LUPUS NEPHRITIS

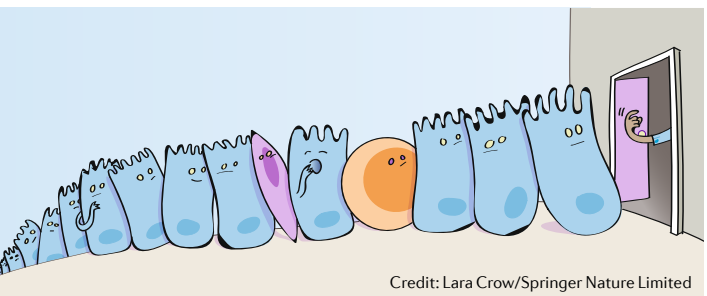
Single-cell analysis identifies key pathways and prognostic markers in lupus nephritis

> these findings might provide an opportunity for further personalization of therapy

Clinical and histopathological findings in lupus nephritis (LN) are heterogeneous and insufficient for accurate prognosis. Now, Jill Buyon, Thomas Tuschl, Chaim Putterman and colleagues have used single-cell RNA sequencing (scRNA-seq) of kidney and skin biopsy samples to identify molecular signatures that could potentially be used to predict treatment responses and enable personalized therapy in LN.

"ScRNA-seq provides a very detailed view of gene expression at high resolution, enabling exploration of the heterogeneity of cell types and states," explains Evan Der, first author of the study. "A major advantage of this approach was that we could generate data from very small pieces of kidney tissue that were obtained during clinically indicated biopsies in patients with suspected LN."

The researchers analysed kidney biopsy samples from 21 patients with LN and skin biopsy samples from 17 of these patients. They identified upregulation of type I interferon (IFN) response pathway genes in renal tubular cells and skin keratinocytes from the patients compared to those from three healthy individuals. The IFN-response signature in the tubular cells was also significantly higher in patients who did not respond to therapy than in those who were partial or complete responders at 6 months after the kidney biopsy. "These data support continuing investigation into how the IFN pathway could lead to scarring in the kidney, which is what we most want to avoid in LN," says Buyon.



Credit: Lara Crow/Springer Nature Limited

Tubular cells from patients who did not respond to therapy also showed significantly higher expression of genes that encode extracellular matrix (ECM) and ECM-interaction proteins — reflecting an active fibrotic pathway — than did those from treatment-responsive patients. As this gene expression signature was present in the tubular cells of some patients whose kidney biopsy samples did not show histological evidence of fibrosis, the researchers suggest that it might predict a fibrotic response. They also identified a tubular cell signature of fibrosis comprising four collagen genes that could predict the response to treatment of patients with LN at 6-months post-biopsy with 92% accuracy. The keratinocytes of patients who did not respond to therapy also showed upregulation of ECM genes, and the fibrotic gene signature of these cells correlated with that of tubular cells in individual patients.

"Identification of gene expression signatures in the fibrosis and IFN pathways with prognostic significance at the time of kidney biopsy is highly significant because if validated, these signatures could be quickly adapted for patient stratification and personalization of treatment and follow-up," comments Putterman. "The identification of similar signatures in the skin raises the possibility that this much more accessible organ could provide a window on pathogenic processes happening in the kidney. Using skin as a surrogate source of biomarkers for renal disease could potentially enable more frequent monitoring without the need for repeat renal biopsies."

The researchers also identified several pathways that were differentially expressed in tubular cells and keratinocytes from patients with different histological classes of LN. These included TNF, IL-1 and IFN signalling pathways. They suggest that these findings might provide an opportunity for further personalization of therapy.

"Ultimately, our research could lead to improved prediction of responses to conventional therapies and enable earlier consideration of more aggressive or alternative treatment approaches to improve patient outcomes," concludes Putterman. "Although we know that scarring is important, current therapies for LN are directed against the inflammatory response," adds Buyon. "I think our findings call into the armamentarium drugs that will stop the scarring process. Dual therapies that target inflammation and fibrosis might be very interesting approaches for the treatment of LN."

Ellen F. Carney

This article is modified from the original in *Nat. Rev. Nephrol.* (https://doi.org/10.1038/s41581-019-0164-1)

ORIGINAL ARTICLE Der, E. et al. Tubular cell and keratinocyte single-cell transcriptomics applied to lupus nephritis reveal type I IFN and fibrosis relevant pathways. *Nat. Immunol.* https://doi.org/10.1038/s41590-019-0386-1 (2019)

IN BRIEF

RHEUMATOID ARTHRITIS

ACPA-negative RA divided into clinical subsets

The multi-biomarker disease activity (MBDA) score, an algorithm based on 12 serological markers, helped subdivide patients with anti-citrullinated protein antibody (ACPA)-negative rheumatoid arthritis (RA) into clinically relevant subsets in a multivariable analysis. Patients with a high or medium MBDA score at the time of diagnosis had a higher likelihood of sustained DMARD-free remission than patients with a low score ($P = 0.115$). This association was independent of clinical baseline characteristics and driven primarily by levels of C-reactive protein, serum amyloid A and matrix metalloproteinase 3.

ORIGINAL ARTICLE Boeters, D. M. et al. ACPA-negative RA consists of subgroups: patients with high likelihood of achieving sustained DMARD-free remission can be identified by serological markers at disease presentation. *Arthritis Res. Ther.* **21**, 121 (2019)

CONNECTIVE TISSUE DISEASES

Pregnancy outcomes in patients with MCTD

In a multicentre retrospective analysis of 94 ever-pregnant women with mixed connective tissue disease (MCTD) who tested positive for anti-U1RNP antibodies, 147 of the 203 pregnancies (72.4%) resulted in live births, 38 (18.7%) ended in a first trimester pregnancy loss and 18 (8.9%) ended in a stillbirth. The fetal outcomes were worse for women who also tested positive for antiphospholipid antibodies or had pulmonary or muscular involvement. Specific counselling was advised for patients with MCTD who are planning a pregnancy.

ORIGINAL ARTICLE Radin, M. et al. Pregnancy outcomes in mixed connective tissue disease: a multicentre study. *Rheumatology.* https://doi.org/10.1093/rheumatology/kez141 (2019)

SYSTEMIC SCLEROSIS

Nintedanib slows ILD progression in SSc

Treatment with nintedanib, a tyrosine kinase inhibitor, slowed the progression of interstitial lung disease (ILD) associated with systemic sclerosis (SSc) in a 52-week randomized placebo-controlled trial. The adjusted annual rate of decline in forced vital capacity was lower in the nintedanib-treated group than in the placebo-treated group ($P = 0.04$), although no clinical benefits for other manifestations of SSc were observed. The adverse-event profile was similar to that reported in previous trials of patients with idiopathic pulmonary fibrosis; the rate of gastrointestinal adverse events, including diarrhoea, was higher in the nintedanib group than in the placebo group.

ORIGINAL ARTICLE Distler, O. et al. Nintedanib for systemic sclerosis–associated interstitial lung disease. *N. Engl. J. Med.* https://doi.org/10.1056/NEJMoa1903076 (2019)

RHEUMATOID ARTHRITIS

Hyperuricaemia or gout in patients with RA

In a large longitudinal study of 1,999 patients with rheumatoid arthritis (RA) in the USA, the prevalence of gout (6.1%) and hyperuricaemia (17%) was similar to previous estimates of these conditions in the general population. The coexistence of hyperuricaemia or gout in patients with RA was not associated with RA disease activity or severity. However, moderate hyperuricaemia in these patients was strongly associated with an increased risk of cardiovascular-disease-related mortality, which was driven by the presence of other comorbidities.

ORIGINAL ARTICLE Chiou, A. et al. Coexistent hyperuricemia and gout in rheumatoid arthritis: associations with comorbidities, disease activity and mortality. *Arthritis Care Res.* https://doi.org/10.1002/acr.23926 (2019)

Credit: Stocktrek Images/Getty

SYSTEMIC LUPUS ERYTHEMATOSUS

Rare variants in SLE risk genes drive disease

New research reveals that most patients with systemic lupus erythematosus (SLE) carry rare, coding variants in genes implicated in SLE risk; moreover, these variants have damaging effects on protein function and ultimately contribute to the development of autoimmunity. Together, the findings of the study by Simon Jiang and colleagues suggest that rare and novel variants are important in the pathogenesis of SLE.

To date, the prevailing view has been that genetic predisposition to SLE arises from the additive effect of many common variants that have been identified over the past few decades using genome-wide association studies and that individually have modest effect sizes. However, emerging evidence has indicated that rare variants with strong effect sizes contribute to SLE in some individuals.

In an initial investigation in two cohorts of patients with SLE (133 patients in total) and 97 healthy elderly individuals, Jiang et al. found that 82% of patients with SLE and 72% of healthy individuals carried at least one rare nonsynonymous single-nucleotide variant (SNV) in SLE-associated genes. In particular, a substantial proportion of patients with SLE carried rare or low-frequency variants in *BLK* and/or *BANK1*.

Further studies demonstrated that the SNVs in *BLK* (encoding tyrosine-protein kinase BLK) identified in patients with SLE altered the kinase activity of BLK, resulting in impaired phosphorylation of interferon regulatory factor 5 (IRF5) and B-cell scaffold protein with ankyrin repeats (BANK1). SLE-associated BLK variants also failed to repress IRF5-mediated type I interferon expression in comparison with wild-type BLK.

Jiang et al. also demonstrated that a *BANK1* SNV promotes type I interferon activity owing to impaired sequestration of TNF receptor-associated factor 6 (TRAF6), which was associated with increased TRAF6-mediated nuclear localization of IRF5.

"We have shown for the first time that rare variants in SLE-associated genes, specifically *BLK* and *BANK1*, have deleterious effects on their translated proteins," says Jiang. "Interestingly, rare variants in these genes in healthy individuals do not affect protein function."

In lupus-prone $Fas^{lpr/lpr}$ mice, introduction of a *Blk* variant orthologous to one found in SLE led to expansion of pathogenic T cells, demonstrating a contribution to the development of disease in vivo.

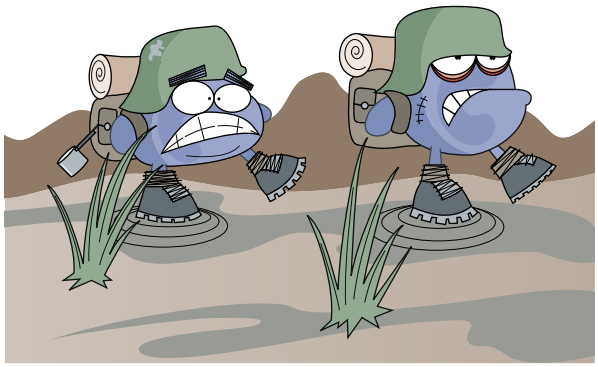
The researchers plan to elucidate the disease-related mechanisms of other rare variants in SLE-associated genes and potential insights into new targets for treatment, such as exploring anti-interferon therapy for patients bearing mutations associated with increased type I interferon activity. "By identifying the genetic mechanisms which contribute to SLE, we may be able to stratify patients further by their genetic variants as well as trial treatments targeted for specific disrupted pathogenic pathways," concludes Jiang.

Sarah Onuora

ORIGINAL ARTICLE Jiang, S. H. et al. Functional rare and low frequency variants in *BLK* and *BANK1* contribute to human lupus. *Nat. Commun.* **10**, 2201 (2019)

LUPUS NEPHRITIS

Patrolling monocytes promote kidney disease



Credit: Springer Nature Limited

The treatment of lupus nephritis with B cell-targeted therapies has so far been mostly unsuccessful, despite a wealth of evidence implicating autoantibodies and immune complexes in the pathogenesis of systemic lupus erythematosus (SLE). The results of a new study suggest that rather than being autoantibody-mediated, early lupus nephritis might instead be promoted by 'patrolling monocytes'.

"In previous work, we identified the protein TNIP1 as part of the Toll-like receptor signalling complex and established gene-deficient mice to study its function in vivo," explains corresponding author Hans Häcker. "We found that these mice spontaneously developed a lupus-like disease characterized by glomerulonephritis, autoreactive antibodies and overall reduced survival." *TNIP1* was subsequently confirmed as an SLE-susceptibility locus, making *Tnip1*$^{-/-}$ mice a good model for human SLE.

Häcker and colleagues found that *Tnip1*$^{-/-}$*Rag1*$^{-/-}$ mice, which lack mature T cells and B cells, still developed glomerulonephritis, indicating that adaptive immune system responses were not necessary for kidney pathology. Turning instead to the innate immune system, the researchers discovered that LY6C^{lo} patrolling monocytes (also known as non-classical monocytes) accumulated in the kidneys of *Tnip1*$^{-/-}$ mice, and also in the kidneys of mice with two other models of lupus (MRL/*lpr* mice and B6.*Sleyaa* mice). CD14^{dim}CD16^{+} monocytes, the equivalent population in humans, were also present in increased numbers in the glomeruli of patients with lupus nephritis.

Genetic deletion of patrolling monocytes in *Tnip1*$^{-/-}$ mice abrogated glomerulonephritis, but did not affect autoantibody production or other disease parameters. "Collectively, these data suggest that different disease symptoms in SLE are mediated by different immune mechanisms," says Häcker. "The early phase of glomerulonephritis seems to be promoted by patrolling monocytes rather than autoreactive antibodies and immune complexes, whereas other, more systemic disease parameters proceed independently of patrolling monocytes," he concludes.

> "patrolling monocytes … accumulated in the kidneys of *Tnip1*$^{-/-}$ mice"

Joanna Collison

ORIGINAL ARTICLE Kuriakose, J. et al. Patrolling monocytes promote the pathogenesis of early lupus-like glomerulonephritis. *J. Clin. Invest.* https://doi.org/10.1172/JCI125116 (2019)

AUTOIMMUNITY

Engineered fusion protein disrupts CD40 signalling

The CD40 pathway has long been an attractive therapeutic target for treating autoimmune diseases; however, early clinical trials of monoclonal antibodies targeting CD40 ligand (CD40L) were halted owing to platelet-related thromboembolic complications. Findings from a new study suggest that VIB4920, a bivalent CD40L-specific Tn3 fusion protein, could suppress the CD40–CD40L axis while avoiding these safety concerns.

> "treatment with high-dose … VIB4920 led to improvements in disease activity"

Credit: S. Harris/Springer Nature Limited

VIB4920 blocks the interaction between CD40 and CD40L by competing with CD40. "This novel construct maintains the specificity of an antibody-based therapeutic, demonstrating specific high-affinity binding to CD40L, but lacks an Fc domain," explains Jodi Karnell, first author of the paper. "As the Fc domain of anti-CD40L antibodies was definitively linked to the safety issues observed in early clinical trials, VIB4920 is predicted to be a safer approach for targeting this pathway."

In preclinical studies, VIB4920 blocked CD40L signalling as well as differentiation and activation of human B cells, but did not induce platelet aggregation in vitro. In healthy volunteers, VIB4920 was well-tolerated and inhibited the humoral immune response to immunization with keyhole limpet hemocyanin in a dose-dependent manner.

In a 12-week phase Ib trial in patients with active rheumatoid arthritis (RA), treatment with high-dose (1,000 mg or 1,500 mg every other week) VIB4920 led to improvements in disease activity as well as reductions in titres of rheumatoid factor autoantibodies and biomarkers related to immune activation. "The results in patients with RA provide a clear demonstration of the potential of a non-antibody based CD40L-targeted therapeutic to provide benefit to patients with autoimmune disease," says corresponding author Jörn Drappa. "Most importantly, we did not see any evidence of platelet activation or any thromboembolic events to date."

Encouraged by these early results, the researchers are planning clinical trials of VIB4920 in various autoimmune and inflammatory diseases in which the CD40–CD40L pathway is implicated.

Sarah Onuora

ORIGINAL ARTICLE Karnell, J. L. et al. A CD40L-targeting protein reduces autoantibodies and improves disease activity in patients with autoimmunity. *Sci. Transl. Med.* **11**, eaar6584 (2019)

OSTEOARTHRITIS

IL-36 inhibition to treat OA

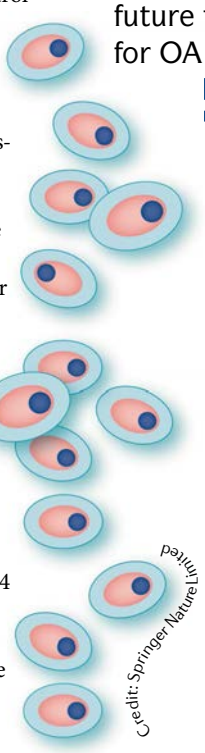
Interfering with transforming growth factor-β (TGFβ) signalling by targeting IL-36 family cytokines could have disease-modifying effects in osteoarthritis (OA), according to new research in mouse models of OA and in tissue from patients with the disease. Li et al. suggest that IL-36 receptor antagonist (IL-36RN in humans; IL-36Ra in mice) might be a future therapy for OA.

Previous studies have implicated altered TGFβ signalling in the development and progression of OA, but global inhibition of TGFβ activity could have negative multi-organ effects, suggesting the need for alternative approaches. The present study demonstrates the feasibility of attenuating OA by targeting a TGFβ type 2 receptor (TGFBR2)–IL-36 signalling axis relevant to joint development and homeostasis.

Li et al. demonstrated that *Tgfbr2*$^{-/-}$ mice spontaneously developed OA and also had more severe post-traumatic OA (induced by destabilization of the medial meniscus (DMM)) than control mice. Genetic ablation of *Tgfbr2* was accompanied by increased expression of IL-36α and IL-36 receptor (IL-36R) and decreased expression of IL-36Ra; similar expression patterns of these cytokines and receptors were observed in wild-type mice treated with a TGFβ inhibitor (SB-505124), in ageing mice with naturally occurring OA and in wild-type mice with DMM-induced post-traumatic OA.

Consistent with these findings, blocking TGFBR2 signalling in primary cultures of human articular chondrocytes with SB-505124 led to dose-dependent increases in IL-36α, IL-36R and matrix metalloproteinase 13 (MMP13) and a decrease in IL-36RN. Furthermore, examination of articular cartilage tissue from human donors (with or without OA-like degenerative changes) revealed a pattern of gradually decreasing TGFBR2 expression and gradually increasing IL-36α, IL-36R and MMP13 expression that correlated with increasing severity of cartilage degeneration.

> IL-36 receptor antagonist… might be a future therapy for OA



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Demonstrating the therapeutic potential of targeting the TGFBR2–IL-36 axis, intra-articular injection of IL-36Ra attenuated OA progression in *Tgfbr2*$^{-/-}$ mice as well as in wild-type mice with DMM-induced OA; by contrast, injection of IL-36α exacerbated OA in both models. In human articular chondrocytes from donors with end-stage OA, treatment with IL-36RN led to a dose-dependent decrease in MMP13 expression in vitro.

Together, the results of the study suggest that targeting IL-36 signalling could attenuate the OA process.

Sarah Onuora

ORIGINAL ARTICLE Li, T. et al. TGF-β type 2 receptor-mediated modulation of the IL-36 family can be therapeutically targeted in osteoarthritis. *Sci. Transl. Med.* **11**, eaan2585 (2019)

EXPERIMENTAL ARTHRITIS

Two targets are better than one

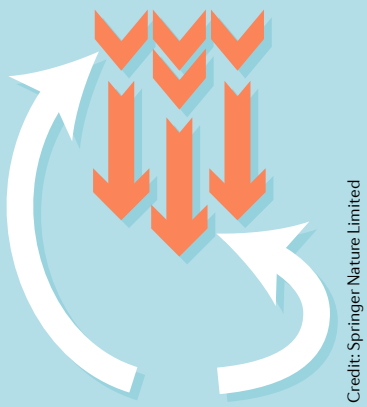
Although several therapeutic options exist for treating rheumatoid arthritis (RA), not even the current gold standard therapies can reliably induce long-term, drug-free remission. Owing to the complex pathogenic processes involved in RA, combination therapies are being developed to target multiple pathways or cytokines at once.

In a new study, one such combination therapy has shown promise in inducing long-term remission in mice with collagen-induced arthritis (CIA). "Given the high costs of biologic therapies and potential for adverse events, we became interested in the therapeutic potential of small molecular weight modulators of TNF receptor signalling," explains corresponding author Richard Williams. "We subsequently started to investigate inhibitors of cellular inhibitor of apoptosis proteins (cIAPs), which have a fundamental role in NF-κB pathway activation by TNF."

The cIAP1 and cIAP2 antagonist GT13072 belongs to a class of inhibitors known as SMAC mimetics that are currently in phase I testing for the treatment of cancer. The researchers used GT13072 to treat mice with CIA and observed a large and rapid decrease in disease severity. Intriguingly, mice treated with GT13072 had reduced numbers of IL-17^{+} T cells compared with mice treated with a structurally related inactive compound. "A more detailed look into the mechanism of action of GT13072 revealed that it decreased NFATc1 expression in human T cells, which is a known regulator of IL-17A expression," says first author Joanna Kawalkowska.

As inhibition of TNF can cause an increase in IL-17^{+} cells in patients with RA, the researchers decided to counter this effect by combining the TNF inhibitor etanercept with GT13072 to treat mice with CIA. Combination therapy had an additive effect on reducing disease severity in these mice and also prevented relapse for longer than treatment with either therapy alone.

> combination therapy had an additive effect on reducing disease severity



Credit: Springer Nature Limited

"Perhaps the most important finding from this study was the long-term therapeutic effect of combination therapy, accompanied by an expansion of regulatory T cells," concludes Williams. "Our objective is to translate these findings into the clinic and to initiate experimental medicine trials in patients with inflammatory diseases."

Joanna Collison

ORIGINAL ARTICLE Kawalkowska, J. Z. et al. cIAP1/2 inhibition synergizes with TNF inhibition in autoimmunity by down-regulating IL-17A and inducing T_{regs}. *Sci. Adv.* **5**, eaaw5422 (2019)

Credit: smartboy10/DigitalVision Vectors/Getty

THERAPY

Can personalized use of NSAIDs be a reality in the clinic?

Michael T. Nurmohamed

The use of NSAIDs in rheumatology could be improved by an appropriate risk scoring system that accounts for adverse events such as bleeding and thrombosis. Such a risk score has now been developed using data from the PRECISION trial, but is this score ready to be applied in clinical practice?

Refers to Solomon, D. H. et al. Validation of a major toxicity risk score among NSAID users based on data from a randomized controlled trial. *Arthritis Rheumatol.* https://doi.org/10.1002/art.40870 (2019).

NSAIDs (including cyclo-oxygenase (COX) inhibitors) are associated with a variety of adverse events, including vascular events, such as bleeding and thrombosis, as well as gastrointestinal, renal and dermal events. NSAIDs are often prescribed for the (initial) treatment of rheumatoid arthritis (RA) and osteoarthritis (OA). Currently, no rational method exists to weigh the analgesic and anti-inflammatory effects of these commonly used drugs against their adverse effects owing to the absence of an algorithm or risk assessment model that takes into account the clinical risk factors for adverse events. However, in a post-hoc analysis of the PRECISION trial[1], Solomon et al.[2] have now put forward a potential risk scoring system for NSAID toxicity, but can its use be translated to the clinic?

NSAIDs inhibit the enzymes COX1 and COX2, which catalyse the conversion of arachidonic acid to prostanoids. COX1 is constitutively expressed in most tissues and regulates prostanoids that are involved in maintaining homeostasis of the stomach mucosa, cartilage and thrombocytes, as well as maintaining renal function[3]. COX1 catalyses the formation of thromboxane, which subsequently leads to vasoconstriction and platelet aggregation. By contrast, COX2 is induced during inflammation or following tissue damage and catalyses the production of prostacyclin, which leads to vasodilatation and the inhibition of platelet aggregation[3]. Nonspecific NSAIDs, such as naproxen, ibuprofen and diclofenac, inhibit COX1 and COX2, which explains their efficacy (a result of COX2 inhibition) and also their adverse effects such as gastrointestinal bleeding (a result of COX1 inhibition).

Approximately 20 years ago, NSAIDs that selectively inhibit COX2 were developed. These drugs produced an ~40–70% reduction in (severe) gastrointestinal adverse events compared with non-selective NSAIDs to give absolute percentages of gastrointestinal adverse events of <2%[4–6]. However, selective COX2 inhibition results in reduced synthesis of prostacyclin, which can lead to thrombosis as thromboxane formation is not inhibited. Indeed, selective COX2 inhibitors had prothrombotic effects in clinical trials for OA and RA, and a subsequent meta-analysis indicated that prothrombotic effects are associated with most NSAIDs (with the possible exception of naproxen)[7]. Therefore, current recommendations from the FDA and European Medicines Agency state that non-selective NSAIDs and selective COX2 inhibitors should be withheld or used cautiously in patients with overt cardiovascular disease or in those at risk of cardiovascular disease[8,9]. However, deciding which patients are most at risk of adverse events when taking NSAIDs has been challenging owing to a lack of appropriate risk assessment models.

Solomon et al.[2] used data from the previously conducted PRECISION trial to develop a new risk score for NSAID toxicity, which was possible owing to the large sample size of the trial[1]. In this randomized double-blind non-inferiority trial, 24,081 patients who required daily treatment with NSAIDs for arthritis pain (90% patients with OA, 10% patients with RA) for at least 6 months were randomly allocated to receive either celecoxib (n = 8,072; mean daily dose 209 mg), naproxen (n = 7,969; mean daily dose 852 mg)

or ibuprofen ($n = 8{,}040$; mean daily dose 2,045 mg)[1]. In addition, all patients received the proton pump inhibitor esomeprazole for gastric protection. An important inclusion criterion in this trial was the presence of confirmed cardiovascular disease or of an increased risk of cardiovascular disease. The mean duration of treatment was 20.3 months and the mean follow-up was 34.1 months, and 69% of patients stopped taking the drug and 27% of patients were lost to follow-up during this 10-year trial[1].

