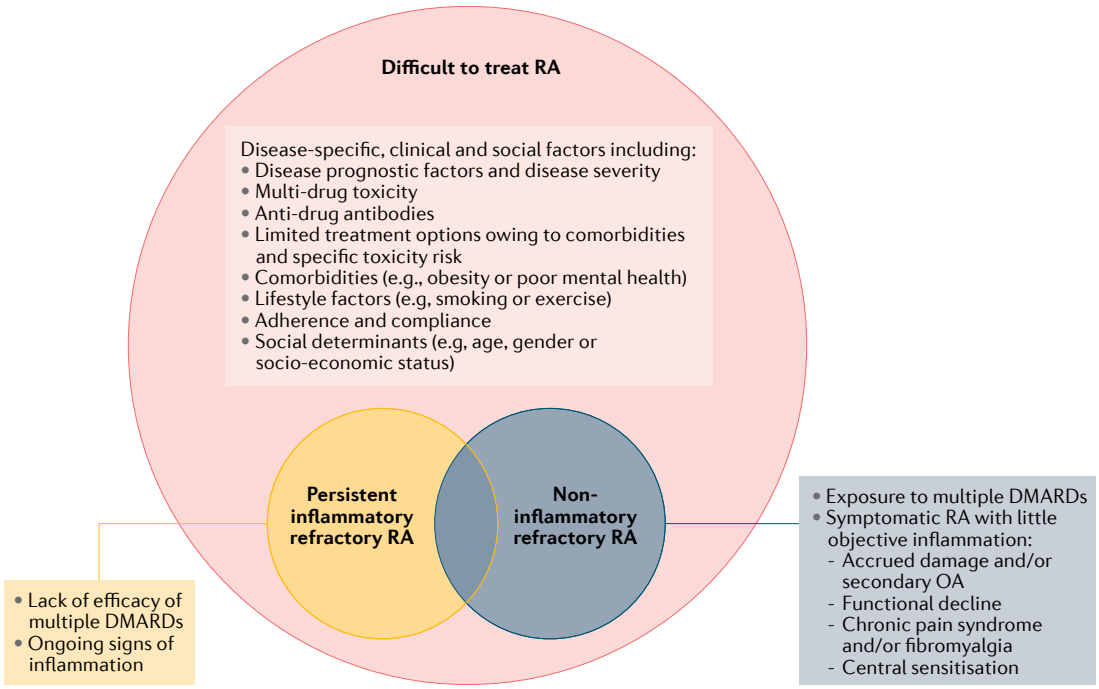


Fig. 1 | **Refractory RA subgroups within the wider context of 'difficult-to-treat' RA.** The term 'difficult-to-treat' rheumatoid arthritis (RA) is often used to describe disease in patients who are exposed to multiple biologic DMARDs and/or targeted synthetic DMARDs, but not necessarily in those for whom they lack efficacy. This group of patients encompasses those with comorbidities that preclude the use of certain therapies, repeated drug toxicity, anti-drug antibody development leading to drug inefficacy, and medication non-adherence and non-compliance. Lifestyle and social determinants might also contribute to lack of treatment efficacy in this group. We propose that the term 'refractory' RA could be defined by the inefficacy (not toxicity) of multiple types of DMARDs. Some individuals in this group will have persistent inflammation in the joints (and potentially systemic inflammation) and would be classified as having persistent inflammatory refractory RA. Other individuals will have symptomatic RA with little objective evidence of ongoing inflammation that can be modulated by DMARDs that principally target innate and adaptive immune system-mediated inflammation. Misdiagnosis of ongoing symptomatology as joint inflammation can lead to individuals in this group cycling though several therapies; such individuals would be classified as having non-inflammatory refractory RA. Importantly, all three groupings can exist in their own right and/or coexist to differing degrees, this latter scenario being the most likely. Although the definitions and terminology suggested here are preliminary, it is clear that the two subtypes of refractory RA would need distinct therapeutic approaches. OA, osteoarthritis.

between anti-citrullinated protein antibody (ACPA) status and clinical manifestations in cohorts of patients with early RA have also suggested possible differences between ACPA-negative and ACPA-positive individuals, including more large-joint involvement in ACPA-negative individuals; however, the data are not altogether conclusive[26,27]. Careful assessment of the site of pathology using ultrasonography can be used to help identify extracapsular disease[28] that would perhaps be more typical of autoantibody-negative RA or SpA-spectrum disorders.

Non-inflammatory refractory RA

With the development of targeted therapies for RA, the expectations of patients and clinicians have changed such that modest levels of inflammation are no longer an acceptable target to settle for. If an individual has only one or two involved joints, local injection or synovectomy can be employed, but for more extensive disease other treatment strategies are clearly needed. Yet some patients designated as having refractory RA can actually have low numbers of (or even no) swollen joints, normal CRP concentrations and erosive disease that is no more extensive than that in individuals who respond well to therapy[20] (in other words, a NIRRA phenotype), raising two questions: what is behind such a clinical profile, and what are the long-term implications for the patient?

Disease activity measures are surrogate indicators of active RA that are used to guide assessment of disease status and treatment response. Refractory RA, including the NIRRA subtype, is identified through a persistently raised disease activity score. Although validated composite disease activity measures have been instrumental in enabling the robust testing of new therapeutics and their introduction into clinical practices, the limitations of such composite measures are well-recognized. The DAS28 is weighted heavily for the tender joint count, yet objective evidence of inflammation does not necessarily correlate with PROMs such as pain[29]. In fact, when patients with RA are split into groups on the basis of the DAS28 and PROMs, a specific phenotype emerges that comprises predominant pain, fatigue and catastrophizing behaviour in the absence of markers of inflammation[30]. The absence of genuine joint and/or systemic inflammation but persistence in measured disease activity and PROMs might be the main factors behind

the futile cycling of DMARDs in this group of patients. Interestingly, in the VEDERA trial, the results of which were reported in 2020, the absence of sonographically determined power Doppler signals in the hands of approximately one-third of participants suggested that in real-world clinical practice, a substantial proportion of even symptomatic individuals with early RA might not have local inflammation to modify[18]. Such patients could be consigned to unnecessary cycling of therapies and falsely thought to be on a refractory disease trajectory.

Individuals with NIRRA are unlikely to require attention from the wider health services in the same way that those with PIRRA would owing to the well-known sequelae that emerge following chronic systemic inflammation[23]. The number of swollen joints, CRP concentration and presence of erosive pathology are the main prognostic determinants of future joint damage and adverse outcomes[31], and are usually low or absent in those with NIRRA. Thus, although PROMs clearly indicate an impaired quality of life that needs addressing for individuals with NIRRA, the long-term prognosis for these patients is likely to be radically different to that for those with PIRRA.

Biological basis for refractory RA

Very few studies have specifically investigated the mechanisms of refractory RA. Nevertheless, current knowledge of the pathogenesis of RA, together with experimental studies that implicate important pathways in the development of pain, provide meaningful insights that can be applied to the development of refractory RA.

Persistent inflammatory refractory RA

In this section, we review the relevance of serological status to the understanding of PIRRA and the distinct genetic associations of autoantibody-positive and autoantibody-negative RA. We discuss epigenetics in relation to two potentially distinct processes: the effect of chronic inflammation on mediating epigenetic changes that thus render RA resistant to treatment; and the increased recognition of age-related epigenetic changes. And finally, we review the immune pathways implicated in RA, including those that have demonstrated redundancy through unsuccessful clinical trials but that might be relevant for specific subgroups of patients, such as those with PIRRA.

Autoantibody status. The fundamental hallmark of RA is the production of autoantibodies such as rheumatoid factor (RF) and antibodies that recognize post-translationally modified proteins, including ACPAs and anti-carbamylated protein antibodies. Autoantibody-positive and autoantibody-negative RA are considered to be distinct disease subtypes that might be associated with specific pathogenic mechanisms[32]. Seropositive RA is associated with severe disease and poor outcomes, including increased mortality[33–35], and several strands of clinical and experimental data indicate that positivity for both RF and ACPAs has an amplifying effect on disease and results in an aggressive phenotype[33,36,37]. However, there is no evidence linking this combination of autoantibodies with the development of PIRRA. A positive autoantibody status is also linked to good responses to rituximab and to other therapies that target B cells and T cells[38,39], further supporting the pathogenic relevance of serological status in RA. Studies looking at synovial tissue and fluid have also suggested discrete tissue characteristics and/or cytokine profiles for seropositive and seronegative disease[40,41], but these results have not yet been translated into personalized medicine approaches, and autoantibody status has not been evaluated in cohorts of individuals with refractory RA of any type, let alone in those with PIRRA (or indeed NIRRA).

Genetics. Autoantibody status (particularly ACPA status) has emerged as the most effective way of stratifying patients with RA owing to distinct genetic and environmental associations with autoantibody-positive and autoantibody-negative disease[42,43]. Clear evidence exists that specific genetic loci, most convincingly those within the HLA region but also those within shared and specific non-HLA genetic regions, contribute to ACPA-positive and ACPA-negative RA[44,45]. For example, specific HLA-DR alleles within the so-called 'shared epitope' (a five-amino acid sequence motif in residues 70–74 of the HLA-DR chain, encoded by several *HLA-DRB1* alleles, that is over-represented among patients with RA) are only associated with the risk of ACPA-positive RA and not with ACPA-negative disease[43,46]. In ACPA-negative RA, associations have been reported with *HLA-DRB1*03* and *HLA-B*08* (REFS[47,48]), and associations with HLA-A alleles have been reported for ACPA-positive RA[44]. The authors of a fine mapping study identified distinct sets of amino acid residues in HLA-DRB1 at position 11 that were either protective for or conferred a risk of ACPA-positive and ACPA-negative RA[44]. Specific residues and amino acid sites explained the HLA associations with ACPA-positive and ACPA-negative RA and the amino acid positions mapped to the peptide binding

Box 2 | **Proposed terminology for refractory RA**

Persistent inflammatory refractory RA

- **Advantages**
  - Confident of active rheumatoid arthritis (RA) pathology in the face of multiple therapies
  - Identifies a group of patients with a poor prognosis
  - Accurate basis for investigation and target validation
- **Disadvantages**
  - Status can change over time as drugs with different mechanisms of action are trialled
  - Could dismiss inflammatory or autoimmune pain mechanisms

Non-inflammatory refractory RA

- **Advantages**
  - Mitigates against unnecessary treatment changes or cycling
  - Identifies a distinct cohort for investigation of residual patient-reported outcomes and alternative pain mechanisms
- **Disadvantages**
  - Risk of missing low-level inflammation
  - Unclear basis
  - Possible overlap with entheseal pathology, osteoarthritis and pain syndromes

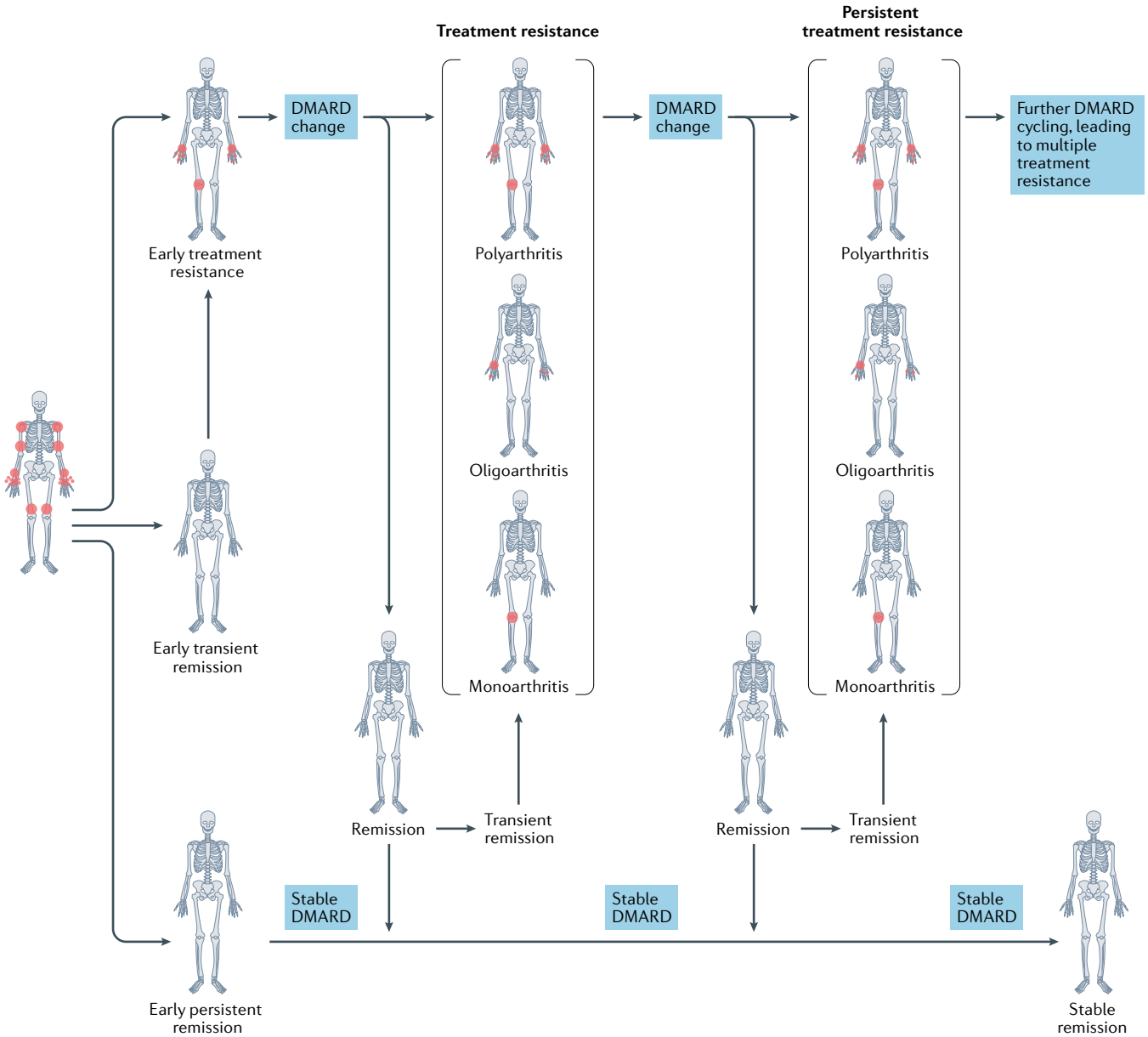


Fig. 2 | **Proposed trajectory and distribution of joint involvement in refractory RA.** Patients with rheumatoid arthritis (RA) who have a refractory disease course can follow this course from the outset, with early failures to successive therapies within the first 2–3 years. Often however, a varying depth of response initially occurs and an acquired loss of response is observed, such that refractory RA emerges after a period of several years. Although patients with untreated RA typically exhibit a symmetrical polyarthritis, individuals with refractory disease of a persistent inflammatory type might have a less extensive pattern of polyarthritis than at the time of diagnosis owing to the partial efficacy of successive therapies. A relatively oligoarticular pattern of disease that includes the small joints of the hands and wrists is also often seen in patients with refractory RA. Very occasionally, patients might have a monoarticular disease with extensive synovitis, although it is debatable if this type of disease would meet any definition of refractory RA, which was historically considered a polyarticular disease. Although some patients can have an intractable refractory disease course, the course of RA more often comprises several periods of stable or partial disease control (or remission) with a particular treatment, followed by a later relapse to active disease. The changes to treatment regimens that are associated with this disease course, often over a prolonged period of time, can also lead to a state of multiple-treatment-resistant refractory RA. In addition, some patients can have stable remission from the earliest time point and maintain this course over a long period of time.

grooves of the HLA molecules, implicating their relevance in antigen recognition and suggesting that different antigens might promote disease in ACPA-positive and ACPA-negative RA. HLA loci thus remain the genetic region with the strongest association with both autoantibody-positive and autoantibody-negative RA, albeit with distinct alleles for each type of disease.

Interestingly, in addition to being associated with ACPA-negative RA, *HLA-B*08* is also associated with susceptibility to psoriatic arthritis (PsA)[49]. Notably, several MHC class I associations have been reported for PsA, pointing to a specific $CD8^+$ T cell immunopathology in this disease[50,51]; supporting this notion, functional studies have shown the production of IL-17A by $CD8^+$

T cells from the synovial fluid of patients with PsA, but not from those with RA[52]. We would speculate that the *HLA-B*08*-associated ACPA-negative subgroup of RA might be similar to PsA and also have a predominant $CD8^+$ T cell-mediated immunopathology[53]. No further direct data exist on this putative subgroup of patients and whether it equates to PIRRA and, more importantly, whether therapies licenced for SpA-type disease might have a role in the treatment of some individuals with refractory RA.

No studies to date have reported a genetic architecture specific to PIRRA (or indeed NIRRA) and, given the relatively small proportion of patients with PIRRA, such an endeavour could be challenging despite the extensive amount of genome-wide association study data available from patients with RA[54]. In the post-genome-wide association study world, whole exome or whole genome sequencing could help to elucidate the genetic basis for PIRRA, which is relatively uncommon[55]. For example, in individuals with PIRRA who are autoantibody-positive and have known RA risk-associated HLA genes, genetic variant associations could be investigated to determine if any novel genetic factors are involved in PIRRA. As with other complex diseases, these variants could then be used to gain insights into the genes, cell types and mechanism of genetic influence in PIRRA, through expression quantitative trail loci and mapping of variants onto gene regions, gene regulators and active chromatin regions[56].

Epigenetic alterations. As discussed in the previous section, genomic studies of populations of individuals with PIRRA could potentially be used to identify a (rare and) distinct genetic trait that explains the treatment-resistant nature of the condition. However, it is likely that PIRRA develops over time. Clinical trial data of targeted interventions in individuals with very early RA show how strikingly good disease control can decrease over time[57,58], raising the possibility that accumulated epigenetic changes in chronic RA might help to determine the PIRRA state.

A number of lines of evidence suggest that epigenetic changes, including methylation and changes in microRNAs (miRNAs) and long non-coding RNAs, occur either before treatment or are induced by ageing and/or treatment[59], and thus might have a role in PIRRA. The epigenetic signature of RA can also change as a result of ageing or environmental factors, and treatment regimens that are initially successful in patients with RA can fail later, suggesting a change in mediators of disease in these individuals. Differential methylation has been observed between RA and osteoarthritis (OA)[60,61], between early and late RA[60], between individuals who respond to treatment and those who do not[62] and between different joint sites[63]. These results give an indication not only of the role of methylation in disease but also its plasticity, and potentially help to explain some of the heterogeneity that occurs in RA in terms of disease course and treatment response. Therefore, the reported unique 'DNA methylome signature' found in the early stages of RA, the ongoing alterations in DNA methylation as the disease progresses[60] and the signatures associated with RA or disease subgroups point towards epigenetic changes contributing to the development of a treatment-resistant pathology. Similar observations have been made for miRNAs and long non-coding RNAs, epigenetic modifiers of gene expression and cell state[64–67]. In particular, the miRNAs miR-146a and miR-155 have been extensively studied and are differentially expressed in patients with RA compared with those with OA or healthy individuals in a wide range of cell types, including cells in the blood, synovial fluid and synovial tissue[65–67].

Therefore, strong evidence now exists for how environmental factors can influence epigenetics, how epigenetics can influence cellular phenotype and how these epigenetic changes can be fluid among different cell types, disease stages and ages. Drug exposure and environmental influences such as smoking[68] are therefore probable contributors to changes in epigenetic states and immunopathogenesis that result in a PIRRA phenotype. A bidirectional relationship is thought to exist between inflammation and epigenetics, with the local inflammatory milieu inducing epigenetic alterations that lead to subsequent immune alterations, and vice versa[69–73]. Specific epigenetic markers contribute to the regulation of gene expression in RA and can be found in both immune and stromal cells[74]. These epigenetic changes potentially explain non-genetic risk factors in RA, and possibly have roles in the chronicity and perpetuation of inflammation. However, it is unclear if such epigenetic alterations occur as stochastic events, in response to specific environmental triggers or as a result of chronic inflammation. The largest study conducted to date suggested that an altered DNA methylation status might partially underlie the genetic effect of HLA risk by acting as an intermediary in the regulation of gene expression by disease variants[73]. Although these observations suggest potential epigenetic mechanisms in continued inflammation, they have not been specifically looked for in patients with PIRRA and thus remain speculative.

De novo mutations that affect epigenetic programming. Epigenetic changes might also be promoted through somatic mutations in RA. These mutations could affect adaptive and/or innate immune cells to contribute to the immunopathogenesis of RA, or could act in a manner that is completely independent of the effects of tissue inflammation. The expansion of haematopoietic clones carrying recurrent somatic mutations has been well described in older individuals, and such clones have also been identified in increased amounts in individuals with myelodysplastic syndrome (MDS) or acute myeloid leukaemia[75,76]. For example, whole-exome sequencing has enabled the identification of somatic mutations in genes involved in epigenetic regulation, including those involved in DNA methylation (*DNMT3A*), DNA hydroxymethylation (*TET2*) and histone methylation and ubiquitylation (*ASXL1*), in individuals with acute myeloid leukaemia[77].

Identical mutations are evident in the dynamic evolution of the haematopoietic system in individuals without clinical haematological disease, but who are at a high risk

of developing MDS and other haematological cancers, in a process known as clonal haematopoiesis of indeterminate potential (CHIP)[78]. Cardiovascular disease is strongly linked to inflammation; therefore, it is of particular interest that CHIP is associated with an increased cardiovascular mortality in individuals without MDS or evidence of tumorigenesis[79]. Thus far, only preliminary reports have been published of common CHIP associations in RA. One study reported the prevalence of CHIP in 59 patients with RA compared with 12 patients with MDS or aplastic anaemia and two healthy individuals and, within the RA group, attempted to ascertain if individuals with severe RA (typically considered a surrogate for a refractory state, although this is not necessarily the case) had a greater degree of CHIP than individuals with milder RA[80]. Overall, the authors of the study noted an expected age-related increase in CHIP in patients with RA that was substantially lower than in those with MDS. No association with severe disease was noted but the need for further studies was acknowledged. CHIP in RA synovial fluid macrophages has also been reported in the preliminary results of a small study of CHIP in patients with arthritis[81].

Certainly, the idea of a genetically evolving somatic mutation burden as a mediator of refractory RA is novel and is supported by evidence of somatic mutations in other RA settings. For example, *STAT3* mutations are associated with Felty syndrome (although this phenotype is not necessarily linked to refractory RA, including PIRRA)[82], and somatic mutations have been reported in $CD8^+$ T cells from patients with newly diagnosed RA[83]. Further studies in well-characterized cohorts of patients with RA are needed to understand the possible functional and mechanistic relevance of somatic mutations in the perpetuation of inflammation and recalcitrant pathology. A French study of inflammatory polyarthritis in patients with MDS showed that for most individuals, disease was ACPA negative, generally non-erosive and responded to steroids, perhaps suggesting that age-related myeloid changes might not be major contributors to refractory RA phenotypes[84]. However, many individuals with MDS and seronegative arthritis probably have a polymyalgia rheumatica-like phenotype, which perhaps is conflated with RA but has a distinctive topographical localization to the synovium[84]. Nevertheless, in a newly described syndrome, somatic mutations in patients with MDS have been linked to chronic inflammatory disease phenotypes including vasculitis and inflammatory arteritis, although a specific RA phenotype was not reported[85]. The association of such phenotypes with multiple simultaneous cytokine perturbations could render disease more refractory, whereby bDMARDs and even tsDMARDs might not provide sufficient suppression of all pro-inflammatory mediators.

Smoking. Smoking is an accepted environmental risk factor for RA, both for the development of the disease and also for poor prognosis and treatment-predictive outcomes[86,87]. Cigarette smoke contains over 4,000 chemicals that can elicit numerous effects on cells of the innate and adaptive immune system[88]. In the lungs, cigarette smoke extract promotes the production of TNF and other cytokines by macrophages[89] and has myriad other effects, including the dysregulation of reactive oxygen species and autophagy, which contribute to the inflammatory milieu[90].

The effects of cigarette smoke on refractory disease could potentially be linked to epigenetic and somatic mutations. In smokers, methylation levels in ACPA-positive individuals were able to account for the interaction between the rs6933349 genotype and smoking, which was not found in ACPA-negative individuals[91]. In addition, an association has been reported between smoking and CHIP[92] that extends the known relationship between smoking and the risk of MDS[93,94]. A relationship between CHIP and atherosclerotic disease, an inflammatory disorder with prominent myeloid cell involvement, has also been shown[76]. These changes most typically occur via somatic mutations in the DNA methylation pathways. Smoking might also epigenetically regulate immunity through other mechanisms, such as changes in DNA methylation status[68], as well as inducing cellular changes through oxidative stress and apoptosis, and promoting ACPA production[88]. Given the independent link between smoking and chronic pain[95], smoking seems to be a common denominator that could also contribute to the persistent and residual symptoms of a subgroup of patients with NIRRA[96]. Such data could, in the future, lend further support to a role for smoking cessation as part of a wider management strategy to improve long-term outcomes.