> deciding which patients are most at risk of adverse events when taking NSAIDs has been challenging

For the post-hoc development of the risk score, data on patients recruited in the first 4 years of the trial were used to derive the scoring system, and data on patients recruited in the last 5 years of the trial were used for validation[2]. The primary outcome for the analyses was a composite of severe cardiovascular adverse events, important gastrointestinal events, renal toxicity and death during the first year of treatment, which occurred at a rate of 3.4% (95% CI 3.1–3.7)[2]. The model derived from the initial cohort also performed well in the validation cohort, and these two cohorts were combined to create the final multivariable model.

Solomon et al.[2] developed a risk score that includes factors such as age, history of cardiovascular disease, hypertension or diabetes, use of tobacco or statins, serum creatinine concentration, haematocrit and whether a patient has RA, then created three 1-year risk groups: low risk (<1%), intermediate risk (1–4%) and high risk (>4%). Subgroup analysis revealed no statistically significant differences in the predicted and observed severe toxicity risks for users of celecoxib, ibuprofen or naproxen[2].

The risk group categorization used by Solomon et al.[2] is an appealing approach that was designed on the basis of input from clinicians. In general clinicians would agree that a 1-year risk of >4% (translating to a number needed to harm (NNH) of <25) is too high, whereas a 1-year risk of <1% (translating to a NNH of >100) would be acceptably low. The middle category of 1–4% risk is more difficult to interpret, and will be an area that requires shared decision-making until more specific models become available. Importantly, a tool for risk calculation, analogous to that for the fracture risk assessment tool FRAX[10], is provided by Solomon et al.[2], which could help clinicians to become familiar with this risk score and enable its use in daily clinical practice.

However, some methodological concerns exist that might preclude direct extrapolation of this new risk score[2] to clinical practice, including the post-hoc nature of the development of the risk model. The fact that diclofenac, another frequently used NSAID that is associated with an increased thrombotic risk, was not studied and the relatively low doses of celecoxib that were used in the PRECISION trial[1], which are often insufficient for patients with inflammatory arthritis, are also causes for concern. Patients in the PRECISION trial also had a high cardiovascular risk and frequently used proton pump inhibitors, the use of which is also associated with an increased cardiovascular risk. Many of these aspects were acknowledged by Solomon et al.[2], including the need to validate their risk model in external populations. However, it will take years before the results of such studies are available.

In the meantime, clinicians could consider using this preliminary risk score[2] in the clinic for patients resembling the participants of the PRECISION trial[1]. The use of this risk score could lead to a more rational use of NSAIDs, at least for these patients, and is an important step towards personalized medicine.

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Competing interests

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VASCULITIS

Should ^{18}F-FDG-PET imaging be used in the diagnosis of GCA?

Dario Camellino and Christian Dejaco

^{18}F-FDG-PET is not currently recommended for use in the diagnosis of cranial giant cell arteritis (GCA). A new study has compared ^{18}F-FDG-PET with temporal artery biopsy and clinical diagnosis as gold standards, but is ^{18}F-FDG-PET accurate enough to be used on temporal arteries?

Refers to Sammel, A. M. et al. Diagnostic accuracy of PET/CT scan of the head, neck and chest for giant cell arteritis: the double-blinded giant cell arteritis and PET scan (GAPS) study. *Arthritis Rheumatol.* https://doi.org/10.1002/art.40864 (2019).

Giant cell arteritis (GCA) is the most common form of primary systemic vasculitis and its classic 'cranial' form is characterized by clinical features of temporal arteritis such as headache, jaw claudication and vision loss[1]. An extracranial large-vessel GCA (LV-GCA) phenotype also exists that is characterized by constitutional symptoms, such as fever

or ibuprofen ($n = 8{,}040$; mean daily dose 2,045 mg)[1]. In addition, all patients received the proton pump inhibitor esomeprazole for gastric protection. An important inclusion criterion in this trial was the presence of confirmed cardiovascular disease or of an increased risk of cardiovascular disease. The mean duration of treatment was 20.3 months and the mean follow-up was 34.1 months, and 69% of patients stopped taking the drug and 27% of patients were lost to follow-up during this 10-year trial[1].

> deciding which patients are most at risk of adverse events when taking NSAIDs has been challenging

For the post-hoc development of the risk score, data on patients recruited in the first 4 years of the trial were used to derive the scoring system, and data on patients recruited in the last 5 years of the trial were used for validation[2]. The primary outcome for the analyses was a composite of severe cardiovascular adverse events, important gastrointestinal events, renal toxicity and death during the first year of treatment, which occurred at a rate of 3.4% (95% CI 3.1–3.7)[2]. The model derived from the initial cohort also performed well in the validation cohort, and these two cohorts were combined to create the final multivariable model.

Solomon et al.[2] developed a risk score that includes factors such as age, history of cardiovascular disease, hypertension or diabetes, use of tobacco or statins, serum creatinine concentration, haematocrit and whether a patient has RA, then created three 1-year risk groups: low risk (<1%), intermediate risk (1–4%) and high risk (>4%). Subgroup analysis revealed no statistically significant differences in the predicted and observed severe toxicity risks for users of celecoxib, ibuprofen or naproxen[2].

The risk group categorization used by Solomon et al.[2] is an appealing approach that was designed on the basis of input from clinicians. In general clinicians would agree that a 1-year risk of >4% (translating to a number needed to harm (NNH) of <25) is too high, whereas a 1-year risk of <1% (translating to a NNH of >100) would be acceptably low. The middle category of 1–4% risk is more difficult to interpret, and will be an area that requires shared decision-making until more specific models become available. Importantly, a tool for risk calculation, analogous to that for the fracture risk assessment tool FRAX[10], is provided by Solomon et al.[2], which could help clinicians to become familiar with this risk score and enable its use in daily clinical practice.

However, some methodological concerns exist that might preclude direct extrapolation of this new risk score[2] to clinical practice, including the post-hoc nature of the development of the risk model. The fact that diclofenac, another frequently used NSAID that is associated with an increased thrombotic risk, was not studied and the relatively low doses of celecoxib that were used in the PRECISION trial[1], which are often insufficient for patients with inflammatory arthritis, are also causes for concern. Patients in the PRECISION trial also had a high cardiovascular risk and frequently used proton pump inhibitors, the use of which is also associated with an increased cardiovascular risk. Many of these aspects were acknowledged by Solomon et al.[2], including the need to validate their risk model in external populations. However, it will take years before the results of such studies are available.

In the meantime, clinicians could consider using this preliminary risk score[2] in the clinic for patients resembling the participants of the PRECISION trial[1]. The use of this risk score could lead to a more rational use of NSAIDs, at least for these patients, and is an important step towards personalized medicine.

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VASCULITIS

Should ^{18}F-FDG-PET imaging be used in the diagnosis of GCA?

Dario Camellino and Christian Dejaco

^{18}F-FDG-PET is not currently recommended for use in the diagnosis of cranial giant cell arteritis (GCA). A new study has compared ^{18}F-FDG-PET with temporal artery biopsy and clinical diagnosis as gold standards, but is ^{18}F-FDG-PET accurate enough to be used on temporal arteries?

Refers to Sammel, A. M. et al. Diagnostic accuracy of PET/CT scan of the head, neck and chest for giant cell arteritis: the double-blinded giant cell arteritis and PET scan (GAPS) study. *Arthritis Rheumatol.* https://doi.org/10.1002/art.40864 (2019).

Giant cell arteritis (GCA) is the most common form of primary systemic vasculitis and its classic 'cranial' form is characterized by clinical features of temporal arteritis such as headache, jaw claudication and vision loss[1]. An extracranial large-vessel GCA (LV-GCA) phenotype also exists that is characterized by constitutional symptoms, such as fever

Credit: Science Photo Library/Alamy Stock Photo

and weight loss, polymyalgia rheumatica and large vessel involvement[2]. EULAR recommendations for the use of imaging in patients with large-vessel vasculitis[3] suggest that an imaging test might be preferable to a temporal artery biopsy (TAB) in patients with suspected GCA. A study by Sammel et al.[4] provides new evidence for ^{18}F-FDG-PET as an imaging modality for use in the diagnosis of cranial GCA.

^{18}F-FDG is a glucose analogue, the uptake of which is increased in cells with a high metabolic rate, enabling the localization of neoplastic proliferation and inflammation. Ultrasonography, CT, MRI and ^{18}F-FDG-PET are all currently recommended by EULAR[3] for the assessment of extracranial arteries, despite a relatively low level of evidence for this recommendation, and concomitant LV-GCA has been identified in 80–90% of patients with temporal arteritis using various imaging modalities[2]. However, ^{18}F-FDG-PET is not currently recommended for the assessment of cranial arteries[3] on the basis of literature claiming that temporal arteries cannot be properly evaluated by this method owing to proximity to the brain and the small diameter of temporal arteries.

Sammel et al.[4] used a prospective cohort of 64 patients with suspected GCA to investigate the accuracy of ^{18}F-FDG-PET for the diagnosis of GCA. The design is one of the strengths of this study, as many previous studies that evaluated the performance of imaging modalities in the diagnosis of GCA had a case–control design and/or included the imaging test in question as part of the reference standard, both of which can artificially increase the diagnostic value of the technique being studied. Sammel et al.[4] used a dedicated time-of-flight ^{18}F-FDG-PET protocol that included assessment of the head, neck and thorax with a 1 mm CT reconstruction. Of the 64 patients with suspected GCA, 21 (33%) had a clinical diagnosis of GCA, and 12 of these patients also had a positive TAB result. Compared with a clinical diagnosis of GCA, ^{18}F-FDG-PET had a sensitivity of 71% and a specificity of 91%, whereas the sensitivity was 92% and the specificity was 85% when TAB was used as a reference standard[4]. Of the seven patients who had a negative TAB result and a positive ^{18}F-FDG-PET scan, one had herpes zoster ophthalmicus and one had metastatic lung cancer. The one patient with a positive TAB result and a negative ^{18}F-FDG-PET scan reported symptoms of visual disturbance, headache and jaw claudication that led to a final diagnosis of GCA.

> “^{18}F-FDG-PET is not currently recommended for the assessment of cranial arteries”

One of the most important results from this study[4] is the observation that, in addition to the aorta and large arteries, cranial arteries could be reliably assessed by ^{18}F-FDG-PET, thereby confirming previously published data[5] and contrasting with the EULAR recommendations[3]. This difference from the EULAR recommendations is probably a result of the high resolution of modern PET scanners and time-of-flight protocols. Another important finding is the high detection rate of other diseases in patients who did not receive a final diagnosis of GCA, in particular malignancy and infections, which were found in 20% of patients with suspected GCA[4].

The most important limitation of this study[4] is that PET scans were limited to supra-diaphragmatic parts of the body. Apart from the possibility that vasculitis, tumours or signs of other diseases in the abdomen or the legs could have been missed, this approach precluded the comparison of tracer uptake in the vessel wall with uptake in the liver. Sammel et al.[4] used the blood pool in the superior vena cava as a reference region instead of the liver; however, these two reference regions are characterized by different kinetics of ^{18}F-FDG (and thus visual appearance) that are related to the blood glucose level at the time of the scan, the possible presence of insulin resistance and the time interval between the ^{18}F-FDG injection and image acquisition[6]. Although no studies have systematically compared different approaches to the visual evaluation of ^{18}F-FDG-PET images in patients with large-vessel vasculitis, robust evidence supports the use of the liver as the reference region for ^{18}F-FDG-PET in patients with lymphoma[7], as is currently also recommended for patients with large-vessel vasculitis[6].

> “^{18}F-FDG-PET is a valid alternative imaging technique for diagnosis in patients with suspected GCA”

Looking forward, will ^{18}F-FDG-PET become a primary imaging tool for the diagnosis of GCA? One argument in favour of this technique would be that all vascular beds, including the thoracic aorta (which is not fully accessible by ultrasonography), could be investigated in one examination, enabling the simultaneous detection of diseases that might mimic GCA, such as infections and tumours. However, problems exist with the cost, radiation exposure and availability of this imaging modality. In one study, ^{18}F-FDG-PET was shown to rapidly lose sensitivity in patients with LV-GCA after glucocorticoid therapy was started, unless it was conducted within the first 3 days of treatment[8]. Although it can be challenging or even impossible to organize an ^{18}F-FDG-PET scan within the first 3 days of treatment, glucocorticoid therapy should not be delayed when GCA is strongly suspected owing to the imminent risk of blindness. Another limitation is the lack of standardization of ^{18}F-FDG-PET

scans; Sammel et al.[4] reported a moderate reproducibility of ^{18}F-FDG-PET that was comparable to that of ultrasonography and histological evaluation[9]. In addition, modern ultrasound machines have a spatial resolution of 0.1 mm, enabling the detection of even small changes of the wall thickness of temporal and other arteries, whereas the positron range of ^{18}F intrinsically limits the resolution of ^{18}F-FDG-PET to ~2.4 mm (REF.[10]).

Ultrasonography could maintain its place as a non-invasive, cost-effective, fast and widely available tool to assist the diagnosis of GCA[3]. When a diagnosis is unclear after ultrasonography, or when ultrasonography is unavailable, ^{18}F-FDG-PET might be a valid alternative. In the study by Sammel et al.[4], only six patients received both ^{18}F-FDG-PET and ultrasonography. Although the results for the two techniques were mostly concordant[4], larger studies are needed to directly compare them.

In conclusion, the study by Sammel et al.[4] showed that ^{18}F-FDG-PET is a valid alternative imaging technique for diagnosis in patients with suspected GCA. However, further standardization and validation against other imaging methodologies is still needed to better evaluate the role of ^{18}F-FDG-PET in the diagnosis of GCA.

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Stratifying management of rheumatic disease for pregnancy and breastfeeding

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Abstract | The management of inflammatory rheumatic diseases during pregnancy and breastfeeding has undergone considerable change in the past few years. Modern therapeutics, including biologic and targeted synthetic DMARDs, have enabled substantial improvements in the control of rheumatic diseases, resulting in more patients with severe disease considering pregnancy. Therefore, management of disease for these patients needs to be discussed with clinicians before, during and after pregnancy and patients need to know what complications they might experience before they become pregnant. This Review summarizes the effects pregnancy has on various rheumatic diseases and the effects these diseases have on pregnancy, as well as providing advice regarding the alteration and monitoring of therapy before, during and after pregnancy.

Intrauterine growth restriction
(IUGR). Reduced fetal growth resulting in an estimated weight below the 10th percentile for gestational age.

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Inflammatory rheumatic diseases, which include systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), other inflammatory arthropathies, axial spondyloarthritis, primary Sjögren syndrome, systemic sclerosis (SSc) and primary systemic vasculitis, can affect women of childbearing age[1–5] (BOX 1). In this context, SLE and various inflammatory arthropathies have been studied the most and are associated with an increased burden of adverse pregnancy outcomes (APOs), such as miscarriage, maternal hypertension, intrauterine growth restriction (IUGR) and/or premature delivery. These women often have disease that is being controlled by use of DMARDs and they are, therefore, increasingly considering pregnancy as a possibility[6].

Management of pregnancy in women with rheumatic diseases is affected by various factors. Active disease is associated with APOs[6]; hence, DMARDs are required during pregnancy to ensure control of maternal disease and satisfactory pregnancy outcomes. However, prescription of many DMARDs is complicated by safety concerns and evidence-based guidelines are unable to provide reliable evidence-based recommendations for all drugs[7–9]. Specific pregnancy concerns exist for various conditions that should be considered when discussing pregnancy planning and disease management with patients.

Conversations between clinicians and patients with rheumatic diseases are vital to ensure that the patient understands the potential effects of the timing of conception (with respect to disease activity), of disease upon pregnancy and of pregnancy upon disease, and the potential need for medication alterations in relation to pregnancy and breastfeeding.

This Review focuses on pregnancy planning in patients with RA, SLE, psoriatic arthritis (PsA), spondyloarthritis, primary Sjögren syndrome or SSc. Pregnancy in patients with primary systemic vasculitis is rare; advice specific to this disease is beyond the scope of this article but it is managed in a similar manner to SLE.

Timing of pregnancy

Patients with inflammatory rheumatic diseases should conceive during a period of disease quiescence to reduce the risk of disease flare in pregnancy[6,9,10]. In patients with well-controlled disease that lacks extra-articular manifestations and organ dysfunction, a 3-month period of disease control on stable medications compatible with pregnancy should suffice, but the precise duration of this period of quiescence is a matter of debate. In SLE, the risk of disease flare during pregnancy is increased in patients with active disease within 4–6 months before conception[11,12], in patients with active disease at conception[13,14], and following discontinuation of hydroxychloroquine (even when disease is quiescent)[15,16]. EULAR recommends that 6–12 months of disease quiescence is needed before conception dependent on various maternal factors, such as degree of residual organ dysfunction[17].

Effect of disease on pregnancy

Fertility and parity. Patients with inflammatory rheumatic diseases have fewer children than other women[6,18]. Explanations for this phenomenon include physical disability[19], renal failure[20], teratogenic medications[21], depression or fatigue, which can lead to

Key points

- Various inflammatory rheumatic diseases carry an increased burden of adverse pregnancy outcomes.
- Pregnancy can exacerbate some but not all inflammatory rheumatic diseases.
- Pre-pregnancy counselling is required to evaluate and reduce risks of adverse pregnancy outcomes for each patient.
- Some therapies must be altered before, during and/or after pregnancy.
- Careful monitoring is required throughout pregnancy by a multidisciplinary team.
- Vigilance for disease flare is required post-partum.

reduced libido or sexual dysfunction in women[22], and increasing maternal age associated with reduced ovarian reserves and oocyte quality[23] (reviewed elsewhere[24]). In particular, cyclophosphamide-induced gonadal toxicity is a substantial problem for patients with severe rheumatic disease[21,24], and strategies have been developed to safeguard against fertility loss before treatment with cytotoxic drugs[25]. These strategies include semen cryopreservation, embryo or oocyte cryopreservation, and the use of gonadotropin-releasing hormone analogues or agonists to suppress ovarian function and utero-ovarian blood flow in order to reduce exposure to gonadotoxic drugs such as cyclophosphamide. However, the use of gonadotropin-releasing hormone analogues is controversial and data from randomized trials of their use in patients receiving chemotherapy for different cancers are inconsistent[25].

Risk of adverse pregnancy outcomes. An increased burden of APOs has been reported in various inflammatory rheumatic diseases, described below and in TABLE 1. Various retrospective studies have shown that women with RA are at an increased risk of hypertensive disorders of pregnancy (gestational hypertension and pre-eclampsia), IUGR, premature delivery, caesarean delivery and increased length of hospital stay for pregnancy in population studies that included >3,500 patients with RA[26–29]. Although a smaller retrospective cohort study (243 women with RA) also confirmed an increased risk of prematurity and caesarean section, an approximately 50% increased risk of hypertensive disorders of pregnancy and reduced fetal growth in patients with RA was not statistically significant compared with population controls[30]. In other small (up to 150 patients) prospective studies of pregnancy in patients with RA, the risk of hypertensive disorders was increased in one study[31], but not in two others[10,32]. Overall, given the consistent finding of an increased risk of hypertensive disorders in RA pregnancy from large population studies it would seem prudent to counsel women with RA about this risk.

In SLE, several large (mostly retrospective) population-based studies of a total of >15,000 pregnancies in patients with SLE have identified an increased risk of hypertensive disorders of pregnancy, pre-term labour and IUGR[14,33–36]. In addition, a meta-analysis including studies published between 2001 and 2016 (including 3,395 patients with SLE) confirmed an increased risk of a range of maternal and fetal APOs, including hypertension (relative risk (RR) 1.99), pre-eclampsia (RR 1.91), pre-term labour (RR 3.05), IUGR (RR 4.44) and small for gestational age (RR 1.69)[37].

High disease activity immediately before and during pregnancy is clearly linked with APOs in RA and SLE[10,38,39]. A cohort study of pregnancy in patients with axial spondyloarthritis found an increased risk of APOs in these patients compared with healthy individuals as well as an association between active disease and preterm delivery[31].

The effect of other inflammatory rheumatic diseases on pregnancy outcome is not well-characterized. Pregnancy in PsA does not seem to be associated with APOs[40,41]. Case–control studies have shown an increase in the rates of spontaneous abortion, preterm deliveries and caesarean section in pregnancies in women with primary Sjögren syndrome compared with those in healthy individuals[42]. A high frequency of pre-term births and small full-term infants has been shown to occur in patients with SSc, with no difference in the frequency of miscarriage and neonatal survival compared with healthy individuals[43].

Effect of pregnancy on disease

Remission and relapse in pregnancy. Reports of improvement in disease activity in as many as 90% of RA pregnancies come mostly from retrospective studies that do not reflect current practice, as the use of biologic therapies now enables many women with severe disease to become pregnant[6,44]. Studies using validated measures of disease activity, such as the 28-joint Disease Activity Score for RA, found less convincing evidence that pregnancy reduces disease activity, with only 48–60% of women with active RA showing signs of reduced disease activity during pregnancy and 39–50% having a disease flare within 6 months post-partum[45].

The effect of pregnancy on SLE disease activity is unclear, as some studies have reported no increased risk of SLE flares during pregnancy, compared with non-pregnant patients with SLE[13,46,47], whereas other studies show that pregnancy is associated with an increased SLE flare rate[48–50]. These results are conflicting, possibly because of small cohort sizes and the use of varying methodologies to assess disease activity and define disease flares, but a systematic review calculated an overall flare rate (mostly mild flare) of ~25% and a severe flare rate of ~5%[51]. These flare rates were obtained from a meta-analysis of 1,842 patients with SLE with 2,751 pregnancies among them, including patients with lupus nephritis[35] and the prospective Predictors of Pregnancy Outcome: Biomarkers in Antiphospholipid Antibody Syndrome and Systemic Lupus Erythematosus (PROMISSE) study of 385 SLE pregnancies[34]. In the PROMISSE study, 12.7% had a mild or moderate flare and 2.5% had a severe flare at 20 to 23 weeks, plus 9.6% had a mild or moderate flare and 3.0% had a severe flare at 32 to 35 weeks[34]. A single-centre observational study[52], which was not included in the meta-analysis[35], of 398 pregnancies in 304 patients with SLE and 1,045 non-pregnant patients with SLE reported an increased flare rate in pregnancy (HR 1.59; 95% CI 1.27–1.96) that was reduced by hydroxychloroquine therapy (HR 1.26; 95% CI 0.88–1.69) in hydroxychloroquine-treated patients,

Cyclophosphamide-induced gonadal toxicity
Gonadal damage induced by cyclophosphamide, leading to reduced ovarian function.

Gestational hypertension
New-onset hypertension presenting after 20 weeks' gestation without significant proteinuria.

Pre-eclampsia
New-onset hypertension presenting after 20 weeks' gestation with significant proteinuria. Indicative of maternoplacental dysfunction.

Small for gestational age
Term used to describe a baby who is smaller than usual for the number of weeks of pregnancy. These babies usually have birthweights below the 10th percentile for gestational age.

compared with HR 1.83 (95% CI 1.34–2.45) in those who were not treated with hydroxychloroquine.

Information on disease activity and pregnancy outcomes in women with other inflammatory rheumatic diseases is limited. Some studies show that disease course and severity are not altered by pregnancy in axial spondyloarthritis[53,54] and similar data are conflicting for PsA[40,41,55].

Presence of autoantibodies. Anti-Sjögren syndrome-related antigen A (anti-SSA)/Ro and anti-SSB/La antibodies are usually detected in patients with SLE and primary Sjögren syndrome, are uncommon in RA and are sometimes detected incidentally in patients who lack other features of autoimmune disease[56]. At around 16 weeks' gestation, these antibodies cross the placental barrier by active transplacental transfer (FIG. 1). This process involves engagement of the Fc region with neonatal Fc receptors on syncytiotrophoblast cells[57] and has a number of implications for the fetus and newborn. The most important risk is that of atrioventricular block (AVB), with congenital complete heart block occurring in 1–2% of babies born to anti-SSA/Ro positive mothers with no previously affected pregnancies, known as neonatal lupus syndrome. This risk increases to ~17% if previous pregnancies have resulted in AVB[58]. Less common in children born to mothers who are positive for anti-SSA/Ro antibodies is late-onset cardiomyopathy leading to congestive cardiac failure[58]. In three case reports, anti-SSA/Ro positivity and anti-SSB/La positivity have also been associated with endocardial fibroelastosis, which has a poor cardiac prognosis including end-stage heart failure and death and can occur in the absence of AVB[59]. Other features of neonatal lupus syndrome include a transient subacute cutaneous lupus rash exacerbated by UV exposure after birth, haematological manifestations (such as cytopenia) and hepatobiliary disease[60]. Most of these manifestations resolve in the first 6–9 months of life as maternal anti-SSA/Ro antibodies are cleared from the baby's circulation[60].

Persistent positivity for anti-phospholipid (aPL) antibodies identifies individuals at risk of various specific APOs (one or more unexplained deaths of a morphologically normal fetus at ≥10 weeks' gestation; one or more premature births of a morphologically normal neonate before 34 weeks' gestation because of eclampsia, pre-eclampsia, or placental insufficiency; or three or more consecutive spontaneous pregnancy losses at <10 weeks' gestation, unexplained by chromosomal abnormalities or by maternal anatomical or hormonal causes) and maternal thrombosis that characterise antiphospholipid syndrome (APS)[61,62]. Common aPL antibodies are detected by lupus anticoagulant, anti-cardiolipin and anti-β2 glycoprotein 1 assays. Lupus anticoagulant positivity seems to be the strongest predictor of APOs in APS[34,63] and triple positivity for all three tests indicates an especially high risk of pregnancy complications and thrombosis[64,65]. The presence of aPL antibodies increases the risk of venous thromboembolism in patients with SLE twofold for anticardiolipin positivity and sixfold for lupus anticoagulant positivity, compared with the absence of aPL antibodies[66]. In patients without an underlying autoimmune disease, venous thrombotic risk is increased 1.5-fold for anticardiolipin positivity and up to tenfold for lupus anticoagulant positivity[67,68], whereas arterial thrombosis is increased threefold and fourfold, respectively[68]. However, these estimates, derived from meta-analyses, are limited by a lack of prospective data, small cohort size, lack of controls and variable aPL antibody assay methodology among the source studies. Patients with a history of thrombotic APS have a lower live birth rate than those without thrombosis[65]. A 2019 survey of data from the European Registry on Obstetric Antiphospholipid Syndrome[69], which studied 1,000 women with obstetric APS as defined by the 2006 Sydney classification criteria[61], has shown that the live-birth rate is only 49.6% without treatment, but rises to 85% with the recommended treatment regimen and to 72.4% with other regimens, including low-dose aspirin or low-molecular-weight heparin (LMWH), but not on the recommended schedule. Recurrent miscarriages were observed before 10 weeks' gestation in 27%, fetal loss in 17% and stillbirth in 18.5% of this cohort of patients with obstetric APS. The survey also reported early pre-eclampsia (before 34 weeks' gestation) in 18.1% and early IUGR (before 34 weeks' gestation) in 16.1% of the cases studied[69].

Presence of organ dysfunction. The presence of organ dysfunction as a complication of an inflammatory rheumatic disease greatly increases the prospect of maternal and fetal morbidity and mortality and should therefore be discussed during pregnancy planning[6,70]. If a patient has active disease and organ dysfunction, conception should be delayed until a period of disease quiescence and improvement or normalization of organ function is achieved. For example, the presence (or even history) of

Box 1 | **Prevalence of rheumatic diseases**[a]

Rheumatoid arthritis
- 120 (age 16 to 44)[1]

Systemic lupus erythematosus
- 80 (age 20 to 49)[2]

Psoriatic arthritis
- 130 (age 18 to 49)[3]

Axial spondyloarthritis
- 117 (age 15 to 44)[4]

Sjögren syndrome
- Data unavailable but probably low, as it usually occurs after the age of 40

Systemic sclerosis
- 7.0[b] (age 16–39)[5]

Primary systemic vasculitis
- Data unavailable but probably low, as onset usually occurs after the age of 40

[a]In women of child-bearing age (per 100,000). [b]Estimated from aggregated male and female prevalence of 8.4, given that 83% of patients with systemic sclerosis are female.

Endocardial fibroelastosis
A rare heart disorder of infants and children that is characterised by a thickening within the muscular lining of the heart chambers due to an increase in the amount of supporting connective tissue (inelastic collagen) and elastic fibres.

Placental insufficiency
Failure of the placenta to deliver sufficient nutrients and oxygen to the fetus during pregnancy.

Minimum tolerable dose of corticosteroid
The minimum dose required to maintain disease control and reduce complications such as steroid-induced diabetes mellitus, hypertension and infections in the mother.

active lupus nephritis at conception is a strong predictor of poor maternal and fetal outcomes[35,71]. However, the risk associated with renal disease is not specific to patients with rheumatic disease and prospective studies involving women with chronic kidney disease of various aetiologies have demonstrated increased risks of pre-eclampsia, preterm delivery, small size for gestational age and increased infant and perinatal mortality rates[72–74]. Furthermore, women with advanced chronic kidney disease (stage 4–5) before pregnancy have an increased risk of an accelerated decline in renal function, potentially leading to end-stage disease and the need for renal replacement therapy either in pregnancy or shortly after[75]. Other relative contraindications to pregnancy in patients with inflammatory rheumatic diseases that require multidisciplinary consultation and management include pulmonary hypertension[76], severe interstitial lung disease[77], advanced heart failure[78] and previous severe gestational hypertensive disorders despite therapy[79].

Medication

Predictive tests to stratify patients at risk of disease relapse and thus requiring intensification rather than withdrawal of therapy in pregnancy are lacking. Therefore, treatment decisions are made on the basis of the pattern of disease activity and manifestations and using standard laboratory markers of disease activity. Medications that are compatible with pregnancy therapy should be continued and intensified appropriately during pregnancy to ensure maintenance of disease control and reduce the risk of APOs (FIG. 2). The British Society for Rheumatology (BSR) and EULAR have published guidance regarding the use of various anti-rheumatic drugs in pregnancy and breastfeeding[7–9]. Evidence-based recommendations are summarized here and in TABLE 2.

Glucocorticoids. Glucocorticoids can be divided into non-fluorinated (such as prednisone, prednisolone, hydrocortisone and methylprednisolone) and fluorinated (such as dexamethasone and betamethasone) formulations. Non-fluorinated glucocorticoids are safe in pregnancy and breastfeeding as they are metabolized in the placenta with less than 10% of the active drug reaching the fetus[80]. Titration to the minimum tolerable dose of corticosteroid is required to reduce complications such as steroid-induced diabetes mellitus, hypertension and infections in the mother[81]. Glucocorticoids are associated with an increased risk of premature birth and some reports have suggested that this increased risk is independent of disease activity[10,82]. Some studies in SLE have detected an association between these drugs and premature rupture of the

Table 1 | **Considerations for rheumatic diseases in pregnancy**

Disease	Disease activity during and after pregnancy	Adverse pregnancy outcomes	Risk factors for adverse pregnancy outcomes
Rheumatoid arthritis	• ~48–60% reduction during pregnancy • ~39–50% flare rate post-partum[44,45]	Pregnancy-induced hypertension, IUGR, pre-term birth, small for gestational age, low birthweight[26–31]	Active disease at conception and during pregnancy
Psoriatic arthritis	• ~40–50% reduction during pregnancy • Variable flare rate post-partum[40,41,55]	No increased risk[40,41]	NA
Axial spondyloarthritis	~80% active and stable during pregnancy and post-partum, with exacerbation most likely in the second and third trimesters[53,54]	Pre-term birth, small for gestational age, emergency or elective caesarean section[31]	Active disease at conception and during pregnancy
SLE	~25% flare rate in pregnancy[51]	Pregnancy loss, pregnancy-induced hypertension, IUGR, pre-term birth, small for gestational age, low birthweight, caesarean section, congenital heart block, neonatal lupus[14,33–37]	Active disease at conception and during pregnancy, hypertension, lupus nephritis, APS, anti-SSA/Ro antibodies, anti-SSB/La antibodies
APS	~2–10-fold increased risk of thrombosis in pregnancy and post-partum[66]	Pregnancy loss, pregnancy-induced hypertension, IUGR, pre-term birth, caesarean section	Antiphospholipid antibodies (particularly triple positivity for aCL, anti-β2GP1 and lupus anticoagulant)
Sjögren syndrome	NA	Congenital heart block, neonatal lupus, pregnancy loss, pre-term birth, caesarean section[42]	Anti-SSA/Ro antibodies, anti-SSB/La antibodies
Systemic sclerosis	NA	Pre-term birth, small for gestational age[43]	Rapidly progressive diffuse disease

APS, antiphospholipid syndrome; aCL, anticardiolipin antibodies; anti-β2GP1, anti-β2 glycoprotein-1 antibodies; anti-SSA, anti-Sjögren syndrome-related antigen A, IUGR, intrauterine growth restriction; NA, not applicable; SLE, systemic lupus erythematosus.

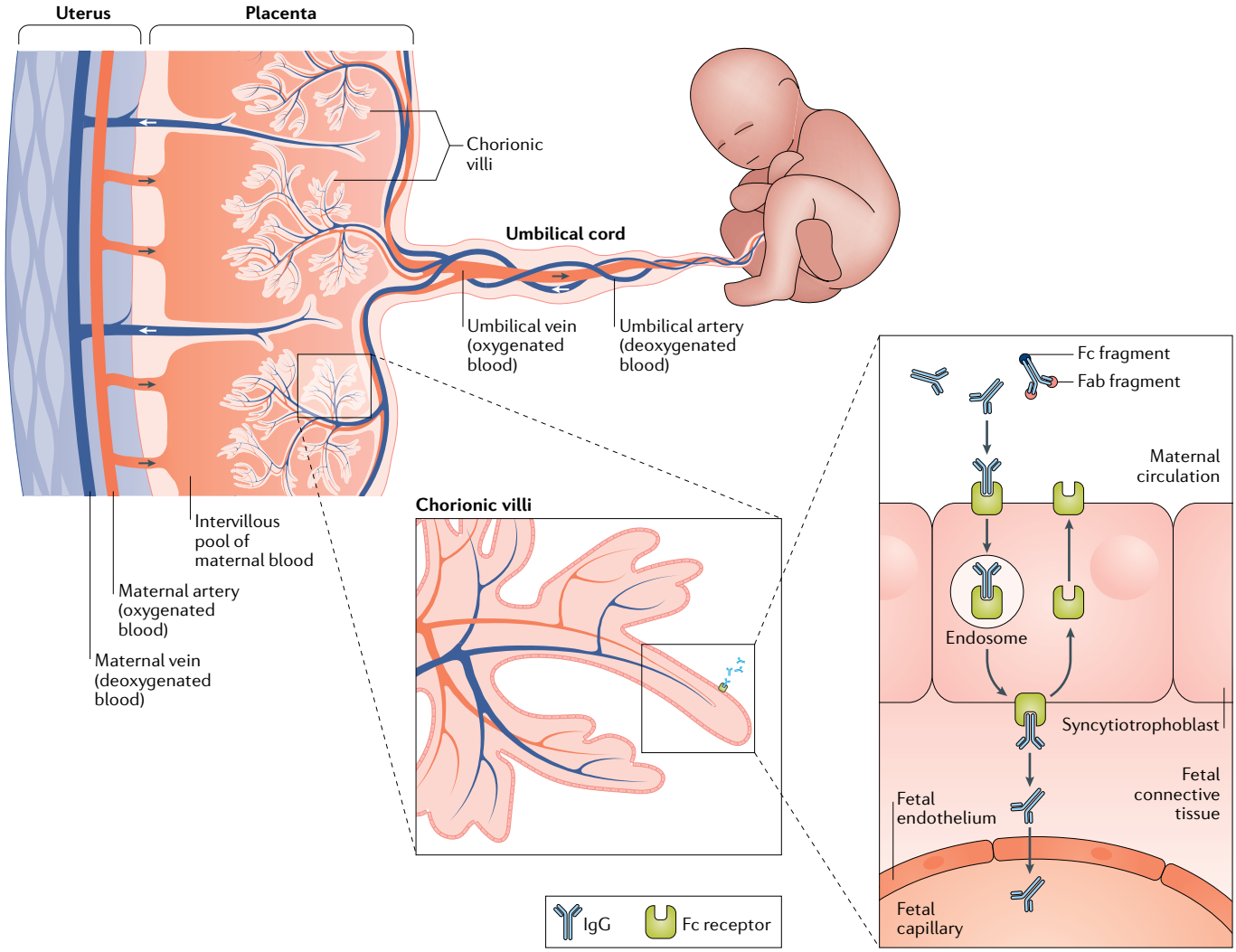

Fig. 1 | **Transplacental transfer of IgG antibodies from maternal blood into the fetal circulation.** Antibodies such as anti-Sjögren syndrome-related antigen A (anti-SSA)/Ro and anti-SSB/La cross the placental barrier by active transplacental transfer via Fc receptor expressed on neonatal syncytiotrophoblast cells. Antibody transfer can have implications for the fetus and baby, including neonatal lupus syndrome. Maternal antibodies are cleared from the baby's circulation within the first 6–9 months of life.

amniotic sac (known as rupture of membranes) surrounding the baby[83], but other studies did not detect this association[84–86]. Fluorinated corticosteroids are not metabolized by the placenta and cross the placental barrier[87], so these drugs should be used for fetal indications only. Dexamethasone has been suggested to cause developmental problems, such as delayed neuropsychiatric development[83], but this conclusion was not confirmed in two other cohorts of children born to anti-Ro/SSA antibody-positive mothers[88,89]. In pregnancy, non-fluorinated corticosteroids are generally administered orally (prednisolone), whereas intravenous administration (for example, with methylprednisolone) is generally used as rescue therapy for severe disease. Compared with prednisolone, parenterally administered methylprednisolone has a prolonged duration of action, with equivalent glucocorticoid (anti-inflammatory) effects at a lower dose (80% of prednisolone dose) and similar rates of transplacental transfer[7].

Rescue therapy
Treatment given after a patient has failed to respond to standard therapy.

Synthetic DMARDs. A number of conventional synthetic DMARDs should be stopped before conception. Methotrexate is teratogenic and should be stopped 3 months before conception[7,9]. Given that leflunomide was teratogenic in animal studies and has a long half-life, a cholestyramine washout to eliminate the drug from the body should be completed pre-conception, despite reassuring data from accidental exposures in human pregnancies[90]. Mycophenolate mofetil is teratogenic and should be stopped 6 weeks before conception[91]. Cyclophosphamide is teratogenic and should be stopped at least 3 months before conception[92].

Conventional DMARDs that can be continued during pregnancy include hydroxychloroquine, sulfasalazine, azathioprine and the calcineurin inhibitors ciclosporin and tacrolimus[7,9]. Women who conceive while being treated with leflunomide and then stop this drug and undergo cholestyramine washout in the first trimester[90,93], or who are exposed to leflunomide at

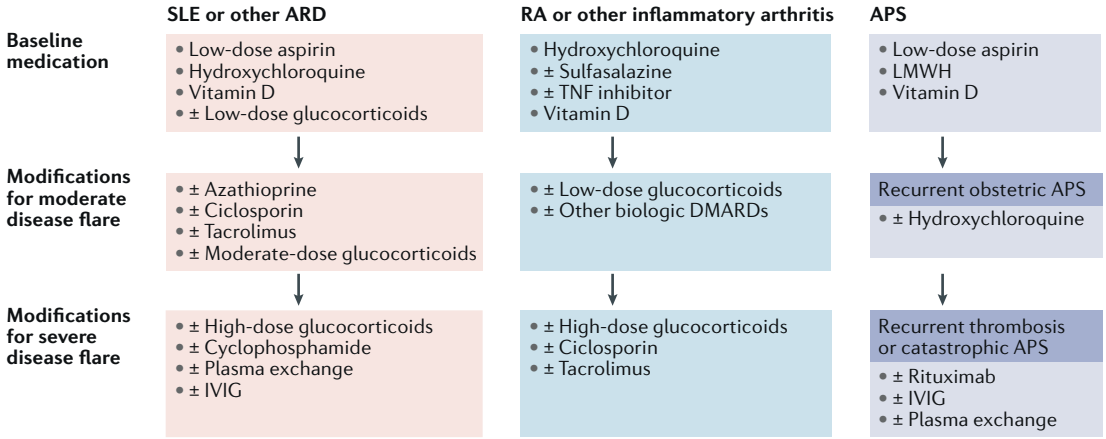

Fig. 2 | **Treat-to-target strategy for management of inflammatory rheumatic diseases in pregnancy.** Treatment during pregnancy is aimed at maintaining disease control and reducing the risk of adverse pregnancy outcomes. Medications that are compatible with pregnancy should be continued and intensified appropriately during pregnancy in the case of disease flare. Appropriate treatment depends on the disease and type of manifestation. ±, and/or; APS, antiphospholipid syndrome; ARD, autoimmune rheumatic disease; IVIG, intravenous immunoglobulins; LMWH, low-molecular-weight heparin; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

various stages of pregnancy without washout[94,95], are not at an increased risk of APOs. Although leflunomide does not seem to be a major human teratogen, more data are required before its use during pregnancy can be safely advised.

Targeted synthetic DMARDs (such as apremilast, tofacitinib and baricitinib) are small-molecule inhibitors that are increasingly used to treat inflammatory rheumatic diseases[96–98]. These drugs should be avoided during pregnancy until data are gathered on the risks associated with their use. The precise timing of when to stop these drugs before pregnancy is unclear, but given their short half-lives (3–12 h[96–98]), stopping each drug 1 month before conception should be sufficient.

Biologic DMARDs. Biologic DMARDs are recombinant proteins, usually either monoclonal IgG1 antibodies or fusion proteins containing the Fc portion of IgG1 joined to receptor-blocking proteins. These drugs share similar structure with maternal IgG, which are large proteins (~150 kDa) that are unable to diffuse across the placenta[99]. Active transplacental transfer of maternal IgG takes place via neonatal Fc receptors on the syncytiotrophoblast[100–102] and occurs rapidly from week 16 of pregnancy onwards[100–102] (FIG. 1). Some biologic DMARDs are fusion proteins containing part or none of the IgG structure, principally etanercept and abatacept. Etanercept is a fusion protein of the soluble TNF receptor 2 and the Fc region of IgG1a and despite the presence of the Fc region has low rates of transplacental transfer[103,104]. Anakinra is a recombinant human IL-1 receptor antagonist that does not contain any immunoglobulin structure; hence, it lacks the Fc region and has not been found to cross full-term human placenta[105]. Abatacept contains the Fc region of IgG1 fused to the extracellular domain of CTLA4 and has not yet been associated with any specific pattern of risk[106]. Therefore, it is important to consider carefully both the structure and the timing of biologic DMARD exposure during pregnancy.

Current guidelines recommend that patients treated with TNF inhibitors should continue with these drugs through to the second or third trimester (depending on drug bioavailability based on the half-life of the drug in the circulation, and depending on transplacental passage based on the structure of the TNF inhibitor). Administration of the TNF inhibitors is usually stopped at appropriate times in pregnancy (TABLE 3) to ensure that there is no TNF inhibitor in the maternal circulation at the time of birth as it would also be present in the baby and persist in the neonatal circulation, possibly putting the baby at risk of infection after administration of live vaccines[7,9]. If there is a concern that an inflammatory rheumatic disease will flare, TNF inhibitors should be continued throughout pregnancy, but live vaccines, such as rotavirus and tuberculosis, should be avoided in the exposed infant until 6 months of age. This advice is based on a case of fatal tuberculosis-like disease reported post-BCG vaccination in an infant who was not breastfed but was exposed throughout pregnancy to infliximab, a TNF inhibitor with a long half-life in infants[107]. Certolizumab pegol, a PEGylated Fab′ that is specific for TNF, has minimal levels of transfer across the placenta[108] and into breastmilk[109], and is therefore licenced by the European Medicines Agency and FDA for use during pregnancy and breastfeeding.

Current British and European guidelines recommend that other biologic DMARDs (such as rituximab, belimumab, anakinra and tocilizumab) are stopped in advance of pregnancy owing to limited data[7,9]. However, some of these drugs (anakinra and tocilizumab) have been recommended for use during pregnancy if other treatment options are limited and the benefits of maintaining disease suppression with these biologic agents outweigh the risks[110,111].

Increasingly, biosimilars are replacing existing originator biologic DMARDs. To date, published evidence on the use of biosimilars in pregnancy is very limited. Given their similarity to originator compounds in terms

Table 2 | **Anti-rheumatic drugs recommended by BSR and EULAR for use at conception and during pregnancy**[7–9]

Drug class	Compatible with pregnancy	Some evidence of lack of harm	Contraindicated
Analgesic	Conventional NSAIDs (up to 32 weeks of pregnancy), amitriptyline, opiates	NA	COX2 inhibitors, gabapentin, pregabalin
Anti-thrombotic	Low-dose aspirin, heparin	NA	Warfarin, apixaban, rivaroxaban, dabigatran, fondaparinux
Glucocorticoids	Glucocorticoids	NA	NA
Conventional DMARDs	Hydroxychloroquine, sulfasalazine, azathioprine, tacrolimus, ciclosporin	Leflunomide	Methotrexate, cyclophosphamide, mycophenolate mofetil
Biologic DMARDs	Certolizumab pegol, infliximab, adalimumab, etanercept, golimumab	Anakinra, canakinumab, tocilizumab, abatacept	Rituximab, belimumab, ustekinumab, rilonacept
Targeted synthetic DMARDs	NA	NA	Apremilast, tofacitinib, baricitinib

BSR, British Society for Rheumatology; COX2, cyclooxygenase 2; NA, not applicable.

of identical molecular target and antibody structure, with variation only in post-translational modifications (as exists between different batches of existing originator biologic DMARDs), it seems reasonable to counsel patients regarding the use of biosimilars in pregnancy on the basis of the existing evidence for each originator compound.

Analgesics. Conventional NSAIDs are generally safe, but should be avoided in the third trimester owing to their effects upon the ductus arteriosus, namely the premature closure of this vessel, leading to progressive right heart dysfunction, congestive heart failure and intrauterine death, but can be used with caution in the first trimester owing to a low risk of miscarriage[8]. However, cyclooxygenase-2-selective NSAIDs are not recommended because of a lack of data and the theoretical risks that these drugs could impair fertilization, implantation and maintenance of pregnancy[112].

Codeine is compatible with pregnancy and during peri-conception for acute pain[8]. British Society for Rheumatology guidelines state that no consistent evidence exists to recommend dose reduction before delivery, but neonatologists should be aware of the maternal use of codeine during breastfeeding owing to the risk of central nervous system depression resulting from the unpredictable metabolism of codeine to morphine[8].

Amitriptyline, gabapentin and pregabalin are commonly used to treat chronic pain. Amitriptyline is safe to use during pregnancy, but gabapentin or pregabalin are not recommended[8].

Co-morbidity medications. A review of the entire drug list prescribed to each patient is important to ensure that all medications prescribed for co-morbidities, particularly those commonly associated with inflammatory rheumatic diseases, are compatible with pregnancy. In the event of pregnancy, patients with pre-existing hypertension should be instructed to switch from angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers and chlorothiazide agents associated with congenital anomalies to alternative antihypertensive drugs, such as labetalol nifedipine or methyldopa[113]. In addition, women at a moderate or high risk of pre-eclampsia should avoid excessive dietary salt intake and should take low-dose aspirin[114,115]. Patients receiving an ACE inhibitor to reduce proteinuria before pregnancy may have greater proteinuria as a result of stopping the ACE inhibitor and the increased glomerular filtration rate (of 50–80%) in pregnancy. Even in patients with diabetic nephropathy, this change does not signify worsening renal disease and proteinuria often returns to baseline post-partum[116]. Warfarin is contraindicated in pregnancy owing to an increased risk of congenital abnormalities if continued after week 6 of gestation; thus, patients taking warfarin should switch to LMWH once pregnancy is confirmed[8].

Management during pregnancy

General principles. Patients with inflammatory rheumatic diseases who are planning pregnancy or who are pregnant should be managed in a multidisciplinary setting with close obstetric and rheumatological monitoring, involving regular clinical, laboratory and obstetric ultrasound evaluations and the advice of other specialists depending on organ involvement. Risk stratification should be performed according to degree and extent of maternal disease in addition to the antibody status of the patient (aPL, anti-SSA/Ro and anti-SSB/La antibodies). The overall goal is to develop an individualized plan for management to suppress disease activity using a treat-to-target approach during pregnancy to optimize the chance of a successful pregnancy outcome (FIG. 3).

Maintenance and monitoring of disease control. Disease control is maintained by continued prescription of medications that are compatible with pregnancy. For patients with inflammatory arthritis, sulfasalazine and hydroxychloroquine are ideal maintenance therapies[7,9] and, with the caveats already discussed, biologic DMARDs might be considered. In patients with SLE, hydroxychloroquine is a mainstay of treatment and discontinuation of this drug is associated with an increased risk of flares and APOs in pregnancy[15,16,52]. Other

Ductus arteriosus
A blood vessel in the fetus connecting the main pulmonary artery to the proximal descending aorta, allowing most blood from the right ventricle to bypass the lungs. Premature closure of this blood vessel leads to progressive right heart dysfunction, congestive heart failure and intrauterine death.

Table 3 | **TNF inhibitors used during pregnancy**

TNF inhibitor	Half-life	Recommended discontinuation time during pregnancy[7,9]
Infliximab	8–9.5 days	16–20 weeks
Etanercept	70 h	24–32 weeks
Adalimumab	10–20 days	20–24 weeks
Certolizumab	14 days	Safe throughout pregnancy
Golimumab	7–20 days	Limited data available; possibly safe in first trimester

suitable DMARDs include azathioprine, ciclosporin and tacrolimus[7,9].

Glucocorticoids can be used to treat any inflammatory rheumatic disease, particularly to treat disease flares, and should be titrated to the minimum tolerable dose to maintain disease control and limit steroid-related adverse effects such as hyperglycaemia and bone loss[17,81]. Concomitant use of calcium and vitamin D until the end of lactation is also particularly important for patients treated with glucocorticoids and/or heparin as they are at increased risk of osteoporosis[17,117]. All pregnant patients with SLE at high risk of pre-eclampsia, including those with lupus nephritis or who are positive for aPL antibodies, should be treated with low-dose aspirin (≤150 mg daily), which has been shown to reduce the risk of pre-eclampsia in non-SLE high-risk pregnancies[17,118].

When monitoring disease activity in pregnancy, awareness of physiological changes of pregnancy that resemble those of disease flare is important. Examples of such changes include proteinuria (up to 300 mg per day), increased erythrocyte sedimentation rate (up to 70 mm/h), a 2–3-fold rise in serum complement concentrations or a fall in serum haemoglobin concentrations (<110 g/l)[119]. In addition, increasing degrees of back pain and swelling of the hands, feet and knees are common in late pregnancy and could be mistaken for arthritis flare. Therefore, C-reactive protein is a more accurate indicator of inflammation than erythrocyte sedimentation rate in pregnancy[119] and should be used in this situation for monitoring, except in SLE for which any fall in complement (C3 or C4) of ≥25% is important, even if into the 'normal' range[17].