Immune pathways. Clinical trials of DMARDs for RA have been enormously instructive and have enabled the development of a more refined understanding of the relative roles of cytokines, their signalling pathways and their hierarchy within the immune system[97,98]. The great success of therapies directed against TNF or IL-6, especially in patients with early RA[57,99], with the achievement of high remission rates, supports their importance in the cytokine network; results that are consolidated by reports of an IL-6-mediated $CD4^+$ T cell signal transducer and activator of transcription 3 (STAT3) signature in the earliest stages of disease[100,101]. Granulocyte–macrophage colony-stimulating factor (GM-CSF) is also implicated in the pathogenesis of RA, as confirmed by positive results in early-phase studies of GM-CSF blockade[102]. However, the inflammation in individuals with PIRRA is likely to be independent of such cytokines, assuming resistance to therapies that target these cytokines is not mediated by neutralizing antibodies or poor drug compliance. Similarly, therapies that target B cells or T cell co-stimulation pathways validate RA as a prototypic autoantibody-mediated disease[103,104]. By contrast, although preclinical studies suggested roles for several cytokines in RA, including IL-17 (REFS[105,106]) and IL-1 (REFS[107,108]), clinical trials and studies of inhibitors for these cytokines lacked the non-redundancy needed to enable effective targeting[109,110]; however, these cytokines might still be relevant in specific subgroups of patients with refractory RA. A commonly cited hypothesis for the development of refractory disease is that of an escape mechanism and the emergence of a new mediator

following chronic blockade of an immune pathway, possibly through alteration of the tissue microenvironment and/or the systemic environment. Aside from a preliminary report of improved treatment response in mice with collagen-induced arthritis by targeting the IL-23–IL-17 axis alongside TNF blockade[111], no studies currently support this theory. Thus, it is unclear which immunological pathways typify PIRRA and whether targets outside of those pathways highlighted above remain to be discovered.

Non-inflammatory refractory RA

Several scenarios, some conjectural, can be considered to explain the pain and joint symptomatology that occurs in patients with NIRRA, which cannot all be comprehensively reviewed here. Coexistent or disease duration-dependent secondary OA is an obvious scenario. Reports of refractory RA associated with swollen joint counts, CRP concentrations and radiographic damage that are no more severe than in individuals with RA who respond well to treatment, and that is more common in young women than in men[20], might be linked to the multifaceted differences in pain perception that have been reported between men and women[112]. The persistence of pain in patients with RA is a well-recognized phenomenon that has not abated with the introduction of powerful anti-inflammatory agents. Studies suggest that such residual pain is attributable to persistent central sensitization and the development of maladaptive pain processing[113] (FIG. 3a). The effects of pain sensitization and poor outcomes in individuals who have delayed initiation of therapy[17,22,114] and secondary fibromyalgia are also obvious factors that could contribute to the development of NIRRA.

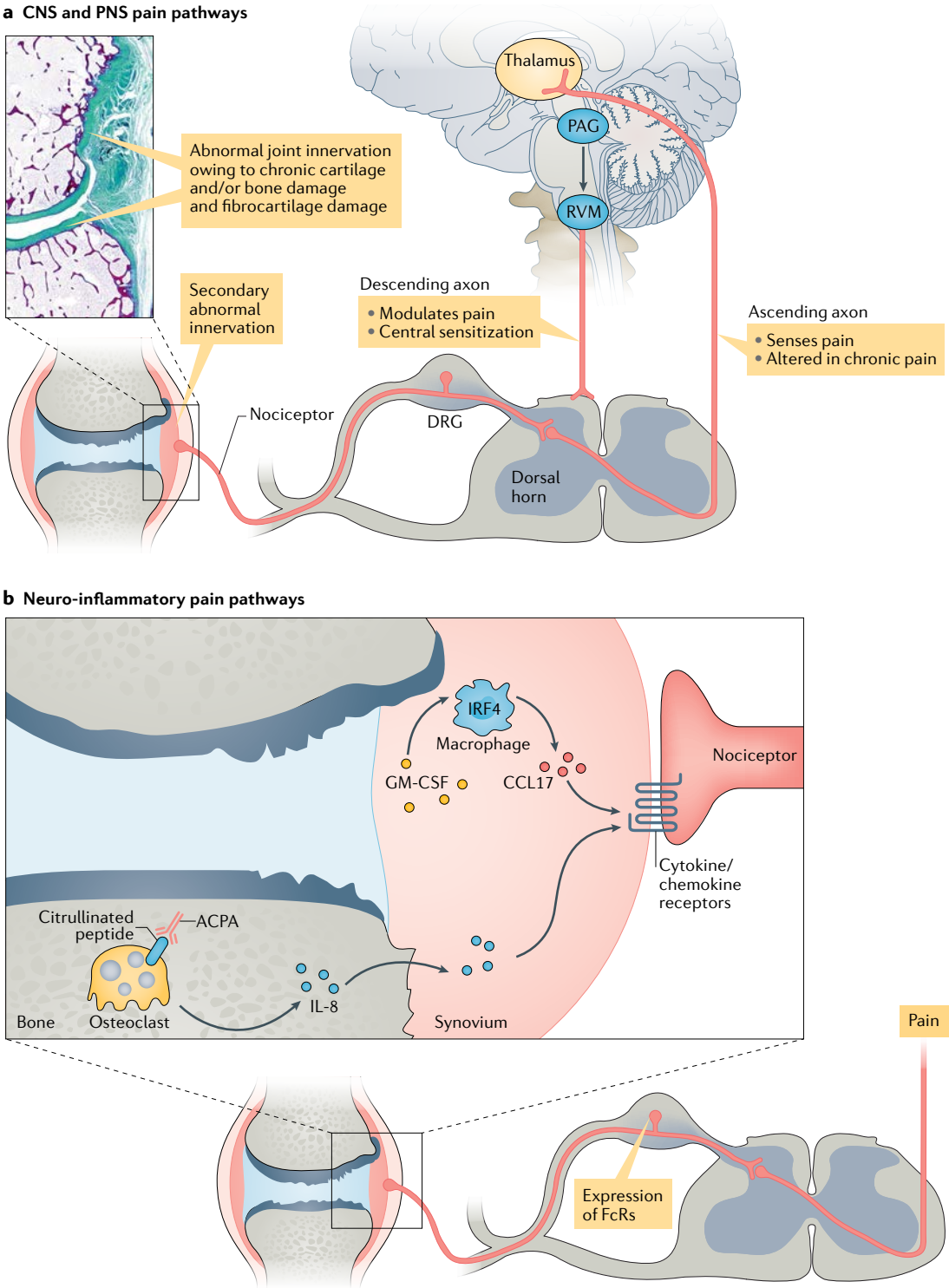
A potential role for autoimmunity and inflammation as an unconventional mediator of pain in RA (not related to acute or chronic joint synovitis) has attracted much research interest (FIG. 3b). ACPAs might directly contribute to osteoclast activation and are associated with bone pain in experimental models of RA[115], a scenario that could conceivably occur without discernible clinically or imaging-defined joint synovitis. A second area of research pertains to type II collagen-related immune complexes, which seem to be capable of activating Fc receptors expressed on dorsal root ganglion afferent nerve fibres. The injection of anti-type II collagen autoantibodies into mice was associated with pain behaviour in advance of discernible joint pathology[116]. Although anti-type II collagen autoantibodies are not specific to RA, this research provides a possible mechanism for peripheral nociception that is linked to joint inflammation in general. Several signalling pathways, including the TNF pathway and phosphoinositide-3 kinases, are known to have direct roles in dorsal root ganglion-related pain and inflammation in experimental arthritis[117,118]. In addition, a GM-CSF axis linked to the CC-chemokine ligand 17 (CCL17) pathway has been described in experimental arthritis, in which CCL17 was involved in pain development both dependently and independently of joint inflammation[119]. Accordingly, if this axis was able to promote pain independently of inflammation in RA, then mavrilimumab, an anti-GM-CSF antibody, would be predicted to improve outcomes in patients with RA, which was not the case[120].

We would speculate that such putative neuroimmunological effects could take place in conjunction with abnormal small joint innervation, secondary to inflammation-mediated damage to the normally avascular, ligamentous, fibrocartilaginous tissue that abundantly lines the proximal interphalangeal and metacarpophalangeal joints. The sites of small joint erosion in RA are actually covered with cartilage and form elaborate synovio-entheseal complexes that, once damaged by inflammation, might contribute to abnormal joint innervation[121,122]. Although the concept of abnormal joint enthesis innervation is well established in the spine in intervertebral disc degeneration disease[123], it remains to be established in small-joint RA.

Experimental studies in mice have also shown a central effect of TNF in mediating pain[124] and, in a pilot brain functional MRI study of patients with RA, treatment with a TNF inhibitor reduced activity in thalamic, limbic and associative areas of the brain before clinical improvements were seen[125]. A sophisticated multi-modal MRI study that included 54 patients with RA found that high levels of peripheral inflammation were associated with an increased number of positive connections between specific areas in the brain, and that these patterns of connectivity could predict fatigue, pain and cognitive dysfunction[126]. Intriguingly, post hoc analysis of a clinical trial of the Janus kinase (JAK) inhibitor baricitinib in RA suggests an effect on pain symptoms that might be independent of clinically evident joint swelling[127]. Comparative trials between JAK inhibition and TNF inhibition suggest that, despite comparable reduction of swollen joint counts and radiographic joint erosion retardation, JAK inhibitors produce superior composite disease activity scores to TNF inhibitors[128,129]. This superiority is thought to be mediated by an as yet poorly understood effect on pain. Curiously, the emergent JAK inhibitor therapies for RA might have serendipitously stumbled into the NIRRA arena to good clinical effect. Although entirely speculative, this apparent benefit of JAK inhibition could indicate an as yet unrecognized systemic neuro-inflammatory component of pain, an autoantibody-mediated mechanism of pain or could implicate JAK–STAT signalling in the pathogenesis of complex pain that is completely independent of inflammation.

Stromal cells in refractory RA

The role of stromal cells, namely synovial fibroblasts, across the refractory RA spectrum is also worthy of comment but is at a speculative stage of research. Stromal cells are relevant in the early stages of RA by virtue of their antigen-presenting capabilities and their ability to interact with the immune system to support the functional roles of adaptive immune cells[130,131]. The production of IL-6, prostanoids and matrix metalloproteinases by stromal cells also perpetuates synovitis and enables destruction of the extracellular matrix and subsequent joint damage[132]. However, these processes could conceivably be part of a stromal cell-mediated pathway



that is shared with other diseases such as OA[133,134], which might exist in isolation but might also be classified as autoantibody-negative RA and/or coexistent RA and OA. Interestingly, some individuals in an early RA inception cohort, in which patients' disease was characterized by synovial tissue pathotype, had a pauci-immune or fibroid 'non-inflammatory' type of disease[135]. Individuals in this fibroid group, approximately half of which were negative for ACPAs and RF, showed the lowest acute phase reactant levels, swollen joint counts and power Doppler ultrasonography scores[135]. This clinical profile, coupled with the near complete absence of immune cells in the synovium, would be consistent with a clinical disease pattern characterized by little or no inflammation that would not be expected to respond to immune-targeted DMARDs, which is indeed what was reported[135,136].

We would speculate that this phenotype of RA with low levels of inflammation might reflect a milder disease state or a post-inflammation resolution state if it

Fig. 3 | **Speculative pain mechanisms in non-inflammatory refractory RA.** Pain in rheumatoid arthritis (RA) arises from the interactions between joint pathology and the processing of pain signals by peripheral, spinal and supraspinal pain pathways. **a** | Dysregulation of central nervous system (CNS) pain pathways can contribute to hyperalgesia and allodynia, which are associated with chronic pain. The primary CNS pain regulatory mechanisms comprise descending modulatory pathways via the periaqueductal grey (PAG), the rostral ventromedial medulla (RVM) and central sensitization. In the joints, chronic inflammation, malalignment and damage to the cartilage, bone, capsule and fibrocartilage can lead to secondary abnormal joint innervation and the development of local pain. This type of pain is more likely to develop in chronic RA. **b** | Theories are emerging of neuro-inflammatory mechanisms of pain in RA. Autoimmune mechanisms contribute directly to pain in settings where there is no measurable synovitis, which might have relevance for non-inflammatory refractory RA (and might also occur in persistent inflammatory refractory RA). Putative mechanisms from experimental models of arthritis include anti-citrullinated protein antibody (ACPA)-mediated activation of osteoclasts and associated bone pain mediated by IL-8, and immune complex-mediated activation of neuronal Fc receptors (FcRs) expressed on the dorsal root ganglion (DRG). Inflammatory mediators such as CC-chemokine ligand 17 (CCL17) can also have central effects on nociceptive pain. A granulocyte–macrophage colony-stimulating factor (GM-CSF)–CCL17 pathway mediated by interferon regulatory factor 4 (IRF4) has been demonstrated in experimental models of arthritis that could have implications for humans. These mechanisms could be pervasive in RA, contributing to pain in both inflammatory and non-inflammatory RA phenotypes. Such mechanisms could operate during the earlier phases of the disease, when minimal clinical joint swelling is present. PNS, peripheral nervous system. Part **a** adapted with permission from McGonagle et al.[121], Wiley. Copyright © 2009 by the American College of Rheumatology.

were to emerge after therapy, or might be a result of diagnostic overlap and/or misclassification with OA. Alternatively, a neat explanation for progression from early RA to later RA, in which responses to therapy are less robust, is the evolution to a non-immune stromal pathology. Such a theory would explain resistance to the currently available anti-inflammatory DMARDs, which fail to address fibroblastic disease. An ongoing study of cyclin-dependent kinase inhibition[137] was conceived on the premise that active stromal pathology underlies the residual ceiling effect of bDMARDs and that additional targeting of fibroblasts might further close the disease activity gap. However, if stromal cells are indeed responsible for refractory RA, stromal pathology might be expected to confer a site-specific disease (such as persistent monoarthritis or oligoarthritis); it is more difficult to conceptualize multi-joint PIRRA in terms of dysregulated stromal biology unless chronic inflammation can elicit abnormal stromal function in multiple joints[138]. A study of patients with RA in which a circulating fibroblast-like cell type was identified during a flare[139] needs to be validated, and other studies have failed to detect stromal cells in the blood[140]. Anatomical and functional heterogeneity in fibroblasts has been reported across different diseases[63,141] and specifically in RA[142], possibly implicating roles for fibroblasts across the refractory RA spectrum. A study in mouse models of resolving and persistent arthritis revealed two distinct subsets within the fibroblast activation protein-α (FAPα)-positive synovial fibroblast population: one that assumed an immune-effector role by sustaining inflammation through its distinct chemokine and cytokine profile; and one that mediated joint damage through bone effector cells[143]. However, it is unclear if these mouse fibroblast phenotypes can be translated to the human setting.

Management of refractory RA

As discussed at the beginning of this Review, we believe that placing the paradigm of persistent joint swelling and raised acute phase reactants (which are clearly linked to progressive joint destruction and poor outcomes)[4,19] at centre-stage helps to identify which patients with refractory RA belong to the two major (but overlapping) sub-categories — PIRRA and NIRRA. The ability to classify these two categories strongly argues for a more attentive approach and for the precise evaluation of persistent disease activity and PROMs (FIG. 4). Careful clinical assessment to demonstrate the absence of extensive joint synovitis, including the use of power Doppler sonography (if available) and a targeted examination of painful and tender joints, can be used to support a clinical impression that DMARDs are appropriately targeting inflammation. These assessments can also serve to reassure patients that their lingering treatment-resistant symptoms might not require a change in DMARD therapy. Where sonography is not available, definite clinically defined 'boggy' joint swelling, raised acute phase reactants and progressive radiographic damage can be used to differentiate PIRRA from NIRRA and to mitigate against the erroneous perception of continued underlying synovitis.

We further believe that an important determinant in the assessment of refractory RA should be in relation to identifying an individual's RF, ACPA and HLA status. As a first line of stratification, PIRRA could be considered to comprise three groups: autoantibody-positive RA that has an HLA association; autoantibody-negative RA (for both RF and ACPA) that has an HLA association; and autoantibody-negative RA with no HLA association (which would be more autoinflammatory in nature).

Given the heterogeneity of refractory RA and the current understanding of the pathogenesis of RA[144], the vital question for therapeutic strategies is whether the inflammation in PIRRA is predominantly humorally mediated, T cell-mediated or innate cell-mediated (autoinflammatory)[53] (FIG. 5). Identifying the principal mechanism of inflammation has obvious ramifications for the choice of therapeutic approach. B cell-targeted therapies and T cell costimulation-targeted therapies need to be considered for autoantibody-positive disease[38,145]. By contrast, autoantibody-negative refractory RA (in particular, potentially non-HLA-associated disease) would be predicted to be more innate immune cell-mediated or autoinflammatory in origin, and might also include other differentials such as crystal deposition disease[146] that can coexist with or be confused with RA. Thus, reappraisal for alternative (and/or coexistent) conditions or other rare innate immunopathologies or autoinflammatory mechanisms should also be undertaken. Cytokines that are seemingly less relevant to RA (such as IL-1) might actually be biologically pertinent[147,148] in a subgroup of patients with refractory RA despite IL-1 antagonism having minimal efficacy in patients with a typical autoimmune pattern RA following TNF inhibitor failure[110].

The possibility also remains that an as yet unidentified treatment target exists that is not covered by current advanced therapies for RA. Experimental studies

to identify new, tractable targets and stratified treatment approaches are therefore urgently needed. For individuals with persistent seronegative refractory RA, a potential role for the IL-23–IL-17 axis could be considered, as the RA phenotype might potentially be a presentation of PsA without psoriasis. Looking further afield, evidence exists that anti-IL-18 strategies might be beneficial for autoinflammatory disease phenotypes such as adult-onset Still's disease[149]. A seronegative refractory RA more akin to autoinflammatory conditions or adult-onset Still's disease could be a future candidate for targeting cytokines such as IL-18, type I interferon[150] and IFNγ[151].

As well as being an important mediator of early RA[99], IL-6 seems to also be relevant in later stages of the disease, as an IL-6-targeted therapy was effective in patients who had previously failed to respond to multiple TNF inhibitors[152]. These results are consistent with a non-redundant role for IL-6 and distinct intracellular signalling pathways for TNF and IL-6. However, it is important to acknowledge that the participants in this trial[152] did not clearly have refractory RA, but instead either had inadequate responses to one or more TNF inhibitors and/or an intolerance to two or more TNF inhibitors. In addition, a genuine form of multidrug-resistant refractory RA that evades targeting of both TNF and IL-6 is clearly seen

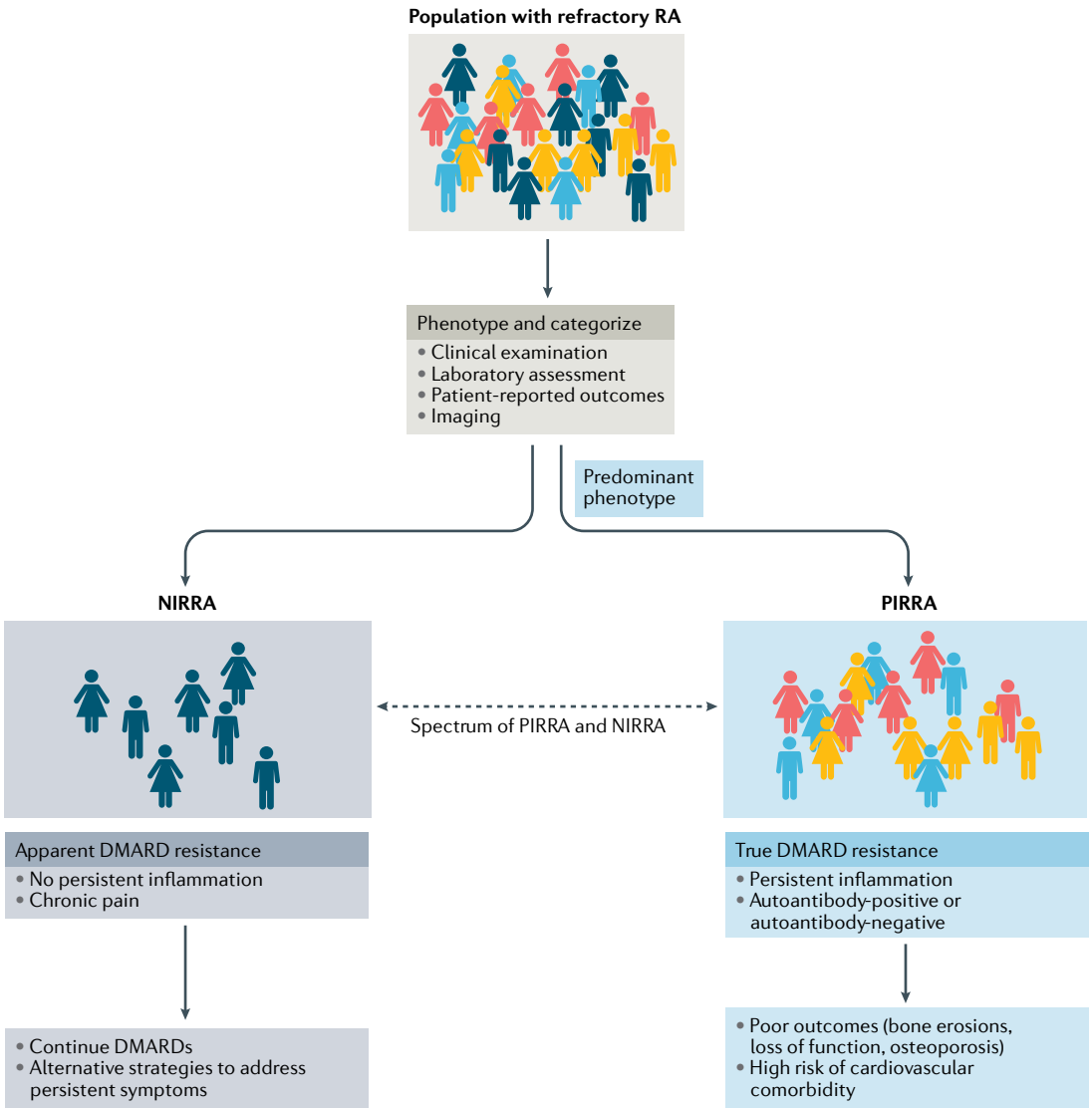


Fig. 4 | **A first step in the stratification of refractory RA.** A fundamental first step in stratifying individuals with refractory rheumatoid arthritis (RA) should be to confirm the presence of a genuine, persistent inflammatory pathology. Clinical examination, biochemical evidence of systemic inflammation and patient-reported outcomes can be supplemented with sensitive imaging (such as ultrasonography, which can be readily used at most disease sites) to verify recalcitrant disease. This strategy splits patients into two broad categories that we have called persistent inflammatory refractory RA (PIRRA) and non-inflammatory refractory RA (NIRRA). Those in the PIRRA group have a poor prognosis and the highest risk of comorbidities such as cardiovascular disease. A greater understanding of PIRRA immunopathogenesis will be central to identifying existing or emergent therapies. Those in the NIRRA group are likely to be receiving appropriate targeted therapy but have poor quality of life and patient-reported outcomes such as pain and fatigue, highlighting the need for alternative strategies for addressing the persistent symptomology that promotes the measured disease burden.

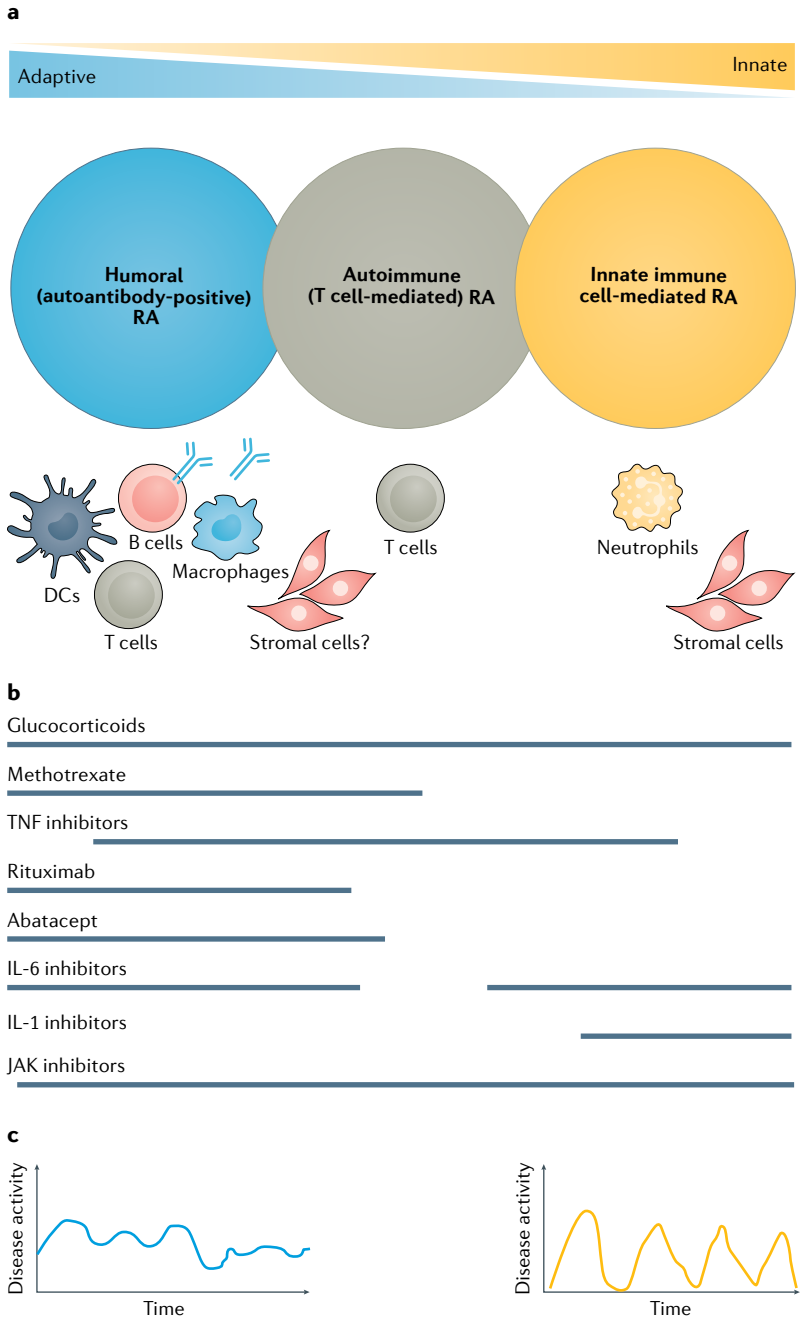


Fig. 5 | **Therapeutic approaches to targeting inflammation in refractory RA. a** | Inflammation in refractory rheumatoid arthritis (RA) can be predominantly humorally mediated, T cell-mediated or innate immune cell-mediated, or can involve a combination of all three owing to the functional integration of innate and adaptive immunity. A cellular hierarchy is likely to exist between more autoimmune RA and more innate immune cell-mediated RA. Humorally mediated disease relies on the triune axis of follicular helper T cells, dendritic cells (DCs) and B cells. Macrophages mediate much of the pathology of autoimmune RA following immune complex activation and also probably have a major role in seronegative (potentially more T cell-mediated) RA. Neutrophilic inflammation is important in innate immune cell-mediated RA. The role of stromal cells or joint fibroblasts in the pathogenesis of RA could be multifaceted and awaits elucidation in refractory RA, so we have placed stromal cells as primarily facilitating innate pathways, although speculate that they might have a role in adaptive processes via epigenetic mechanisms. **b** | Therapeutic approaches to refractory RA such as targeting B cells (rituximab), T cells (abatacept) or the IL-6 pathway affect adaptive immune cell-mediated disease, as does the use of Janus kinase (JAK) inhibitors. TNF inhibitors are effective across most of the range of RA. JAK inhibitors are beneficial in innate immune cell-mediated diseases, as are glucocorticoids, which are effective across a wide range of both innate and adaptive immune pathways. IL-1 blockade is licensed for use in RA, but has proven ineffective at a group level; however, IL-1 blockade might be beneficial in refractory RA that is innate immune cell-mediated. It remains to be seen whether some individuals with seronegative refractory RA actually have a psoriatic arthritis-type phenotype, and therefore if inhibiting the IL-23–IL-17 axis warrants consideration. **c** | Different disease activity courses can be observed over time for adaptive immune cell-mediated and innate immune cell-mediated RA. Whereas adaptive immune cell-mediated RA might typically demonstrate persistent, albeit varying, levels of disease activity, innate immune cell-mediated RA might show the episodic spikes in inflammation (disease activity) that are characteristic of autoinflammatory pathology.

in the clinic. Nevertheless, previous drug exposure and the stage of disease at which these cytokines are targeted might be relevant in mitigating against future refractory disease[1]. Delayed treatment and suboptimal targeting of RA near onset might favour the development of refractory RA, which, in humorally mediated PIRRA, might be mediated by secondary lymphoid organ development and an accompanying increased autoantibody titre in the joint[153]. Planned exploratory analysis of a trial of methotrexate with or without a TNF inhibitor in patients with early RA[18] suggested a reduced responsiveness to TNF inhibition following methotrexate exposure, implicating a change in the biology of the disease to a more refractory form. If credible, the importance of disease suppression at the very earliest stages of RA in mitigating against the development of refractory disease could alter the current perspective that methotrexate should be used as a generic first-line treatment for all patients.

The comparable response profiles to JAK inhibitors in patients with established RA who have previously failed to respond to bDMARDs with those observed in patients with an earlier stage of disease[154–156] make a persuasive argument for the additional benefit of simultaneously inhibiting multiple cytokine signalling pathways with JAK inhibition. The use of JAK inhibitors, combined with effective targeting of RA-related inflammation at an early stage, could limit epigenetic changes and somatic mutations, and might preclude the development of PIRRA. The ability of JAK inhibitors to target the multiple cytokines that mediate autoantibody-positive and autoantibody-negative RA, as well as the overlapping connective tissue diseases that can coexist, provides the coverage needed to capture the heterogeneity of RA and refractory RA (FIG. 5). For example, JAK inhibition with tofacitinib[157] might be effective in adult-onset Still's disease, which overlaps with seronegative or autoinflammatory types of RA, suggesting that JAK inhibition might

overcome treatment resistance in individuals with these types of refractory RA. Although at an early stage of evaluation, and in the absence of data in individuals with clearly confirmed PIRRA, it is still tempting to speculate that JAK inhibition could help to treat this group of patients.

Aggressive treatment strategies might also have a role in severe PIRRA. Historically, the use of TNF inhibitors and anakinra (which targets IL-1) to treat severe RA was limited by toxicity and no clear efficacy[158]. Nevertheless, the potential for combinatorial cytokine antagonism has been reinvigorated by the possibility of antagonism of the synergistically acting cytokines TNF and IL-17A[159] or TNF, IL-17A and IL-17F[160]. This approach offers a potential future option for treating individuals with PIRRA. Finally, radical strategies that reset the 'immunostat', such as autologous haematopoietic stem cell transplantation, have proved disappointing in RA[161]. Whether an allogeneic bone marrow transplant might be more fruitful for inducing long-term remission in individuals with truly refractory RA who otherwise have good health remains speculative[162,163].

Future research needs

On the basis of the literature discussed in this Review, patients with genuine refractory RA are likely to be genetically heterogeneous and to even show complex overlaps between innate and adaptive immune mechanisms[147]. We would postulate that ostensible non-autoantibody-associated disease is likely to be molecularly heterogeneous and to involve unrecognized humorally mediated disease, $CD8^+$ T cell-mediated disease and predominantly innate immune cell-mediated disease. By contrast, the basis for resistant seropositive refractory RA pathology in individuals with PIRRA despite the use of current humorally targeted approaches remains unclear. Single-cell RNA sequencing and mass cytometry have been used to delineate the transcriptomic and cellular basis of joint synovitis in RA, although the samples used for these studies were procured from arthroplasty, as well as from ultrasound-guided biopsy[164]. Systems biology, although powerful, has thus far only pointed towards broad homogeneity in cell populations and cytokine biology in RA, potentially reflecting the diversity of the patient populations studied to date. Modern technologies have enabled the discovery of rare disease-relevant subpopulations of cells in patients with RA[165], but the relevance of these cells to refractory RA remains unclear. Also, the cardinal molecular events that mediate autoantibody-positive PIRRA might take place in the primary and secondary lymphoid organs outside of the joint, which are not investigated by taking tissue samples by synovial biopsy. Nevertheless, characterization of individuals with PIRRA using an integrated omics approach involving serological status, whole genome sequencing, RNA sequencing and epigenetic modifications could help to identify underlying endotypes and inform a personalized medicine approach to multiple therapy-resistant RA.

Conclusions

Overall, although a substantial amount of the ongoing symptomatology of individuals with refractory RA might be related to persistent pain mechanisms, prototypic autoimmune and inflammatory mechanisms that lead to ongoing synovitis in patients with refractory RA clearly exist. In this Review, we suggested that distinguishing those with multiple therapy-resistant refractory RA (PIRRA) from those with persistent measured disease activity and cycling of therapies in the absence of inflammation (NIRRA) is fundamental to understanding and managing refractory RA. Refined clinical phenotyping will be essential to aid in the identification of those with genuine persistent inflammatory disease and, thus, to reveal specific molecular pathways associated with disease subtypes. Epigenetic mechanisms and acquired somatic mutations, together with environmental cues such as smoking, might influence the dynamic genetic and epigenetic landscape of RA, leading to changes in the biology and relative roles of immune and non-immune pathways. Suboptimal or delayed treatment of RA near onset might favour the development of refractory RA that is generally resistant to DMARD strategies. Therapies that target TNF or IL-6 are of central importance for the treatment of RA and, when used early, lead to highly impressive outcomes. But whether the sequence in which targeted therapies are used is relevant to the development of refractory RA (including persistent symptoms in the absence of clear inflammation) is not clear. For individuals with truly treatment-resistant disease, the tools are now available for interrogating immune and stromal cells in the joint and beyond in order to explore therapy resistance mechanisms, and for interrogating the genomic architecture and epigenetic changes in distinct cell types at single-cell resolution. Precise clinical phenotyping to identify genuine refractory RA, which we have termed PIRRA, combined with a multi-omics approach, should lead to greater mechanistic insights into the cellular heterogeneity and differentiation behind the development of refractory disease.

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The evolution of nerve growth factor inhibition in clinical medicine

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Abstract | Nerve growth factor (NGF) is a neurotrophin that activates nociceptive neurons to transmit pain signals from the peripheral to the central nervous system and that exerts its effects on neurons by signalling through tyrosine kinase receptors. Antibodies that inhibit the function of NGF and small molecule inhibitors of NGF receptors have been developed and tested in clinical studies to evaluate the efficacy of NGF inhibition as a form of analgesia in chronic pain states including osteoarthritis and chronic low back pain. Clinical studies in individuals with painful knee and hip osteoarthritis have revealed that NGF inhibitors substantially reduce joint pain and improve function compared with NSAIDs for a duration of up to 8 weeks. However, the higher tested doses of NGF inhibitors also increased the risk of rapidly progressive osteoarthritis in a small percentage of those treated. This Review recaps the biology of NGF and the studies that have been performed to evaluate the efficacy of NGF inhibition for chronic musculoskeletal pain states. The adverse events associated with NGF inhibition and the current state of knowledge about the mechanisms involved in rapidly progressive osteoarthritis are also discussed and future studies proposed to improve understanding of this rare but serious adverse event.

Chronic pain, especially from musculoskeletal conditions such as osteoarthritis (OA) and chronic low back pain (CLBP), affected more than 100 million individuals in the USA in 2008 and, in 2010, had an estimated annual cost of over US$600 billion[1]. These estimates underscore the considerable public health burden of chronic pain and remind the medical community that diseases that cause pain are all too common. The currently available treatments for these ailments, such as paracetamol (acetaminophen), NSAIDs, opioids, tramadol and anti-depressant medications, can be effective but also have substantial limitations. In addition, the incidence of opioid-related hospitalizations among patients with musculoskeletal disorders has increased over the past two decades and continues to increase in individuals with OA[2]. Although important advances have sharpened our understanding of the pathophysiology of musculoskeletal pain, the majority of new pharmaceuticals have failed when translated from the laboratory to clinical trials.

Over the past two decades, nociceptive pain induced by neurotrophins via peripheral sensory nerve pathways has been carefully studied. This work led to the development of inhibitors of the neurotrophin nerve growth factor (NGF), which were initially studied in preclinical and clinical non-musculoskeletal conditions. NGF inhibitors, in the form of anti-NGF monoclonal antibodies that bind NGF and render it inactive, have also been evaluated for efficacy in the reduction of pain in musculoskeletal and non-musculoskeletal disorders. Despite initial phase II and III clinical trials with NGF inhibitors demonstrating efficacy in reducing joint pain and improving function, reports of rapidly progressive OA (RPOA) of both the knee and hip joints emerged[3]. The incidence of RPOA resulted in the FDA halting the clinical trials for a period; a review of the clinical trials found that RPOA was associated with the higher doses of the anti-NGF antibodies used in the studies, and with the combined use of an anti-NGF antibody and an NSAID[3]. The clinical trial development programmes subsequently resumed using lower doses of the anti-NGF antibodies and, at the time of writing, a new drug application for the anti-NGF antibody tanezumab has been submitted to the FDA for review and approval.

In this Review, we cover the biology of NGF, the clinical studies performed to evaluate the efficacy of inhibiting NGF in chronic musculoskeletal pain states, the adverse events that subsequently developed and the investigations that have been performed to explain those adverse events. We also recommend future studies to improve the understanding of the rare but serious adverse event of RPOA.

The biology of NGF

The discipline of neuroscience dates back to the late nineteenth century, when novel microscopy techniques became available that enabled the detailed study of the central nervous system (CNS). In work that resulted

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Key points

- Chronic pain from osteoarthritis (OA) is highly prevalent, and effective non-opioid medications are few.
- Nerve growth factor (NGF) is an important neurotrophin that activates nociceptive neurons to transmit pain signals from the peripheral to the central nervous system.
- Treatment with anti-NGF antibodies inhibits joint pain and improves function in individuals with moderate to severe knee and hip OA.
- NGF inhibition is associated with rapidly progressive large joint OA; many theories exist as to why but the exact mechanisms involved remain unknown.
- Anti-NGF antibody treatments, if approved, should reduce pain and improve quality of life for individuals with knee and hip OA; however, safety monitoring programmes will be necessary.

in the 1906 Nobel Prize in Physiology or Medicine, Santiago Ramón y Cajal used silver nitrate staining techniques developed by Camillo Golgi to examine the CNS and found it to be composed of a network of depolarizing neurons interconnected with synapses[4]. This fundamental understanding paved the way for the development of the field. NGF was first described in 1951 and was initially found to control the growth and differentiation of embryonic sympathetic and sensory neurons[5]. Decades later, NGF was discovered to be present in adults and to have a role in tissue injury and pain[6], which led to the study of NGF in health and various diseases. Important milestones in the history of NGF from the onset of neuroscience to the development of therapeutic antibodies are outlined in FIG. 1.

NGF as a neurotrophin. NGF belongs to a group of structurally related neurotrophins in the peripheral nervous system (PNS) and CNS. Important neurotrophins include NGF[5], brain-derived neurotrophic factor (BDNF)[7], neurotrophin 3 (NT3)[8], NT4 (also known as NT5)[9], NT6 (REF.[10]) and NT7 (REF.[11]). The nature and mechanism of action of neurotrophins is complex and thus not described here in detail (reviewed elsewhere[12]). Briefly, neurotrophins regulate neuron survival, growth and differentiation in the PNS and CNS during embryonic development. For example, BDNF mediates embryonic placode development of CNS sensory neurons. In addition, neurotrophins have an important role in the physiology of the nervous system in adulthood and are upregulated under pathological conditions.

Of all the neurotrophins, NGF has been studied in the greatest detail. The NGF molecule is composed of three subunits, called α, β and γ, and regulates the embryonic development of PNS sensory and sympathetic neurons from the neuronal crest: embryonic neuroblasts that lack NGF undergo apoptosis[13]. However, the presence of NGF is also required in adulthood; phenotypic knockout of NGF in adult mice (via the induction of anti-NGF antibodies) produces animals with skeletal muscle dystrophy and a reduced number of splenocytes[14]. Furthermore, these mice have smaller superior cervical ganglia and a reduced number of dorsal root ganglia (DRG) neurons compared with wild-type mice. Regarding the CNS in these mice, neurons that stained positive for anti-choline acetyl transferase were diminished in number and the learning capacity of the mice was impaired[14]. Thus, the presence of NGF seems to be obligatory for both the PNS and the CNS, and perhaps also for the immune system of adult organisms.

NGF signalling. NGF binds to two separate receptors; p75 and tyrosine kinase A (TrkA)[15]. The low affinity receptor p75 is not necessary for NGF to achieve its biological function and might serve as a co-receptor[16]. By contrast, TrkA has a high affinity for NGF and belongs to a group of transmembrane receptors that have overlapping specificities for several other neurotrophins[17,18]. For example, TrkB selectively binds BDNF and NT4 (REF.[19]), and TrkC has a high affinity for NT3 (REF.[20]). When NGF binds to TrkA, the receptor complex is endocytosed and translocated to the nucleus of the DRG by retrograde axoplasmic transport. Within the DRG nucleus, phosphorylation of the NGF–TrkA complex induces gene transcription[18,21,22]. In adults, NGF induces the overexpression of other neuronal molecules, such as substance P[23] and calcitonin gene-related peptide (CGRP)[24], in response to pain stimuli (including thermic, mechanical, electrical and UV irradiation) originating from nociceptors (FIG. 2a). These neurotransmitters are transported to spinal cord synapses for the transmission of action potentials to the CNS. However, they can also be released from the nociceptor itself after antidromal transport. In this situation, the neurotransmitters can then act as pro-inflammatory molecules to induce vasodilation and chemotaxis, causing subsequent local inflammation[25] (FIG. 2b). In addition, primary afferent nerve fibres have an increased excitability in response to NGF when acid-sensing ion channels[26], transient receptor potential cation channel subfamily V member 1 (REF.[27]) and other receptors are activated. The result of this activation is an increase in the excitability of these fibres, termed peripheral sensitization. By contrast, changes to the CNS induced by ongoing pain stimuli lead to hyperexcitability and reduced neuronal inhibition, a phenomenon termed central sensitization[28]. In a clinical context, afferent nerve stimuli can cause an increased sensitivity to heat or touch stimuli, which can induce allodynia. Thus, stimulation of the nociceptor and internalization of the NGF–TrkA complex is converted to local inflammation and further pain sensation in a process called neurogenic inflammation (FIG. 2b).

In adult rats, 44% of low-calibre (<30 µm) sciatic DRG neurons express TrkA, 27% express TrkB and 17% express TrkC[29]. The vast majority of visceral pelvic neurons express TrkA and TrkB, but express TrkC to a lesser extent. By contrast, afferent motor neurons express TrkB (50%) and TrkC (73%) but rarely TrkA (20%)[29]. These data suggest that TrkA and its ligand NGF are crucially important for pain perception in adult rats. However, TrkA is not only found on neurons but also exists on a variety of non-neuronal cells including human keratinocytes[30], synovial fibroblasts[31], mast cells[32] and all major types of peripheral leukocytes[33]. These data suggest that several distinct non-neuronal mechanisms are linked to NGF.

NGF in joint tissues. As a modulator of chronic pain, NGF represents a promising target for the treatment of pain associated with musculoskeletal diseases.

Placode
Ectodermal structures in embryonic development that give rise to several different sensory systems.

Dorsal root ganglia
The cell bodies of sensory nerves that transmit action potentials to the spinal cord.

Retrograde axoplasmic transport
A process in which signalling molecules are moved from the periphery towards the cell body of an axon.

Antidromal transport
Axoplasmic transport of signalling molecules from the nucleus to nociceptors.

Allodynia
Painful sensation in response to non-painful stimuli.

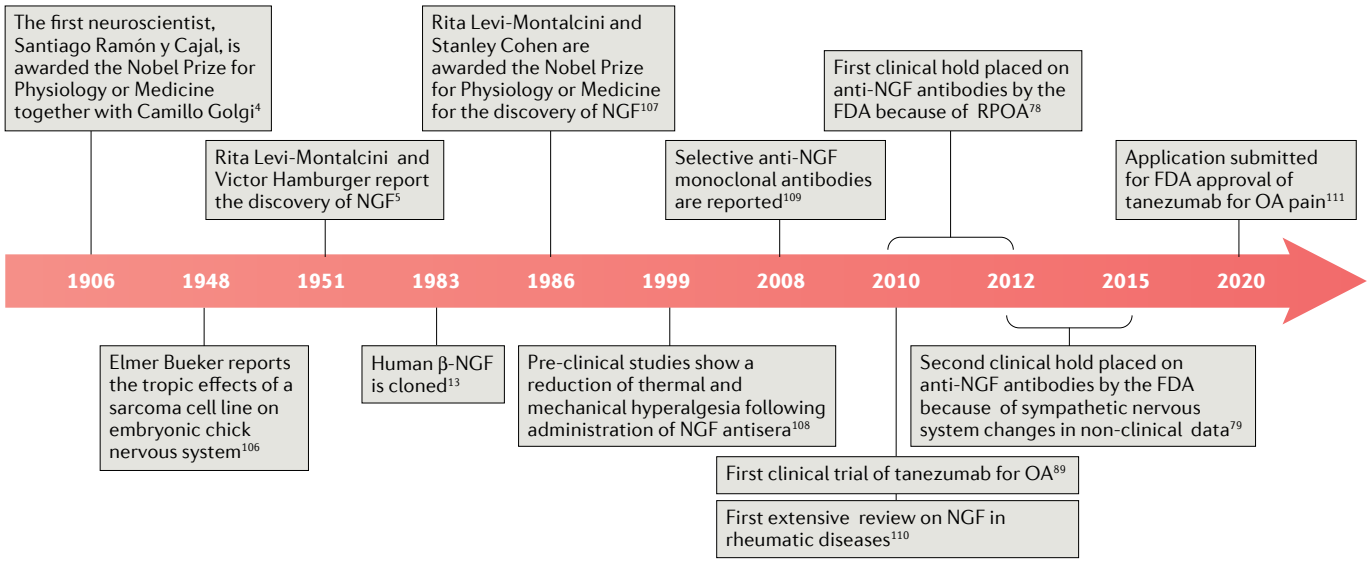

Fig. 1 | **The evolution of NGF inhibition in clinical medicine.** A timeline showing some important steps in the development of nerve growth factor (NGF) inhibitors for use in clinical medicine, from the birth of neuroscience when Santiago Ramón y Cajal and Camillo Golgi were awarded the Nobel Prize for Physiology or Medicine in 1906, to the submission of tanezumab to the FDA for approval for use in the treatment of osteoarthritis (OA) in 2020 (REFS[4,5,13,78,79,89,106–111]). RPOA, rapidly progressive osteoarthritis.

In addition, in the past decade, it has become clear that NGF is a pleiotropic molecule that affects the nervous system, bone and many other tissue compartments. Knowledge of NGF-mediated mechanisms beyond the nervous system is therefore crucial for understanding how systemic NGF inhibition might work and its potential adverse effects in patients with chronic pain. However, most of what is known about the effects of NGF on the musculoskeletal system comes from animal studies and contributes only indirectly to an understanding of the human musculoskeletal system.

NGF has an important role in bone metabolism and regeneration in animal studies. In healthy C57BL/6 mice, NGF is present in endothelial cells in the subchondral bone layer adjacent to the articular surface and scattered throughout the bone marrow[34]. Furthermore, TrkA and p75 expression is mostly limited to nerve fibres that are in close proximity to NGF-positive blood vessels. NGF also regulates sensory pain signals in the bone of rats in similar way to other parts of the periphery[35]; however, this signalling is a rapid, independent process that occurs before retrograde transport mechanisms and gene transcription can take effect. Experimental fracture or joint distraction models permit a detailed analysis of regenerative bone metabolism. In unfractured rat bone, osteoprogenitor cells express NGF[36]. After fracture, bone marrow stromal cells, osteoblasts and endothelial cells within newly formed capillaries are positive for NGF, and during subsequent callus formation, the periosteal matrix also gains positivity for NGF. In mice with tibial fractures, NGF also stimulates the formation of the callus by increasing the number of osteoblasts[37]. Topical application of β-NGF to cranial defects in rats induced the expression of β-III-tubulin and vascular endothelial growth factor, suggesting a regulatory role for neuronal growth and angiogenesis[38]. However, although NGF inhibitor treatment did not inhibit callus formation in a closed femur fracture pain model in mice, it did reduce fracture-induced pain-related behaviour by ~50%[39]. These data suggest a regulatory and probably pro-osteogenic effect of NGF in murine models.

Preclinical models can also provide insight into potential favourable outcomes of clinical trials in humans[40]. For example, vaccination against NGF produced a substantial reduction in pain behaviour in mice with partial meniscectomy-induced OA[41], providing further evidence that a decrease or depletion of NGF can be a powerful tool in reducing musculoskeletal pain. However, in human disease, the situation is similar in some ways and different in others. Results from experimental models of disease are sometimes difficult to interpret because they often involve an experimental procedure that does not exactly resemble human pathology. In addition, immune responses, connective tissue metabolism and pain perception mechanisms in animals can differ considerably from human physiology. The results from such animal studies thus resemble the human situation, but might not be identical. Therefore, the results from these studies should form the basis for experiments with human tissue.