For most inflammatory rheumatic diseases, the same outcome assessment as for non-pregnant patients is used during pregnancy, but modified disease activity scores are recommended in some conditions. For example, a modified 28-joint disease activity score for RA includes a measure of C-reactive protein (DAS28-CRP) and unlike the standard DAS28 lacks the global health score, which can be affected by pregnancy itself[15]. Owing to the complexity of SLE assessment in pregnancy, a disease activity index has been developed and validated by modifying the British Isles Lupus Assessment Group (BILAG2004) to create the BILAG2004-Pregnancy index so that physiological changes of pregnancy do not influence the score[120].

Uterine artery Doppler ultrasound
A technique used to measure uterine artery blood flow between mother and baby.

Monitoring for pregnancy complications. Women with inflammatory rheumatic diseases should seek antenatal care early (before 12 weeks of pregnancy) and this care should be managed in a multidisciplinary setting. In addition to routine pregnancy monitoring, clinical assessment of the mother and the baby should include measurement of blood pressure, urinalysis and blood tests, such as for autoantibody status and disease activity. In addition, obstetric ultrasound scans are required at specified intervals to record fetal anatomy, growth and development as recommended for healthy pregnancy with additional monitoring in the third trimester for SLE and APS pregnancies[17,69].

Prevention and treatment of pre-eclampsia. There is an increased risk of pre-eclampsia in patients with inflammatory rheumatic diseases, particularly in patients with SLE, previous renal disease or patients treated with concomitant glucocorticoids[14,17,33–36]. Risk factors for pre-eclampsia and APOs in SLE pregnancy include a high level of disease activity at conception and during pregnancy, a history of lupus nephritis, maternal hypertension, presence of aPL antibodies, low serum complement concentrations and thrombocytopenia[121]. All patients at increased risk of pre-eclampsia should be treated with low-dose aspirin until delivery[17,51]. In addition, they should be monitored for development of pre-eclampsia, which includes measurement of blood pressure and proteinuria at each visit and fetal ultrasound and uterine artery Doppler ultrasound flow studies, as ordered by obstetric consultants. The presence of bilateral uterine notching, indicating abnormal blood flow, between 23 and 25 weeks' gestation can be used to predict early-onset pre-eclampsia and gestational hypertension[122]. In addition, measurement of circulating angiogenic factors (including soluble Fms-like-tyrosine kinase 1, placental growth factor and soluble endoglin), which are dysregulated in pre-eclampsia in non-autoimmune pregnancies[123], can also be used to predict the risk of various APOs, including pre-eclampsia in patients with SLE[124].

First-line treatment of hypertension in pregnancy is labetalol, with alternatives being methyldopa and nifedipine; ACE inhibitors, angiotensin II receptor blockers and chlorothiazide diuretics are associated with congenital malformations[125].

Distinguishing between lupus nephritis and pre-eclampsia is challenging but important, as the management of each condition is different[126]. Both conditions are characterized by proteinuria, oedema, renal impairment, hypertension and thrombocytopenia. Indicators of lupus nephritis include C3 and C4 levels that are falling (or failing to increase), a rising titre of antibodies specific for double-stranded DNA, active urinary sediment and other clinical indicators of SLE activity, such as skin disease, arthritis and cytopenia[126]. Anti-C1q antibodies are also associated with renal involvement in SLE[127] and may therefore function as a biomarker of active SLE. By contrast, many of these features are lacking in pre-eclampsia and unlike lupus nephritis, in pre-eclampsia, C3 and C4 levels often increase[126]. If these tests are indecisive a renal biopsy is required to differentiate between lupus

nephritis and pre-eclampsia. Depending on the clinical circumstances and the gestation of the pregnancy, premature delivery of the fetus may be mandated, as the only cure for pre-eclampsia is delivery of the baby and potent immunosuppression cannot be given for serious renal lupus flare until after delivery owing to the increased risk of adverse events that would affect the mother and baby in this situation. Multidisciplinary care is critical in this situation as inappropriate management could result in the death of the baby and/or mother[118,128].

Thromboprophylaxis and anticoagulation. Pregnancy itself is a pro-coagulant state with alterations in both coagulation and fibrinolysis, presumably to reduce blood loss at delivery[119], and this procoagulant risk is increased in various inflammatory rheumatic diseases[129], particularly APS[61,63,69]. Thromboprophylaxis is required with therapeutic anticoagulation in APS and is a consideration with anti-platelet therapy in other conditions, principally SLE[17,63,69]. Treatment to prevent recurrent early miscarriage is the only management in obstetric APS supported by clinical trial data[63,69]. Dual treatment with low-dose aspirin and LMWH is the standard-of-care for all patients with APS. Warfarin is contraindicated in pregnancy owing to its teratogenic effects and patients being treated with this anticoagulant drug should be switched to a therapeutic dose of LMWH once pregnancy is confirmed[8]. Evidence is lacking regarding the safety of the direct oral anticoagulants apixaban, rivaroxaban, dabigatran and fondaparinux in pregnancy and use of these drugs is therefore not recommended[130].

Anti-SSA/Ro and anti-SSB/La complications. Using fetal cardiac ultrasound, screening for congenital heart block in pregnancies of anti-SSA/Ro-positive and/or anti-SSB/La-positive mothers with a previously affected child should begin at week 16 of pregnancy[17]. For anti-SSA/Ro-positive and/or anti-SSB/La-positive women with no previous congenital heart block, most fetal cardiologists recommend an initial fetal cardiac ultrasound scan at 16–20 weeks with a repeat at 28 weeks if the initial scan is normal. Once established, complete AVB is irreversible and cardiac pacing is almost always required, but specialist management of any associated cardiac disease may improve fetal outcome[131]. Fluorinated glucocorticoids have been used to treat the early stages of AVB[132], but the benefit of this therapy has not been proven. Hydroxychloroquine, however, is associated with a reduction in the rate of recurrent AVB in future pregnancies after an affected pregnancy[133] and a lower rate of AVB in babies born to mothers with anti-SSA/Ro antibodies with or without SLE[134,135]. Non-cardiac neonatal lupus manifestations are transient[136] and specific treatment is not required.

Management of flares and organ dysfunction. Standard management of inflammatory rheumatic disease flares in pregnancy is to treat with systemic glucocorticoids and add other DMARDs depending upon the disease and type of manifestation (FIG. 2). In inflammatory arthritis, addition of sulfasalazine and/or hydroxychloroquine is suitable to maintain disease control[7,9].

Pre-conception

Clinical review
• Aim to achieve disease remission for at least 4 months before conception • Avoid pregnancy if patient has severe pulmonary hypertension, renal failure or history of stroke within the past 6 months
Drug therapy
• Ensure adequate washout of teratogenic drugs
Antibodies
• Assay antiphospholipid, anti-SSA/Ro and anti-SSB/La antibodies
Pre-pregnancy counselling

↓

During pregnancy

Clinical review
• Dictated by disease activity/manifestations and obstetric complications • Check blood pressure, urine protein, full blood count, kidney and liver function and disease biomarkers at each visit
Drug therapy
• Do not discontinue hydroxychloroquine or other appropriate treatments for disease • For SLE, all patients require treatment with low-dose aspirin • For thrombotic APS, switch oral anticoagulation to heparin when pregnancy is confirmed
Antibodies
• If anti-SSA/Ro or anti-SSB/La-positive, perform fetal cardiac ultrasound scan at 16–20 weeks and monitor fetal heart rate; repeat ultrasound if AVB develops

↓

Post-partum

Clinical review
• Monitor for disease flare up to 4 months post-partum • Consider contraception
Drug therapy
• Ensure compatibility with breast feeding
Antibodies
• For thrombotic APS, switch from heparin to warfarin

Fig. 3 | **Optimization of management of inflammatory rheumatic diseases in pregnancy.** For patients with inflammatory rheumatic diseases, the chance of a successful pregnancy outcome is optimized by the development of an individualized plan to suppress disease activity using a treat-to-target approach. Patients who are planning pregnancy or who are pregnant should be managed in a multidisciplinary setting with close obstetric and rheumatological monitoring, with risk stratification according to the degree and extent of maternal disease as well as the antibody status of the patient. Anti-SSA, anti-Sjögren syndrome-related antigen A; APS, antiphospholipid syndrome; AVB, atrioventricular block; SLE, systemic lupus erythematosus.

For connective tissue disease or vasculitis, azathioprine is preferred[7,9] and in the case of lupus nephritis or cytopenia, tacrolimus can be used alone or in combination with glucocorticoids and/or other DMARDs in pregnancy[137]. In the case of severe maternal inflammatory rheumatic disease, intravenous immunoglobulins and plasma exchange may be therapeutic[138,139]; premature delivery of the fetus should also be considered, to reduce the adverse consequences of chronic intrauterine asphyxia that can arise from impaired placental function caused by maternal disease[140].

Thromboprophylaxis
In this context, the prevention of thromboembolic disease by pharmacological means.

Fetal cardiac ultrasound
Technique used to evaluate the structure of the fetal heart.

Screening for congenital heart block
The use of fetal cardiac ultrasound at the 16th to 20th week of pregnancy in anti-SSA/Ro-positive mothers.

Cardiac pacing
Technique used to regulate heart rate involving the fitting of a pacemaker.

Table 4 | **Anti-rheumatic drugs recommended by BSR and EULAR for use during breastfeeding**[7–9]

Drug class	Compatible with breastfeeding	Consider if benefits outweigh potential risks	Contraindicated
Analgesic	Conventional NSAIDs, amitriptyline	Opiates	NA
Anti-thrombotic	Low-dose aspirin, heparin, warfarin	NA	Apixaban, rivaroxaban, dabigatran, fondaparinux
Glucocorticoids	Glucocorticoids	NA	NA
Conventional DMARDs	Hydroxychloroquine, sulfasalazine, azathioprine, tacrolimus, ciclosporin	NA	Methotrexate, cyclophosphamide, mycophenolate mofetil
Biologic DMARDs	Certolizumab pegol, infliximab, adalimumab, etanercept, golimumab	Anakinra, canakinumab, abatacept, tocilizumab, rituximab, belimumab, ustekinumab	NA
Targeted synthetic DMARDs	NA	NA	Apremilast, tofacitinib, baricitinib

BSR, British Society for Rheumatology; NA, not applicable.

Post-partum management

Risk of post-partum flare. Post-partum inflammatory rheumatic disease flares are common but vary considerably in severity and timing, from several days to 3–6 months after delivery[45,46,52,54]. Evidence that increasing the dose of glucocorticoids prevents flare does not exist, but it is possible that flares are worsened by patients discontinuing therapy for fear of harming their baby when breastfeeding.

Drugs and breastfeeding. Drugs that can be used in pregnancy are usually compatible with breastfeeding, but few trials have tested this compatibility (TABLE 4). Certolizumab pegol is the only TNF inhibitor licensed by the European Medicines Agency and FDA for use during breastfeeding as it has minimal transfer into breast milk[109]. Most inflammatory rheumatic disease flares during breastfeeding are treated by starting or increasing the dose of existing oral prednisolone or intramuscular methylprednisolone[126]. Hydroxychloroquine is compatible with pregnancy and, although it enters the breast milk[7,9,141], evidence does not exist that this drug can harm the baby. Similarly, sulfasalazine is commonly used by mothers with various inflammatory conditions. However, 5 mg daily folic acid should be taken during pregnancy and breastfeeding to prevent folate deficiency in the baby[7]. Patients treated with LMWH in pregnancy should continue this drug for 6 weeks post-partum[142] and patients previously taking warfarin can re-start this drug during breastfeeding[8,142] The safety of the direct oral anticoagulants (such as apixaban, rivaroxaban, dabigatran and fondaparinux) during breastfeeding is unknown and these drugs are therefore not recommended during breastfeeding[130].

Contraception. Contraception should be discussed soon after delivery for women with inflammatory rheumatic diseases and at risk of post-partum flare to ensure that they do not have a further pregnancy that is unplanned[17]. Many women still think that breastfeeding protects them from further pregnancy. The most appropriate contraception method is probably the method they used before pregnancy. An intrauterine device, such as a hormone-releasing coil, is a viable and safe option for a patient to consider and discuss with their general practitioner. Risk of infection is low in women using intrauterine devices and DMARDs, except in individuals whose behaviour, independent of their disease and its treatment, puts them at increased risk.

Conclusions

Pregnancy in patients with inflammatory rheumatic diseases has the best outcomes if women conceive during disease remission and with appropriate therapy. Persistent disease activity and disease flares in pregnancy are associated with an increased risk of IUGR, resulting in babies that are small for gestational age and premature delivery. During pregnancy, screening and managing active disease and the increased risk of pre-eclampsia are important, particularly in patients with a history of hypertension, RA or SLE (especially lupus nephritis and APS). Women with inflammatory rheumatic diseases require specific advice about drug therapy while trying to conceive, during pregnancy and while breastfeeding and should be aware of the risk of post-partum flares. However, with careful planning, monitoring and treatment, most women with these diseases can have successful pregnancies.

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All authors made substantial contributions to the research and discussion of the content. I.G. drafted the article and C.-S.Y. and C.G. edited/reviewed the article.

Competing interests

I.G. declares that he has received honoraria and travel grants from UCB and Lupus Academy to speak at educational meetings on topics related to pregnancy in rheumatic disease. C.S.Y. declares that he has consulted for Bristol Myers Squibb, Immupharma and EMD Serono. C.G. declares that she has consulted for and received honoraria from Bristol-Myers Squibb, GlaxoSmithKline, EMD Serono and UCB in relation to lupus clinical trial design and analysis, and has been a member of the speakers' bureau for GlaxoSmithKline and UCB. C.G. also declares that she has participated in clinical trials sponsored by UCB and funded by Arthritis Research UK with drugs supplied by GlaxoSmithKline.

Publisher's note

New therapies for systemic lupus erythematosus — past imperfect, future tense

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Abstract | The failure of many new, mostly biologic, drugs to meet their primary end points in double-blind clinical trials in patients with systemic lupus erythematosus (SLE) has caused a profound sense of disappointment among both physicians and patients. Arguably, the success of B cell depletion with rituximab in open-label clinical trials, the approval of belimumab (which blocks B cell-activating factor (BAFF)) for use in patients with lupus nephritis in the USA and in difficult-to-treat patients with SLE in the UK and the recognition that clinical trial design can be improved have given some cause for hope. However, changes to therapies in current use and the development of new approaches are urgently needed. The results of the latest studies investigating the use of several new approaches to treating SLE are discussed in this Review, including: fully humanized anti-CD20 and anti-CD19 monoclonal antibodies; inhibition of tyrosine-protein kinase BTK; CD40 ligand blockade; interfering with the presentation of antigen to autoreactive T cells using a peptide approach; a receptor decoy approach using an analogue of Fcγ receptor IIB; dual blockade of IL-12 and IL-23; and inhibition of Janus kinases.

The outlook for patients with systemic lupus erythematosus (SLE) improved from a 4-year survival rate of ~50% in 1950 to a 15-year survival rate of ~85% by 2013 (REF.[1]). However, patients continue to die prematurely, and morbidity in SLE, such as osteoporosis[2], an increased risk of infection[3] and atherosclerosis[4], is often substantial. An analysis of patients with lupus nephritis (potentially the most harmful disease manifestation) indicated that there had not been a major improvement in outcome in the 30 years to 2011 (REF.[5]), suggesting that conventional drugs are unlikely to produce any further clinically important beneficial effects in these patients. Hopes had been high that, as with patients with other autoimmune rheumatic diseases, those with SLE would benefit from biologic therapies. However, biologic therapy for the treatment of SLE has been relatively unsuccessful and several biologic agents have failed to meet their primary end points in large-scale clinical trials[6,7]. Thus, physicians treating patients with SLE currently cannot choose between several highly successful approved biologic drugs when conventional therapies fail, as is the case for those treating patients with rheumatoid arthritis (RA), psoriatic arthritis (PsA) or ankylosing spondylitis[8]. Hence, a clear unmet need exists for targeted biologic therapies, particularly for aspects of disease such as lupus nephritis that have a limited number of proven therapies.

In this Review, we consider the current use of biologic therapies to treat patients with SLE and provide some discussion about why previous trials have failed. We also outline several potential new therapies, indicating the pathways that each approach seeks to block. Many therapeutic targets are currently under investigation, and several ongoing clinical trials for SLE have been discussed elsewhere[7] so in this Review, we focus only on those approaches that we consider to be particularly encouraging.

Current use of biologic therapy

In SLE, evidence exists of a general breakdown in both B cell and T cell tolerance, and a number of aspects of B cell biology have been implicated in its pathogenesis[9]. Perhaps the most obvious pathogenic function of B cells in SLE is the production of autoantibodies that target self-antigens such as DNA and extractable nuclear antigens. The contribution of B cells to disease initiation and perpetuation in SLE is complex, but it is probable that they help to prime autoreactive T cells, function as antigen-presenting cells (APCs) and are a rich source of the cytokines involved in immune dysregulation in SLE[9]. Not surprisingly, many of the therapeutic agents that have been trialled in SLE target B cell pathways[10]. The approaches of these therapeutic agents vary, from targeting B cell-selective cell surface molecules (such

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Key points

- The approval of new therapies, especially biologic drugs, for systemic lupus erythematosus (SLE) has been scarce in comparison to rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis.
- Belimumab (FDA approved) and rituximab (National Health Service England approved) are available for use in some countries, although the cost (particularly of belimumab) mitigates their universal uptake.
- Clinical trial design for SLE is problematic, and success in phase II trials is not often followed by success in phase III trials.
- Several new approaches are under investigation that target B cells, cytokines or intracellular signalling pathways, providing hope that new therapies will be approved for SLE.

as CD22 or CD20), to inhibiting B cell survival by targeting cytokines and signalling molecules (such as B cell-activating factor (BAFF), IL-6, IL-17 and IL-21), to interfering with B cell antigen presentation by targeting co-stimulatory molecules (such as CD40–CD40 ligand (CD40L) interactions and inducible T cell co-stimulator (ICOS)–ICOS ligand (ICOSL) interactions). Many of these therapies, including rituximab (an anti-CD20 monoclonal antibody (mAb)), epratuzumab (an anti-CD22 mAb), abatacept (which stops APCs from interacting with T cells via CD80 and CD86) and tabalumab (an anti-BAFF mAb), have not shown a statistically significant benefit in clinical trials for SLE[11,12]. However, despite the disappointing results of these (mostly) biologic therapies in clinical trials, not all of the approaches attempted in the past few years have failed completely.

Rituximab and belimumab (an anti-BAFF mAb) are the biologic drugs most commonly used to treat SLE in clinical practice. The results of a large number of open-label studies of rituximab[11] and the encouraging data from national registries[12,13] were sufficient for both the ACR[14] and EULAR[15] to recommend rituximab as a treatment for lupus nephritis and for the National Health Service England to sanction its use in difficult-to-treat patients[16]. For example, in the Lupus Clinic at University College Hospital, London, UK, ~170 patients have been treated with rituximab since 2000 owing to the inefficacy of treatment or adverse events following immunosuppression with steroids, azathioprine, mycophenolate mofetil (MMF) or cyclophosphamide (D.A.I., unpublished observations). Importantly, although rituximab is regarded as being generally effective, its use is associated with hypogammaglobulinaemia[17] (which causes an increased risk of infection), and allergy-like responses (ranging from a mild cutaneous rash with flushing and pruritus to symptomatic bronchospasm with dysphonia, hypoxia and wheeze) were reported at one centre in 16% of patients treated with rituximab[18,19].

Following successful clinical trials[20,21], belimumab was approved by the FDA in 2012 for use in the USA in patients with SLE and by the National Institute of Clinical Excellence in 2016 for use in the UK in patients with SLE who have active skin and joint disease. Belimumab thus became the first drug to be approved by the FDA for the treatment of SLE in more than 50 years. Encouragingly, a 2018 trial[22] of intravenous belimumab that included 677 patients from China, Japan and South Korea reported a response rate (using the SLE Responder Index (SRI)-4 end point) of 53.8% in the belimumab-treated group versus 40.1% in those given placebo in addition to standard-of-care treatment. However, this trial[22] excluded patients who had renal disease or central nervous system (CNS) disease. The efficacy and safety of a subcutaneous form of belimumab has also been reported. In a study of 839 patients with SLE, 556 of whom were given belimumab and 280 of whom were given placebo, 61.4% of those taking belimumab met the primary end point of achieving an SRI-4 response compared with 48.4% of those taking placebo[23].

Although limited by regulatory bodies and cost, 'real-life' data on belimumab use are also emerging. For example, the results of a study from Italy[24] of 188 patients with SLE treated with belimumab who were followed up for a mean of 17.5 months have been reassuring in terms of both efficacy and safety. In this population, the most common disease manifestations that required belimumab to be started were polyarthritis and skin rashes[24]. The results of a trial of belimumab in patients with renal disease[25] are still awaited, and more detailed knowledge of the effectiveness of belimumab in SLE manifestations, such as pleuropericarditis, gastrointestinal disease and CNS disease, is also desired.

Despite the clinical practice and, in the case of belimumab, clinical trial evidence supporting the use of belimumab and rituximab, they are not panaceas, and a proportion of patients remain whose disease is not controlled by existing B cell-targeting strategies. Thus, there remains a 'gap in the market' for successful and relatively adverse-effect-free biologic therapies to treat SLE.

Challenges for SLE clinical trials

Assessment of disease activity

Assessing disease activity in SLE can be challenging, not least because it is essential to distinguish clinical features resulting from disease activity from those resulting from concomitant diseases or damage. Several disease activity assessment systems have been developed and validated[26]. The best-known disease activity measures are probably the SLE Disease Activity Index 2000 (SLEDAI-2K) and the British Isles Lupus Assessment Group (BILAG)-2004 disease activity index. The SLEDAI-2K provides a simple comprehensive score that is easy to calculate but that does not distinguish features of clinical activity that are only partly improved from those that have not changed[26]. This index also misses out some important clinical features of SLE, including gastrointestinal disease, ophthalmic disease and haemolytic anaemia. By contrast, the BILAG-2004 disease activity index is more comprehensive and is able to distinguish between different disease states, but takes longer to complete when the disease is active[26]. A BILAG A or B score refers to new severe (A) or moderate (B) disease activity within a particular domain that typically leads to a change in therapy.

Several composite end points have also been developed, such as the SRI and the BILAG-based Composite Lupus Assessment (BICLA), both of which include components of the BILAG-2004 disease activity index and the SLEDAI-2K. Both the SRI and the BICLA aim

Table 1 | **Deaths in clinical trials of biologic therapies for systemic lupus erythematosus**

Drug	Total number of patients in the trial	Deaths in the placebo group (*n* (%))	Deaths in the low-dose treatment group (*n* (%))	Deaths in the medium-dose treatment group (*n* (%))	Deaths in the high-dose treatment group (*n* (%))	Refs
Atacicept	455	0 (0.0)	0 (0.0)	NA	2 (1.4)	27
Tabalumab	1,164	2 (0.5)	2 (0.5)	NA	3 (0.8)	30
	1,124	3 (0.8)	2 (0.5)	NA	1 (0.3)	31
Belimumab	865	3 (1.0)	2 (0.7)	NA	4 (1.0)	21
	819	0 (0.0)	2 (0.7)	NA	1 (0.4)	22
Sifalimumab	431	2 (1.9)	0 (0.0)	2 (1.9)	2 (1.9)	78

NA, not available.

to capture clinical features in patients with SLE that are caused by active SLE and not by concomitant disease (~30% of patients with SLE have one or more additional autoimmune rheumatic diseases), damage (for example, a painful hip might be caused by active synovitis or by avascular necrosis, and the treatment differs accordingly) or the adverse effects of other drugs (such as steroids, which can cause proximal muscle weakness)[26].

SLE is a complicated disease, and the majority of pharmaceutical companies perform two kinds of trials: renal and non-renal. Those that focus on lupus nephritis have the advantage of hard end points, such as the measurement of protein-to-creatinine ratios, serum creatinine concentrations and glomerular filtration rates, which are not dependent on subjective interpretation, as is the case with non-renal SLE. As discussed elsewhere[26], the use of composite end points, such as the SRI and the BICLA, in addition to the Physicians Global Assessment (PGA) is demanding for clinicians, and whether or not such assessments are better performed without consideration of medication changes is an ongoing debate. Ideally, clinicians who participate in international clinical trials should receive formal training in the use of these disease activity measures and be assessed to ensure that they understand the important principles behind these measures. The addition of an independent review panel (separate from the assessors at individual centres and central monitors) to review the data from different centres on a regular basis throughout the trial should also be encouraged. Such an addition makes it easier to highlight individual centres and clinicians whose disease activity assessment results differ substantially from those of other centres and individuals, and to therefore correct any problems during the trial.