OA has a pro-inflammatory cytokine profile similar to that found in rheumatoid arthritis (RA), but at a lower intensity. NGF, TNF and IL-6 are all present in knee synovial and meniscal tissue following injury[42]. In synovial fluid, NGF expression is present and higher in RA than in OA[43]. $CD3^+$ T cells and $CD14^+$ monocytes and macrophages from RA synovial fluid stain positive for NGF[43], and NGF expression co-localizes with fibroblasts and some macrophages in synovium from patients with OA[44]. In vitro, substance P induces NGF overexpression both alone and in combination with IL-1β in OA synovial cells cultured in serum-free media[45]; a similar

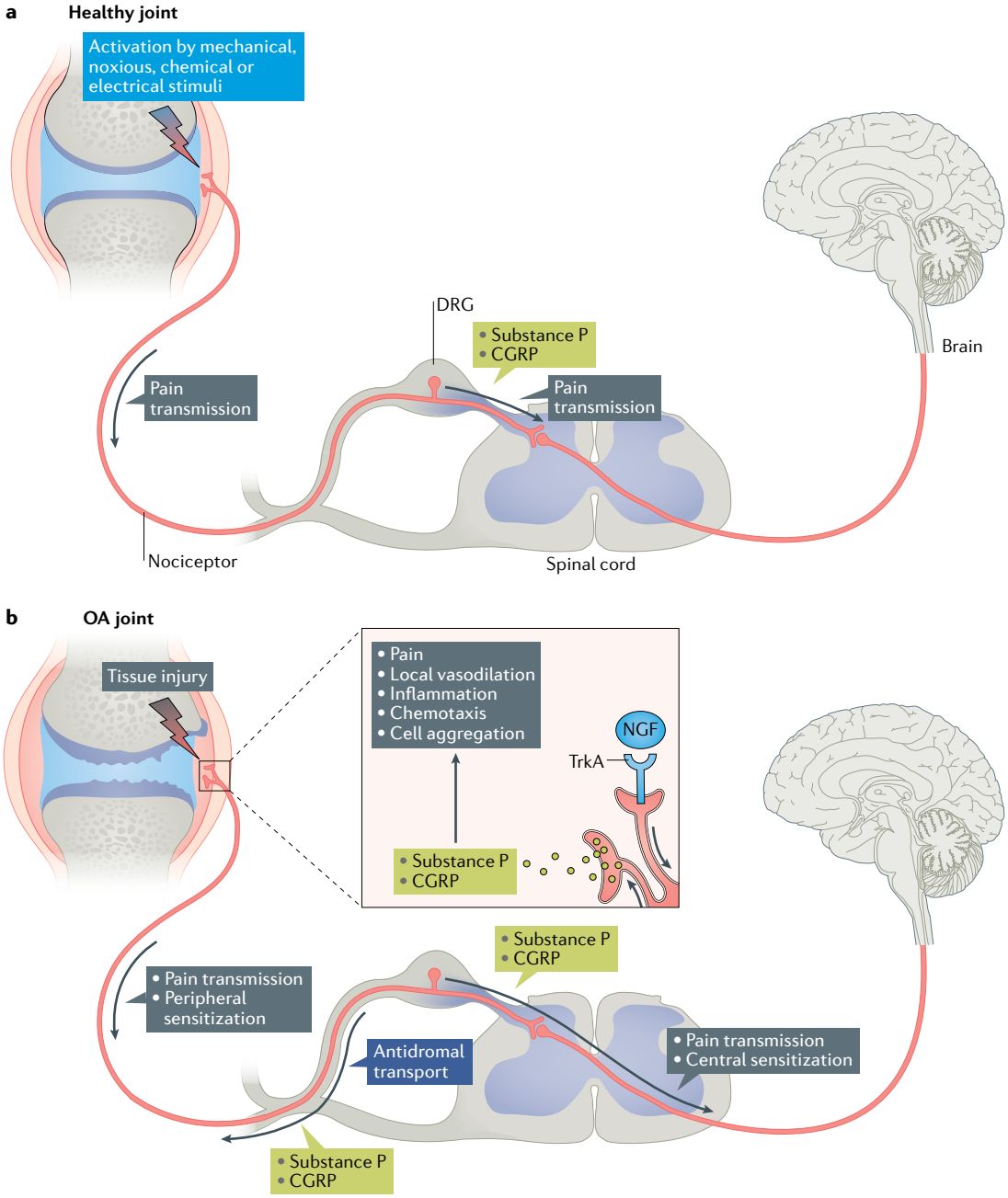

Fig. 2 | **Principles of neurogenic inflammation in joint pain. a** | Occasional pain stimuli (mechanical, noxious, chemical or electrical) are transmitted from nociceptors in the joints to the nucleus of dorsal root ganglia (DRG) via action potentials, which trigger the transportation of neurotransmitters such as substance P and calcitonin gene-related peptide (CGRP) to the spinal cord. **b** | Chronic painful stimuli in the joints (such as those that occur in osteoarthritis (OA)) induce an increase in nerve growth factor (NGF), which binds to the high affinity receptor tyrosine kinase A (TrkA). The NGF–TrkA complex that is formed is translocated to the DRG nucleus and induces the overexpression of substance P and CGRP. These neurotransmitters convey pain signals to the spinal cord, but are also transported back towards the joints via antidromal transport and released at the nociceptor. In the joints, substance P and CGRP function as strong inducers of local inflammation. At the same time, NGF also induces increased excitability in the neuron by activating acid-sensing ion channels, resulting in peripheral hypersensitization. Chronic pain stimuli also change neuronal activity in the central nervous system by increasing membrane excitability or reducing axonal inhibition, known as central sensitization.

effect also occurs with TNF in combination with IL-1β[31]. In both RA and OA, osteochondral angiogenesis is accompanied by subchondral bone marrow replacement and NGF expression within vascular channels[46]. NGF is also expressed in subchondral mononuclear cells, osteoclasts and chondrocytes in tissue from patients with knee OA[47]. In these individuals, NGF expression was associated with age and synovitis scores, suggesting an association with symptomatic OA and pain[47]. Preliminary data also suggest that NGF, TrkA and

Zygapophyseal joints
Vertebral (facet) joints that interconnect the vertebral bodies.

other inflammatory mediators are present in human zygapophyseal joints, with NGF predominantly expressed in capsular synovial tissue and to some extent in the bone marrow, and TrkA mostly expressed in the bone marrow[48]. These studies demonstrate the presence of NGF in different target tissues and suggest that inhibitors of NGF might be a suitable and robust tool for reducing site-specific neurogenic inflammation and thus chronic pain.

NGF in the CNS. In rodents, NGF mediates the homeostasis of adult CNS neurons and is found in the hippocampus and cortex[49]. Treatment with NGF protects the CNS from degeneration in mice[50]. Conversely, anti-TrkA antibodies reduce the number and size of basal forebrain cholinergic neurons in rats[51]. This effect is transient, reversible and dependent on the stage of postnatal development. NGF might also have a role in Alzheimer disease, as there seems to be a degeneration of cholinergic neurons in the basal forebrain and hippocampus in mice with an experimental model of this disease[52]. Furthermore, the results of a 2018 clinical trial showed that intranasal administration of NGF could improve cognitive function in two patients with frontotemporal dementia[53], suggesting a role for NGF in adult human CNS function. The results of these studies[49–53] imply that an intact blood–brain barrier is a prerequisite for the treatment of elderly individuals with NGF inhibitors. Therefore, any patient population with an impaired blood–brain barrier, such as after stroke and in those with multiple sclerosis, Alzheimer disease or neuroinflammatory disorders[54], should be excluded from treatment with NGF inhibitors. Patients with chronic pain syndromes such as OA are mostly advanced in age and thus develop considerable comorbidities that can include the cardiovascular system or the CNS. The latter is frequently characterized by neuronal degeneration and the loss of CNS function, such as Alzheimer disease. Therefore, these patients should be evaluated and carefully monitored before and during NGF inhibitor treatment.

NGF inhibition in clinical trials

The importance of NGF in chronic pain has prompted the development of antagonists directed against NGF or neurotrophin receptors. A variety of small molecule inhibitors or antibodies have been investigated in both preclinical[55–59] and clinical studies[60–63] with varying degrees of success (TABLE 1). Larotrectinib, a small molecule inhibitor that targets TrkA, TrkB and TrkC, has been approved for the treatment of solid tumours[64], whereas another small molecule inhibitor, ASP7962, which is an oral selective TrkA antagonist, did not show efficacy in a phase IIa trial in patients with knee OA[63]. Although several monoclonal antibodies have been studied extensively in human OA and other chronic pain conditions, the clinical development of most molecules has been discontinued for a variety of reasons. Only tanezumab and fasinumab are currently under clinical investigation for OA and CLBP and are discussed in the following sections. The relationship of these agents with RPOA and joint destruction and replacement is complicated and is addressed in a later section.

Hip and knee osteoarthritis. Monoclonal antibodies that bind to NGF have been tested for efficacy in reducing pain in both knee and hip OA in phase II and

Table 1 | **Inhibitors of NGF and NGF receptors**

Name	Chemical properties	Specificity	Investigations	Refs
ALE-0540	Non-peptidic molecule	TrkA and p75	Studied in allodynia in rats	55
TrkAd5	Soluble receptor protein	TrkA	Studied in an experimental OA model in mice	56
MNAC13	Recombinant mouse anti-TrkA antibody Fab fragment	TrkA	Studied in basal forebrain cholinergic neurons in rats	94
K252a	Small molecule inhibitor	TrkA	Studied in experimental psoriasis using a SCID mouse–human skin transplantation model	58
ABT-110 (PG110)	Humanized mAb	NGF	Studied in hypersensitivity in rats; clinical trials discontinued	59,95
Larotrectinib (ARRY-470)	Small molecule inhibitor	TrkA, TrkB and TrkC	FDA approved for malignant solid tumours	64
ASP7962	Small molecule inhibitor	TrkA	Phase II RCT in knee OA	63
Tanezumab	Humanized mAb	NGF	Clinical trials in hip and knee OA, chronic low back pain, acute bunionectomy, chronic prostatitis/chronic pelvic pain syndrome, interstitial cystitis, neuropathic pain and pain from bone metastases	96
Fasinumab	Fully human mAb	NGF	Clinical trials in OA, acute sciatic pain and chronic low back pain	97
Fulranumab	Fully human mAb	NGF	Clinical trials in post-herpetic neuralgia, post-traumatic neuropathy, cancer-related pain, hip and knee OA, interstitial cystitis, chronic low back pain and diabetic peripheral neuropathy; investigations discontinued	98

mAb, monoclonal antibody; NGF, nerve growth factor; OA, osteoarthritis; RCT, randomized controlled trial; SCID, severe combined immunodeficiency; Trk, tyrosine kinase receptor.

phase III clinical trials. In this section, we review trial reports and meta-analyses of the anti-NGF antibodies tanezumab, fulranumab and fasinumab that have been published since our previous review of the topic in 2013 (REF.[65]). The salient points from the studies are summarized in TABLE 2, and the study details are listed fully in Supplementary Table 1.

NGF inhibition has been studied in knee and hip OA both together and separately. The primary end points that have been almost universally utilized in these studies are the Western Ontario and McMaster Universities Arthritis Index (WOMAC) pain and function subscales, which are combined with physician's global assessment (PGA) scales in many studies. The WOMAC is a well-validated measure that is widely used by the OA research community; thus, the use of it in the majority of these studies allows for comparison and facilitates meta-analysis of the data. In general, these studies show that anti-NGF antibodies produce a significant improvement in pain, function and PGA scores compared with placebo for both knee and hip OA (Supplementary Table 1). However, anti-NGF antibodies carry an increased risk of adverse events compared with placebo that are primarily of a peripheral neurological nature. Meta-analyses performed on data from the anti-NGF antibody clinical trials have shown that these agents have a significant but modest effect and are superior to placebo for the main study end points, but are variable in terms of superiority compared with active NSAID treatment. These meta-analyses also reaffirmed the safety findings of the individual studies: anti-NGF antibodies increased peripheral neuropathy and sensation adverse events, but there were no significant differences in serious adverse events compared with either placebo or NSAID treatments. Overall, the number of clinical trials and the relative consistency of their findings with regard to pain and function outcomes, along with multiple meta-analyses that have reported similar findings, provide relatively robust support for the efficacy of anti-NGF antibodies for the treatment of painful knee or hip OA. By contrast, a single phase IIa trial of a TrkA inhibitor reported no effect of the agent when compared with placebo and inferiority to naproxen for WOMAC pain scores[63]; given the paucity of data related to the use of TrkA inhibitors for the treatment of pain in OA, it is probably too early to draw definite conclusions.

The number of studies that have been performed using NGF inhibitors enables some interesting observations to be made. One point of interest is the time course of efficacy for pain inhibition in knee and hip OA using these agents, which was reported in some but not all of the studies in TABLE 2. In many of the tanezumab studies, it seems that clinical efficacy (as measured by the WOMAC pain score) begins at week 4 after initiation of treatment[66–70] and persists through to either week 16 (REFS[67,69,70]) or week 24 (REFS[66,68]). Of the two studies in which fulranumab was investigated for the treatment of knee or hip OA, one provided no information about outcomes at multiple time points[71] and the other was difficult to interpret owing to the large number of study groups[72]. The authors of the single study of fasinumab for this indication reported significant pain improvement that was superior to placebo starting at week 2 (least squares mean change from baseline −0.7 in the placebo group and −1.4 to −1.6 in the fasinumab groups) and persisting through to week 16 (REF.[73]) (Supplementary Table 1). The fact that pain relief is consistently reported to begin at around 2–4 weeks in these studies might be informative for a discussion of expectations with patients, if one or more of the agents are eventually approved and used in clinical practice.

When considering the efficacy of anti-NGF antibodies for the indications of knee and hip OA, it is important to consider whether the medications improve function in addition to whether they improve pain. In many of the clinical trials, function was a co-primary end point along with pain outcomes, most commonly measured using the WOMAC function score. Notably, in each study in which the anti-NGF antibody demonstrated superiority to the comparator (placebo or an NSAIDs) for pain measures, there was also superiority over the comparator for functional improvement.

Interestingly, the most recent phase III study of tanezumab for knee or hip OA (published in 2020) found that the higher dose of subcutaneously administered tanezumab (5 mg) was associated with improvements in all three co-primary endpoints (WOMAC pain score, WOMAC function score and PGA), but the lower dose (2.5 mg) was not associated with an improvement in PGA[74]. A previous study of subcutaneously administered tanezumab (using doses of 2.5, 5 and 10 mg) that was published in 2018 was terminated owing to an FDA hold on clinical trials and was therefore underpowered compared with the intended recruitment goal[67]. Studies prior to these[67,74] used an intravenous formulation of tanezumab and demonstrated efficacy in all end points at lower doses (2.5 mg or 5 mg) as well as doses up to 10 mg (TABLE 2). The results of the 2020 phase III subcutaneous tanezumab study suggests that the lowest doses of subcutaneous tanezumab might be at the lower limits for achieving a valuable clinical reduction in pain and improvement in quality of life[74].

Chronic low back pain. Two clinical trials and one meta-analysis have been published since 2013 on the use of NGF inhibition (specifically tanezumab) for CLBP, another painful musculoskeletal condition (Supplementary Table 1). Overall, an effect for NGF inhibition was detected for this indication, but was only small to moderate in magnitude and was, in some studies, only present for the higher doses of the agent. Whether this result represents a different degree of efficacy for tanezumab than that observed for hip or knee OA is currently unclear.

The results of a phase IIb study in which three doses of tanezumab (5, 10 or 20 mg every 8 weeks) were compared with either naproxen (500 mg twice daily) or placebo were reported in 2013 (REF.[75]). In the study, the change in daily average low back pain intensity was evaluated between baseline and week 16. The two higher doses of tanezumab (10 mg and 20 mg) were superior to both naproxen and placebo, but the lowest dose of tanezumab (5 mg) was only superior to placebo.

Table 2 | **Trials and meta-analyses of anti-NGF antibody therapy for osteoarthritis**

Study	Target joint (number of participants)	Agent (comparator)	Study conclusions	Adverse events	Ref
Clinical trials					
Brown et al. (2012)	Knee (690)	IV tanezumab 2.5 mg, 5 mg or 10 mg (placebo)	Tanezumab was superior to placebo for all end points	More common in tanezumab groups than placebo groups, mostly paraesthesia and hypoaesthesia; RPOA not reported	68
Birbara et al. (2018)	Knee or hip (379)[a]	SC tanezumab 2.5 mg, 5 mg or 10 mg (IV tanezumab 10 mg or placebo)	All tanezumab groups had greater improvement than the placebo group at all time points; final analyses not performed owing to FDA clinical hold	Marginally more TJRs in tanezumab groups ($n = 3$) than in placebo groups ($n = 2$); only 1 TJR with imaging was reviewed, which was judged to show normal progression of OA	67
Birbara et al. (2018)	Knee or hip (678)[a]	SC tanezumab 2.5 mg, 5 mg or 10 mg (not controlled)	All tanezumab doses resulted in improvements in all outcomes	34 TJRs; majority in tanezumab 10-mg group; of the adjudicated TJRs, half were judged to be normal OA and half RPOA	67
Schnitzer et al. (2015)	Knee or hip (2,700)	IV tanezumab 5 mg or 10 mg with or without an NSAID (placebo with an NSAID)	Pain and function improved more in all tanezumab groups than in the placebo with NSAID group; tanezumab with NSAID was superior to placebo with NSAID for PGA; tanezumab monotherapy was equivalent to tanezumab with NSAID for pain and function	Higher in all tanezumab groups than in the placebo with NSAID group; highest in the tanezumab with NSAID group (specifically paraesthesia and hypoaesthesia); worsening OA and osteonecrosis more common in tanezumab with NSAID groups; TJRs twice as common in tanezumab with NSAID group than in tanezumab monotherapy or placebo with NSAID groups; RPOA reported in 34 participants, more common in tanezumab groups than in the placebo with NSAID group	99
Spierings et al. (2013)	Hip or knee (610)	IV tanezumab 5 mg or 10 mg (placebo or oxycodone)	Both tanezumab doses had more improvement in pain than either the placebo or the oxycodone group	Highest rate in the oxycodone group; 2 TJRs (hip) in the higher dose tanezumab group; 1 TJR was judged to be normal OA and the other RPOA	100
Balanescu et al. (2014)	Knee or hip (604)	IV tanezumab 2.5 mg, 5 mg or 10 mg with diclofenac (placebo with diclofenac)	All tanezumab groups were superior to placebo with diclofenac for all co-primary outcomes	TJR more common in all tanezumab groups (1.3–2.1%) than in the placebo with diclofenac group (0.7%); serious adverse events were similar in tanezumab groups (5.3–7.6%) and in the placebo with diclofenac group (5.3%); adjudication confirmed one case of RPOA, but some cases did not have sufficient radiographs for a judgement to be made	66
Ekman et al. (2014)	Knee (828)	IV tanezumab 5 mg or 10 mg (placebo or naproxen)	Tanezumab at both doses was superior to placebo for all co-primary endpoints; tanezumab 5 mg but not 10 mg was superior to naproxen for pain and PGA; both tanezumab doses were superior to naproxen for function	Serious adverse events were not more common in the tanezumab groups (2.9–3.4%) than in the placebo (3.8%) or naproxen (2.4%) group; 3 TJRs reported, only one of which was in the tanezumab 5-mg group; 2 TJRs were judged to be worsening OA, but there was insufficient information to make a decision about RPOA	69
	Knee or hip (840)	IV tanezumab 5 mg or 10 mg (placebo or naproxen)		Serious adverse events were not more common in the tanezumab groups (1.4–1.9%) than in the placebo (1.9%) or naproxen (4.3%) groups; 3 TJRs reported, none of which was in the tanezumab groups; 1 TJR was judged to be worsening OA, but unclear whether it could have been RPOA	
Schnitzer et al. (2019)	Knee or hip[b] (698)	IV tanezumab 2.5 mg or 2.5 mg then 5 mg (placebo)	Both tanezumab groups had a greater reduction in all co-primary end points than the placebo group	Similar across all groups, except that abnormal peripheral sensation adverse events were more common in the tanezumab groups than in the placebo group; TJRs more common in both tanezumab groups than in the placebo group and showed a dose–response pattern; RPOA noted in the tanezumab 2.5-mg group (2.2%) and in the tanezumab 2.5-mg then 5-mg group (0.4%); no RPOA in the placebo group	83
Schnitzer et al. (2020)	Knee or hip[b] (696)	IV tanezumab 2.5 mg or 2.5 mg then 5 mg (placebo)	Both tanezumab groups had a greater reduction in all co-primary end points than placebo at week 2 and at week 16		101
Berenbaum et al. (2020)	Hip or knee (849)	SC tanezumab 2.5 mg or 5 mg (placebo)	The tanezumab 5-mg group had a greater reduction in all end points than the placebo group; the tanezumab 2.5-mg group was only superior to the placebo group for WOMAC outcomes	Both tanezumab groups had more hypoaesthesia than the placebo group; the tanezumab 5-mg group had more paraesthesia than the placebo group; RPOA in 1.4% of the tanezumab 2.5-mg group, 2.8% of the tanezumab 5-mg group and none in the placebo group; TJRs were similar across all groups (6.7–7.8%)	74

Table 2 (Cont.) | **Trials and meta-analyses of anti-NGF antibody therapy for osteoarthritis**

Study	Target joint (number of participants)	Agent (comparator)	Study conclusions	Adverse events	Ref
Clinical trials (cont.)					
Mayorga et al. (2016)	Knee (196 randomized and 65 completed 12 weeks)[c]	Fulranumab 3 mg or 9 mg (placebo or oxycodone)	Both fulranumab groups had superior outcomes to the oxycodone group but not to the placebo group	Neurological adverse events were higher in the fulranumab groups than in the placebo group, but similar to the oxycodone group; 4 TJRs, 3 in the fulranumab groups and 1 in the oxycodone group; no TJRs were judged to be RPOA	71
Sanga et al. (2017)	Knee or hip (401)	Fulranumab 1 mg or 3 mg every 4 weeks or 6 mg or 10 mg every 8 weeks (placebo)	Long-term improvement in the two fulranumab 4-week groups and in the fulranumab 10-mg 8-week group compared with the placebo group for all outcomes	Neurological adverse events were more common in the fulranumab groups than in the placebo group; 81 TJRs in 71 individuals, including 25 in non-index joints; 21% of TJRs were judged to be RPOA, all of which were in participants receiving fulranumab and also taking NSAIDs	72
Dakin et al. (2019)	Knee or hip (342)	Fasinumab 1 mg, 3 mg, 6 mg or 9 mg (placebo)	All fasinumab groups had greater improvements at all end points than the placebo group	More common in the fasinumab groups than in the placebo group; 25 arthropathies noted, primarily in the fasinumab groups and showing a dose-related pattern; 18 TJRs occurred that were evenly distributed across all groups; 16 cases of RPOA were detected, all in fasinumab groups	73
Systematic reviews and meta-analyses					
Schnitzer and Marks (2015)	Knee or hip (8,606)	Tanezumab, fulranumab and fasinumab (placebo)	Tanezumab at all doses was superior to placebo for all end points with no difference in effect size across the doses; fulranumab and fasinumab seemed superior to placebo overall	Withdrawals owing to adverse events for tanezumab were generally similar to placebo; fulranumab and fasinumab were not different from placebo for withdrawal owing to adverse events as there were too few adverse events for analysis; for all anti-NGF antibody groups combined, there was borderline statistical significance for increased withdrawal owing to adverse events compared with placebo; RPOA was not discussed	102
Kan et al. (2016)	Knee (1,839)	Tanezumab (placebo)	Tanezumab was superior to placebo for all outcomes	Serious adverse events were similar for tanezumab and placebo; tanezumab was associated with increased peripheral neuropathy and withdrawal owing to adverse events compared with placebo; RPOA was not discussed	103
Chen et al. (2017)	Knee and hip (7,665)	Tanezumab (placebo or placebo with an NSAID)	Tanezumab was superior to placebo or placebo with an NSAID for all outcomes	Serious adverse events were similar for tanezumab and placebo or placebo with an NSAID; tanezumab was associated with increased paraesthesia and hypoaesthesia and withdrawal owing to adverse events than placebo or placebo with an NSAID; RPOA was not discussed	104
Tive et al. (2019)	Knee or hip (7,491)	IV tanezumab 2.5 mg, 5 mg or 10 mg with or without an NSAID (placebo or placebo with an NSAID)	Tanezumab was superior to placebo for all end points; only the two higher doses of tanezumab were superior to placebo with an NSAID for all outcomes	Tanezumab was associated with an increased incidence of abnormal peripheral sensation adverse events; overall incidence of adverse events was stated to be similar across groups but no statistical analysis was reported; RPOA not evaluated for the different groups in the studies	105

IV, intravenous; NGF, nerve growth factor; OA, osteoarthritis; PGA, physician's global assessment; RPOA, rapidly progressive osteoarthritis; SC, subcutaneous; TJR, total joint replacement; WOMAC, Western Ontario and McMaster Universities Arthritis Index. [a]Trial was underpowered owing to FDA clinical hold. [b]Excluded radiographic 'joint safety conditions' (RPOA, fracture or osteonecrosis). [c]Trial halted early owing to FDA clinical hold.

Adverse events were more common in participants who received tanezumab than in those who received placebo or naproxen; in particular, arthralgias, headaches and paraesthesia were noted in those who received tanezumab. Interestingly, there were no total joint replacements (TJRs) for any reason in this study, despite the relatively large samples size ($n = 1,347$).

An uncontrolled randomized trial has also been performed to evaluate the long-term safety and efficacy of tanezumab for CLBP[76]. 848 participants were drawn from a parent study for inclusion in the trial and received 10 mg or 20 mg tanezumab every 8 weeks as three rounds of intravenous administration followed by four rounds of subcutaneous administration. Outcomes were the change from parent study baseline in Brief Pain Inventory Short Form, Roland Morris Disability Questionnaire and PGA for low back pain. Both tanezumab doses were associated with persistent and similar efficacy for all the defined outcomes. The most common adverse events were arthralgia, paraesthesia and hypoaesthesia, which occurred at frequencies similar to those in other studies. Thirteen patients had TJRs, and adjudication of eight of those TJRs revealed one instance of RPOA.

Paraesthesia
Abnormal skin sensation without stimulation.

Hypoaesthesia
Numbness of the skin with a reduction of sensations to sensory stimuli.