Adverse outcomes

Given the failures of many trials, it is encouraging that pharmaceutical companies are still willing to 'engage' with SLE. As with many new forms of therapy, biologic drugs used to treat patients with SLE are monitored very closely for adverse events, including infection, allergic responses, malignancies and deaths (TABLE 1). In particular, the risk of infection has been a concern in patients with SLE. For example, atacicept (which blocks BAFF and a proliferation-inducing ligand (APRIL)) was first used in a flare prevention study[27]. In this study, patients with active SLE (defined by the presence of one or more BILAG A or B scores) were initially treated with glucocorticoids that were sharply tapered once the active disease had been brought under control and were then treated with either a high (150 mg) or low (75 mg) dose of atacicept or placebo. The aims of this study were to look for the time to first flare and the number of flares in the 1-year follow-up period[27]. However, the safety committee became concerned after two deaths caused by infection in the high-dose group, which was subsequently suspended. Despite this setback, atacicept continued to be developed for SLE and, reassuringly, a trial of 300 patients with active SLE reported no deaths due to atacicept and a serious infection rate of 7% in the placebo group, 8% in the 75 mg atacicept group and 1% in the 150 mg atacicept group[28]. Additionally, a trial of ocrelizumab[29] (an anti-CD20 mAb) was terminated early owing to an increase in the infection rate when combined with MMF; hence, toxicity in patients on background immunosuppressive therapy is an important concern. In the design of future trials, due consideration should be given to the potential for background immunosuppressive therapies to increase the risk of adverse events (particularly infections) when used in combination with the study drug, and thought should be given to how to minimize background therapy where possible.

Glucocorticoid use

The use of glucocorticoids and other immunosuppressive drugs in therapeutic trials in the past 10 years seems to have been liberal. In effect, the consequence has been to raise the bar so high that it has become almost impossible for the test drug to really show its merits. For example, two trials of tabalumab, each involving ~1,100 patients, came to different conclusions regarding the efficacy of the drug, because in one trial[30] the primary end point was not met, whereas in the second study it was[31]. The critical difference between the trials was that, in the first trial[30], a stipulation was included that any alteration in the steroid dose implied a failure of the drug. On reflection, this stipulation meant that, in patients whose disease had improved while taking tabalumab and whose dose of steroids was subsequently reduced, tabalumab was deemed to have failed. Despite setbacks such as these, detailed post-hoc analyses of some trials have revealed encouraging results even when the primary outcomes were not achieved. Clear reporting of concomitant glucocorticoid use and the consideration of necessary deviations from pre-defined dosing

strategies in the final statistical analysis need to be taken into account in the design of future trials.

Promising new therapeutic approaches

The history of SLE therapeutics is littered with agents that seemed promising in preclinical or early-phase clinical studies but then failed in late-phase trials. Although some of the challenges surrounding trial design will have contributed to these failures, the issues involved are complex, and preclinical success does not guarantee success in clinical practice. Likewise, success in a phase II trial does not guarantee success in a phase III trial. The complexity and heterogeneity of the underlying immune dysregulation in SLE probably also contributes to the failure of trials, and targeting particular cytokines or cell-specific pathways within defined patient subgroups will probably be beneficial in the future.

FIGURE 1 shows the targets of interventions aimed at immune cells thought to be involved in the pathogenesis of SLE. Accurately predicting which (if any) of these approaches might ultimately prove to be successful is extremely difficult and, for several therapies, trial results are still awaited (TABLE 2). Given the complex nature of the aetiopathogenesis of SLE, more than one approach will probably be required. Nonetheless, it is hoped that one or more of the agents discussed below will prove successful for patients with SLE.

Targeting B cells

Anti-CD20 antibodies. The high rate of allergy-like responses[19] to rituximab in patients with SLE seems to be related, at least in part, to the fact that rituximab is not fully humanized. A number of alternative, fully humanized, anti-CD20 mAbs are becoming available. Two types of anti-CD20 mAbs (known as type I and type II) have been identified according to various functional properties[32] (TABLE 3).

Ocrelizumab has been studied in two clinical trials in patients with SLE. BEGIN, a phase III study[33] in patients with non-renal SLE, was terminated early when the sponsor decided not to pursue this indication. BELONG, a phase III study in patients with lupus nephritis who were treated with ocrelizumab and either cyclophosphamide or MMF was terminated early owing to a high serious infection rate in patients receiving ocrelizumab and MMF[34]. An assessment of the 32-week data from this trial revealed renal response rates of 63% and 51% in the ocrelizumab and placebo groups, respectively, and an apparent benefit for those patients receiving additional cyclophosphamide[34]. Another fully humanized anti-CD20 mAb, obinutuzumab, induced better B cell

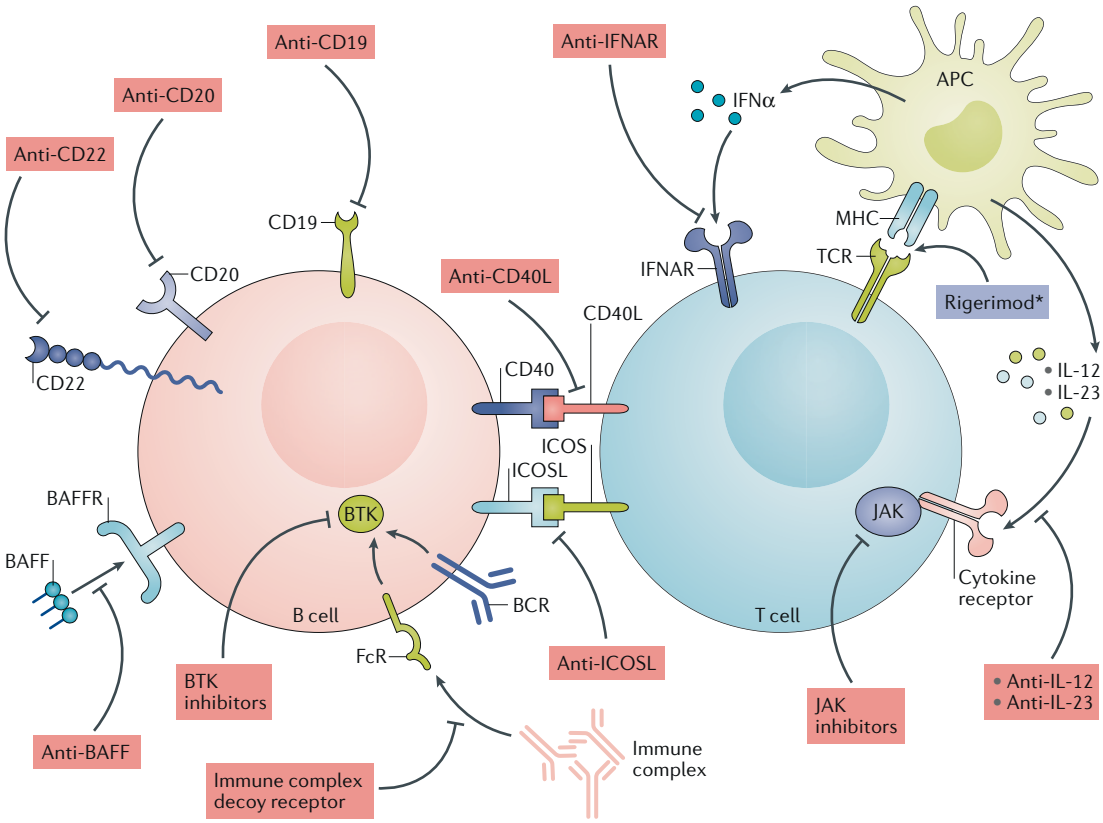

Fig. 1 | **Therapeutic targets in systemic lupus erythematosus.** Various immune cells and molecules interact during the pathogenesis of systemic lupus erythematosus and are the target of monoclonal antibodies and other treatments that have the potential to offer therapeutic advantage. *The mechanism of action of rigerimod is not fully elucidated. APC, antigen-presenting cell; BAFF, B cell-activating factor; BAFFR, BAFF receptor; BCR, B cell receptor; BTK, tyrosine-protein kinase BTK; CD40L, CD40 ligand; FcR, Fc receptor; ICOS, inducible T cell co-stimulator; ICOSL, ICOS ligand; IFNAR, type I interferon receptor; JAK, Janus kinase; TCR, T cell receptor.

Table 2 | **Ongoing clinical trials of new therapies for systemic lupus erythematosus**

Therapy	Target(s)	Trial phase	Status	Size	Primary outcome	Refs
Obinutuzumab	CD20	II	Active, not recruiting	127 participants	Percentage of patients with complete renal response at 52 weeks	36
Combination therapy with rituximab and belimumab	CD20 and BAFF	II	Recruiting	Target of 30 participants	Reduction in disease-relevant autoantibodies at 28 weeks	41
		III	Recruiting	Target of 200 participants	Proportion of patients with a SLEDAI-2K score of <2 without the use of additional immunosuppression	40
		II	Active, not recruiting	Target of 50 participants	Reduction in anti-dsDNA antibodies at 52 weeks	42
GDC 0853	BTK	II	Active, not recruiting	240 participants	SRI-4 response at 48 weeks	49
Dapirolizumab pegol	CD40L	II	Active, not recruiting	182 participants	Proportion of patients with a BICLA response at 24 weeks	59
Anifrolumab	IFNAR	II	Recruiting	Target of 150 participants	Relative change from baseline in urine protein-to-creatinine ratio	82
IFNα kinoid	B cells to stimulate the production of anti-IFNα antibodies	II	Active, not recruiting	178 participants	• Change from baseline in expression of IFN-induced genes at 36 weeks • Treatment response as assessed by BICLA at 36 weeks	85
Baricitinib (BRAVE I)	JAK1 and JAK2	III	Recruiting	Target of 750 participants	Percentage of patients achieving an SRI-4 response at 52 weeks	90
Baricitinib (BRAVE II)	JAK1 and JAK2	III	Recruiting	Target of 750 participants	Percentage of patients achieving an SRI-4 response at 52 weeks	91
Tofacitinib	JAK1 and JAK3	I/II	Complete	34 participants	Safety of tofacitinib in patients with mild-to-moderate disease activity	96
Ustekinumab	IL-12 and IL-23	III	Recruiting	Target of 500 participants	Percentage of patients achieving an SRI-4 response at 52 weeks	95

BAFF, B cell-activating factor; BICLA, BILAG-based Composite Lupus Assessment; BTK, tyrosine-protein kinase BTK; CD40L, CD40 ligand; dsDNA, double-stranded DNA; IFN, interferon; IFNAR, type I interferon receptor; JAK, Janus kinase; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; SRI-4, SLE Responder Index 4.

cytotoxicity than rituximab in patients with RA or SLE[35]. An ongoing phase II trial that is due to last for 1 year aims to investigate the efficacy and safety of this drug in lupus nephritis, with complete renal response as the primary outcome[36]. Although it is unlikely that all of the new anti-CD20 agents will reach the market, ofatumumab (an IgG1)[37] has been approved for the treatment of chronic lymphocytic leukaemia, and has also been used to treat autoimmune haemolytic anaemia and immune-mediated thrombocytopenia, and lupus nephritis[38] in a small number of patients. These agents could have a particular use in patients for whom rituximab has shown efficacy, but allergy-like responses have led to its discontinuation.

Combination rituximab and belimumab therapy. The combination of B cell depletion with rituximab and inhibition of B cell survival with belimumab is based on the premise that the production of BAFF following B cell depletion might facilitate the maturation of autoreactive B cells[39]. Several groups have reported preliminary data from small studies that outline the efficacy of such a strategy. The largest of these studies, the CALIBRATE trial, assessed the effect of rituximab with one pulse of cyclophosphamide followed by monthly intravenous belimumab infusions beginning at 4 weeks ($n = 21$) compared with rituximab and cyclophosphamide alone ($n = 22$) in patients with active lupus nephritis[40]. No significant difference in renal response was noted between the groups, although the addition of belimumab did lead to a delay in B cell repopulation without an increase in hypogammaglobulinaemia[40]. The results of the SYNBIOSE study, an open-label proof-of-concept study using a similar infusion protocol without the additional cyclophosphamide, have also been reported[41]. Clinical improvement was noted in a cohort of previously refractory patients who had improved SLEDAI scores at week 24 (renal responses were noted in 11 out of 16 patients)[41], and the results of phase III studies are awaited. In this study[41], clinical improvement was also mirrored by a reduction in autoantibodies, including anti-double-stranded DNA (dsDNA) antibodies, as well as a reduction in neutrophil extracellular trap formation, a process implicated in SLE pathogenesis. A multi-centre, double-blind, placebo-controlled phase III trial, BEAT-Lupus, investigating the safety and efficacy of starting belimumab 4–8 weeks after rituximab has completed enrolling patients[42]. Given the conflicting evidence to date, further clarity is needed about the usefulness of combination strategies such as this in treating SLE.

Anti-CD19 antibodies. A novel humanized anti-CD19 antibody called obexelimab (XmAb5871) that has been engineered to have an increased affinity for Fcγ receptor IIB (FcγRIIb) has been used to treat SLE in a phase II

Table 3 | **The characteristics of type I and type II anti-CD20 monoclonal antibodies**[32]

Type of antibody	Examples	Redistributes CD20	Internalization of anti-CD20 monoclonal antibody complexes	Complement-dependent cellular cytotoxicity	Antibody-dependent cellular cytotoxicity	Antibody-dependent cell phagocytosis	Method of direct cell death
Type I	Rituximab, ofatumumab, ocrelizumab and veltuzumab	Yes	Yes, but highly variable	Potent	Yes	Yes	Apoptosis
Type II	Obinutuzumab and tositumomab	No	Yes, to a small extent	Weak	Yes	Yes	Non-apoptotic lysosome-mediated cell death

study of 104 patients with moderate-to-severe disease[43]. Low disease activity was first achieved by a short course of disease-suppressing intramuscular steroids, after which background immunosuppression was stopped, and those with the required disease activity improvement were randomly allocated 1:1 to XmAb5871 or placebo. Patients were followed up until day 225, and the preliminary results showed that disease activity levels were maintained with no 'loss of improvement' (defined as an increase in SLEDAI of >4 or a new BILAG A or B score (indicating a substantial increase in disease activity)) in 42% of patients treated with XmAb5871 compared with 23% of patients treated with placebo[43]. Given the clinical success of other B cell-targeting strategies, the results of phase III studies of this agent are awaited with interest.

Targeting BTK. Tyrosine-protein kinase BTK is expressed by many immune cells, including macrophages, monocytes and B cells, and regulates signalling downstream of the B cell receptor, Fc receptors and, possibly, Toll-like receptors[44]. The loss of BTK activity ameliorated lupus-like disease in mice[45], whereas overexpression of BTK in cells from mice with lupus-like disease caused an increase in anti-dsDNA antibody production[46]. A number of BTK inhibitors have been developed, including ibrutinib and GDC-0853. Ibrutinib is an irreversible tyrosine kinase-selective inhibitor that binds to BTK and causes increased B cell apoptosis. A preclinical trial in a mouse model of lupus nephritis[47] showed that ibrutinib treatment reduced the amount of some autoantibodies, including anti-nucleosome antibodies and anti-histone antibodies, but not anti-dsDNA antibodies, and improved renal disease. GDC-0853 (REF.[48]) is currently being used in an ongoing phase II trial of SLE that aims to assess the efficacy and safety of this therapy in patients with a SLEDAI score of >6 (REF.[49]). As with many other agents, confirmation that strong preclinical evidence can translate into clinical success is awaited.

Targeting CD40–CD40L interactions. Interest in CD40–CD40L interactions in the pathogenesis of SLE and the potential to therapeutically target this interaction has been reignited in the past few years. CD40L is a member of the TNF superfamily that engages with its receptor CD40 on B cells, leading to B cell differentiation, isotype switching and the formation of germinal centres[50]. Owing to their centrality in the induction of a robust immune response, CD40–CD40L interactions are thought to be an important mechanism in the development of autoimmunity. In SLE, both $CD4^+$ T cells and $CD8^+$ T cells overexpress CD40L during active disease, and CD40L is also aberrantly expressed by monocytes and B cells from patients with SLE[51]. Moreover, transgenic mice that ectopically express CD40L on B cells develop lupus-like disease[52]. The results of preclinical studies suggest that inhibition of the CD40–CD40L pathway might help to ameliorate lupus-like disease. Specifically, lupus-prone NZB/W mice had delayed onset or prevention of proteinuria, improved survival and less severe renal disease when treated with an anti-CD40L mAb before the onset of symptoms[53].

Unfortunately, initial clinical studies of anti-CD40L mAbs were not promising. Ruplizumab, a humanized anti-CD40L antibody, produced a partial therapeutic response in patients with lupus nephritis in an early-phase, open-label study[54]; however, an increased incidence in thrombosis in patients receiving ruplizumab led to the early termination of this study. Another humanized anti-CD40L mAb, toralizumab, was also used in a phase II study in patients with SLE but produced no statistically significant improvements in disease[55]. Similarly, further development of this agent was stopped owing to increased thrombosis in trials of toralizumab in patients with Crohn's disease[56].

The thromboembolic effects of ruplizumab and toralizumab transpired to be mediated by the Fc portions of these antibodies, resulting in the formation of immune complexes that caused platelet aggregation and activation[57]. Dapirolizumab pegol, a polyethylene glycol-conjugated anti-CD40L Fab fragment, has been designed to circumvent these issues and showed no evidence of prothrombotic effects in preclinical studies. This therapeutic agent was evaluated in a 32-week phase I study[58] of 24 patients with SLE that was primarily designed to explore the safety, tolerability and pharmacokinetics of the repeated intravenous dosing regimen. The results of this study revealed potential improvements in disease activity in patients who had high baseline disease activity scores, although the study was not powered to address this question. Treatment with dapirolizumab pegol resulted in an SRI-4 response in 41.7% of patients with SLE, compared with 14.3% of patients in the placebo group[58]. A higher incidence of non-serious infection was noted in the dapirolizumab pegol group than in the placebo group, but there was no increase in serious infection and, notably, no evidence

of thromboembolism[58]. The initial results of a phase II study[59] have been announced in a press release[60]. Few data have been provided, but the primary end point of establishing a dose response with $P \leq 0.055$ at week 24 was not met ($P = 0.06$), although "strong evidence of histological activity and improvement in the majority of clinical endpoints" was reported in patients given dapirolizumab pegol[60]. The full results of this study and a decision as to whether further trials of this agent will be pursued in SLE are still awaited.

Targeting ICOS–ICOSL interactions. ICOS is a T cell-specific molecule that is expressed on the cell surface upon T cell activation and interacts with ICOSL, which is a constitutively expressed molecule on APCs, including B cells[61]. Functionally, ICOS is a co-stimulatory molecule similar to CD28 that causes T cell activation and contributes to B cell differentiation[61]. Increased numbers of ICOS-expressing T cells and B cells with reduced expression of ICOSL are found in the blood of patients with SLE[62], indicating that T cell–B cell interactions might have just taken place. The results of a phase II trial to assess the safety profile and tolerability of AMG 557, an anti-ICOSL mAb, in patients with mild SLE was reported in 2016 (REF.[63]). AMG 557 had an acceptable safety profile and the anticipated pharmacokinetic profile[63]. Further trials are awaited to assess the clinical efficacy of anti-ICOSL antibody therapy in SLE.

Targeting immune complexes. The Fc region of IgG is recognized by FcγRs, transmembrane proteins that are expressed on B cells and dendritic cells[64]. The binding of immune complexes to FcγRs triggers intracellular signalling pathways, which ultimately causes an immune response. FcγRIIB is an inhibitory receptor, unlike most other FcγR molecules, which tend to be activatory, and is an important regulator of activated B cells. Notably, patients with SLE have a reduced expression of FcγRIIB[65].

FcγRIIB has a limited degree of polymorphism in humans and is not immunogenic. An extracellular version of human FcγRIIB has been developed (known as SM101), which acts as a decoy receptor by binding to immune complexes and thereby preventing FcγR-mediated signalling. In an encouraging 24-week phase IIa trial, 51 patients with SLE were randomly allocated to receive weekly doses of SM101 or placebo for 4 weeks[66]. SLEDAI, BILAG and PGA scores were recorded, as well as global response and renal parameter measurements, even though this was primarily a safety study. No serious unexpected adverse events occurred and the SRI-4 response was doubled in the SM101 group compared with the placebo group; results were particularly encouraging in patients with lupus nephritis[66]. Given the encouraging results of the phase II study, it will be interesting to see if this is a viable agent in phase III studies, particularly for the treatment of renal disease.

Rigerimod. Rigerimod is a therapeutic agent that is theoretically appealing for the treatment of SLE. Rigerimod is a 21-amino-acid linear peptide derived from the small nuclear ribonucleoprotein U1-70K that has the addition of phosphorylation at Ser140 (REF.[67]). Rigerimod causes the depletion of autoreactive T cells via apoptosis without affecting the ability of T cells and B cells to respond to antigens, making it immunomodulatory rather than immunosuppressive, although the mechanism of action is not completely understood. In lupus-prone MRL/lpr mice, rigerimod treatment reduced disease activity (particularly vasculitis, protein excretion and skin disease) and anti-dsDNA antibody production[68]. Phase II clinical studies of rigerimod have shown some promise. In a 2012 phase IIb study, 149 patients with active SLE (SLEDAI-2K score of ≥6, patients with an A score in any BILAG domain excluded at screening) were randomly allocated to receive placebo or subcutaneous rigerimod every 2 or 4 weeks in addition to standard-of-care therapy[69]. 53% of patients treated with monthly rigerimod attained an SRI-4 response at week 12 compared with 36% of those treated with placebo ($P = 0.048$)[69]. A post-hoc analysis of a subpopulation of patients who had a clinical SLEDAI score of ≥6 at baseline revealed an even greater magnitude of response between the monthly rigerimod group and the placebo group ($P < 0.025$)[69]. Similar to belimumab, it seems that the greatest clinical benefit occurs in patients with predominant articular and cutaneous disease. This study[69] also included an analysis at 24 weeks, but the beneficial effects of rigerimod at the end of this additional 12-week treatment-free period were less evident. However, the initial results of a phase III study of rigerimod[70] (reported in a press release)[71] showed that, although rigerimod demonstrated a good safety profile and a superior response rate to placebo (68.8% versus 59%) in the 153 patients who completed the trial (the difference was greatest among anti-dsDNA antibody-positive patients), the difference was not statistically significant. The equivocal and non-significant response to rigerimod in phase III trials means that the usefulness of rigerimod as a treatment for SLE is unclear. Interestingly, an open-label extension of the phase III study was announced in 2018 and is yet to be reported[72].

Targeting the interferon pathway

Many patients with SLE have an increased expression of genes regulated by type I interferons in peripheral blood cells (known as the interferon gene signature), the products of which have diverse effects on the innate immune system and the adaptive immune system[73]. Evidence also exists to support a genetic association between SLE and type I interferon-associated genes[74], and a high prevalence of 'drug-induced SLE' occurs in patients receiving therapeutic IFNα[75]. Together, these findings have promoted a strong interest in developing agents targeting type I interferons for use in SLE. Importantly, although most studies to date have focused on the inhibition of IFNα, the type I interferon family comprises 13 subtypes of IFNα, as well as IFNβ, IFNε, IFNκ and IFNω, which mediate their biological effects by binding to the common type I interferon receptor (IFNAR)[76].

Contrary to expectations, there have been conflicting results from studies of type I interferon pathway inhibition. Rontalizumab and sifalimumab are mAbs that directly inhibit IFNα. In a phase II study of patients with SLE, rontalizumab did not meet the primary or

secondary end points, although the results surprisingly suggested a benefit for patients with a low baseline interferon gene signature in their peripheral blood cells[77]. By contrast, sifalimumab met its primary end point in a phase II study of patients with SLE, and the results suggested a benefit for patients with a high interferon signature; however, the clinical benefits were modest compared with placebo (56% and 58% of patients in the two sifalimumab groups achieved an SRI-4 response compared with 45% of patients in the placebo group)[78].

The fully human IgG1κ antibody anifrolumab antagonizes IFNAR, thereby downregulating the effects of all type I interferons. In a 2017 phase IIb study[79], in addition to standard-of-care therapy, intravenous anifrolumab was superior to placebo in patients with moderate-to-severe SLE treated over a 48-week period. The primary end point of this study was the percentage of patients attaining an SRI-4 response at 24 weeks in addition to a sustained reduction of oral glucocorticoids from weeks 12–24, which was achieved in 34% of patients receiving 300 mg/month anifrolumab compared with 17.6% receiving placebo[79]. The advantage over placebo was less pronounced for patients receiving 1,000 mg/month anifrolumab (28.8% of patients achieved an SRI-4 response), suggesting a possible plateau effect[79]. Similar to sifalimumab, in this study[79], the greatest benefit was noted in patients with a high baseline interferon gene signature; 75% of patients had a high baseline interferon gene signature, and it was the response rate in this subpopulation that caused the difference between the treatment and placebo groups in the study, suggesting that selecting this cohort of patients for treatment with type I interferon inhibition could be beneficial. Similar to other studies of type I interferon inhibitors, an increase in viral infections (particularly herpes zoster infections) was noted in the anifrolumab groups[79], consistent with the mechanism of action of these agents. However, despite the optimism generated by the results of the phase II trial[79], a phase III study (TULIP1)[80] of 463 patients with SLE who have mucocutaneous and/or musculoskeletal disease did not meet its end point of reducing disease activity (SRI-4 response)[81]. A further phase II study specifically addressing the efficacy of anifrolumab in patients with active proliferative lupus nephritis is ongoing[82].

Indirect inhibition of the type 1 interferon pathway by means of an IFNα kinoid vaccine has also been studied in patients with SLE. This vaccine comprises IFNα2b coupled to a carrier protein, which together induce native, polyclonal neutralizing anti-IFNα antibodies[83]. This vaccine substantially reduced the interferon gene signature in patients with SLE in a phase I study[84]. A larger phase IIb study is ongoing to address the efficacy, safety and immunogenicity of this agent in SLE[85].

Targeting the JAK–STAT pathway

The Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway is the primary signalling mechanism downstream of type 1 and type 2 cytokine receptors. Polymorphisms in genes encoding JAK and STAT proteins increase susceptibility to SLE[86], and inhibition of the JAK–STAT pathway is already used to treat many autoimmune diseases (including RA and PsA)[87].