Finally, a single meta-analysis of the use of anti-NGF antibodies for the treatment of CLBP has been published[77]. The authors identified only randomized controlled trials that met their criteria, two using tanezumab, one using fasinumab and one using fulranumab. The quality of the evidence generated by this meta-analysis was low or very low for pain relief, functional improvement and adverse effects using the Grading of Recommendations Assessment, Development and Evaluation criteria, indicating that the reader should be cautious when interpreting the available findings. Overall, the authors of this meta-analysis reported a small effect for pain (0.29 standard deviations below placebo) and for functional improvement (0.21 standard deviations below placebo) and an increased number of adverse events compared with placebo at 12–16 weeks (relative risk (RR) 1.13; 95% CI 0.98–1.29), primarily for neurological adverse events (RR 1.93; 95% CI 1.41–2.64)[77]. The difference in magnitude of effect size of anti-NGF antibodies between CLBP and knee OA could potentially relate to the fact that CLBP includes multiple disease entities, including facet joint OA, discogenic pain and muscle-related pain, and the efficacy of anti-NGF antibodies for these various entities could differ considerably.

Adverse effects of anti-NGF antibodies

During the phase II and III clinical trials of the tanezumab development programme, unexpected adverse events (including osteonecrosis and rapid destruction of joints) were reported by the study site investigators, such that the FDA placed a partial clinical hold on studies of tanezumab for all indications other than cancer pain between 2010 and 2012 (REF.[78]). This clinical hold was eventually extended to cover all anti-NGF monoclonal antibodies that were in clinical development.

Another partial clinical hold was instituted by the FDA on all anti-NGF antibody programmes from 2012 to 2015 after a report of reductions in the size and number of neurons in the sympathetic nervous system of adult mice[79]. Investigations were subsequently instituted to determine the aetiology and potential clinical relevance of these findings, including a series of studies in which cynomolgus monkeys were treated with tanezumab[80] and a systematic review of clinical records from participants in tanezumab clinical trials, which was presented at the American Academy of Neurology conference in 2015 (REF.[81]). The investigations found no evidence of sympathetic nervous system dysfunction and the FDA allowed the clinical studies of tanezumab to resume with sympathetic function disorder as a new exclusion criterion.

Independent adjudication of anti-NGF antibodies. To try to understand the risks associated with the use of anti-NGF antibodies, adjudication was performed by independent committees of experts. The adjudication committee formed for the studies of tanezumab undertaken by Pfizer reviewed all of the information for cases of adverse events and developed validated definitions for assessments of the radiographs that included osteonecrosis, worsening OA, another condition or insufficient information to determine if the case was OA or osteonecrosis[3]. Overall, 386 study participants experienced an adverse event and underwent TJR in the tanezumab phase III studies in OA ($n = 373$) and in the phase II study in CLBP ($n = 13$). In the OA studies, the TJRs were in the index joint in 216 participants and in a non-index joint in 170 participants; however, 74.7% of those who received TJR in a non-index joint had evidence of OA in the affected joint and the remaining ~25% had either insufficient information (20%), another joint abnormality (3.5%) or a normal joint or minimal OA (1.8%)[3]. Of the 13 participants who underwent TJR in the phase II CLBP study, OA was present in the affected joint in 11 participants and there was insufficient information for 2 participants. In total, adverse events were adjudicated in 249 participants from the tanezumab studies: 47.8% ($n = 119$) of the events were labelled as normal OA progression, 27.3% ($n = 68$) as RPOA and 0.8% ($n = 2$) as osteonecrosis[3]. The committee determined that there was no association between TJR and the dose of tanezumab monotherapy; the overall TJR rate for tanezumab monotherapy was similar to that of the comparators, and both of those rates were similar to the placebo and not statistically significant[3]. However, when tanezumab was administered in combination with an NSAID, the rate of TJRs increased in line with increasing doses of tanezumab and was about two to three times the rate of TJR in those receiving placebo. The time to a TJR was not associated with the dose of tanezumab used; however, the time to TJR decreased when tanezumab was combined with an NSAID, especially with the 5-mg or 10-mg doses of tanezumab[3].

The adjudication committee also reviewed those participants who developed RPOA ($n = 68$; hip (56%), knee (40%) and shoulder (4%))[3]. RPOA was subclassified as either type 1 or type 2, with type 1 indicating a ≥1-mm loss of joint space width in less than 1 year and type 2 indicating bone loss or destruction at a level not normally associated with end-stage OA, including catastrophic bone failure and joint destruction. RPOA of both types occurred in 67 participants from the OA studies and in 1 participant from the CLBP study. 43 instances of RPOA (63%) occurred in the index joints and 25 (37%) in non-index joints; of the non-index joints, 15 (60%) had definitive OA at a pre-study visit, 9 (36%) had unknown status of the joint at a pre-study visit and 1 (4%) had another abnormality[3]. The participants who developed RPOA were more likely to be women and to have increased joint pain after the baseline study visit. Importantly, the incidence of RPOA was associated with the dose of tanezumab monotherapy used; 2.5 mg tanezumab was associated with 0 events per 1,000 patient years, whereas 10 mg tanezumab was associated with 11 events per 1,000 patient years[3]. The incidence of RPOA in participants who received tanezumab with an NSAID was significantly increased compared with the comparator, with hazard ratios ranging from 8.76 (95% CI 1.05–73.40) for 2.5 mg tanezumab with an NSAID to 17.50 (95% CI 2.37–129.40) for 10 mg tanezumab with an NSAID[3]. These data clearly demonstrate a dose–response relationship between

tanezumab and RPOA and an added contribution from NSAIDs.

In addition to tanezumab, adjudication of TJRs was also performed for phase II studies of fulranumab for OA. In these studies, 108 joints were replaced, of which 64% were from normal progression of OA, 18% from RPOA, 14% had insufficient information to make a diagnosis, 4% were revision TJRs and none had osteonecrosis[72]. Safety results have also been reported for a phase IIb/III study of fasinumab in OA ($n = 342$)[73]. Adjudicated arthropathies were detected in 25 joints (13 index joints and 12 non-index joints) from 23 participants, totalling 7% of those who received fasinumab and 1% of those who received placebo. The joint-related adverse events were dose dependent. 14 patients developed type 1 RPOA and 2 patients developed type 2 RPOA following fasinumab treatment, whereas no patients developed RPOA with placebo treatment. In addition, subchondral insufficiency fractures occurred in 1.8% of patients who received fasinumab at any dose and in 1.2% of those who received placebo. On the basis of these data[73], the sponsor of these studies modified their clinical development plan to only include doses of 1 mg fasinumab every 4 weeks and 1 mg fasinumab every 8 weeks in an ongoing phase III study, the results of which should be available within the next year.

Tanezumab follow-up studies. Once the FDA hold was released, tanezumab studies recommenced in individuals with painful knee or hip OA, but at reduced doses (2.5 mg and 5 mg). The preliminary results of one study that included 2,996 patients with OA have been reported, in which tanezumab was administered by subcutaneous injection every 8 weeks and compared with oral NSAID use for 56 weeks with 24 weeks of follow-up[82]. During the 80 weeks in which the participants were monitored, the time-adjusted rate of events per 1,000 patient years for the primary composite joint safety end point was higher for those receiving 2.5 mg tanezumab (37.4 events per 1,000 patient years) and 5 mg tanezumab (71.5 events per 1,000 patient years) than for those receiving NSAIDs (14 events per 1,000 patient years). Rates of type 1 and 2 RPOA were higher in those receiving tanezumab treatment than in those receiving NSAIDs, as were the rates of TJRs, which ranged from 25.7 events per 1,000 patient years with NSAID treatment to 51.8–79.7 events per 1,000 patient years with tanezumab treatment. These results show that even when using lower doses, the risks of joint deterioration remain greater with tanezumab treatment than with NSAID treatment[82].

In another phase III study, subcutaneous tanezumab (two doses of 2.5 mg (2.5-mg tanezumab group) or one dose of 2.5 mg followed by one dose of 5 mg (2.5/5-mg tanezumab group)) was compared with placebo for pain reduction in individuals with knee or hip OA ($n = 582$)[83]. No adjudicated joint safety events occurred between weeks 0 and 16; however, over the 40-week treatment and post-treatment follow-up period, a total of 25 joint safety adverse events occurred in participants receiving tanezumab and 5 in participants receiving placebo. RPOA was diagnosed in 5 individuals in the 2.5-mg tanezumab group, in 1 individual in the 2.5/5-mg tanezumab group and in no-one receiving placebo. Abnormal peripheral sensation adverse events were reported up until the end of the study, including paraesthesia (11 in those receiving tanezumab versus 1 in those receiving placebo) and hypoaesthesia (11 in those receiving tanezumab versus 6 in those receiving placebo)[83]. Other clinical trials of subcutaneous tanezumab at 2.5-mg and 5-mg doses for OA have not yet been published[84,85]; thus, a more complete picture of the efficacy and adverse event profile for this treatment is still pending.

The mechanisms that underlie the RPOA associated with tanezumab treatment, either alone or in combination with NSAIDs, are currently unclear. Possible explanations include neuropathic neuropathy, in which the loss of ability to feel pain leads to abnormal joint loading, and analgesic neuropathy, in which reduced joint pain could lead to overloading of the joint and rapid deterioration. The latter explanation had been previously proposed for a similar situation involving indomethacin, following the results of a study in which individuals waiting for hip replacements were randomly allocated to receive azapropazone or indomethacin[86]; after ~2 years of follow-up, participants who received indomethacin had more radiographic joint destruction and joint pain than those who received azapropazone. However, given that the risk of TJR increased when tanezumab was used in conjunction with an NSAID, mechanisms related to changes in inflammation, pain reduction and reduced prostaglandin E2 production within the joint have been proposed. Another possible cause might be related to changes in the mass and architecture of the subchondral bone, as individuals with knee and hip OA who have atrophic radiographic changes have accelerated joint destruction and more joint replacements than those who have a greater amount of juxta-articular bone mass[87].

Additional studies have been performed in an attempt to refine the phenotype of those who will go on to receive TJR, determine the effects of NSAIDs and discover potential bone, cartilage, soft-tissue or inflammatory biomarkers that were associated with TJR. A post hoc analysis of data from clinical trials of tanezumab in OA that included 47 participants who developed RPOA and 92 who did not aimed to discover biomarkers by comparing those who used NSAIDs for <90 days with those who used NSAIDs for ≥90 days over a 10-month period[88]. Two serum biomarkers, C3M (a marker of synovial tissue inflammation) and C2M (a marker of cartilage degradation) predicted type 2 RPOA in those who used NSAIDs for <90 days with an accuracy of 71%, and individuals with this biomarker phenotype had an 8-fold higher risk of developing RPOA than patients with OA without this phenotype[88]. These results are intriguing; however, additional validation is needed before these biomarkers can be recommended for identifying individuals at high risk of RPOA.

Unanswered questions concerning RPOA

The adverse events of RPOA and peripheral sensation changes were not anticipated in either the preclinical studies or phase I clinical studies of anti-NGF antibody therapies. Although many hypotheses exist around how RPOA occurs, to date there is no clear understanding

of the risk profile of patients with OA who are likely to develop RPOA. The changes in peripheral sensation might be linked to the underlying mechanism of NGF inhibition reducing nociceptor activity; however, more work is needed to refine this idea. Neurological sensory adverse events are generally reversible upon discontinuation of the medication, although some individuals reported that analgesia was still present at the termination of the studies[89]. By contrast, RPOA is not reversible. Preclinical studies that determine the fate of nociceptors during anti-NGF antibody treatment and clinical studies that refine the phenotype of patients with OA who might be at risk of RPOA will help clinicians to identify those patients who would benefit the most from these novel analgesic therapies.

The few preclinical studies that have specifically evaluated the effects of NGF inhibition on both pain behaviour and joint structure were described in a 2017 review[90]. These studies evaluated the effects of treatment with soluble NGF receptors, small molecule inhibitors of TrkA or anti-NGF antibodies. The studies that only assessed pain demonstrated a reduction in pain using reduced weight-bearing asymmetry as an end point[90]. Studies that assessed both pain and histological or radiographic joint changes reported reduced gait imbalances following NGF inhibitor treatment compared with controls that were maintained up to 35 days, and an increased knee diameter in NGF inhibitor-treated animals that differed from control-treated animals[90]. A study that used a model of rat medial meniscal injury in which treatment with a humanized anti-NGF antibody (tanezumab) was initiated at the time of the injury and continued for 28 days reported that tanezumab-treated animals were protected against gait deficiency; however, rats treated with tanezumab at any dose had increased cartilage damage, subchondral bone sclerosis and tibial osteophytes compared with those treated with control substances[91]. In another study in rats with monosodium iodoacetate-induced OA, treatment with an anti-NGF antibody at the time of injury prevented weight-bearing asymmetry, but there was increased cartilage damage in the treated knee at day 28 compared with vehicle controls[92]. When anti-NGF antibody treatment was delayed to either 14 or 21 days after injury, the treated rats had a decrease in weight-bearing asymmetry and mechanical allodynia at day 28, and although there was no clear difference in the amount of cartilage damage, there was a decrease in osteoclast numbers at the tibial plateau in anti-NGF antibody-treated rats compared with saline-treated rats[92]. Overall, these studies confirm the considerable analgesia observed in the clinical trials of anti-NGF antibodies and that these therapies are effective at treating different stages of OA. However, these studies also provide evidence of cartilage degeneration, synovitis and osteoclast activity in the subchondral bone that is different in NGF inhibitor-treated animals than in control-treated animals. The joint damage reported in the animal studies was greater when the NGF inhibitor treatment was initiated in the early stages of the disease[91,92].

Although NGF inhibitors are effective at reducing pain in animal models of OA and in patients with OA, a gap still exists in our knowledge of how the joint can rapidly degenerate so, on this point, we are speculating about the mechanisms. NGF signals through the TrkA and p75 receptors on nociceptors, thereby promoting the expression of ion channels and neuropeptides in neurons that contribute to the innervation of the joint microenvironment. Thus, inhibition of NGF signalling and subsequent deficits in neuronal signalling and innervation could potentially alter the microenvironment within the joint, which might then result in accelerated joint degeneration. Given that nerves and blood vessels grow in congruence with each other and nociceptors regulate blood flow, a relatively rapid reversal from enhanced NGF signalling in OA to a near complete loss of NGF signalling could potentially also cause a dramatic change in synovial innervation and blood flow, thereby compromising the joint. In addition, as bone is loaded, osteocytes within the bone signal to the bone surface to direct remodelling of the tissue to accommodate the loads. Bone remodelling is associated with NGF; thus, inhibition of NGF signalling could also potentially interfere with normal loading signals, further altering the structural integrity of the joint[46]. In fact, mice that lack TrkA have reduced bone formation under loading conditions compared with wild-type mice, suggesting that this receptor is required for load-induced bone formation[93]. Clearly, more research into the interaction between the nerves, vasculature and the rest of the joint microenvironment is needed to explore this issue.

Conclusions

Over the past 70 years, our understanding of the biology of the neurotrophin NGF has expanded from a factor that stimulates the growth of embryonic sensory and sympathetic neurons to a factor with an important role in arthritis and in modulating the PNS. Early-phase and late-phase clinical trials have determined that NGF inhibition with subcutaneous tanezumab or fasinumab is an effective form of analgesia for knee and hip OA and for CLBP. The analgesic efficacy of these anti-NGF antibodies is noteworthy because of their completely novel mechanism of action that lacks the adverse effects associated with conventional NSAIDs, opioids and steroids, and their demonstrated efficacy in patients with painful large joint OA. However, adverse events including RPOA and insufficiency fractures of the tibia have been reported that have to be carefully considered. Although the aetiology of these events is not yet fully understood, it is reasonable to expect that if these medications are approved for the treatment of pain associated with knee and hip OA, clinicians will need to inform their patients about these risks. Moving forwards, it will be crucial to identify patient characteristics that increase the risk of RPOA during anti-NGF antibody treatment. If we are able to identify risk factors for RPOA, the use of anti-NGF antibodies in clinical practice for large joint OA and other treatment-resistant chronic pain syndromes would be safer and more appealing.

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B.L.W. declares that he received a research grant from Pfizer from 2011 to 2012. M.F.S. declares that he has been a consultant for Eli Lilly and Pfizer, and that he has received educational grants from these companies. N.E.L. declares that she has performed phase II and phase III clinical trials for Pfizer (2010–2012 and 2016–2019) and has been a consultant for Pfizer (2011–2019).

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Review criteria
A search focused on the biology of nerve growth factor was performed in Medline, Medpilot, the Information Centre for Life Sciences in Cologne, Germany and libraries in Basel and Bern, Switzerland. The search terms used were "nerve growth factor", "pain", "neurogenic inflammation", "neurotrophin", "substance P", "brain-derived neurotrophic factor", "calcitonin gene-related peptide", "osteoarthritis", "collagen-induced arthritis", "low back pain", "therapy" and "cytokines" alone and in combination. A further search for clinical studies published between 2012 and 2020 was performed in PubMed and Embase. The search terms used were "knee or hip osteoarthritis", "nerve growth factor", "NGF", "tropomyosin kinase receptor" and the names of individual agents alone or in combination. Relevant reports of trials and meta-analyses were included and conference abstracts, animal studies, publications in languages other than English and articles discussed in our 2013 review[65] were excluded. We also searched the reference lists of the identified articles for further relevant papers.

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Mechanisms and therapeutic implications of cellular senescence in osteoarthritis

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Abstract | The development of osteoarthritis (OA) correlates with a rise in the number of senescent cells in joint tissues, and the senescence-associated secretory phenotype (SASP) has been implicated in cartilage degradation and OA. Age-related mitochondrial dysfunction and associated oxidative stress might induce senescence in joint tissue cells. However, senescence is not the only driver of OA, and the mechanisms by which senescent cells contribute to disease progression are not fully understood. Furthermore, it remains uncertain which joint cells and SASP-factors contribute to the OA phenotype. Research in the field has looked at developing therapeutics (namely senolytics and senomorphics) that eliminate or alter senescent cells to stop disease progression and pathogenesis. A better understanding of how senescence contributes to joint dysfunction may enhance the effectiveness of these approaches and provide relief for patients with OA.

Osteoarthritis (OA), the most common form of arthritis, is a disease of the synovial joints that is characterized by cartilage degradation and bony overgrowth in the form of osteophytes and subchondral thickening[1]. OA is also associated with varying degrees of synovitis and damage to other joint structures, including ligaments and the menisci in the knee[1]. OA progresses gradually and eventually leads to debilitating pain and loss of mobility, especially in older adults[2]. Although risk factors such as obesity, joint injury and genetics have all been linked to OA, the most prevalent risk factor is age[3]. With the ageing baby boomer generation (that is, individuals born between 1946 and 1964), the number of people in the USA afflicted with OA is estimated to rise from 30 million to 67 million by the year 2030, with over half of those cases predicted to be in individuals aged 65 years and older[4,5]. Along with the burden of pain and disability suffered by patients with OA, treatment and care for this disease was estimated in 2013 to cost the US health-care system $27 billion annually[6] and even more in lost workforce productivity. Accordingly, researchers in the ageing and pharmaceutical fields have taken great interest in designing novel therapeutics to alleviate the symptoms of OA and slow its progression.

Within the past 5 years, researchers have begun to explore a novel approach to treating OA through the targeting of chondrocytes and other joint tissue cells that have undergone cellular senescence. Senescence, one of the hallmarks of ageing[7], is a cell fate characterized by permanent cell cycle arrest and the release of harmful pro-inflammatory molecules into the surrounding microenvironment, a feature known as the senescence-associated secretory phenotype (SASP). Senescent cells accumulate as an organism ages, resulting in reduced cellular proliferation and impaired tissue regeneration and function[8]. For these reasons, senescence has been implicated in the pathogenesis and progression of a myriad of ageing-associated diseases, including OA[9,10]. Although age correlates with both OA and cellular senescence, the exact mechanism linking senescence to OA pathology remains unclear. Nevertheless, clinical trials are underway to test a pharmacotherapeutic approach to treating OA by eliminating senescent cells using senolytics, a class of drugs that selectively induce the death of senescent cells. This approach has shown promising early results by ameliorating other ageing-related diseases in murine models, such as idiopathic pulmonary fibrosis, atherosclerosis and cancer[11]. Additionally, enzymes linked to the progression of OA have been identified as SASP factors, and the selective inhibition of these factors with therapeutics called senomorphics (also known as SASP inhibitors and senostatics) could one day provide relief for patients with OA. However, evidence of the benefit of senomorphics in treating OA is currently limited by a lack of studies testing the specificity and efficacy of these drugs for treating joint diseases.

In this Review, we explore several common phenotypes associated with cellular senescence and their links to OA pathology. Additionally, we examine several

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Key points

- Osteoarthritis (OA) pathology overlaps with the senescence of cells in joint tissue and the senescence-associated secretory phenotype.
- Several hallmarks of senescence are associated with OA, but it is unclear which of these cause disease progression.
- Ageing, DNA damage and oxidative stress can induce senescence in cells in joint tissue.
- The complexity of the senescent cellular phenotype necessitates the careful use of biomarkers to identify senescent cells.
- Targeting senescence for OA therapy is a promising new approach that deserves further investigation.

therapeutic strategies that target senescent cells directly and are being tested as a means of preventing the disease or improving patient outcomes.

Cellular senescence and the SASP

Since its discovery by Hayflick and Moorhead over a half century ago[12], cellular senescence has been commonly defined as irreversible cell cycle arrest in response to replicative stress and ageing. However, studies from the past decade have expanded this definition beyond simply a reduction in proliferative capacity. For example, senescent phenotypes have been detected in postmitotic cells, such as damaged neurons and aged osteocytes[13,14]. Furthermore, senescence can be induced independently of replicative stress and ageing, such as by DNA damage, oncogenic signalling and oxidative stress[15–17]. Senescence is best described as a complex process involving the metabolic, morphological, and physiological transformation of cells in response to a multitude of cellular stresses[18]. Additionally, this process can affect neighbouring cells by altering paracrine signalling pathways, a discovery that has compelled researchers to investigate how senescent cells transform their microenvironments, a process that can have systemic effects on the entire organism[19].

Much of the research on senescence has been devoted to understanding its pleiotropic role as both a tumour suppressor and a driver of ageing-related disease. In its role as a tumour suppressor, senescence involves the upregulation of cell cycle inhibitor genes in response to oncogenic signals, resulting in permanent growth arrest and the prevention of neoplastic proliferation[20]. In its role as a driver of disease, senescence hinders long-term tissue regeneration and normal cell function and has been linked to pathologies such as sarcopenia, osteoporosis, macular degeneration, neurodegeneration and OA[21]. Furthermore, novel roles for senescence include critical functions in the early stages of wound healing and in embryogenesis[22,23].

Although senescent cells live in a state of permanent growth arrest, they are not dormant within tissues. Instead, senescent cells remain metabolically active and undergo dynamic transformations in their physiology, which can include alterations to paracrine signalling. The SASP is characterized by the increased secretion of particular bioactive molecules by senescent cells, including chemokines, cytokines, proteases and growth factors; these molecules can induce a range of physiological responses in the surrounding microenvironment, including inflammation, growth arrest and tumorigenesis[24]. Mechanistically, mTOR is a key regulator of the SASP owing to its ability to differentially regulate the translation of MAP kinase-activated protein kinase 2 (MAPKAPK2, also known as MK2)[25] and IL-1α[26]. MK2 is phosphorylated by p38 and deactivates ZFP36L1, a zinc-finger protein that degrades the mRNA of many pro-inflammatory SASP factors. IL-1α promotes NFκB signalling, which has been linked to the upregulation of many SASP genes. Accordingly, inhibition of mTOR by rapamycin reduces SASP factor expression[25,26].

Furthering the complexity of this phenotype, different senescence-inducing stimuli produce distinct secretory proteomes that can result in different biological outcomes depending on the tissues affected[24]. Much of the research on the SASP has focused on its role in disease pathogenesis and progression and on how SASP factors might be targeted for therapeutic intervention[27]. Diseases linked to the expression of SASP factors include atherosclerosis, cancer, cardiac dysfunction, myeloid skewing, kidney dysfunction, OA and a general decrease in health span. Identifying how specific SASP factors contribute to different pathological outcomes in patients with ageing-related diseases could help further the development of therapeutics that attenuate disease development. To this end, repositories such as the SASP Atlas[24] are helpful tools that allow researchers to search and catalogue the discovery of novel SASP factors and their contextual effects on tissue phenotypes.

Cellular senescence and OA

Although chondrocytes are hypo-replicative during homeostasis, they do maintain the potential to proliferate in some settings. For example, chondrocytes proliferate in the form of 'clusters' during the early stages of OA, which is commonly viewed as an attempt to repair damaged matrix[28]. Chondrocytes also initiate cell division when plated in tissue culture[28]. The relationship between quiescence (that is, reversible cell cycle arrest) and senescence is complex, with evidence that mitogenic stimulation of damaged, quiescent cells can actually contribute to the induction of senescence upon re-entry into the cell cycle[29].

Like other organs, joint tissues are subject to senescence and decay over time, and the number of senescent chondrocytes and synovial fibroblasts correlates strongly with age[30,31]. Given the important role of bone–cartilage crosstalk, increased osteocyte senescence during ageing might also contribute to OA[14]. Senescence is also a feature of post-traumatic OA, as joint injury can accelerate chondrocyte senescence and stimulate cartilage degradation[32]. Abnormal mechanical loading could be one cause of premature senescence after injury, as catabolic shear stress has been found to initiate senescence in young cartilage explants[33]. Additionally, lifestyle factors that increase susceptibility to OA have been found to overlap with cellular senescence. For example, mice placed on calorie-dense and nutrient-poor diets exhibited increased senescence in adipose tissue, while exercise reduced this outcome[34]. Furthermore, OA can induce phenotypic changes in joint cells that correlate

with senescent signatures. For example, the cell surface protein urokinase plasminogen activator surface receptor (uPAR) is induced broadly in senescent cells[35], as well as in chondrocytes derived from osteoarthritic cartilage[36].