In a preclinical study, tofacitinib (a JAK1 and JAK3 inhibitor) reduced both kidney disease and the concentration of pathogenic autoantibodies in lupus-prone mice[88]. The results of a phase II trial of baricitinib[89] (an oral JAK1 and JAK2 inhibitor) in 314 patients with SLE who have active cutaneous disease or musculoskeletal activity were reported in 2018. 67% of patients receiving 4 mg/day baricitinib achieved a SLEDAI-2K response at 24 weeks, which was significantly more than those receiving placebo (53%; $P = 0.04$)[89]. Treatment with 4 mg/day baricitinib also reduced the proportion of patients with 'worst joint pain' compared with placebo and improved PGA and low disease activity scores; however, the 2 mg/day dose of baricitinib did not show any benefit compared with placebo[89]. The phase III BRAVE I[90] and BRAVE II[91] studies, which aim to assess the effects of baricitinib in patients with SLE, are currently recruiting. Whether JAK inhibitors are more efficacious for non-organ-threatening disease (in particular, active joint disease or cutaneous disease) is unclear, and the results of these studies are keenly awaited.

Targeting IL-12 and IL-23

Blockade of IL-12 and IL-23 is already used to successfully treat psoriasis and PsA[92], and evidence suggests that these cytokines might be involved in some aspects of SLE pathogenesis[93]. The results of a phase II, placebo-controlled trial of ustekinumab[94] (an antibody against IL-12 and IL-23) in 102 seropositive patients with SLE were reported in 2018. All patients had a SLEDAI-2K score of ≥6 and/or two BILAG B scores and were receiving standard-of-care therapy to which was added either a single infusion of intravenous ustekinumab followed by subcutaneous ustekinumab every 8 weeks, or a single infusion of intravenous placebo followed by subcutaneous placebo every 8 weeks[94]. 60% of patients treated with ustekinumab achieved the primary end point of an SRI-4 response at 6 months compared with 31% of the placebo-treated group ($P = 0.0046$)[94], which was a very encouraging result. The risk of a new flare (one BILAG A score or two new BILAG B scores) was significantly lower in the ustekinumab-treated group than in the placebo-treated group ($P = 0.0078$)[94]. Particularly encouraging results were also observed for patients with active cutaneous disease and articular involvement at baseline, and the safety profile of ustekinumab in this study was similar to the safety profile in studies for other indications. Patients are currently being recruited for a phase III study to assess the efficacy of ustekinumab as a therapy for SLE[95], the results of which are required to determine its true potential in the clinic.

Conclusions

The development and implementation of new therapies for SLE has lagged behind that of other rheumatic diseases, but many new molecular pathways and targets have been studied in the past two decades, some of which show promise for SLE. Given the problems encountered in previous clinical trials, most notably those of rituximab, it is clear that the design of trials for

SLE needs to be revisited to decide the most objective indicator of response for this complex condition and to enable a clear distinction between the active treatment and, often quite substantial, background immunosuppression. In this Review, we have highlighted a number of promising targets and pathways but, increasingly, success in phase II trials has not been followed by the achievement of primary end points in phase III trials. In general, clinical trials for SLE should aim to minimize background therapy (particularly glucocorticoids), use individual organ or system outcome measures rather than relying solely on composite measures and have stringent requirements for the selection of trial sites. Such measures would help to maximize the chances of the therapies in development being successful. Although there is room for some optimism, the challenges of bringing successful new biologic therapies into everyday clinical practice for SLE remain daunting.

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Mouse models for human hyperuricaemia: a critical review

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Abstract | Hyperuricaemia (increased serum urate concentration) occurs mainly in higher primates, including in humans, because of inactivation of the gene encoding uricase during primate evolution. Individuals with hyperuricaemia might develop gout — a painful inflammatory arthritis caused by monosodium urate crystal deposition in articular structures. Hyperuricaemia is also associated with common chronic diseases, including hypertension, chronic kidney disease, type 2 diabetes and cardiovascular disease. Many mouse models have been developed to investigate the causal mechanisms for hyperuricaemia. These models are highly diverse and can be divided into two broad categories: mice with genetic modifications (genetically induced models) and mice exposed to certain environmental factors (environmentally induced models; for example, pharmaceutical or dietary induction). This Review provides an overview of the mouse models of hyperuricaemia and the relevance of these models to human hyperuricaemia, with an emphasis on those models generated through genetic modifications. The challenges in developing and comparing mouse models of hyperuricaemia and future research directions are also outlined.

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Hyperuricaemia is a metabolic disorder characterized by an excessively increased serum urate concentration that can occur as a result of overproduction and/or gut underexcretion and/or renal underexcretion of urate[1,2] (FIG. 1). Hyperuricaemia is the major risk factor for the development of gout[3] and is associated with multiple metabolic disorders including diabetes, hypertension, atherosclerosis and renal disease[4,5]. Hyperuricaemia is common, and although the prevalence has been increasing globally[6–9], there are some indications that the prevalence is stabilizing[10]. For example, the US National Health and Nutrition Examination Survey reported a prevalence of hyperuricaemia of 19.1% in 1988–1994 (REF.[7]), 25.1% in 2007–2008 (REF.[7]) and 20% in 2015–2016 (REF.[10]).

Hyperuricaemia and gout occur mainly in higher primates, including in humans, because of inactivation of the gene encoding uricase (also known as urate oxidase), the first in a sequence of three enzymes that metabolize urate into water-soluble allantoin (FIG. 1). During the course of primate evolution, multiple independent mutations in the promoter region and later in the protein coding sequence of this gene led to gene silencing[11–13]. The gradual loss of uricase might have enabled the ancestors of higher primates to gradually adapt to major biological changes caused by the loss of uricase (such as acute urate nephropathy and renal failure, which occur in uricase-deficient mice)[14]. Various human physiological processes have been attributed to increased urate levels, including antioxidant and neuroprotection effects, maintenance of blood pressure and stimulation of the innate immune system[11,15,16].

Serum urate is regulated by genetic, intrinsic and environmental (extrinsic) factors and interactions between these factors[1,17,18] (FIG. 1). Genetic studies, particularly genome-wide association studies (GWASs), have identified ~40 loci that are reliably associated with serum urate concentrations in humans[19–21]. These loci jointly explain ~7% of the variation in serum urate concentrations, of which ~4% is accounted for by two major loci: *SLC2A9* and *ABCG2* (REF.[20]). Some of the associated loci include genes encoding urate transporters, such as *SLC2A9*, *ABCG2*, *SLC17A1*, *SLC22A11*, *SLC22A12*, *SLC16A9* and *PDZK1* (REFS[17,18]). With the exception of *ABCG2* (REF.[22]) and *PDZK1* (REF.[23]), the causal variants underlying these associations have not been identified and the genetic architectures underlying hyperuricaemia and gout, including in particular the progression from hyperuricaemia to gout[17], remain largely obscure (BOX 1).

Mouse models are being developed to help dissect the regulatory mechanisms underlying human hyperuricaemia. In this Review, we summarize the properties of mouse models published for hyperuricaemia and their relevance for human hyperuricaemia, and we discuss the advantages and shortfalls of these models. We focus on the challenges of using mouse models of hyperuricaemia, including the high mortality and difficulty of

Key points

- Hyperuricaemia occurs mainly in higher primates, including in humans, primarily owing to inactivation of the uricase gene during primate evolution, which resulted in subsequent evolution of human-specific physiology to tolerate this inactivation.
- Mouse models of hyperuricaemia have been widely used to provide valuable insights into urate biology but do not yet reliably and consistently simulate the urate-mediated hyperuricaemia that occurs in humans.
- Such models are potentially valuable resources for dissecting the mechanisms underlying hyperuricaemia as well as the progression from hyperuricaemia to gout and associated comorbidities.
- A key challenge is to develop uricase-disabled model mice that can survive with increased urate levels and remain healthy and fertile.
- Community-wide efforts are needed to reach consensus about the definition of hyperuricaemia in mice, to develop protocols for generating suitable models of hyperuricaemia and to adhere to a standard protocol for urate measurements.

comparing urate levels between models. Finally, we suggest approaches for developing a suitable mouse model to replicate hyperuricaemia in humans.

Mouse models of hyperuricaemia

Mouse models of hyperuricaemia can be divided into two main categories: mice with genetic modifications that result in hyperuricaemia (genetically induced models) (TABLE 1) and mice that have been exposed to environmental factors that induce hyperuricaemia (environmentally induced models). Several features should be considered when assessing data from mouse models of hyperuricaemia. A widely used definition of hyperuricaemia in humans is a serum urate concentration above 6.8 mg/dl (408 μmol/l), at which point monosodium urate (MSU) crystals form in vitro at pH 7.0 and temperature 37 °C (REF.[24]). However, the circulating urate concentrations in wild-type mice are much lower than in humans, and the appropriate urate concentration for defining 'hyperuricaemia' in mice is unknown. The reported serum urate concentrations in different mouse models vary (TABLE 2). Notably, genes associated with hyperuricaemia are expressed differently across human and mouse tissues (TABLE 3). For example, the gene that encodes uricase, *UOX*, is silenced in humans[13], but the mouse orthologue, *Uox*, is expressed in multiple tissues in mice (including at high levels in the liver); *SLC2A9* is expressed mainly in the kidneys in humans, but the mouse orthologue *Slc2a9* is expressed primarily in the liver; and *ABCG2* is expressed mainly in the small intestine in humans, but the mouse orthologue *Abcg2* is expressed primarily in the kidneys.

Genetically induced mouse models

Uricase-related models. Given that the gene encoding uricase is inactivated in humans but not in mice, genetic modification of the mouse orthologue *Uox* was an obvious target to generate a 'human-like' model to study hyperuricaemia. Researchers have used two *Uox*-knockout approaches to generate mice with markedly high serum urate concentrations.

Wu et al.[14] generated the first *Uox*-knockout model ($Uox^{-/-}$), in which disruption of the *Uox* gene was mediated by insertion of a neomycin selection cassette into exon 3 of *Uox* (FIG. 2), shifting the open reading frame of the gene. This cassette was inserted in embryonic stem cells (ESCs) derived from 129/SvEv mice, which (after homologous recombination and ESC selection) were then injected into C57BL/6J mouse blastocysts to create chimaeras; the $Uox^{-/-}$ mouse line was established by mating heterozygous $Uox^{+/-}$ mice, resulting in mice with a hybrid genetic background (C57BL/6J*129Sv). The average serum urate concentration of the $Uox^{-/-}$ mice was 11.0 ± 1.7 mg/dl, which is ~12 times higher than that in the wild-type controls (0.9 ± 0.3 mg/dl). The $Uox^{-/-}$ mice were viable and fertile but had a high mortality of 65% at 4 weeks of age because of severe nephropathy (including uric acid crystal deposition, multiple cysts, tubular atrophy and collapse of the nephron). Furthermore, the percentage of the $Uox^{-/-}$ mice born from the heterozygous mating was 7.1%, a percentage far below the expected rate of 25%, suggesting that the $Uox^{-/-}$ genotype is associated with embryonic lethality.

Using the transcription activator-like effector nuclease (TALEN) technique to delete a region of 28 base pairs in exon 3 of the *Uox* gene, Lu et al.[22] generated another *Uox*-knockout mouse model on a C57BL/6J background[25]. These $Uox^{-/-}$ mice had moderately increased serum urate concentrations that were similar to those in humans with hyperuricaemia (that is, 8.7 ± 2.3 mg/dl in males and 7.1 ± 1.6 mg/dl in females), and ~40% of the mice survived up to 62 weeks. The rate of live births of $Uox^{-/-}$ mice from heterozygous mating was 15.9% (55 out of 345), which was much lower than the expected Mendelian frequency of 25%, reiterating the issue of embryonic lethality. The mice developed renal dysfunction including uric acid crystal deposition and glomerular or tubular lesions. The male $Uox^{-/-}$ mice also developed metabolic disorders associated with compromised insulin secretion and increased susceptibility to streptozotocin-induced diabetes, whereas the female mice developed hypertension accompanied by aberrant lipo-metabolism.

In addition to these two knockout models, researchers have also generated another uricase-related mouse model using caesium irradiation to induce a paracentric inversion (In(3)55Rk) on mouse chromosome 3, which resulted in a mutation in *Uox* and uricase deficiency (referred to as $Uox^{In/In}$ mice)[26]. Mice with homozygous inversions had extremely high serum urate concentrations: 21.1 ± 8.0 mg/dl in males and 23.2 ± 9.0 mg/dl in females. These mice had nephropathy with glomerular and tubular dilatation; 63% of mice died by 14 days of age, but those mice that survived to adulthood generally lived a normal breeding lifespan[26]. Compared with the specifically engineered *Uox*-knockout mice[14,25], the $Uox^{In/In}$ mice have gross genetic changes, with the paracentric inversion probably disrupting the 3D nuclear structure and possibly having other nonspecific effects on urate metabolism[26].

SLC2A9-related models. In humans, *SLC2A9* encodes a transporter (solute carrier family 2, facilitated glucose transporter member 9, SLC2A9; also known as GLUT9) that functions as a major urate reabsorption transporter in the kidney[27,28]. Genetic variation in *SLC2A9*

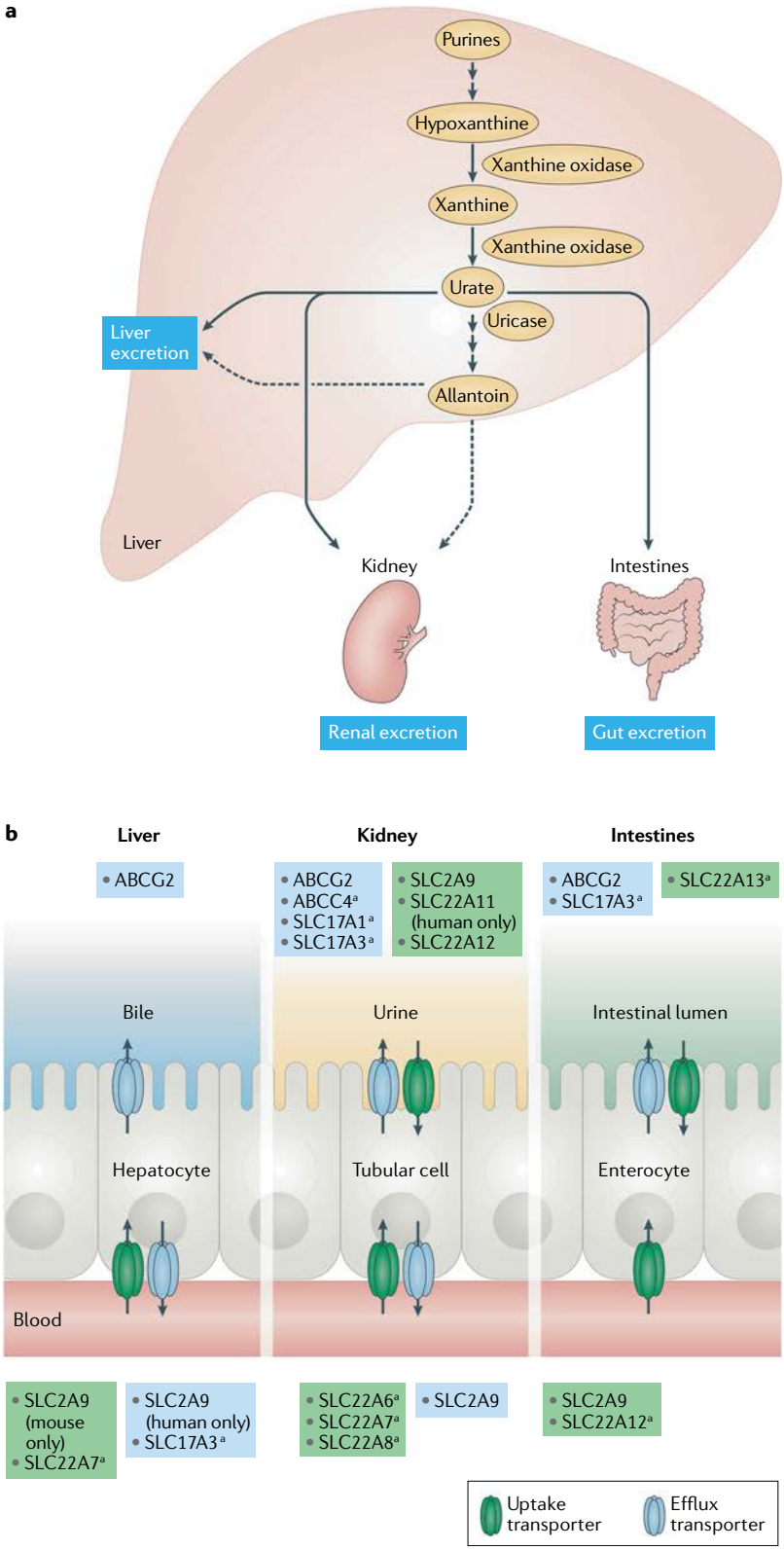

Fig. 1 | **Urate metabolism in mammals. a** | Urate (in humans) and allantoin (in mice) are the end products of the purine metabolism pathway in hepatocytes of the liver. Urate can be excreted by the liver (in bile) or pass through the circulation and be excreted by (most commonly) the kidneys (in urine) or the intestines. **b** | Urate transporters enable the transport of urate into cells (uptake transporters) or out of cells (export transporters) during urate excretion or re-uptake. [a]Expressed in humans, but the expression in mice is unknown.

is strongly associated with serum urate concentrations, explaining ~3% of variance in phenotype[20], an extremely large effect size for a locus associated with a complex (polygenic) phenotype such as urate levels. Researchers have generated a total *Slc2a9*-knockout mouse model (G9KO) and a liver-specific *Slc2a9*-knockout model (LG9KO) on a mixed C57BL6/J and C57BL6/N genetic background[29]. The rate of live births of G9KO mice was approximately half of the expected Mendelian frequency, indicating some embryonic lethality in the absence of SLC2A9. Compared with the average plasma urate concentrations of wild-type mice (0.4-0.8 mg/dl), all the *Slc2a9*-knockout mice had increased plasma urate concentrations: 1.5 mg/dl and 1.8 mg/dl in male and female G9KO mice, respectively, and 2.0 mg/dl and 3.1 mg/dl in male and female LG9KO mice, respectively. The G9KO mice had early-onset nephropathy because of renal structural abnormalities that became apparent from 2 weeks of age. The LG9KO mice were free from urate nephropathy and structural abnormalities in the kidneys but had higher serum urate concentrations that were attributed to SLC2A9-mediated re-uptake of urate in the kidney, which did not occur in G9KO mice[29]. Additional increases of serum urate concentrations caused by inosine administration and high-fat diets in the LG9KO mice induced chronic inflammation and acute renal failure[30] but not hypertension[31]. These data emphasized the important function of SLC2A9 in urate homeostasis in mice via uptake in the liver.

In addition to the liver and kidneys, SLC2A9 is also expressed on the apical and basolateral gut enterocyte membranes in mice[32]. Gut enterocyte-specific *Slc2a9*-knockout mice (G9EKO) have been generated by crossing mice harbouring a floxed *Slc2a9* allele with mice overexpressing Cre recombinase driven by the enterocyte-specific *Vil1* promoter on a C57BL/6 background[32]. The rate of live births of G9EKO mice followed the expected Mendelian ratios and the mice were fertile and had moderately increased serum urate concentrations (2.9 mg/dl) compared with age-matched wild-type mice (2.3 mg/dl)[32]. The G9EKO mice had impaired enterocyte urate transport kinetics and resulting metabolic syndrome, including spontaneous hypertension, dyslipidaemia and increased body fat. These findings support an important function for gut enterocytes in regulating urate and whole-body metabolism.

A kidney-specific *Slc2a9*-knockout mouse model (kiKO) on a C57BL/6N background has also been generated using a tetracycline-inducible system in which *Slc2a9* is specifically deleted along the entire nephron[33]. The kiKO mice were morphologically identical to their wild-type littermates and had no spontaneous kidney stones. The urinary excretion rate and the fractional excretion of urate were both approximately tenfold higher in kiKO mice than in wild-type mice. Interestingly, the mice did not have hypouricaemia (as is observed with inactivating mutations of *SLC2A9* in humans[34]) and had unchanged plasma urate concentrations. This finding is an important distinction from the other *Slc2a9*-knockout models in which urate concentrations were increased. The reason for this difference is unclear; the unchanged urate concentrations in kiKO

Box 1 | Progression from hyperuricaemia to gout

Gout is a painful inflammatory arthritis that affects 10–20% of people with hyperuricaemia[115]. Three pathophysiological stages are required for the development of gout: hyperuricaemia, deposition of monosodium urate (MSU) crystals and innate immune responses to MSU crystals (known as flares). Progression through each of the stages is complex and is regulated by inherited genetic variation and environmental exposures that are not well understood. Central to gout flares is the production of bioactive IL-1β through activation of the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome by MSU crystals and a poorly understood Toll-like-receptor-mediated second signal[116]. The flare is self-limiting and its resolution involves the neutrophil extracellular trap apparatus[117]. Furthermore, given the important functions of urate and the innate immune system in human metabolism and other biological processes, post-hyperuricaemia progression to gout could causally contribute to other metabolic comorbidities such as diabetes and cardiovascular diseases, although there is little evidence for a causal relationship between hyperuricaemia and metabolic comorbidities[38]. Understanding the progression trajectories and underlying mechanisms is critical to develop better treatment of gout and prevention strategies.

mice could be explained by compensatory maintenance of urate homeostasis by other kidney urate transporters and/or background *Slc2a9*-knockout in the liver[33], which would be predicted to increase the circulating urate concentrations. Furthermore, SLC2A9 is expressed in different places in the kidney: SLC2A9 is expressed in the distal convoluted tubule in mice, whereas SLC2A9 is expressed exclusively in the proximal tubule in humans, which could contribute to the unexpected findings.

In humans, establishing whether soluble circulating serum urate and hyperuricaemia has a causal relationship with metabolic comorbidities has been of much interest in the past decade[35,36]. The balance of evidence, including the fact that (by Mendelian randomization) genetic variation in *SLC2A9* does not associate with other metabolic conditions[37], suggests that there is no direct causal relationship with hyperuricaemia[38]. However, the development of a metabolic syndrome-like phenotype in the SLC2A9-deficient G9EKO model complicates this notion. Data from the various animal models suggest that SLC2A9 has organ-specific effects, whereas data from human epidemiological and Mendelian randomization studies evaluate circulating serum urate, the concentrations of which will reflect the activity of SLC2A9 in multiple organs. The different relative levels of SLC2A9 expression in various tissues between humans and mice also complicate the comparison (TABLE 3).

ABCG2-related models. In humans, *ABCG2* encodes a secretory urate transporter that regulates urate excretion primarily in the gut[2,39,40]. Data from *Abcg2*-knockout mice (*Abcg2*$^{-/-}$) have provided important insights into urate excretion in the intestinal tract as an 'extra-renal' urate excretion pathway[2,41]. For example, male *Abcg2*-knockout mice treated with oxonate (a urate oxidase inhibitor[42]) have an increased amount of renal urate excretion but a reduced amount of gut urate excretion compared with wild-type mice, resulting in higher serum urate concentrations (2.8 mg/dl) than in wild-type mice (2.2 mg/dl)[2]. In humans, inactivation of ABCG2 transport activity and blockage of gut excretion caused by polymorphisms in *ABCG2* (Q141K and Q126X) leads to increased renal urate excretion because of an overload of the renal excretion machinery in some[2,43] but not all[20,44] studies.

Data from mice with the *Abcg2* polymorphism Q140K (equivalent to the risk variant Q141K in humans) have provided insights into the relationship between ABCG2-mediated gut excretion of urate and renal excretion of urate[45]. In Q140K-*Abcg2* mice, which were generated using the CRISPR–Cas9 system on a C57BL/6 background, serum urate concentrations are increased by ~50% compared with wild-type mice (TABLE 2). However, the fractional excretion of urate was unchanged despite an ~50% (albeit statistically nonsignificant) compensatory increase in total urinary excretion of urate. The unchanged fractional excretion of urate conflicts with the data from *Abcg2*-knockout mice[2], potentially because of the differing genetic backgrounds (FVB mice versus C57BL/6 mice) and/or the additional use of potassium oxonate treatment in the *Abcg2*-knockout mice and/or the lack of expression of ABCG2 in the kidneys of *Abcg2*-knockout mice. Interestingly, in Q140K-*Abcg2* mice, the expression and function of ABCG2 is lost in the gastrointestinal tract, in a sex-specific manner (that is, the loss is greater in males than in females), but not in the kidneys[45]. These findings emphasize the gut as an important site of urate excretion regulation.

Other genetically induced models. Additional mouse models have been generated by genetically modifying mouse orthologues of genes that are associated with hyperuricaemia in humans[20,46,47]. For example, the hyperuricaemia-associated gene *SLC22A12* encodes a urate transporter (SLC22A12; also known as URAT1) expressed in the kidney that reabsorbs urate from the filtered urine. *Slc22a12*-knockout mouse models have been generated on a C57BL/6 background (Eraly et al.[48]) or on a C57BL/6J*129Sv background (Hosoyamada et al.[49]), but neither resulted in changes of serum urate concentrations, although the fractional excretion of urate was higher in the knockout mice than in the wild-type mice in both models[48,49]. Mice that are deficient in both UOX and SLC22A12 (*Uox*$^{-/-}$*Slc22a12*$^{-/-}$ mice) have similar serum urate concentrations to *Uox*-knockout mice[50]. Following treatment with the urate-lowering drug allopurinol, an inhibitor of xanthine oxidase (the enzyme that catalyses the xanthine to urate reaction), the serum urate concentration of *Uox*$^{-/-}$*Slc22a12*$^{-/-}$ mice was ~50% lower than that in *Uox*-knockout mice[50]; hence, the urate-lowering efficiency of allopurinol was doubled in the absence of SLC22A12. This finding suggests a function for SLC22A12 in regulating bioactive levels of allopurinol and/or the active metabolite of allopurinol (oxypurinol).