Senescence induces metabolic reconfigurations in cells that, over time, can contribute to the pathogenesis of OA. In fact, the transplantation of senescent fibroblasts into the knee joints of mice induced cartilage erosion, osteophyte formation and loss of mobility, suggesting that senescent cells alter the synovial microenvironment and induce OA-like arthropathy[37]. Senescent joint cells exhibit common hallmarks, such as telomere erosion, increased expression of p53 and of the cyclin-dependent kinase (CDK) inhibitors p21 and $p16^{INK4a}$ (p16), enhanced generation of reactive oxygen species (ROS) via mitochondrial dysfunction, and increased senescence-associated heterochromatin[38]. Notably, chondrocytes, osteocytes and synovial fibroblasts can also exhibit the SASP[14,30,31]. As noted above, a hallmark of the SASP is the secretion of pro-inflammatory cytokines, such as IL-6, IL-17, IL-1β, oncostatin M and TNF[19,24], and several SASP factors induce OA-related changes, including inflammation, bone growth and degradation of the extracellular matrix (ECM) (FIG. 1). Therefore, a better understanding of OA pathogenesis will include identifying the phenotypic consequences of SASP factors in joint tissues.

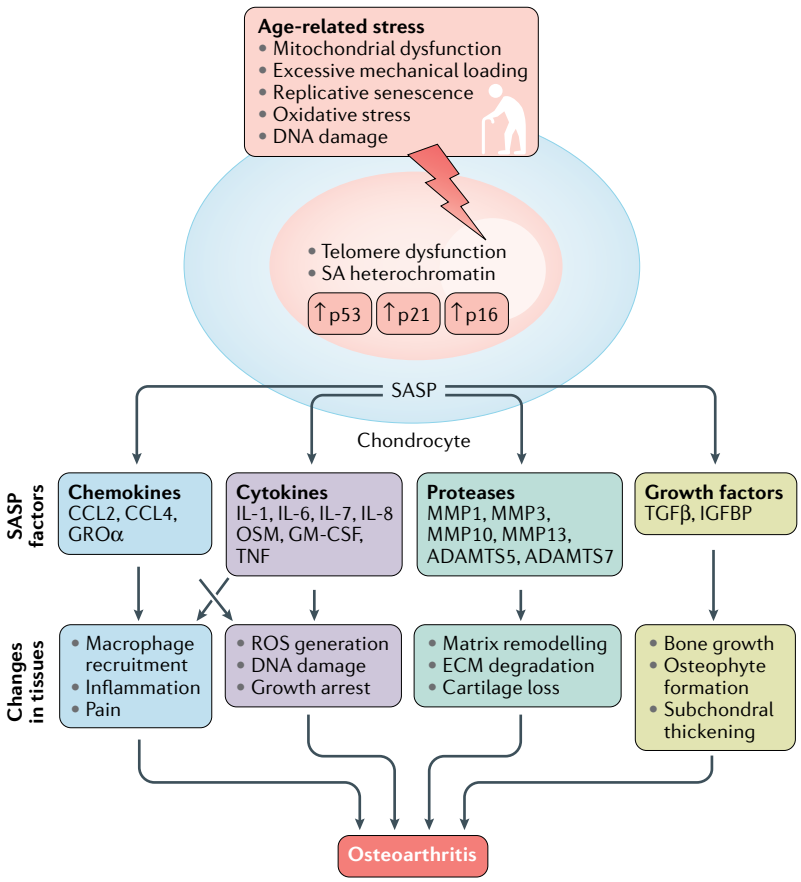

Fig. 1 | **Associations between age-related stress, senescence and OA.** Multiple age-related stresses converge on the induction of senescent hallmarks in articular joint cells. These cells can exhibit the senescence-associated secretory phenotype (SASP) and secrete factors (including chemokines, cytokines, proteases and growth factors) that act independently or together to induce changes commonly found in osteoarthritic tissues. ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; CCL, CC-chemokine ligand; ECM, extracellular matrix; GM-CSF, granulocyte–macrophage colony-stimulating factor; GRO, growth-regulated alpha protein; IGFBP, insulin-like growth factor binding protein; MMP, matrix metalloproteinase; OA, osteoarthritis; OSM, oncostatin M; ROS, reactive oxygen species; SA heterochromatin, senescence-associated heterochromatin; TGFβ, transforming growth factor-β.

Cytokines such as IL-6 are elevated in the synovial fluid of patients with OA[39]. The IL-6–STAT3 signalling pathway induces premature senescence in normal human fibroblasts, suggesting that these cells might trigger a bystander effect that drives further senescence and SASP in surrounding cells[40,41]. Furthering this hypothesis in cartilage, chondrocytes have been shown to facilitate intercellular communication via the production of extracellular vesicles (EVs), the levels of which were greatly upregulated in patients with OA compared with those in healthy individuals and resulted in the induction of a senescent state in nearby cells[42]. The role of EVs in cellular senescence and OA is discussed in more detail later in this Review.

Cytokines can upregulate the expression of a family of enzymes known as matrix metalloproteinases (MMPs)[1]. Like cytokines, MMPs, such as MMP13 (also known as collagenase-3), and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), such as ADAMTS-5, are secreted by cells into the ECM. The catabolic activity of MMPs and ADAMTS can degrade ECM proteins in cartilage, including sulfated proteoglycans, collagen and fibronectin[1]. Loss of cartilage ECM is a key early feature of OA, which further implicates the senescence of chondrocytes and other cells of the synovial joints as drivers of OA pathogenesis.

Senescence processes and biomarkers

Several phenotypic transformations occur during cellular senescence (BOX 1). Here we discuss three of them (senescence-associated β-galactosidase (SA-β-gal) production, p16 expression and EV secretion), and their relevance to osteoarthritis pathogenesis.

Senescence-associated β-galactosidase

The cytochemical staining of β-galactosidase activity to detect senescent cells, known as SA-β-gal staining, is one of the most commonly used techniques in both cell culture and tissue samples[43,44]. Positive staining is caused by the upregulation of β-galactosidase activity in lysosomes, which is optimally detected at pH 4.0 but detectable in senescent cells at pH 6.0 (REF.[45]). In articular cartilage, the number of SA-β-gal-positive chondrocytes was higher in old mice than in young mice[30]. However, a few precautions must be observed when using SA-β-gal as a marker for senescent cells in the joint. First, the enzymatic activity of lysosomes is regulated by the autophagy pathway, and isolating and culturing primary cells in monolayer can increase both basal autophagy[46] and senescent phenotypes[47]; thus, SA-β-gal staining in cultured chondrocytes can represent an increase in autophagy rather than a senescent state. Second, silencing *GLB1*, the gene encoding β-galactosidase, eliminates SA-β-gal staining but does not inhibit senescence,

Box 1 | **Common changes seen in cellular senescence**

- Increased production of β-galactosidase
- Increased expression of $p16^{INK4a}$
- Irreversible growth arrest
- Increased secretion of extracellular vesicles (EVs)
- Alterations in the microRNA content of EVs
- Genomic instability
- Increased levels of heterochromatin
- Telomere attrition
- Loss of proteostasis
- Dysregulated nutrient sensing
- Mitochondrial dysfunction
- Increased production of reactive oxygen species and reactive nitrogen species
- Increased secretion of senescence-associated secretory phenotype factors
- Upregulation of urokinase plasminogen activator surface receptor (uPAR)

demonstrating an indirect link between positive staining for SA-β-gal and senescence[45]. Third, senescence-independent β-galactosidase staining was observed in vivo in the neurons of young rodents and correlated with increased expansion of lysosomes during cell growth[48]. Finally, fibroblasts from patients with autosomal recessive G(M1)-gangliosidosis, a disease in which lysosomes are dysfunctional, were negative for SA-β-gal after undergoing replicative senescence[45]. Taken together, these studies suggest that changes to autophagy and the lysosomal activity of a cell, rather than senescence, determine the results of SA-β-gal staining.

Accordingly, changes in autophagy that occur with ageing and OA should be considered when performing SA-β-gal staining. Autophagy and lysosomal function decrease in patients with OA, whereas stimulation of autophagy (for example, with rapamycin), can confer protective homeostatic effects on normal human cartilage[49–51]. Autophagy can also be stimulated by a multitude of cellular stresses that can occur independently of senescence, including nutrient deprivation, hypoxia, ROS, DNA damage, protein aggregates, damaged organelles or intracellular pathogens[52,53]. Hypoxia-induced autophagy is of particular concern because chondrocytes reside naturally in low oxygen conditions due to a lack of blood vessels in cartilage[54]. Metabolism and homeostasis in this environment are maintained through autophagy, which recycles intracellular amino acids and clears dysfunctional mitochondria. Consequently, chondrocytes express the autophagy markers ULK1, Beclin1 and LC3 under normal physiological conditions, suggesting that autophagy is constitutively active in these cells[50].

For these reasons, in studies using cellular senescence as an indicator of OA progression, SA-β-gal experiments should ideally be performed on joint tissues rather than on cultured cells, and the studies should incorporate one or more additional biomarkers of senescence. Furthermore, when inducing or treating OA-like phenotypes, careful consideration should be given to how the treatment being applied effects autophagy.

p16

p16 induces cellular senescence by binding CDK4 and CDK6 and preventing the downstream inhibition of the cell cycle repressor protein retinoblastoma-associated protein (Rb). p16 is upregulated in response to cellular stress, such as DNA damage from radiation or telomere shortening, ROS or oncogenic stress[55]. As a tumour suppressor, p16 mutations have been linked to an increased risk of several cancers, including cutaneous malignant melanoma and pancreatic cancer[56,57]. Notably, p16 expression is highly correlated with age, and measuring cellular p16 levels has been proposed as a biomarker both for cellular senescence and for determining the biological age of an organism[58]. In addition to its role as a biomarker, the selective removal of p16-high cells can extend the lifespan and healthspan of mice, demonstrating that p16-expressing cells influence the onset of ageing-related pathologies[59].

Importantly, higher p16 expression was found to correlate with age in murine and human articular chondrocytes[30]. Chondrocytes expressing high levels of p16 also displayed lower expression of cartilage-related ECM proteins, such as aggrecan, but increased expression of ECM-degrading SASP factors such as MMP13 and MMP1. These initial results suggest that chondrocyte senescence not only correlates strongly with age, but also results in a metabolic transformation that contributes to the further destruction of cartilage. Given that p16 and the SASP can be independent arms of the senescence phenotype[60], the group also assessed whether p16 itself contributed to OA pathology and found that it did not[30]. Indeed, somatic inactivation of p16 in chondrocytes of adult mice did not inhibit the SASP, nor did it alter the rate at which OA occurred in response to physiological ageing or induced joint injury. Together, these results demonstrate that p16 can be utilized as a biomarker of chondrocyte ageing but chondrocyte p16 does not appear to play a causal role in OA.

Extracellular vesicles

Understanding how ageing contributes to changes in tissue structure is a major focus of ageing research, but how ageing affects circulating factors, which are crucial for maintaining tissue homeostasis and function, is also important. In a landmark study, aged mice exposed to factors present in young mice through parabiosis exhibited restored regenerative capacity in skeletal muscle progenitor cells[61]. Moreover, a study in which young mice were exposed to the blood of aged mice resulted in impaired tissue function and repair[62]. Parabiosis has not been widely used to study cartilage function, but an experiment described this year demonstrated that mice had less severe OA if they shared circulation with young mice as opposed to older mice for the past 4 months before they were killed[63]. Further experiments in this study showed that daily systemic injection with the rejuvenating factor growth/differentiation factor 11 increased chondrocyte proliferation and protected mice from joint tissue degradation. Given these results, identifying specific circulating factors that contribute to the promotion or deterioration of joint tissue health could be important

for understanding the mechanisms underlying OA as well as other age-related diseases.

EVs such as exosomes are small lipid membrane-bound particles that facilitate intercellular communication via the transport of proteins and RNA[64]. Like SASP factors, EV secretion is upregulated in senescent cells[65,66], which can induce premature senescence in neighbouring cells, for example, through the transfer of microRNAs that activate senescence pathways[67]. Interestingly, a cross-sectional and longitudinal study found that EV concentration in plasma decreases with advancing age[68]. However, this decrease was accompanied by increased vesicle internalization and activation of B cells and monocytes, suggesting that EVs might enhance pro-inflammatory immune responses with age. Together, these studies highlight the emerging role of EVs in cellular and organismal senescence.

In another study, both senescent chondrocytes and EV concentrations were enriched in cartilage from patients with OA relative to cartilage from healthy individuals[42]. Furthermore, exposing non-senescent chondrocytes to EVs derived from patients with OA increased senescent phenotypes and decreased proteoglycan production. Fluorescent labelling and tracking of EVs confirmed that these vesicles were internalized by chondrocytes within 6 h of exposure. MicroRNAs were also differentially expressed between senescence-associated EVs and EVs not associated with senescence; the former displayed a decrease in miR-140-3p, the depletion of which was associated with cartilage dysfunction[69], and an increase in miR-34a-5p that was linked to the upregulation of senescence-associated proteins[70]. The selective removal of senescent cells using the senolytic compound UBX0101 (see below) reduced the number of EVs in cultured chondrocytes from patients with OA, and EVs isolated from the synovial fluid of UBX0101-treated mice contained features associated with cartilage growth, such as increased aggrecan and decreased proteases[42]. Together, this work suggests that increased EV secretion and internalization, along with changes to vesicular RNA and protein content, should be investigated as potential biomarkers for both chondrocyte senescence and OA. Importantly, the authors of this study also found differences in the expression of microRNAs in EVs from the synovial fluid between aged healthy donors and donors with clinical OA[42]. Examining EV microRNA profiles could help distinguish cartilage loss caused by OA and other arthropathies rather than by ageing.

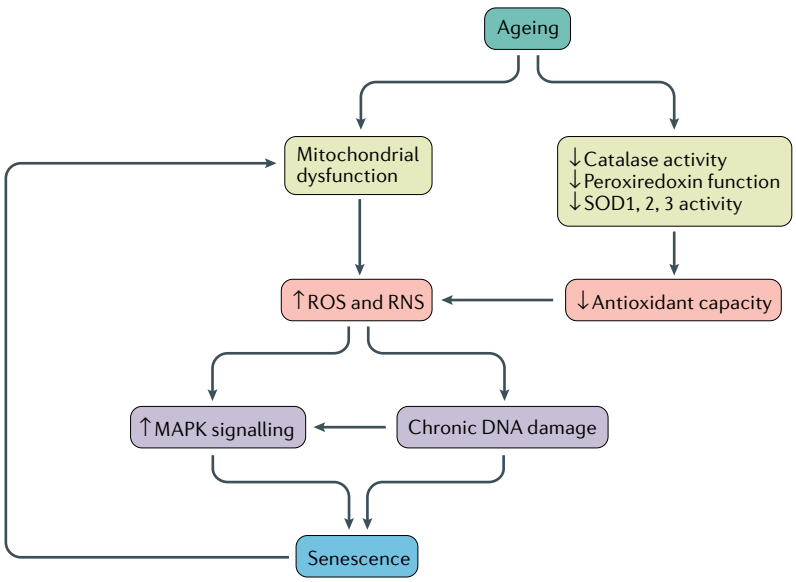

Fig. 2 | **Model for oxidative stress-induced senescence in joint cells.** Aged chondrocytes and synovial cells exhibit mitochondrial dysfunction, as well as a reduction in antioxidant capacity, via a decrease in the activity of catalase and superoxide dismutase (SOD) and decreased peroxiredoxin function. These phenotypes increase the generation of reactive oxidative species (ROS) and reactive nitrogen species (RNS), which induce chronic DNA damage and increase MAPK stress signalling, both of which can act independently or together to induce senescence. Senescence itself can cause further mitochondrial damage, causing positive feedback.

Oxidative stress drives OA and senescence

Another hallmark of ageing is mitochondrial dysfunction, which causes oxidative stress by increasing cellular levels of ROS. ROS-induced DNA damage has been linked to the pathogenesis of many age-related conditions, including cardiovascular, pulmonary, kidney and neurodegenerative diseases[71]. Additionally, increased oxidative stress and a decrease in the antioxidant capacity of mitochondria can disrupt physiological cell signal transduction, which might promote ageing by gradually causing loss of cellular integrity and tissue homeostasis[72,73].

With regard to OA, oxidative stress has been proposed as a driver of the catabolic and anabolic signalling imbalance in cartilage that results in progressive matrix degradation[74] (FIG. 2). For example, survival and tolerance of oxidative stress is regulated by members of the mitogen-activated protein kinase (MAPK) pathway, such as c-Jun N-terminal kinases (JNKs) and p38. It has been suggested that cytokine-mediated activation of JNK signalling worsens OA-associated phenotypes by activating pro-inflammatory and ECM degradation pathways in joint tissue cells[75]. However, oxidative stress in cultured human chondrocytes inactivated JNKs while p38 remained active[76]. Deletion of JNK1 and JNK2 in mice resulted in more severe age-related OA than in wild-type mice, as well as increased senescence in cartilage and particularly in the synovium[77], suggesting that JNK is a negative regulator of joint senescence.

In addition to ageing, senescence itself has been shown to induce mitochondrial dysfunction and stimulate ROS production[78]. Overproduction of hydrogen peroxide and reactive nitrogen species, including nitric oxide (NO), has been detected in aged cartilage and OA cartilage from both humans and monkeys[79]. Cells from human cartilage explants cultured in the presence of hydrogen peroxide exhibited hallmarks of chondrocyte senescence, including shortened telomeres, reduced replicative capacity and lower production of glycosaminoglycan[80]. Loss of antioxidant enzymes, such as superoxide dismutase (SOD), is known to correlate with premature senescence and accelerated ageing phenotypes[81,82]. All three SOD family members (SOD1, SOD2 and SOD3) are abundantly expressed in human articular cartilage, but their activity is markedly decreased in cartilage from patients with OA[83,84].

Similarly, peroxiredoxins and catalases are antioxidants that are critical in the regulation of redox signalling and the protection against oxidative stress by controlling levels of H_2O_2 (REF.[85]). Chondrocytes isolated from older adults were noted to have hyperoxidized (and thus inactive) peroxiredoxins, whereas overexpression of catalase targeted to the mitochondria reduced the severity of OA in 24-month-old mice[86]. Together, these results suggest a correlation between increased oxidative stress and the induction of senescence in cartilage, which might drive OA. They also support the strategy of using antioxidants to prevent ROS-induced senescence, which could be a useful approach to the treatment of OA.

Senolytics and senomorphics for OA

Senolytics and senomorphics are two classes of therapeutics that have been reported to alleviate ageing-associated pathologies in murine models and are currently being investigated in trials in humans. Senolytics induce apoptosis preferentially in senescent cells, whereas senomorphics inhibit the SASP factors linked to pro-inflammatory paracrine signalling and tissue damage[87] (FIG. 3). Given the correlations between senescence, SASP and OA, these drugs are attractive candidates for targeting OA pathogenesis and slowing its progression (TABLE 1).

Senolytics

Development of senolytics for OA. In pioneering preclinical studies, an inducible transgene was developed that allowed the targeted killing and clearance of senescent cells expressing high levels of p16 (REFS[59,88]). Mice expressing this transgene demonstrated increased median lifespan and delayed onset of ageing-associated pathologies compared with wild-type mice. When a similar transgenic technique was used to clear senescent cells locally in mouse articular cartilage, the development of post-traumatic OA was substantially decreased[32].

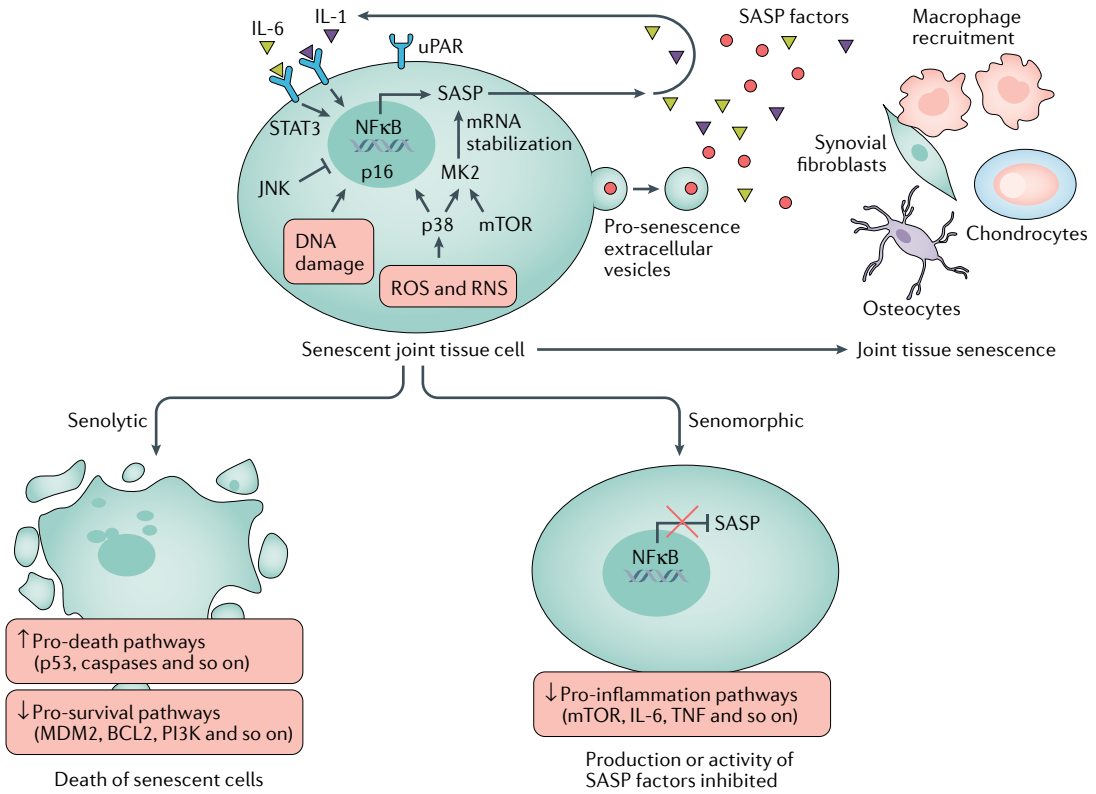

Fig. 3 | **Model for cellular senescence in joint tissue and potential treatments.** Cytokines such as IL-6 promote senescence via the transcription factor STAT3, and IL-1 can induce NFκB-driven expression of genes encoding senescence-associated secretory phenotype (SASP) factors. Senescent joint cells are characterized by increased oxidative stress (owing to the generation of reactive oxidative species (ROS) and reactive nitrogen species (RNS)), DNA damage, increased expression of urokinase-type plasminogen activator surface receptor (uPAR), and upregulation of stress proteins such as p38, c-Jun N-terminal kinase (JNK) and mTOR. p38 induces senescence and the expression of p16, while JNK negatively regulates senescence in cells in joint tissue. mTOR and p38 promote the SASP by upregulating the translation of (mTOR) and phosphorylating (p38) MK2 (also known as MAPKAPK2), which stabilizes mRNA transcripts encoding SASP factors. SASP factors (including IL-1 and IL-6) and senescence-inducing extracellular vesicles are secreted by these cells into the extracellular matrix, promoting macrophage recruitment to, and driving further senescence in, the surrounding joint tissue. Senolytic drugs aim to prevent senescence-associated disease by inducing apoptosis specifically in senescent cells via the upregulation of p53, caspases and other proteins in death-associated pathways, while repressing pathways associated with cell survival (for example, pathways involving MDM2, BCL2 and PI3K). Senomorphic drugs do not kill senescent cells, but repress the SASP by inhibiting the activity of proteins related to inflammation, such as mTOR, or by directly inhibiting the activity or production of SASP factors such as IL-6 and TNF.

Although these experiments utilized transgenic mice to induce apoptosis in cells undergoing senescence, other studies have tested whether senolytics can mimic this effect therapeutically. In one study, which compared the gene expression profiles of senescent cells and proliferating cells, senescence was found to upregulate genes encoding proteins in anti-apoptotic signalling networks, such as BCL-2 family members and proteins in the PI3K–AKT pathway[89]. Many senolytics induce apoptosis selectively in senescent cells by suppressing pro-survival pathways that are activated in senescent, but not healthy, cells. For example, treatment of irradiated or normally aged mice with navitoclax (ABT-263), a BCL-2 and BCL-X_L dual inhibitor, depleted senescent haematopoietic stem cells in bone marrow and senescent muscle stem cells, and promoted cellular rejuvenation[90]. Furthermore, in mouse cartilage explants, navitoclax reduced the senescence burden by eliminating chondrocytes expressing high levels of p16 through apoptosis[91]. Another example is the senolytic cocktail of dasatinib and quercetin, which effectively eliminates senescent cells and is being investigated in clinical trials for treating idiopathic pulmonary fibrosis, a potentially fatal disease associated with senescence[92,93]. Dasatinib inhibits multiple tyrosine kinases, including BCR-ABL, SRC, c-KIT, ephrin A receptor and platelet-derived growth factor-β receptor kinases[94], whereas quercetin is a plant flavonol that inhibits PI3K and inhibitors of serine proteinases called serpins[89]. In another study, senescent cells were transplanted into young and old mice, and caused physical dysfunction and decreased lifespan[95]. However, treating these mice with dasatinib and quercetin attenuated the harmful effects of senescence and increased healthspan and lifespan. Similarly, treating aged mice with dasatinib and quercetin reduced the number of senescent osteocytes in bone, decreased osteoclast formation and bone loss, improved mineral reabsorption and thickness, and substantially improved the trabecular and cortical bone microarchitecture[96].

Although these drugs have yet to be tested in humans for the treatment of joint tissue disease, several other senolytics are currently being investigated in clinical trials for OA, including UBX0101 (REFS[97–99]), which inhibits the interaction between p53 and mouse double minute 2 homologue (MDM2), the E3 ubiquitin protein ligase that targets p53 for degradation. Local intra-articular injection of UBX0101 in mice with post-traumatic OA selectively cleared senescent cells, decreased proteoglycan loss, and alleviated OA-related disease outcomes of pain and articular cartilage degradation[32]. In another study, pro-inflammatory stress in chondrocytes induced cathepsin B-mediated cleavage of the NAD-dependent deacetylase sirtuin-1 (SIRT1)[100], an enzyme that was found to play a critical role in chondrocyte survival and ECM homeostasis[101]. Cleavage of SIRT1 resulted in an N-terminal fragment that lacks deacetylase activity, and an elevated ratio of N-terminal to C-terminal SIRT1 fragments in serum correlated with both early-stage OA and chondrosenescence[100]. The researchers demonstrated that anterior cruciate ligament transection increased the ratio of N-terminal to C-terminal SIRT1 in serum and that clearance of senescent cells by the combined application of systemic navitoclax and intra-articular UBX0101 lowered this ratio.

Table 1 | **Senolytics and senomorphics with potential as therapeutics for OA**

Drug name	Target of action	Refs
Senolytics		
Dasatinib	BCR-ABL, SRC, c-KIT, ephrin A receptor	92–96
Quercetin	PI3K and serpins	89,92,93,95,96
Fenofibrate	PPARα	102
Fisetin	SIRT1, IL-1β	103–105
UBX0101	MDM2	32,97–100
Navitoclax (ABT-263)	BCL-2, BCL-X_L	90,91,100
Senomorphics		
Lutikizumab	IL-1α, IL-1β	120
Canakinumab	IL-1β	122
Tocilizumab	IL-6 receptors	123,124
Etanercept	TNF	121
CL82198	MMP13	130

MDM2, mouse double minute 2 homologue; MMP13, matrix metalloproteinase 13; PPARα, peroxisome proliferator-activated receptor alpha; SIRT1, NAD-dependent deacetylase sirtuin-1.

High-throughput drug screening can be utilized to find new senolytics that work on chondrocytes and synovial cells, as well as to discover novel mechanisms that contribute to OA pathology. For example, in one study, over 1,000 compounds were screened for senolytic activity in a human chondrocyte cell line[102]. Fenofibrate, a flavonoid and agonist of peroxisome proliferator-activated receptor-α (PPARα) that is used to treat dyslipidaemias, was found to induce apoptosis in SA-β-gal-positive chondrocytes. This discovery led the authors to investigate PPARα expression in the context of OA, and they found that it was reduced in the blood and knee cartilage of patients with OA[102]. Flavonoids that activate sirtuins, such as fisetin, are linked to longevity and inhibit IL-1β-induced inflammation in osteoarthritic chondrocytes[103,104]. Fisetin is currently being evaluated in clinical trials for efficacy in alleviating OA symptoms by reducing senescence burden in cartilage[105].

Concerns associated with the use of senolytics in OA. Although pharmacological approaches to treating age-related diseases appear promising, the potential for side effects and disparities in drug potency remain a concern. Regarding the treatment of joint disease, it is unknown if promoting cell death with senolytics will compromise tissue integrity and exacerbate cartilage and bone loss seen in patients with OA. Interestingly, killing chondrocytes in the superficial zone of articular cartilage in mice, using diphtheria toxin produced by cells expressing proteoglycan 4 (also known as superficial zone proteoglycan), did not induce further cartilage damage[106]. In fact, the death of chondrocytes in the superficial zone appeared to improve injury outcomes following surgical destabilization of the medial meniscus. The authors proposed that catabolism from intact chondrocytes,

rather than chondrocyte death, drives further cartilage loss following joint injury. Given that senescence is a feature of post-traumatic OA, this evidence suggests that killing senescent chondrocytes with senolytics might help to prevent injury-induced cartilage loss caused by catabolic SASP factors that are secreted by senescent chondrocytes. It will be important to perform similar studies in patients with age-related OA to determine the capacity of cartilage to maintain long-term homeostasis after cell death is induced.

Another consideration for the use of senolytics in OA strategies is that, while a plethora of evidence implicates cellular senescence as a driver of ageing and disease pathology, some studies have suggested a beneficial role for senescence in various physiological processes, including tissue remodelling and wound healing[107]. For example, senescence was found to be induced during the intermediate stages of limb regeneration in salamanders[108]. After amputation, senescent cells accumulated in the cartilage and muscles of the developing limb but were subsequently cleared naturally by macrophages before full regrowth. Macrophage depletion prevented the clearance of senescent cells[108], and was found, in another study, to stunt regeneration[109]. Importantly, the proportion of cells that became senescent after amputation was not influenced by age, suggesting an ageing-independent role of senescence in tissue repair. Although more research into this concept is needed, the authors of this study postulated that efficient immunosurveillance of senescent cells might have allowed macrophages to be recruited to areas of damaged tissue, which was necessary for regeneration. In a study in mice, senescent fibroblasts and endothelial cells were found to accumulate near sites of cutaneous wounds and to accelerate healing through the secretion of platelet-derived growth factor AA (PDGF-AA; that is, PDGF composed of two A subunits), which induced myofibroblast differentiation[110]. This study suggests that secretion of growth factors and remodelling enzymes by the SASP might help to stimulate cell growth, which can aid tissue renewal and wound closure. Accordingly, more research is needed to establish if the wholesale elimination of senescent cells from joints causes side effects that could further contribute to tissue loss in OA.

Senomorphics

Overview of senomorphic candidates. The therapeutic targeting of pathways and molecules linked to inflammation and disease is not a new strategy, and a wide array of senomorphic candidates have been shown to inhibit pathways linked to the SASP without inducing apoptosis. These senomorphic candidates include inhibitors of IκB kinase and NFκB (such as NEMO-binding domain peptides)[111], inhibitors of the tyrosine protein kinase JAK (such as ruxolitinib)[112], ATM inhibitors (such as KU-60019)[113], compounds that block progerin–lamin A/C binding (such as JH4)[114], activators of PDGF and fibroblast growth factor signalling (for example, conditioned medium from embryonic stem cells)[115], inhibitors of TGFβ receptor type 2 and p21 (such as Mmu-miR-291a-3p)[116], and more[117]. Given the correlation between the expression of SASP factors and OA-like pathology, the inhibition of these factors is an attractive treatment approach. However, choosing the right target is necessary to ensure therapeutic efficacy and specificity.

Cytokine inhibition. In cartilage, TNF combined with the release of other SASP factors such as IL-1β stimulates the production of damaging MMPs and inhibits tissue repair[118,119]. Clinical trials of TNF or IL-1 inhibition for the treatment of OA have been somewhat disappointing. For example, in a phase II trial of lutikizumab, a dual inhibitor of IL-1α and IL-1β, in patients with knee OA and synovitis, lutikizumab treatment led to a very limited improvement in pain and had no effect on synovitis[120], and in a trial of etanercept, a TNF inhibitor, in patients with inflammatory hand OA, etanercept treatment failed to improve pain and had a limited effect on structure[121]. However, a recent exploratory analysis of data from a trial designed to examine the efficacy of the anti-IL-1β antibody canakinumab on cardiac events in an at-risk population (that is, patients with previous myocardial infarction and elevated C-reactive protein) found a lower incidence of knee and hip replacement in the canakinumab-treated groups than in a placebo-treated control group[122].

IL-6 has been implicated in the pathogenesis of rheumatoid arthritis (RA), and the IL-6 receptor inhibitor tocilizumab is effective in clinical therapy for RA[123] and is currently in phase III trials for hand OA[124]. Although RA is an autoimmune disease, it shares common features with OA, including the release of pro-inflammatory cytokines and degradation of the cartilage matrix. Surprisingly, however, *Il6* knockout mice exhibit more severe OA in response to physiological ageing than wild-type mice[125], suggesting that OA pathogenesis is complex and requires a multifaceted approach to treatment.

Targeting MMPs. MMPs are another class of SASP factors to consider as targets for pharmacological intervention due to their known catabolic effects on cartilage. Specifically, MMP13 is the most highly expressed MMP in connective tissue[126] and the most specific enzyme for the degradation of type-II collagen found in articular cartilage[127]. Human chondrocytes from patients with OA were found to express higher levels of MMP13 than chondrocytes from donors with healthy cartilage[128]. Furthermore, postnatal overexpression of MMP13 in transgenic mice induced OA-like arthropathy, implicating MMP13 as a primary driver of OA pathogenesis[129]. In another study, chondrocyte-specific deletion of MMP13 reduced the severity of OA induced by meniscal-ligamentous injury (MLI)[130]. To test the effects of senomorphics on OA progression, the researchers also treated wild-type mice with CL82198, a selective inhibitor of MMP13, after MLI. CL82198 treatment reduced OA severity, increased levels of type II collagen and inhibited chondrocyte death.

Together, these data suggest that the inhibition of SASP factors via senomorphics might be a promising therapeutic approach to treating OA. However, more research is needed to determine precisely which

SASP factors contribute to OA pathology, and if their inhibition slows or prevents disease progression.

Conclusions

The evidence implicating cellular senescence in joint tissues as a primary driver of OA pathogenesis and progression is compelling, but further investigation is needed to identify the precise mechanisms by which senescence causes specific disease phenotypes. Most likely, the thread tying ageing, senescence and OA pathology together is the accumulation of senescent cells over time combined with gradual changes in cellular metabolism, morphology and function, all of which contribute to loss of joint tissue homeostasis and integrity. Effective OA treatment strategies will require first establishing the underlying mechanisms that drive these changes to cell physiology, and then designing therapies directed towards these mechanisms.

Additionally, the common biomarkers used to identify senescence are insufficient for diagnosing OA. SA-β-gal staining is not necessarily an indicator of chondrocyte senescence and can be influenced by changes in autophagy and lysosome function, both of which are reduced in OA[49,50]. Also, the expression of p16 in chondrocytes, which is used in many studies using senolytics to identify senescent cells, is not required for the SASP or OA pathogenesis[30]. Therefore, other biomarkers should be considered for the therapeutic targeting of cells involved in OA to ensure specificity and prevent unintended effects. Recent evidence has demonstrated that chondrocyte senescence and OA are linked to changes in the secretion of EVs and their cargo[42]. Accordingly, EVs, as well as the expression of uPAR (which is present on senescent chondrocytes[36]), should be further investigated to determine if they are accurate clinical markers for joint disease.

Senescence in joint tissues is driven by several stress-related pathways that converge on the SASP, and techniques that suppress inflammatory cytokines or selectively eliminate senescent cells while leaving healthy cells unharmed are attractive candidates for use in anti-ageing strategies (FIG. 3). However, although the preclinical evidence for using senolytics and senomorphics to treat OA phenotypes looks promising, these approaches have not yet demonstrated efficacy in eliminating or preventing the disease. Additionally, although SASP inhibitors, such as CL82198, have been proven effective in reducing the severity of post-traumatic OA in mice[130], the same effect has yet to be demonstrated on aged or diseased chondrocytes and other synovial joint cells in humans.

Furthermore, the progression of these therapies from the laboratory to the clinic is hindered by the lack of evidence implicating a specific cell type as the primary driver of OA. Chondrocytes, synovial fibroblasts, osteocytes and probably other joint tissue cells not yet studied, are all capable of becoming senescent and secreting SASP factors into the joint environment. Without knowing which cells are responsible for each OA phenotype, drug specificity for disease treatment will be difficult to evaluate.

Finally, further investigation is needed into the potential harmful effects of killing or altering senescent cells in an organ. Recent studies have demonstrated that senescence stimulates early wound healing and tissue regeneration via macrophage recruitment[108–110]. Even if senescent cells are responsible for the progression of OA after injury, eliminating these cells or preventing paracrine signalling too early might prevent the initial healing of damaged cartilage and other tissues, which could have devastating consequences for the entire joint. For this reason, studies using senolytics and senomorphics must include comparisons of disease outcomes from different treatment timings to ensure that drug efficacy can be properly inferred.

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Non-surgical management of knee osteoarthritis: comparison of ESCEO and OARSI 2019 guidelines

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Abstract | Knee osteoarthritis (OA) is a heterogeneous disease associated with substantial effects on quality of life, and its clinical management is difficult. Among the several available guidelines for the management of knee OA, those from OARSI and ESCEO were updated in 2019. Here, we examine the similarities and differences between these two guidelines and provide a narrative to help guide health-care providers through the complexities of non-surgical management of knee OA. OARSI and ESCEO both recommend education, structured exercise and weight loss as core treatments, topical NSAIDs as first-line treatments and oral NSAIDs and intra-articular injections for persistent pain. Low-dose, short-term acetaminophen, pharmaceutical grade glucosamine and chondroitin sulfate are recommended by ESCEO whereas OARSI strongly recommends against their use (including all glucosamine and chondroitin formulations). Despite this difference, the two guidelines are consistent in the majority of their recommendations and provide useful treatment recommendations for individuals with OA and health-care providers.

Osteoarthritis (OA) is the most common chronic joint disorder, is characterized by local inflammation and joint structural change, and is associated with painful symptoms and loss of function leading to considerable impairment of quality of life[1]. Globally, hip and knee OA are leading contributors to disability in terms of years lived with disability[1,2]. With population ageing and the increasing prevalence of obesity across the globe, it is widely accepted that the burden of OA will continue to increase[3], leading to an increased strain on health-care systems. Given the current absence of effective disease-modifying treatments for knee OA, attention has turned to providing effective guidance on the medical management of OA; over the past decade, several sets of recommendations have been published[4–9]. Clinical practice guidelines help assist decision-making and are therefore a vital source of information for health-care providers.

Recommendations for OA treatment are often separated into non-pharmacological, pharmacological and surgical interventions[10,11], as well as categorized by disease severity and joint site. Other variances in treatment guidelines include the target readership (for example, some taking a more patient-centred approach) and geographical focus (international versus national). These differences have led to some confusion, evidenced by the limited uptake of published guidelines by patients[12] and within primary and secondary care[13,14].

Most guidelines, however, agree in their core treatment recommendations for knee OA[15], which include the provision of education, physical therapy and encouraging weight loss. The guidelines then typically either outline a sequential, staged approach to the management of knee OA beyond core treatments[6,16,17] or outline treatment recommendations by disease and/or comorbidity group[4,7,9]. Treatment typically includes the use of analgesics, including NSAIDs and intra-articular corticosteroid injections, with joint replacement surgery recommended for more severe cases. While knee joint replacement has been shown to be effective in the management of knee OA symptoms[18], this surgery might not be suitable for all patients as up to 20% report dissatisfaction and/or persistent symptoms postoperatively[19,20]. Furthermore, knee replacement is conventionally performed in end-stage disease[18], after years of painful symptoms and loss of function and despite correctly conducted medical treatment.

In 2014, the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) published recommendations for the management of knee OA, which summarized expert opinion and the most relevant, high-quality data[6] and outlined a staged treatment algorithm to help assist health-care providers in prioritizing treatments[6]. Similarly, the Osteoarthritis Research Society International (OARSI), which has a history of publishing highly cited guidelines for OA[21–23], published recommendations in 2014 that also outlined an algorithm for the non-surgical management of knee OA[4]. In 2019, both of these international organizations updated their recommendations for the non-surgical management of knee OA[16,17]. In November 2019, a working group comprising selected authors of the 2019 OARSI (N.K.A, R.R.B., I.K.H. and T.E.M.) and ESCEO (N.K.A., O.B., C.C. and J.-Y.R.) publications as well as independent members (T.A.P., M.C.H. and A.M.) convened and jointly reviewed these latest guidelines. In this Perspective article, the members of that working group highlight the similarities and differences between the treatment algorithms and the methodological approaches used to formulate recommendations in the OARSI and ESCEO guidelines.

Comparison of aims and objectives

The membership of OARSI, an international not-for-profit research society, comprises both health-care professionals and researchers focused on the prevention and treatment of OA. OARSI supports the international growth of OA-related research leading to the dissemination of expert resources and knowledge.

ESCEO, a not-for-profit organization that operates within Europe, specializes in the provision of care and musculoskeletal research through supporting networks of academic scientists, prescribing physicians, not-for-profit organizations, regulatory authorities and corporate partners; ESCEO is tasked with providing practitioners with the most current, clinical, and economic evidence-based information to assist in the delivery of care. Both the 2019 OARSI and ESCEO guidelines were constructed to provide a practical algorithm to help guide clinicians in their decision-making for the management of knee OA[16,17]. In addition, both guidelines aimed to deliver patient-centred recommendations.

The OARSI guidelines[17] update and expand upon previously reported OARSI guidelines[4]. Similarly, the 2019 ESCEO guidelines[16] sought to update their previously published algorithm[6] by including new evidence published since 2014. Whereas the 2019 ESCEO guidelines focus only on the evaluation of treatments for knee OA, the OARSI guidelines include recommendations for knee OA, hip OA and polyarticular OA. Furthermore, OARSI exclusively formulated recommendations for the non-surgical management of knee OA whereas ESCEO developed recommendations for both non-surgical and surgical treatments. In this article, we focus exclusively on guidelines related to the non-surgical management of knee OA as it is generally regarded that surgical intervention remains the most effective and cost-effective treatment modality for end-stage disease[18,24,25].

Comparison of the methodologies

In this section, we examine the similarities and differences between the methods used by OARSI and ESCEO to develop the treatment algorithms and recommendations in their respective 2019 guidelines. Briefly, the methods used were largely similar, with both organizations using well-characterized procedures for the reporting of the guidelines. However, key differences exist in the constitution of the panels, literature search strategies, voting procedures and scaling of the treatment recommendations, which need to be carefully considered. The methodological similarities and differences are summarized in TABLE 1.

Assessing quality of evidence

The working groups that developed the 2019 OARSI and ESCEO guidelines both followed the Grading of Recommendations, Assessment, Development and Evaluations (GRADE)[26] methodology, which combines an objective review of the literature with expert consensus. OARSI evaluated the methodological rigour of meta-analyses and systematic reviews using the Assessment of Multiple Systematic Reviews Tool and randomized controlled trials using the

Table 1 | **Comparison of methodologies used to develop recommendations for the non-surgical management of knee OA**

Method	OARSI	ESCEO
Objectives	To perform an updated review of the literature, to assess the harms and benefits of 67 pre-specified non-surgical treatments for knee OA and to develop a treatment algorithm for the non-surgical management of knee OA	To perform an updated review of the literature, to assess the efficacy of a selected group of medications and to develop a set of treatment recommendations for the surgical and non-surgical management of knee OA in patient-specific scenarios
Panels	The OARSI working group included specialists in rheumatology, orthopaedics, primary care, pharmacology, sports medicine, clinical epidemiology, evidence-based medicine, rehabilitation and physical therapy, as well as patient representatives A core expert panel of six members supervised the project; the voting panel comprised 13 members, and five individuals made up the literature review panel	The ESCEO working group included specialists in rheumatology, rehabilitation, orthopaedics, clinical epidemiology, geriatrics, pharmacology, public health and health economics, as well as patient representatives A single panel comprised 18 members, of whom four conducted the literature search
Literature search	Databases searched included Medline, EMBASE, Cochrane databases, PubMed, Google Scholar and the reference lists of relevant systematic reviews and meta-analyses Modified GRADE criteria were used to rate the quality of evidence; the literature search covered the period until December 2017 (with no start date; the search was updated on 12 July 2018) Search terms included, but were not limited to, 'osteoarthritis', 'arthrosis', 'randomized controlled trials', 'crossover', 'controlled trial', 'double-blind', 'single-blind', 'arthroscopy' and 'arthroplasty' Meta-analyses of the reviewed manuscripts were performed	Databases searched included Medline, EMBASE and Cochrane databases GRADE criteria were used to rate the quality of evidence The literature search covered the period included in the previous guidelines (that is, 2000 to February 2014) plus a new search that covered publications from 2014 to 30 September 2018 Search terms included keywords and controlled terms for the study types and OA; the exact search strategies used were not published Meta-analysis was not performed
Voting procedure	Voting on recommendations was carried out online using an anonymous survey application In stage 1, the initial vote was to select core treatments[a] from a pre-specified list of candidates Stage 2 consisted of three further voting rounds	Votes were submitted by e-mail and were anonymous; the number of voting rounds was not reported
Strength of recommendations	Recommendations were determined to be 'strong' (if ≥75% of the panel voted either for or against) or 'conditional' (if 26–74% of the panel voted for or against and vice versa) Core treatments[a] were given a strong recommendation by default	Consensus was defined as ≥75% of the panel members voting either 'strongly' or 'weakly' in favour of or against a recommendation; the strength of the recommendation was determined to be 'strong' rather than 'weak' if ≥75% of the panel rated a recommendation as 'strong'

GRADE, Grading of Recommendations Assessment, Development and Evaluation; ESCEO, European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International. [a]Core treatments were defined as treatments appropriate for use by the majority of patients in nearly any scenario and deemed to be safe for use in conjunction with first-line and second-line treatments.

Cochrane Risk of Bias Assessment method. Although several systems exist for the grading of clinical evidence and the creation of clinical practice guidelines[27], GRADE has been widely used owing to its balance between simplicity and effectiveness for quality assessment[28,29]. A major difference in the development of the OARSI and ESCEO recommendations is that the OARSI working group performed new meta-analyses to inform their GRADE assessments, whereas ESCEO used published meta-analyses. For the ESCEO guidelines, the findings of network meta-analyses were assessed using GRADE only if 'direct comparisons' were performed[16]; the results from all remaining network meta-analyses were reported descriptively.

Selection of the expert panels

In their 2019 reports[16,17], both OARSI and ESCEO recognize the need for input from multiple disciplines; thus, health-care providers and patient representatives contributed to the development of the respective treatment guidelines. The structure and the duties of the panels, however, differed. Firstly, the ESCEO working group comprised European members only, whereas the OARSI panels included members from the UK, Europe, Asia, North America, South America and Australasia.

ESCEO gathered a single panel of 18 members comprising specialists in rheumatology, rehabilitation, orthopaedics, clinical epidemiology, public health and health economics, as well as patient representatives, to oversee all aspects of the project; four individuals were tasked with conducting the literature search. By contrast, OARSI recruited a core expert panel of six members who supervised the project; a separate voting panel consisted of 13 members considered representative of the wider OARSI membership, including specialists in rheumatology, orthopaedics, primary care, pharmacology, sports medicine, physical therapy and rehabilitation. In addition, a literature review team comprised five individuals with methodological expertise, and a patient panel comprised three patient representatives who were invited to participate in formulating the OARSI recommendations during a meeting at the 2018 OARSI convention. A key difference between the task forces was that the OARSI literature review panel pre-selected specialists in statistical methods whereas the ESCEO panel did not; this difference is most likely attributable to the fact that OARSI conducted new meta-analyses as part of the assessment process while the ESCEO assessment was restricted to a systematic review.

Declaring competing interests

For OARSI, conflicts of interests were managed by adherence to OARSI Ethics Committee guidelines and by independent review of disclosures by the Ethics Committee. Individuals with high-level competing interests (for example, close involvement with a manufacturer of a product) were ineligible, whereas those with a lower level related to a specific intervention (for example, consulting) were prohibited from participating in discussions, evidence synthesis and/or review of the corresponding sections[17]. By contrast, ESCEO permitted panel members to participate provided they were transparent regarding any potential conflicts of interest.

Literature searches

Both the OARSI and ESCEO panels conducted extensive systematic reviews and adhered to a predefined consensus methodology to develop their recommendations. Both systematic searches identified systematic reviews, meta-analyses and relevant randomized controlled trials. However, the review methodologies differed in some respects.

A key difference between the OARSI and ESCEO literature search strategies was that the OARSI core expert panel developed a list of a priori questions formulated using the PICO (population, intervention, control and outcomes) framework before commencement of the systematic search. The PICO question list consisted of 67 knee OA-related questions focused on evaluating the benefits and harms of 31 non-pharmacological, 24 pharmacological and 12 nutraceutical treatments. The ESCEO panel did not adopt PICO methodology prior to conducting their systematic search, but rather focused on the evaluation of a number of selected treatments in specific, patient-centred scenarios. Using the PICO framework to inform the systematic literature search was a key advantage of the OARSI guidelines methodology, as the development of focused clinical questions, modelled using the PICO framework, is considered the most effective approach to identifying high-quality evidence[30] with data from empirical studies suggesting this approach yields more precise search results[31]. Furthermore, the use of PICO questions to evaluate the benefits and harms of non-surgical treatments for knee OA helped to ensure that the search strategy was patient-focused.