Other genetically induced mouse models relating to hyperuricaemia-associated genes, including *Pdzk1* (REFS[51,52]), *Aldh16a1* (REF.[53]), *Abcc4* (REFS[54,55]) and *Maf*[56–58], have been generated to study gene or renal function or for other purposes. The serum urate concentrations of these mice have not been reported; however, with respect to renal function, a naturally occurring mutation in the DNA-binding domain of the transcription factor MAF causes renal tubule nephritis[58].

Mendelian ratios
The expected ratios of genotypes at a locus observed in offspring under Mendel's law of independent assortment; if one allele is embryonically lethal, the ratio will be skewed.

Mendelian randomization
The use of genetic variation in genes of known function to examine the causal effect of an exposure on disease in observational studies.

Researchers have also studied renal function of models of other diseases that have a hyperuricaemic phenotype. A mouse model of Lesch–Nyhan syndrome (also known as juvenile gout, caused by deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT1; also known as HGPRT), an enzyme involved in the purine salvage pathway[59]) has been generated on a B6.129P2 background by deleting the promoter and first two exons of *Hprt1*. In this model, the serum urate concentrations of the knockout mice were ~1.25 mg/dl, which was approximately threefold higher than in wild-type mice[60]. The mice also developed nephropathy, inflammatory and fibrotic changes of the renal interstitium and renal xanthine crystals, consistent with the phenotype of Lesch–Nyhan syndrome in humans.

In humans, mutations in *UMOD* (the gene encoding uromodulin; also known as Tamm-Horsfall protein) cause a group of autosomal dominant kidney diseases characterized by juvenile-onset hyperuricaemia, gout and progressive kidney failure. The introduction of a hyperuricaemia-causing mutation in transgenic mice (referred to as THP-C217G mice) caused not only kidney fibrotic structural changes but also upregulation of SLC22A12 expression in the proximal tubule[61]. Upregulation of SLC22A12 and increased renal uptake of urate could contribute to the higher urate concentrations observed in this mouse model.

Finally, the enzyme 5-hydroxyisourate hydrolase (HIU hydrolase) catalyses the second step of the conversion of urate into allantoin in most vertebrate species (although this enzyme is inactive in humans)[62]. A p.Tyr98Cys mutation in exon 3 of the gene encoding mouse HIU hydrolase (*Urah*) prevents the expression of HIU hydrolase and results in hepatomegaly and hepatocellular carcinoma in mice. Mice homozygous for the mutation ($Urah^{Plt2/Plt2}$ mice) develop increased serum urate concentrations and platelet counts[63].

Environmentally induced mouse models

Chemical inhibition of uricase has been a popular approach used to generate mice with increased serum urate concentrations. Potassium oxonate, a selectively competitive uricase inhibitor, blocks the effect of hepatic uricase. Oral administration of oxonate (250 mg/kg per day) in mice results in 1.5-fold to 2.1-fold increased concentrations of serum urate within 7 days, with accompanying urate nephropathy[64–67]. However, the various inhibitor doses, induction periods, administration methods and technical issues with measuring serum urate concentrations[68] across different studies make the resultant models virtually impossible to compare[69–73].

Modification of the diet is also widely used to induce increased serum urate concentrations in mice, especially in the context of other comorbidities associated with hyperuricaemia. For example, the effect of high-fat diets on serum urate concentrations in mice has been examined in several studies[31,74–76]. In one study, male *Nlrp3*-knockout mice and wild-type mice were fed with a control diet (11% energy in the form of fat), a western diet (43% energy from fat and 0.15% from cholesterol) or water with 15% fructose for 16 weeks[76]. Deficiency of NOD-, LRR- and pyrin domain-containing 3 (NLRP3) disrupts the formation of the NLRP3 inflammasome, preventing the production of bioactive IL-1β. Plasma urate concentrations in the wild-type mice increased from ~0.7 mg/dl on the control diet to 1.7 mg/dl on the fructose diet and 2.0 mg/dl on the western diet, whereas these effects were completely diminished in *Nlrp3*-knockout mice, suggesting that NLRP3 is an important modulator for diet-induced hyperuricaemia and nephropathy (including renal inflammation and fibrosis)[76]. On the basis of the clinically established situation in humans in which nephropathy causes hyperuricaemia (as evidenced, for example, by the phenotype of individuals with kidney disease caused by mutations in *UMOD*), nephropathy is speculated to cause the hyperuricaemia in the *Nlrp3*-knockout model. In a study of the relationship between hyperuricaemia and non-alcoholic fatty liver disease, supplementation of a high-fat diet in mice for 8 weeks to induce non-alcoholic fatty liver disease induced the expression of hepatic xanthine oxidase at both the mRNA and protein levels without changing the expression and activity of uricase, increasing the serum urate concentrations from 2.0 to 3.0 mg/dl (REF.[74]). Similar trends were observed in a different non-alcoholic fatty liver disease model induced by a methionine-deficient and choline-deficient diet for 2 weeks[75].

Hyperuricaemia-prone mouse strains

Some mouse strains that have been engineered for a separate purpose, such as to study other metabolic phenotypes, also have increased urate concentrations. In L-$G6pc^{-/-}$ mice (mice with a liver-specific deficiency of the catalytic subunit of glucose-6-phosphatase enzyme), a model of glycogen storage disease type 1a, serum urate concentrations increase during the first month after gene deletion and then decrease to wild-type levels after 6 months[77]. KK-A^{y}/Ta mice, a model of type 2 diabetic nephropathy[78], develop hyperuricaemia and have been proposed as a model of hyperuricaemia[79]. Obese mice, such as $Lepr^{db\text{-}db}$ mice, have increased serum urate concentrations[80]. However, although mice that are obese owing to a high-fat diet have increased subclinical inflammation orchestrated by non-adipose tissue-resident macrophages, this pro-inflammatory environment does not exacerbate MSU crystal-induced inflammation[81]. New insights might be gained from generating new mouse models of hyperuricaemia by crossing genetically induced models of obesity (for example, *ob/ob* mice) with existing hyperuricaemia models (TABLE 1) to possibly accelerate the development of hyperuricaemia. The effect of diet on existing genetically induced mouse models of hyperuricaemia is also worth noting. For example, LG9KO mice had a normal kidney phenotype with a standard-chow diet but developed nephropathy after inosine administration and a high-fat diet[30].

Mouse models of spontaneous gout

No mouse model of spontaneous gout currently exists, despite the availability of mice with stable and increased serum urate concentrations. The limited lifespan of mice might hinder developing such a model as gout is a chronic metabolic disease and MSU crystal

Table 1 | **Genetically induced mouse models of hyperuricaemia: mouse phenotypes**

Name	Modification	Background	Extended phenotype	Survival profiles	Refs
Uox$^{-/-}$ (Wu et al.[14])	*Uox* knockout	• C57BL/6J • 129Sv	• Nephropathy with renal uric acid crystals • Hyperuricosuria • Decreased urinary allantoin • Decreased urine osmolality • Urate stones in dilated bladder • Copious pale urine • Moderate azotaemia • Exploratory and novelty-seeking behaviour	65% died by 4 weeks	14,110,120
Uox$^{-/-}$ (Lu et al.[25])	*Uox* knockout	C57BL/6J	• Nephropathy with renal uric acid crystals • Hypertension • Metabolic disorders • Vulnerability to streptozotocin-induced diabetes • Aberrant lipid metabolism • Increased concentrations of inflammatory response proteins in atherosclerosis • Prone to carotid atherosclerosis when collared	~40% died by 5 weeks	25,121
Uox$^{In/In}$	Paracentric inversion (In(3)55Rk) on chromosome 3 (including *Uox*)	• DBA2J • C57BL6/J	• Nephropathy with chronic polyuria • Ammonium and potassium urate calculi in urine sediment	63% died postnatally at 12–14 days; surviving adults had normal breeding lifespan	26
G9KO	*Slc2a9* knockout	C57BL6/J C57BL6/N	• Nephropathy with renal uric acid crystals • Hyperuricosuria • Polyuria • Increased 24 h water intake • Increased sodium concentration in urine • Increased fractional excretion of urate • Decreased urine osmolality • Lower spot urine pH • Normal body weight	Birth rate was half of the expected Mendelian ratio	29
LG9KO	Liver-specific *Slc2a9* knockout	C57BL6/J C57BL6/N	• Nephropathy and renal uric acid crystals when fed on high-fat diet • Hyperuricosuria • Polyuria • Decreased urine osmolality • Increased fractional excretion of urate • No hypertension	No information	29,30
G9EKO	Enterocyte-specific *Slc2a9* knockout	C57BL/6	• Nephropathy • Increased lipids and body fat • Hyperinsulinaemia; hypertension • Increased hepatic triglyceride • A trend towards increased collagen type 1α and fatty acid synthase • Increased basal heart rate • Decreased diastolic left ventricular internal diameter with increased relative wall thickness	Expected postnatal Mendelian ratio	32
kiKO	Inducible, kidney-specific *Slc2a9* knockout	C57BL6/N	• Polyuria without renal morphological changes • Normal serum urate concentrations • Decreased urine osmolality • Decreased blood pressure with increased heart rate	No information	33
Abcg2$^{-/-}$	*Abcg2* knockout	FVB.129P2	• Increased renal fractional urate excretion • Decreased intestinal urate excretion	No information	2,41
Q140K-*Abcg2*	Glutamine-to-lysine point mutation at position 140 of *Abcg2*	C57BL/6	Decreased intestinal urate excretion	No information	45
Slc22a12$^{-/-}$ (Hosoyamada et al.)	*Slc22a12* knockout	• C56BL/6J • 129Sv	• Grossly normal • Increased urinary urate to creatinine ratio • Higher fractional excretion of urate	No information	49
Uox$^{-/-}$*Slc22a12*$^{-/-}$	*Slc22a12* and *Uox* knockout	• C57BL/6J • 129Sv	• Increased urinary urate excretion compared with *Uox*$^{-/-}$ mice • No difference in plasma urate concentration compared with *Uox*$^{-/-}$ mice	No information	50

Table 1 (cont.) | **Genetically induced mouse models of hyperuricaemia: mouse phenotypes**

Name	Modification	Background	Extended phenotype	Survival profiles	Refs
Slc22a12$^{-/-}$ (Eraly et al.)	*Slc22a12* knockout	C56BL/6J	• Grossly normal • Increased urinary urate excretion • No difference in plasma urate concentration	No information	48
Pdzk1$^{-/-}$	*Pdzk1* knockout	129SvEv	• Grossly normal • Fecund • Increased serum cholesterol concentrations • Defect in basal net sodium absorption in small intestinal epithelium	No information	51,52
Hprt$^{-/-}$	*Hprt* knockout	B6.129P2	• Nephropathy with liver showing yellowish appearance • Inflammatory and fibrotic changes of the interstitium • Xanthine tubular crystals	No information	60
THP-C217G	A cysteine-to-glycine point mutation at position 217 of the gene encoding uromodulin (also known as THP) (transgenic mice)	FVB/N	• Polyuria with marked oliguria • Fibrotic kidneys • Reduced creatinine clearance • Increased serum urate and allantoin concentrations	No information	61
Urah$^{Plt2/Plt2}$	A tyrosine-to-cysteine point mutation at position 98 of *Urah*	C57BL/6	• Increased platelet counts • Hepatocellular carcinoma	No information	62,63

growth requires sufficient urate load[24] (and is hence a concentration-dependent and time-dependent process). Injections of MSU crystals in mice can elicit an immune response in an NLRP3 inflammasome-dependent and IL-1β-dependent manner[82]. Hence, commonly used mouse models of gout currently focus on acute inflammatory responses induced by exogenous MSU crystal injection[83–86]. Crucially, none of these mouse models were hyperuricaemic, which would be the basis for a model of spontaneous gout, not only to study MSU crystal formation but also to study immune hyperresponsiveness to MSU crystals, as is observed in humans[87]. Spontaneous deposition of MSU crystals in the joints is also an important feature of gout models, which is not observed in current mouse models of hyperuricaemia (BOX 2). MSU crystal deposition would require suitable conditions (such as a suitable local temperature, pH, ion concentration and connective tissue factor concentration as well as a sufficient time period of high urate load). The development of a mouse model of spontaneous gout presents a difficult challenge.

Other animal model species

Although this Review focuses on mouse models of hyperuricaemia, other animal models of hyperuricaemia could also be considered. Administration of a uricase inhibitor (oxonic acid or potassium oxonate) either by diet supplementation[88] or intraperitoneally[89,90] is a commonly used method to induce mild hyperuricaemia in rats. Zebrafish are increasingly being used to model human diseases owing to their short breeding cycle and genomic homogeneity with humans. The zebrafish has already been used for investigating the molecular mechanisms underlying the association between some genetic variants and urate regulation[23,91]. For example, zebrafish models of hyperuricaemia have been generated by treating larvae with potassium oxonate and xanthine sodium salt[92] or introducing *uox* mutations[93]. The zebrafish model has also been used as a model of acute gouty inflammation by injecting MSU crystals into 2-day-old larvae[94]. As in humans, the gene encoding uricase is also inactive in avian species[95]. In Korean native broilers, exposure to excess sodium bicarbonate in drinking water causes MSU crystal deposition in the joints, muscles and kidney and can also lead to an articular gout-like phenotype[96]. Other avian models of hyperuricaemia have been described, including red-tailed hawks (who become hyperuricaemic after feeding)[97] and quail (fed a purine-rich diet)[98]. However, in addition to the practical and economic difficulties of avian models, the unstable urate levels and fundamental biological differences with humans hamper their wide application. As the tree shrew (*Tupaia belangeri*) is closely related to non-human primates, there is interest in using these small mammals as an alternative animal model in human medical research[99]. Interestingly, intraperitoneal treatment with potassium oxonate induces acute hyperuricaemia in the tree shrew[100]. Whether the tree shrew can be used to model spontaneous gout remains to be seen. In pigs, jugular injection of urate induces hyperuricaemia[101] — in this model, urate is excreted via the gut. Although not a research model of gout, gout can occur in the Dalmatian dog breed and is caused by variations in *SLC2A9* (REF.[102]). Finally, skeletal evidence suggests that tyrannosaurs had gout[103], although, even if it became possible to resurrect tyrannosaurs in the future[104], they are unlikely to be used to model gout.

Challenges of hyperuricaemia models

Serum urate concentrations

Measuring the serum urate concentrations of mice can be challenging. Notably, one study that reviewed the urate concentrations of mice across 103 studies (published between 1949 and 2014) reported an extremely

Table 2 | **Genetically induced mouse models of hyperuricaemia: serum urate measurements**

Name	Mean serum urate concentration in mouse model (mg/dl)	Mean serum urate concentration in wild-type mice (mg/dl)	Urate measured in living or dead animal?	Plasma and serum separated immediately?	Refs
Uox$^{-/-}$ (Wu et al.[14])	11	0.9	Not reported	Not reported	14,110,120
Uox$^{-/-}$ (Lu et al.[25])	• 8.7 (male) • 7.1 (female)	• 2.6 (male) • 2.5 (female)	Living	No	25,121
Uox$^{ln/ln}$	• 21.1 (male) • 23.2 (female)	<5.0	Not reported	Not reported	26
G9KO	• 1.5 (male) • 1.8 (female)	0.8	Living	Not reported	29
LG9KO	• 2.0 (male) • 3.1 (female)	• 0.4 (male) • 0.6 (female)	Living	Not reported	29,30
G9EKO	2.9	2.3	Not reported	Not reported	32
kiKO	2.9	2.7	Not reported	Not reported	33
Abcg2$^{-/-}$	2.8	2.2	Not reported	Not reported	2,41
Q140K-*Abcg2*	3.2	2.1	Not reported	Not reported	45
Slc22a12$^{-/-}$ (Hosoyamada et al.)	0.20	0.18	Living	Not reported	49
Uox$^{-/-}$*Slc22a12*$^{-/-}$	5	4.2 (*Uox*$^{-/-}$ control mice)	Living	No	50
Slc22a12$^{-/-}$ (Eraly et al.)	2.2×10^{-3}	1.9×10^{-3}	Living	Yes	48
Pdzk1$^{-/-}$	No information	No information	Living	Not reported	51,52
Hprt$^{-/-}$	1.25	0.4	Not reported	No	60
THP-C217G	1.2	0.4	Living	No	61
Urah$^{Plt2/Plt2}$	4.2	2.5	Living	No	62,63

wide variation — from 0.1 to 760 µmol/l (2×10^{-3} to 12.8 mg/dl)[68]. This variation is probably driven by differences in blood sampling protocols rather than by inherent variations in urate concentrations between mice with different genetic backgrounds housed in disparate environments. The investigators found that the urate concentration measurements for mice that were euthanized before blood sampling were 19 times higher than for mice that were anaesthetized before sampling[68]. If plasma or serum was not immediately separated upon sampling, the urate concentrations were four times higher[68]. This difference is a notable issue for quantitative analysis of studies of mouse models of hyperuricaemia. Comparing urate concentrations between different studies and different investigative teams is, essentially, not possible. However, comparisons within studies are still possible, assuming that standard urate measurement processes are used within a study by the same investigative team. Interstudy variability also poses difficulties for defining normouricaemia (the normal range of serum urate concentrations) in mice.

A suitable mouse model of hyperuricaemia should have a consistent, stable and high serum urate concentration. Interpreting data from different mouse models is complex as the mice have different genetic backgrounds and interstudy variability will influence the reported urate concentrations[68]. Nevertheless, modifying the activity of uricase is essential if the goal of a mouse model is to generate mice with equivalent urate concentrations to hyperuricaemia in humans. Although knocking out a mouse orthologue of any non-uricase gene might be useful to study the functions of the gene, this approach is unlikely to increase the serum urate concentrations to the equivalent concentrations that occur in human hyperuricaemia.

Ideally, an animal model of hyperuricaemia should have a sex bias in terms of the serum urate concentration, with higher concentrations in males. Insufficient data are available to confirm the sex-specific concentrations of urate in mice, but collectively the available data indicate no obvious sex-specific effects (TABLE 2). Interestingly, male *Uox*-knockout mice have 20% higher serum urate concentrations than their female counterparts[25], which is a similar relative difference to that reported for humans (~30% higher in males[105]). By contrast, among the SLC2A9-related models, there is a trend towards higher urate concentrations in female G9KO and LG9KO mice than in their male counterparts[29]. In humans, the association of urate levels with genetic variants in *SLC2A9* is stronger in females than in males[20], with this effect linked to menopausal status[106]. The sex-specific differences in urate concentrations of these mouse models are intriguing and, if confirmed, could shed light on the molecular reasons for sex differences in urate concentrations in humans.

Table 3 | **Expression patterns of genes associated with hyperuricaemia in humans and mice**

Gene	Tissue	Human			Mouse		
		FANTOM5	BioGPS	Genevestigator	FANTOM5	BioGPS	Genevestigator
UOX	Kidney	n/a	n/a	n/a	<0.5	5.0	8.5
	Liver	n/a	n/a	7.2	1993	18,815.0	17.7
	Small intestine	n/a	n/a	n/a	<0.5	4.7	8.8
	Colon	n/a	n/a	n/a	n/a	4.8	9.4
	Pancreas	n/a	n/a	6.6	<0.5	4.7	10.3
SLC2A9	Kidney	56.8	7.95	12.6	n/a	6.3	10.1
	Bladder	3.8	n/a	10.8	n/a	35.1	9.0
	Liver	17.6	6.3	12.1	n/a	98.5	11.7
	Small intestine	19.3	3.9	11.0	0.5	59.1	10.4
	Colon	15.2	4.05	10.3	0.5	5.9	9.5
ABCG2	Kidney	5.4	2.9	10.6	279	16,088.2	15.4
	Bladder	6.4	n/a	11.2	n/a	581.1	13.4
	Liver	14.7	4.8	13.6	61	4,415.7	14.5
	Small intestine	251.9	98.55	15.4	136	9,399.7	15.0
	Colon	51.8	3.7	12.7	n/a	4,139.0	14.3
	Pancreas	0	2.95	10.0	2	108.8	11.7
SLC22A12	Kidney	124.5	6.51	13.0	48	12,860.8	14.7
GCKR	Liver	61.2	7.54	13.6	16	1,948.1	15.3
SLC17A1	Kidney	110.3	2.69	15.0	232	9,950.7	15.0
SLC16A9	Kidney	61	13.3	15.7	21	539.8	13.5
SLC17A3	Kidney	70.8	13.68	15.1	32	18,788.4	15.1
	Liver	4	5.53	13.1	19	2,260.5	14.6
PDZK1	Kidney	376	18.46	15.5	n/a	11,771.1	15.5
	Liver	55.7	12.24	14.2	n/a	556.7	13.7
	Small intestine	182	n/a	13.1	<0.5	1,729.3	14.6

Data were obtained from the Functional ANnoTation Of the Mammalian genome 5 (FANTOM5)[122,123], for which the data were obtained by RNA sequencing (tags per million); BioGPS[124,125], for which the data were obtained by microarray (signal intensity); and Genevestigator v3 (REFS[126,127]), for which data were obtained by microarray (log_2 scale of signal intensity). Only tissues with clear difference in expression patterns are listed. n/a, not available.

For genetically induced mouse models, a single-gene knockout model is unlikely to simulate the entire hyperuricaemia phenotype given that urate control in humans is clearly regulated by many loci that individually have a relatively weak effect[20]. In fact, only *Slc2a9*-knockout and *Abcg2*-knockout mice have clearly increased serum urate concentrations, whereas the increases in urate levels in mice caused by deficiency in other hyperuricaemia-associated genes (such as *Slc22a12* (TABLE 2)) are small. Notably, the effect of hyperuricaemia-associated loci on urate concentrations in humans is heterogeneous and population-dependent. For example, genetic variation in *SLC2A9* and *ABCG2* has a consistently stronger effect on serum urate concentrations in European individuals than other hyperuricaemia-associated loci including *SLC22A12* (REF.[20]), mirroring the mouse model data. However, *SLC22A12* has the strongest effect of all urate-associated loci on serum urate concentrations in East Asian populations[107]. Thus, it will be informative to test the effects of knocking out other hyperuricaemia-associated loci in existing *Aldh16a1, Pdzk1* and *Abcc4* knockout mouse lines and in mice with different genetic backgrounds to determine whether the effect is strain-dependent. However, we recommend simultaneously modifying uricase expression (for example, $Uox^{-/-}Slc22a12^{-/-}$ mice[50]) so that functional evaluations are performed in a urate environment similar to humans.

Pseudogenization
The process of generating a pseudogene, which is a gene that has DNA segments related to a real gene but has lost some or all functionality during evolution.