The OARSI and ESCEO searches identified relevant manuscripts in the Medline, EMBASE and Cochrane databases; the OARSI search also included PubMed, Google Scholar and the reference lists of systematic reviews and meta-analyses. The ESCEO panel performed a systematic literature search for publications from 2014 through to 30 September 2018 using a combination of keywords and controlled search terms[16]; the specific terms used in the search strategy were not published by ESCEO. The aim was to identify the most relevant literature related to treatments listed in the previous 2014 guidelines[6] and any other interventions subsequently approved or available for the management of knee OA[16]. The OARSI literature review panel searched the aforementioned databases using search terms including, but not limited to, 'osteoarthritis', 'arthrosis', 'trial', 'comparative study', 'arthroplasty', 'single-blind' and 'double-blind', with no start date specified. In the first instance, a PICO-informed systematic review of the literature from inception to December 2017 was performed and was later updated on 12 July 2018 (REF.[17]). By specifying the inclusion of 'approved' medications, the search strategy employed by the ESCEO panel could have yielded fewer publications than the OARSI strategy. More importantly, this restriction might have excluded informative data; for instance, data from phase 0–III trials in knee OA in which the medication under investigation had not yet been approved by the FDA or the EMA.

Both the OARSI and ESCEO teams screened the abstracts and full texts of the identified publications. When relevant data were available, both the ESCEO and OARSI teams performed 'quality of literature' assessment using the GRADE criteria to assign literature a score of high, moderate, low or very low.

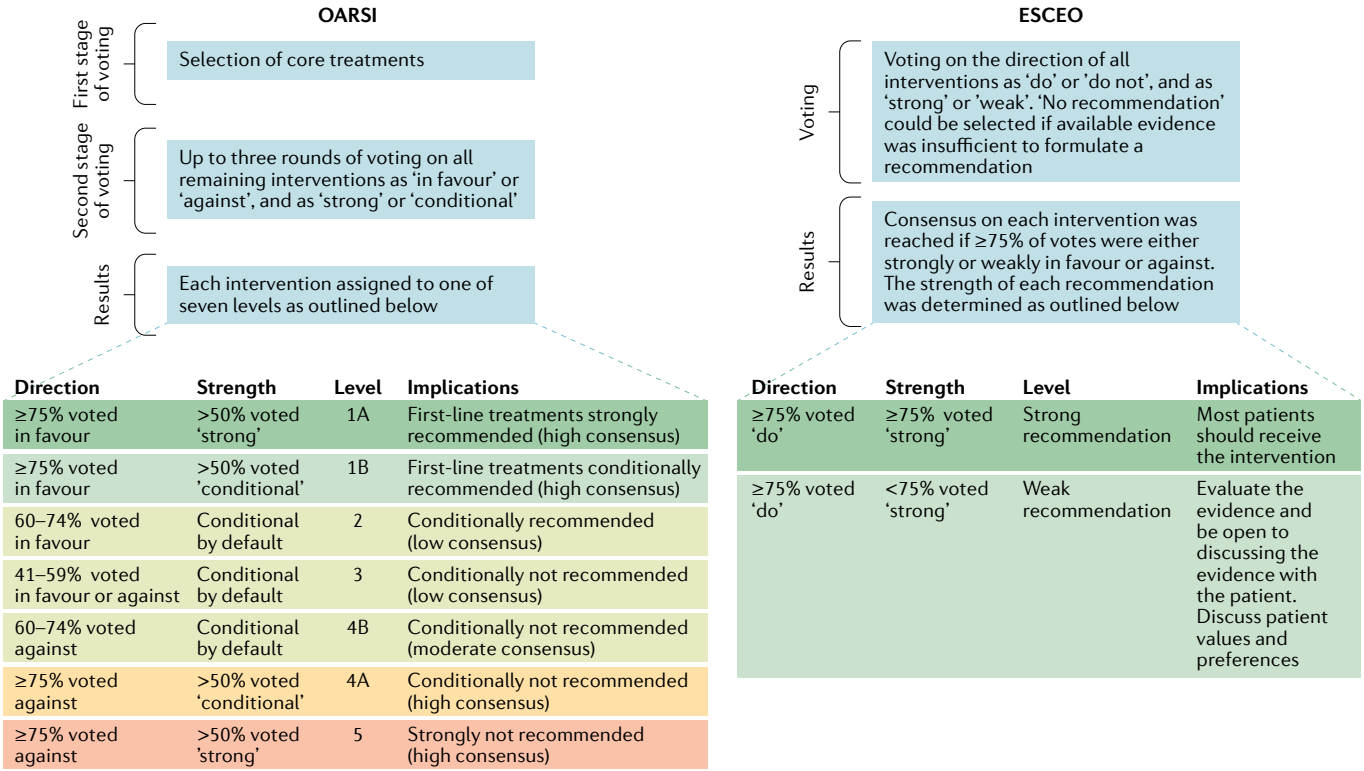
Voting procedures

As part of the OARSI methodology, prior to panel voting, the core expert panel reviewed all relevant documentation synthesized from the systematic literature search and GRADE evidence tables for each intervention. Once this review was completed, the dedicated voting panel, which had access to all the supplementary background materials (including primary data, analyses and GRADE tables), voted on the recommendations formed to address the PICO questions. All voting on recommendations was done using an online, electronic survey system with all votes kept completely anonymous. All contentious

issues were discussed and debated in an online discussion forum before re-voting. By contrast, all members of the ESCEO working group were provided with more detailed work packages, which included details of the 2014 algorithm, selected detailed summaries of the results of the updated literature search (2014–2018), GRADE evidence tables that included summaries of the quality of the evidence, and details of the magnitude of the effect for each respective intervention; reference lists were also provided. Voting by the ESCEO working group was completed anonymously via e-mail with panellists voting on their recommendation for each respective intervention.

Both the OARSI and ESCEO working groups invited their voting panels to provide a recommendation for each question and/or intervention, as outlined in FIG. 1. Votes by the panels were cast on the direction and strength of the recommendations. For the ESCEO guidelines, all treatment recommendations were assessed according to the following criteria: current and past evidence; balance between the benefits and harms of each intervention; magnitude of treatment effects; quality of the evidence; value and preferences; costs (informed by clinical experience and formal cost assessments); and the position of an intervention within the treatment algorithm[16]. Similarly, the OARSI recommendations were based on modified GRADE criteria, which included the criteria listed above as well as the assessment of estimates of treatment effect size, confidence in such estimates and clinical preference. Unlike the ESCEO working group, the OARSI panel conducted a two-stage vote. In the first stage, the expert panel voted on the inclusion or exclusion of a few selected interventions that were put forward by the expert panel; interventions that remained after the first stage were termed 'core treatments', defined as those appropriate for use in almost all patients and safe to use in combination with first-line and second-line treatments. In the second stage, which consisted of three voting rounds, all remaining interventions (including those that were excluded in the first stage) were voted on. The voting panel were asked to vote on the directionality ('in favour' or 'against') and strength ('strong' or 'conditional') of their recommendation in line with modified GRADE criteria.

One of the key differences between the ESCEO and OARSI recommendations was that OARSI specified that in the event that no adequate evidence could be found for a specified intervention, the evidence quality score for that given intervention was designated as 'very low' by default[17]. In the event that the ESCEO panel members thought the available evidence was balanced (that is, between 'do' and 'do not'), they could vote 'no recommendation'. Of the 14 non-surgical recommendations proposed by ESCEO, five are 'strong' and nine are 'weak'; the OARSI panel made nine 'strong' recommendations (core treatments and topical NSAIDs) and 13 'conditional' recommendations. Only core treatments and



Direction	Strength	Level	Implications
≥75% voted in favour	>50% voted 'strong'	1A	First-line treatments strongly recommended (high consensus)
≥75% voted in favour	>50% voted 'conditional'	1B	First-line treatments conditionally recommended (high consensus)
60–74% voted in favour	Conditional by default	2	Conditionally recommended (low consensus)
41–59% voted in favour or against	Conditional by default	3	Conditionally not recommended (low consensus)
60–74% voted against	Conditional by default	4B	Conditionally not recommended (moderate consensus)
≥75% voted against	>50% voted 'conditional'	4A	Conditionally not recommended (high consensus)
≥75% voted against	>50% voted 'strong'	5	Strongly not recommended (high consensus)

Direction	Strength	Level	Implications
≥75% voted 'do'	≥75% voted 'strong'	Strong recommendation	Most patients should receive the intervention
≥75% voted 'do'	<75% voted 'strong'	Weak recommendation	Evaluate the evidence and be open to discussing the evidence with the patient. Discuss patient values and preferences

Fig. 1 | **Summary of voting procedures for the OARSI and ESCEO working groups.** This schematic illustrates the voting procedures used by the working groups of the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) and the Osteoarthritis Research Society International (OARSI) to reach consensus on their respective recommendations for the management of knee osteoarthritis (OA). The OARSI panel voted in two stages. In the first stage, they voted on the inclusion or exclusion of core treatments (that is, treatments appropriate for use in almost all patients and safe to use in combination with first-line and stage 2 treatments). Stage 2 involved up to three rounds of voting on the direction and strength of all remaining interventions. Contentious issues were discussed in an online forum before re-voting. All interventions were assigned to one of seven levels, which determined the ordering of treatment provision and strength of the recommendations. The ESCEO panel voted on the direction and strength of proposed recommendations in a single stage of voting. All interventions were given either a strong recommendation or a weak recommendation. OARSI table adapted with permission from REF.[17], Elsevier.

level 1A and level 5 recommendations made by OARSI are 'strong' whereas all remaining recommendations are 'conditional' (see FIG. 1).

Comparison of the recommendations

We have discussed the similarities and the differences in methods used in the development of the 2019 OARSI and ESCEO recommendations. Despite such differences, the joint OARSI–ESCEO working group found, as outlined in this section, that many aspects of the recommendations for the non-surgical management of knee OA are in agreement.

Similarities

Core treatments appropriate for use in the majority of patients. In both the OARSI and ESCEO stepwise treatment algorithms, patient education and/or access to information, exercise and weight loss (if a patient is overweight) should form the core treatment approach prior to the commencement of first-line and stage 2 treatments (as shown in FIG. 2)[16,17]. In line with their 2014 recommendations[6], the 2019 ESCEO recommendations endorse aerobic, strengthening and resistance exercises. Similarly, the OARSI guidelines recommend structured, land-based exercise programmes of strengthening, cardiovascular, balance and/or neuromuscular exercises, but also add mind–body exercise including Tai Chi and yoga. The only subtle difference between the two guidelines is that the ESCEO recommendation includes all types of exercise, stating that the evidence to differentiate between different modalities is not available, whereas the OARSI recommendation excludes aquatic exercise from core treatments owing to concerns about accessibility.

First-line treatments. Following core treatments, both the OARSI and ESCEO guidelines strongly recommend the use of topical NSAIDs in the first-line management of knee OA, owing to their proven efficacy and a low risk of gastrointestinal, cardiovascular and renal adverse effects[16,17]. Both guidelines advise against the long-term use of paracetamol (acetaminophen) as a first-line treatment for knee OA, with the OARSI guidelines strongly recommending against its use in both the short term and long term, and the ESCEO guidelines making a 'weak' recommendation for its use in the short term. Both guidelines do not recommend the use of supplementation with non-pharmaceutical grade glucosamine hydrochloride, glucosamine sulfate and/or chondroitin sulfate.

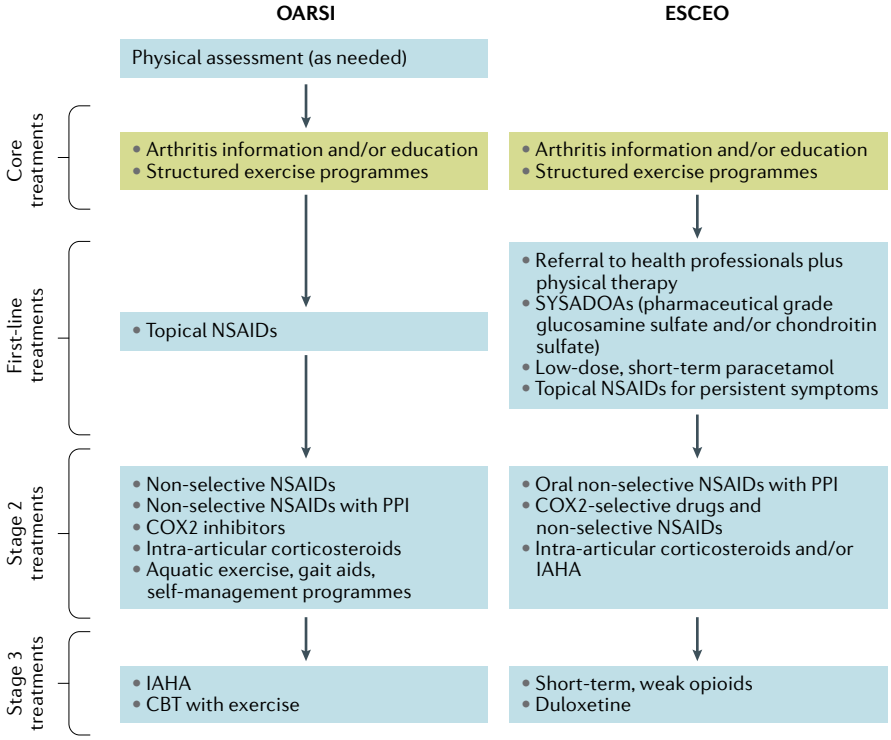


Fig. 2 | **Simplified OARSI and ESCEO treatment algorithms for the non-surgical management of knee OA in patients without comorbidities.** The list of treatments shown conforms to the recommended ordering of treatment provision in the updated 2019 guidelines for the management of knee osteoarthritis (OA) issued by the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) and the Osteoarthritis Research Society International (OARSI). For the OARSI recommendations, physical assessments are performed as needed, before entry into the treatment algorithm. The treatments in stage 3 of the ESCEO algorithm represent the last pharmacological option before knee replacement surgery. CBT, cognitive behavioural therapy; COX2, cyclooxygenase 2; IAHA, intra-articular hyaluronic acid; PPI, proton pump inhibitor; SYSADOAs, symptomatic slow-acting drugs for osteoarthritis.

Pharmacological management of persistent symptoms. As a stage 2 therapy, both the OARSI and ESCEO guidelines recommend the use of oral NSAIDs in patients with persistent OA symptoms after the use of first-line treatments, personalized according to a patient's gastrointestinal and cardiovascular risk profile[16,17]. Both sets of guidelines are in agreement that oral NSAIDs should only be used intermittently for the shortest period of time and at the lowest possible dose to control pain, owing to their known adverse cardiovascular, hepatic and renal effects. Specifically, for patients with normal gastrointestinal function both sets of guidelines recommend the use of non-selective oral NSAIDs, preferably in combination with a proton pump inhibitor (PPI), or selective cyclooxygenase 2 (COX2) inhibitors. In those with gastrointestinal complications, selective COX2 inhibitors and non-selective NSAIDs in combination with a PPI are recommended in both sets of guidelines, with the ESCEO guidelines further suggesting that celecoxib may be the 'preferred' oral NSAID[16]. In those with an increased risk of cardiovascular events, both the ESCEO and OARSI guidelines are very cautious: the former suggests limiting the use of COX2 inhibitors to 30 days and of non-selective NSAIDs to 7 days, whereas the latter recommends against the use of any oral NSAIDs in this group of patients. In the OARSI guidelines NSAIDs are not recommended for use in patients with frailty; the ESCEO guidelines make no such recommendation as this comorbidity was not assessed. Age is a major risk factor in its own right for cardiovascular, cerebrovascular and gastrointestinal adverse outcomes and should be taken into account when assessing the benefit-to-risk ratio of NSAID usage[32,33]. There is also evidence to suggest that age increases the relative risk of adverse effects of NSAIDs; thus, it has been recommended that oral NSAIDs should not be used in persons aged 65 years and above[34]. The ESCEO guidelines recommend the use of topical NSAIDs over oral NSAIDs in patients with OA aged ≥75 years and in those at increased

risk of renal adverse events; the OARSI guidelines make no such recommendation because this age group was not considered separately.

Both the OARSI and ESCEO guidelines support the use intra-articular injections of corticosteroids and state that this intervention might be more effective in the short term (~2–4 weeks) than in the long term (≥6 weeks)[16,17]. Specifically, the ESCEO guidelines recommend the use of intra-articular corticosteroids in patients with persistent pain after first-line treatments and oral NSAIDs, suggesting that this approach is more effective in those with more severe pain, which might be a predictor of its short-term efficacy. Similarly, the OARSI guidelines recommend the use of intra-articular corticosteroids in patients in whom symptom relief is not achieved after treatment with core treatments, topical NSAIDs and/or non-selective NSAIDs. Neither the OARSI nor ESCEO guidelines recommend the use of the presence of effusion as a predictor of a positive response to intra-articular corticosteroids. Intra-articular hyaluronic acid (IAHA) is recommended in both guidelines. The OARSI guidelines conditionally recommended IAHA for all patients at different stages of treatment depending on their comorbidity profiles. For example, in patients with knee OA who have no comorbidities, IAHA is recommended after failure to respond to core treatments, topical NSAIDs and oral NSAIDs (including COX2 inhibitors). The ESCEO guidelines recommend the use of IAHA in patients with contraindications to NSAIDs or those who are still symptomatic despite use of NSAIDs.

Differences

The OARSI and ESCEO treatment algorithms differ in several ways, as summarized in TABLE 2. In this section, we expand upon the differences in recommendations beyond core treatments.

First-line treatments. The ESCEO recommendations advise that patients should be referred to a physical therapist or other medical professional to determine if varus or valgus correction is needed following adherence to core treatments[16]. Alternatively, the OARSI guidelines recommend an initial physical assessment prior to entry into the treatment algorithm. As part of first-line treatment, the ESCEO and OARSI guidelines both recommend the use of topical NSAIDs. OARSI recommends their use as the first pharmacological intervention in all patients except those with chronic widespread pain disorder. The ESCEO guidelines, however, recommend topical NSAID use if painful symptoms persist following short-term rescue analgesia with paracetamol (at doses of no greater than 3 g per day), treatment with symptomatic slow-acting drugs for OA (SYSADOAs), which include pharmaceutical grade (microcrystalline) glucosamine sulfate and chondroitin sulfate, and physical therapy. Background therapy with these products is recommended by ESCEO prior to the use of topical NSAIDs based on their interpretation of the evidence base[16,35–38], and probably because of the inferred excellent safety profile of SYSADOAs and long-lasting symptomatic effects. OARSI evaluated the same literature base and made negative recommendations for all glucosamine and chondroitin products (including pharmaceutical grade).

The ESCEO guidelines provide recommendations for the use of SYSADOAs, including strong recommendations for pharmaceutical grade crystalline glucosamine sulfate and chondroitin sulfate and weak recommendations for avocado soybean unsaponifiables and diacerein; they also make a weak recommendation against the use of combined glucosamine and chondroitin sulfate. Another difference between the ESCEO and OARSI guidelines is that the former includes separate recommendations for pharmaceutical grade and non-pharmaceutical grade products whereas the OARSI recommendations are generalizable to all such products. The ESCEO guidelines provide negative recommendations for non-pharmaceutical grade glucosamine and chondroitin formulations while OARSI strongly recommends against the use of all formulations (including pharmaceutical grade products) because of a lack of efficacy or low quality evidence and high risk of bias[16,17].

Final pharmacological treatment before surgery. As the last attempt to manage symptoms pharmacologically before surgical intervention, the ESCEO guidelines recommend the short-term use of weak opioids (such as tramadol) because of their efficacy in relieving pain and providing small improvements in function. However, the adverse effects of these drugs, which include drowsiness, dizziness, nausea, constipation and an increase in the risk of falls (especially in elderly patients) are well known; hence, they should be used only for short periods of time. As an alternative to opioids, the ESCEO guidelines further recommend the use of duloxetine (a serotonin–norepinephrine reuptake inhibitor), particularly in patients with central pain sensitization, despite an increased risk of adverse events including dizziness and risk of falls. The OARSI guidelines, however, make a negative recommendation for the use of opioids owing to their unfavourable efficacy and/or safety profile[17], and recommend duloxetine only for patients who have knee OA and widespread pain and/or depression.

Consideration of comorbidities. Both the OARSI and ESCEO 2019 guidelines tailor their treatment recommendations to specific comorbidities. Specifically, both include treatment recommendations for patients with knee OA who have no comorbidities

Table 2 | **Differences in OARSI and ESCEO recommendations for the non-surgical management of knee OA**

Level or stage	Intervention	OARSI	ESCEO
First-line treatments	Topical NSAIDs	Recommend use as the first pharmacological intervention	Recommend use after short-term rescue analgesia with paracetamol (acetaminophen), SYSADOAs and physical therapy
	Paracetamol	Conditionally recommend against the use of paracetamol both in the short and long term	Recommend short-term use (≤3 g/day) and strongly advise against use in the long term
	SYSADOAs	Strongly advise against the use of all glucosamine and chondroitin formulations (including pharmaceutical grade)	Recommend the use of pharmaceutical grade glucosamine sulfate and chondroitin sulfate
Treatment in patients with persistent symptoms	Opioids	Strongly recommend against the use of oral and transdermal opioids	Recommend the short-term use of weak opioids such as tramadol

ESCEO, European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International; SYSADOAs, symptomatic slow-acting drugs for osteoarthritis.

and those with or at an increased risk of adverse gastrointestinal and cardiovascular outcomes. In addition, the OARSI recommendations are tailored for patients with frailty or widespread pain and/or depression; the ESCEO recommendations are not, although they are tailored to those at increased risk of renal adverse events. The OARSI working group set out to evaluate treatments in the context of comorbidities a priori and the treatment recommendations were informed by the systematic literature searches, whereas it was unclear from the ESCEO manuscript whether making recommendations in the context of comorbidities was a primary or secondary objective. In addition, a key difference between the two sets of guidelines is that the OARSI guidelines include 'Good Clinical Practice Statements' to accompany the recommendations, which were written to help support the treatment recommendations and were informed by expert experience.

Implications and perspectives

The 2019 recommendations proposed by OARSI and ESCEO outline two informative treatment algorithms for the non-surgical management of knee OA. Both sets of recommendations provide health-care providers with evidence-based and expert-reviewed advice. Overall, the two publications provide very similar recommendations, particularly with regard to the core treatments that all patients should receive. They both provide similar, progressive management algorithms, although some differences exist, particularly in the ordering of treatments along the treatment algorithm. Both attempt to 'personalize' the treatment algorithms to patient characteristics, which is essential when considering the use of oral NSAIDs and COX2 selective inhibitors. Specifically, the OARSI recommendations are tailored for groups with particular comorbidities including those at increased risk of gastrointestinal or cardiovascular adverse events, those with frailty and those with widespread pain and/or depression. The ESCEO recommendations are personalized by considerations for gastrointestinal, cardiovascular, hepatic and renal risk and for specific age groups. Neither set of recommendations specifically discusses age as a factor by which to personalize treatment; however, it is likely that the consideration of comorbidities, especially frailty, partially take age into account.

The OARSI and ESCEO guidelines differ in their recommendations for the use of topical NSAIDs and SYSADOAs. The ESCEO guidelines recommend the use of pharmaceutical grade glucosamine and chondroitin sulfate as first-line therapies prior to the use of topical NSAIDs in those with persistent symptoms. The OARSI guidelines, however, strongly recommend against the use of all glucosamine and chondroitin formulations (including pharmaceutical grade); the OARSI guidelines recommend the use of topical NSAIDS as the first-line treatment. A possible explanation for the conflicting recommendations made by the ESCEO and OARSI groups regarding the use of glucosamine are most likely attributable to differences in the interpretation of the quality of the evidence, including risk of bias, and in the synthesis of that evidence by the expert panels. Lastly, both guidelines strongly recommend against the long-term use of paracetamol owing to its low efficacy and notable adverse effect profile; however, the ESCEO guidelines do suggest short-term use of doses limited to 3 g per day. Again, the assessment of different study literature could explain this difference. Specifically, in their updated literature search (2014–2018) the ESCEO panel evaluated four reviews and/or meta-analyses that examined the safety and efficacy of paracetamol, which covered both randomized trials and observational studies, whereas the OARSI panel examined only the results of five randomized trials. The ESCEO panel reported that while they found no evidence for the use of paracetamol in the short term as a rescue analgesic on a background of other treatments (for example, SYSADOAs), they comment that "this is its traditional use"[16]. Consequently, the recommendation for the short-term use of paracetamol might be informed more by clinical opinion than by the clinical evidence.

The differences in the treatment recommendations proposed by the OARSI and ESCEO working groups can be explained, in part, by methodological differences. Despite evaluating similar data, both groups made several different treatment recommendations, which would suggest that a degree of uncertainty regarding the available evidence remains. Hence, there is a need for more robust evidence.

Conclusions

Overall, the 2019 OARSI and ESCEO treatment algorithms for the non-surgical management of knee OA overlap considerably, which should provide confidence and clarity for practising clinicians regarding the treatment of patients with knee OA. The differences between the two sets of recommendations might be attributable, in part, to methodological issues, highlighting the importance of refining and harmonizing guideline methodology and ideally producing unified guidelines that are endorsed by multiple societies and non-governmental organizations. Furthermore, harmonization could be achieved through the encouragement of cross-collaboration between both national and international organizations. Practising clinicians would also benefit from the future development of online educational programmes specifically designed for health-care practitioners with input from all the major societies and stakeholders, with the subsequent distillation of a consistent set of recommendations for patients with OA and the lay public.

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Author contributions

T.A.P., N.K.A., R.R.B. and O.B. researched data for the article. T.A.P. and N.K.A. wrote the manuscript. All authors made a substantial contribution to discussion of content and review/editing of the manuscript before submission.

Competing interests

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