Survival

In addition to the aforementioned embryonic lethality, low survival is the major hurdle of developing knockout mouse lines (particularly $Uox^{-/-}$ mice) for studying hyperuricaemia[14,25]. This issue is not surprising given that the silencing or pseudogenization of the *Uox* gene in humans is likely to be the result of multiple, independent evolutionary events that would have enabled adaptation to gradually increasing urate levels[108]. During primate evolution, transcription of *UOX* is thought to have decreased gradually because of accumulating mutations in the *UOX* gene, leading to pseudogenization of *UOX*[13], along with adjustment of the function of urate transporters such as SLC22A12 (REF.[109]). Therefore,

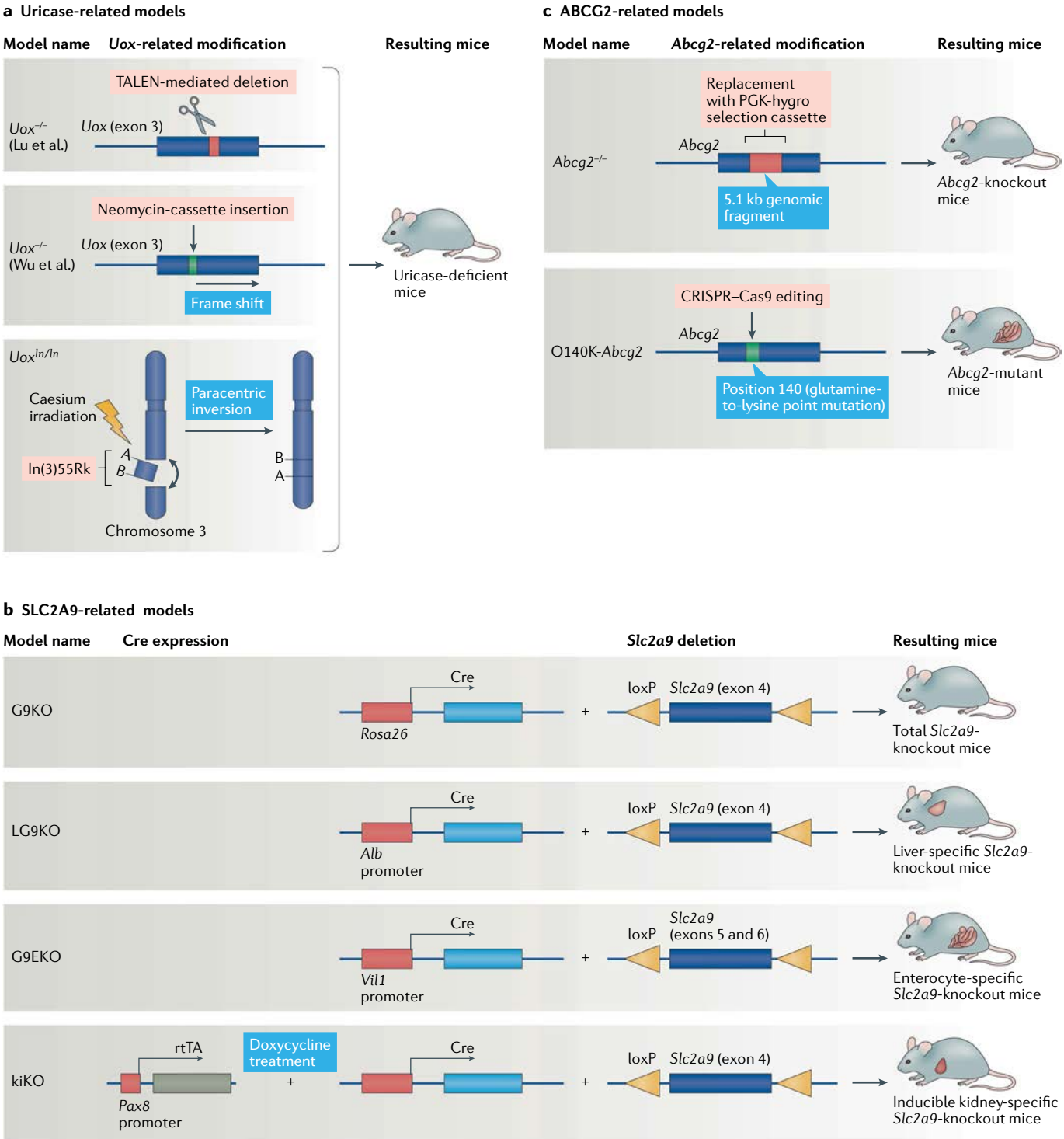

Fig. 2 | **Generation of mouse models of hyperuricaemia. a** | Two *Uox*-knockout (*Uox*$^{-/-}$) mice have been generated: one in which *Uox* is disrupted by insertion of a neomycin selection cassette into exon 3 of the gene, shifting the open reading frame, and one in which exon 3 of the *Uox* gene has been deleted using a transcription activator-like effector nuclease technique. In another model (*Uox*$^{ln/ln}$ mice), a paracentric inversion (In(3)55Rk) on mouse chromosome 3 was induced following exposure of the mice to caesium irradiation, resulting in inversion (and silencing) of *Uox*. **b** | In SLC2A9-related models, Cre-mediated recombination is used to delete a floxed exon of *Slc2a9*. In mice with total knockout of *Slc2a9* (G9KO), Cre recombinase is under the control of a ubiquitous promoter, *Rosa26*. In tissue-specific *Slc2a9* mice, Cre recombinase is under the control of a tissue-specific promoter (*Alb* for liver-specific knockout mice (LG9KO) and *Vil1* for enterocyte-specific knockout mice (G9EKO)). In inducible kidney-specific knockout mice (kiKO), a reverse tetracycline transactivator (rtTA) is under the control of a kidney-specific promoter (*Pax8*). In the presence of doxycycline, rtTA can mediate the expression of Cre recombinase. **c** | In *Abcg2*-knockout mice (*Abcg2*$^{-/-}$), a fragment containing exon 3 to exon 6 of *Abcg2* (encoding the majority of the ATP-binding domain) has been replaced with a PGK-hygro selection cassette. In Q140K-*Abcg2* mice, a glutamine-to-lysine point mutation has been introduced at position 140 (Q140K) of *Abcg2* using CRISPR–Cas9 genome editing, leading to the loss of expression and function of ABCG2 in the small intestine. TALEN, transcription activator-like effector nucleases.

Box 2 | **Uric acid crystal deposition in the mouse**

The formation of monosodium urate (MSU) crystals follows three steps: a reduction in urate solubility, crystal nucleation and crystal growth[118]. The factors involved in regulating urate solubility and crystal nucleation are temperature, pH, other ions and connective tissue factors, whereas crystal growth is dependent on urate load[24]. Mice with hyperuricaemia can have uric acid crystals in the kidneys but, in contrast to in humans, MSU crystals are not deposited in other areas such as the joints and tendons[14,25,29]. Several reasons could explain this phenotype. Urate is filtered at the glomerulus into the proximal tubule, an important site of urate regulation in the kidneys[119]. In mice with hyperuricaemia, a high urate load resulting from increased urinary urate concentrations, and the presence of other ions or low urinary pH, will reduce the solubility of urate and promote uric acid crystal formation in the kidneys. Concerning MSU crystal formation in extremities of the body, a combination of increased temperatures (compared with humans) and differing connective tissue factors in the body extremities might explain the difference in gout phenotype between humans and mice. Another possibility is that the urate load in mice with hyperuricaemia is not sufficient for crystal nucleation and growth in the peripheral tissues and/or there is insufficient time for crystals to nucleate and grow in the mouse, given their short lifespan, especially in an experimental setting.

it seems necessary to ensure that a genetically induced mouse model reproduces and evolves for a number of generations before the issue of survival can be reliably examined. This method is technically possible as ~40% of *Uox*$^{-/-}$ mice survive for a 62-week period and remain fertile during the breeding lifespan[25]. By maintaining and breeding these mice, changes in urate-related physiology and in the genome and epigenome can be measured over generations.

Understanding the major causes of mortality would be useful for developing a suitable and sustainable mouse model of hyperuricaemia. A number of factors are associated with mortality in these models. Very high urate concentrations in the blood and/or urine are obvious possible triggers of mortality (for example, urine urate concentrations were fivefold to tenfold higher in SLC2A9-deficient mice than in their heterozygous and wild-type counterparts)[29]. In the *Uox*-related models, kidney damage was evident in the first 2 weeks[14,110] or in the first 4 weeks of life[25] and was associated with high mortality. The mice that survived to adulthood, however, survived despite kidney damage. Understanding these findings would yield important insights into the relationship between urate and renal function. For example, soluble urate can prime monocytes to develop a pro-inflammatory phenotype[87], which might contribute to renal insufficiency.

Technological advances (for example, CRISPR–Cas9 genome editing[45,111]) will enable more precise genetic modification to generate mice that have serum urate concentrations that are not too high but remain valid to model human hyperuricaemia. The Q140K-*Abcg2* model[45] is the first example of such an approach. RNA interference is an approach that has been used to knock down uricase in a mouse hepatic cell line and resulted in a 66% reduction in uricase mRNA (rather than a 100% reduction, as occurs in knockout models)[112]. Nonetheless, how to maintain the small interfering RNA knockdown effects in recipient mice and to establish these effects as heritable is unclear. Multiple technologies and multiple steps will likely be needed to generate ideal mouse models of hyperuricaemia.

Congenic
A congenic mouse strain has a defined segment from a donor strain introduced into its genome.

Mouse genetic background

The importance of controlling for the mouse genetic background when studying human disease models has been well documented[113,114]. However, the effect of the genetic background on the phenotype of mouse models of hyperuricaemia is largely unexplored. As the goal of these models is to generate a human-comparable environment, those models with a genetic background that is relatively resistant to hyperuricaemia might have an advantage over other models. From this perspective, outbred mouse strains might be better than inbred lines owing to the inherent heterozygosity and increased robustness of outbred strains. If an inbred line is unavoidable, an ideal background must be selected. For example, the *Uox*$^{-/-}$ mice with a C57BL/6J*129Sv background[14] have higher serum urate concentrations and a lower survival than the *Uox*$^{-/-}$ mice with a C57Bl/6J background[25] (TABLES 1,2); as the same exon is knocked out in both strains, this difference is possibly attributable to the different genetic backgrounds (although differences in urate measurement protocols between the different investigative teams might also have contributed).

Given the issues discussed above, another approach to consider is the use of hyperuricaemia-specific congenic strains. The development of such strains should follow a consensus protocol derived from community-wide efforts including guidelines about design, genetic modification, breeding, testing of urate concentrations, validation and maintenance. This approach would require initial linkage studies to identify naturally occurring mouse alleles that influence urate concentrations. The congenic strains could become important resources for the entire community to greatly advance future investigations of the regulatory mechanisms of hyperuricaemia.

Conclusion

The use of mice to model hyperuricaemia in humans is a feasible approach. Previous efforts using such models have resulted in valuable insights into urate biology in both species. Clearly, the alteration of uricase during human evolution is the major contributor to hyperuricaemia and led to a urate-mediated biological system that is specific to humans. Such systemic differences between humans and mice might partially explain the differences in patterns of expressions of genes associated with hyperuricaemia between mice and humans (TABLE 3). Hence, genetic modification of uricase is an essential first step to develop mouse models suitable for hyperuricaemia. Models that have a single gene modification (for example, *Slc2a9*-knockout and *Abcg2*-knockout mice) have been informative (TABLE 1), but the presence of uricase in these mice might lead to effects not seen in humans. Similarly, results from diet-induced hyperuricaemic mouse models should be interpreted with caution owing to the physiological differences between humans and mice and the absence of a standard protocol for the measurement of urate concentrations. However, combining genetic manipulation, particularly the use of *Uox*-knockout mice, with dietary intervention is an attractive strategy.

The development of mouse models of hyperuricaemia is still in the early stages. A major challenge is to

develop uricase-deficient mice that can survive with increased urate concentrations, remain healthy and fertile and be maintained for many generations. Such models should not only help dissect the causal mechanisms underlying hyperuricaemia, gout and associated comorbidities but also will probably provide insights into how humans adapted to increased urate concentrations. However, community-wide efforts are needed to reach a consensus for the definition of hyperuricaemia in mice and to develop protocols for generating suitable models of hyperuricaemia. Such efforts should include criteria or recommendations for the design, genetic modification, breeding, management, assessment and reporting of these models. Finally, an important challenge is to adhere to a standard protocol for measuring urate concentrations.

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The immunobiology of MIF: function, genetics and prospects for precision medicine

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Abstract | The role of macrophage migration inhibitory factor (MIF) in autoimmunity is underscored by data showing that common functional polymorphisms in *MIF* are associated with disease susceptibility or clinical severity. MIF can regulate glucocorticoid-mediated immunosuppression and has a prominent function in cell survival signalling. Further specific functions of MIF are now being defined in different autoimmune diseases and MIF-targeted biologic therapeutics are in early-stage clinical trials. The unique structure of MIF is also directing the development of small-molecule MIF antagonists. Together, these efforts could provide a means of selectively intervening in pathogenesis and overcoming MIF-related genetic susceptibility to many rheumatic diseases.

The function of the cytokine macrophage migration inhibitory factor (MIF) in the immune response and the pathogenesis of autoimmune inflammatory disorders spans both disease initiation and progression. Variant *MIF* alleles, which occur commonly in the population[1], affect the host response to infection[2–11] and are now recognized to modulate autoimmunity. Accruing human genetic data support the role of high-expression *MIF* alleles in the clinical severity and end-organ complications of a number of autoimmune rheumatic diseases, including rheumatoid arthritis (RA)[12–14], juvenile idiopathic arthritis (JIA)[15,16], systemic sclerosis (SSc)[17,18], systemic lupus erythematosus (SLE)[19–21] and systemic vasculitis[22]. Beyond the genetic association between individual rheumatic disorders and functional *MIF* alleles, mechanistic data implicate immunopathogenic MIF pathways in RA, SLE and spondyloarthritis (SpA).

In this Review, distinctive features of MIF structure and function, including its unique inter-relationship with glucocorticoids, are described, as is MIF signalling and opportunities for pharmacological targeting of these pathways. New information, including the description of a second MIF cytokine superfamily member, D-dopachrome tautomerase (D-DT, also known as MIF-2), and the function of MIF in the regulation of the inflammasome, is also discussed. We summarize findings from experimental, human genetic and clinical studies, and suggest therapeutic opportunities for modulating MIF activity, which may lead to a *MIF*-allele-specific, precision-medicine approach to treating autoimmune rheumatic diseases.

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MIF structure and function

MIF is a pleiotropic cytokine that contributes to the pathogenesis of many autoimmune diseases through its upstream immunoregulatory function and its polymorphic genetic locus. MIF is a highly conserved protein of 12.5 kDa, with evolutionarily ancient homologues in plants, protozoans, nematodes and invertebrates[23]. MIF originally attracted attention owing to its eponymous function in tissue macrophage retention, an activity first reported in 1932 when the emigration of inflammatory cells from the lymphoid tissue of a *Mycobacterium*-sensitized animal was found to be impaired in the presence of antigen[24]. MIF-dependent migration inhibition assays were widely used in studies in the 1960s as an in vitro surrogate for delayed-type hypersensitivity and provided insights into the first non-immunoglobulin mediator (or lymphokine)[25,26]. However, the definitive molecular cloning of MIF occurred only after the serendipitous discovery of its function as a regulator of glucocorticoid immunosuppression, following a search for endogenous regulators of adrenal glucocorticoid action[27,28]. The reader is cautioned that several publications that emanated from a preceding human MIF sequence have been retracted (see for instance REF.[29]). On the basis of X-ray crystallography data, MIF was subsequently classified as the defining member of a new structural superfamily[30,31], and the biologically active form of MIF was observed to be a homotrimer (FIG. 1). The description of the second MIF family member, D-DT, followed in 1997 (REF.[32]).

MIF was cloned from anterior pituitary cells as a result of experiments prompted by the observation that,

Key points

- Functional *MIF* polymorphisms are associated with autoimmune and rheumatic disease susceptibility and severity.
- MIF regulation of glucocorticoid immunosuppression and a prominent function in cell survival signalling place MIF in a unique position in the host response.
- Biologic and small-molecule therapies targeting MIF are in clinical evaluation.
- Structural features of MIF make this cytokine suitable for small-molecule antagonism in rheumatic diseases.

in contrast to other physiological regulators of carbohydrate (insulin–glucagon), mineral (calcitonin–parathyroid hormone) or vascular wall (acetylcholine or adrenaline) homeostasis, no circulating mediators that regulate the immunological action of glucocorticoids were evident or previously described. The discovery of a novel protein secreted by the corticotropic pituitary cells of endotoxin-sensitive but not endotoxin-resistant mice led to the sequencing of the *MIF* gene and it was considered that pituitary-derived MIF might influence systemic inflammatory responses and counter-regulate endogenous glucocorticoids, which increase in the circulation in response to the pituitary release of adrenocorticotrophic hormone (ACTH)[27]. Immunogold labelling and electron microscopy analysis showed MIF to be localized in the secretory granules of corticotropic cells that also contain ACTH[33]. MIF accounts for 0.5% of the pituitary protein content, which is comparable to the classical pituitary hormones ACTH and prolactin (0.2% and 0.8%, respectively)[27]. Corticotropin-releasing factor induces the expression and release of MIF from pituitary cells, and corticotropin-releasing factor-induced *MIF* gene transcription is mediated through a cAMP-dependent pathway[33,34]. Mice receiving an inflammatory challenge of Gram-negative endotoxin show a rapid decrease in the pituitary content of MIF protein concurrent with an increase in plasma MIF content[27,33]. Circulating MIF concentrations in rats also rise 3–4 hours after exposure to handling stress, similar to the stress-related increase in circulating ACTH and glucocorticoid levels[35]. MIF also circulates normally at levels (2–6 ng/ml) that follow a circadian rhythm[36]. The phase relationship between plasma cortisol and MIF has been studied in an hypophysectomized patient (that is, with surgical removal of the pituitary gland) on cortisone replacement therapy, showing plasma MIF levels to be phase advanced by 2–3 hours relative to plasma cortisol levels[36]. Notably, studies of hypophysectomized mice revealed that an additional source of MIF protein resides within the monocyte/macrophage population[37]. Like anterior pituitary cells, macrophages contain substantial quantities of MIF within intracellular pools that are rapidly released upon cell stimulation. Rat studies have shown that MIF is released from sources in the pituitary gland, adrenal gland, lung, liver, spleen, kidney and skin within 6 hours of systemic inflammatory stimulation[38]. Much of this release response is from preformed cellular stores, and tissue MIF content falls before the induction of a *Mif* transcriptional response[39].

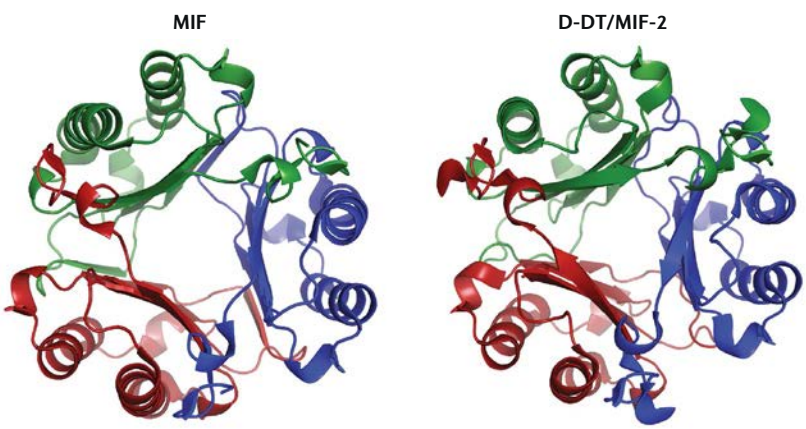

Fig. 1 | **Structure of two MIF cytokine superfamily members.** X-ray crystallographic representation of human macrophage migration inhibitory factor (MIF) and its homologue D-dopachrome tautomerase (D-DT; also known as MIF-2), illustrating their common homotrimeric structures, axial symmetry and 3D similarity that is representative of the MIF cytokine superfamily[70].

Interactions with glucocorticoids

Glucocorticoids downregulate inflammation by affecting a broad range of signalling pathways and, when used pharmacologically, are a keystone in the rheumatology armamentarium because of their incomparable efficacy in rapidly downregulating life-threatening inflammation[40]. Indeed, were it not for dose-limiting adverse effects, which include not only extension of their immunosuppressive properties but also impairment of wound repair, osteoporosis, hypertension, insulin resistance and growth retardation, glucocorticoids would provide ideal control of excessive and tissue-damaging inflammatory responses. Glucocorticoids function primarily by activating a cytosolic receptor that binds DNA-responsive elements, leading to suppression of pro-inflammatory and activation of anti-inflammatory gene transcription[41]. Direct protein–protein interactions of the glucocorticoid receptor with other transcription factors also occur and could be an even more important mechanism for the suppression of inflammation[41].

Initial observations that MIF is secreted from corticotropic anterior pituitary cells, which release ACTH to stimulate adrenal glucocorticoid secretion, led to studies aimed at elucidating functional interactions between these mediators. MIF counteracts the glucocorticoid-induced suppression of inflammatory cytokine secretion by activated macrophages in vitro (for example, TNF, IL-1, IL-6 and IL-8), and in vivo, MIF fully abrogated the protective effect of glucocorticoids in an endotoxin model of lethal inflammation[35]. The MIF–glucocorticoid interaction was examined in experimental arthritis by studying adrenalectomized rats (that is, lacking glucocorticoids), which led to fulminant and ultimately lethal disease. Immunoneutralization of MIF fully protected rats from lethal arthritis, supporting the proximate action of MIF in upregulating inflammation when unopposed by endogenous glucocorticoids[42].

Glucocorticoids also regulate leukocyte trafficking between tissue compartments. In a model of acute stress in which plasma corticosterone concentrations increase 80-fold within 1 hour, treatment with anti-MIF antibody enhanced the stress-induced egress of peripheral

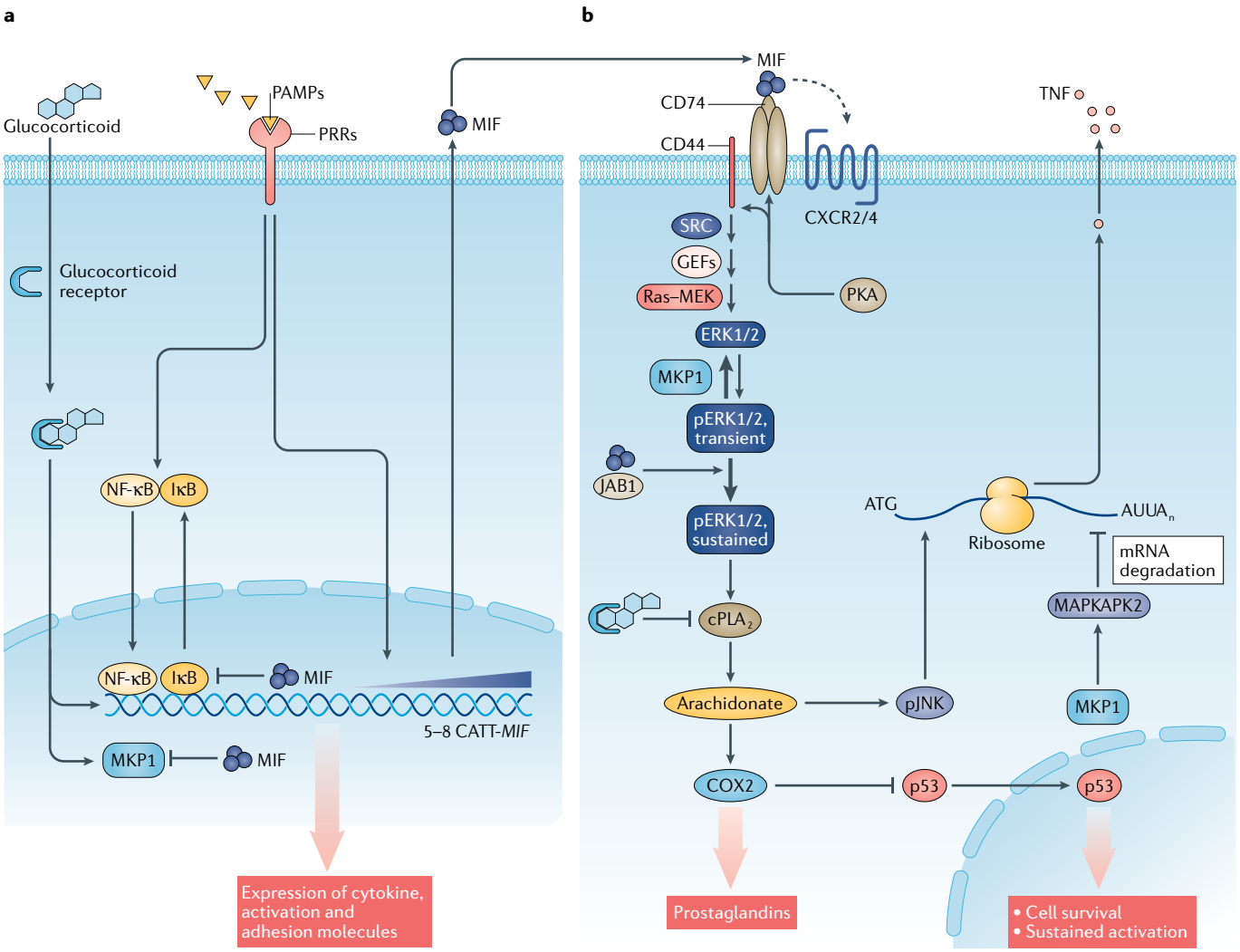

Fig. 2 | **MIF expression and signalling.** This integrated scheme is based on data in monocytes/macrophages and focused on the regulation of glucocorticoids. **a** | Diverse activating stimuli including pathogen-associated molecular patterns (PAMPs) stimulate pattern recognition receptors (PRRs) to activate macrophage migration inhibitory factor (MIF) transcription in a *MIF* allele-dependent manner (5–8 CATT-*MIF*). **b** | MIF activates a multicomponent receptor comprising the CD74 ligand-binding protein and the CD44 signal transducer. MIF also activates CXCR2 and CXCR4-dependent chemotactic responses. CD74 signalling leads to the phosphorylation of the extracellular-signal-regulated kinase 1/2 (ERK1/2), with sustained phase activation mediated by JUN activation domain binding protein-1 (JAB1). MIF upregulates cytoplasmic phospholipase A_2 ($cPLA_2$) activity, which is downregulated by glucocorticoids, leading to arachidonate production and prostaglandin E2 release. Intracellular arachidonate increases the post-transcriptional stability of mRNAs for inflammatory cytokines (including TNF) by activating JUN N-terminal kinase/stress-activated protein kinase (pJNK), which is suppressed by glucocorticoids. MIF overrides glucocorticoid-induced expression of the nuclear factor-κB (NF-κB) inhibitor IκB and mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP1), which downregulates the inflammatory response by dephosphorylating MAPKs. MKP1 targets also include MAPK-activated protein kinase 2 (MAPKAPK2), which mediates the degradation of pro-inflammatory mRNAs. COX2, cyclooxygenase-2; GEF, guanine nucleotide exchange factor; pERK, phosphorylated ERK; PKA, protein kinase A. Adapted from Flaster, H., Bernhagen, J., Calandra, T. & Bucala, R. The macrophage migration inhibitory factor-glucocorticoid dyad: regulation of inflammation and immunity. *Mol. Endocrinol.* **21**, 1267–1280 (2007), by permission of the Endocrine Society (REF.[54]).

leukocytes from the circulation, consistent with a counter-regulatory effect of MIF on glucocorticoids[36].

Mice lacking MIF have lower concentrations of glucocorticoids in the circulation, which might be expected in the absence of an endogenous counter-regulator, and such mice show impaired fetal lung maturation, which is a developmental function of glucocorticoids[43]. Notably, in experimental autoimmune encephalomyelitis (a model of multiple sclerosis), dexamethasone was substantially more efficacious in *Mif*$^{-/-}$ mice than in wild-type mice and was more effective at ameliorating disease induced by the adoptive transfer of MIF-deficient CD4$^+$ T cells than wild-type cells[44].

MIF antagonizes the immunosuppressive effect of glucocorticoids by acting at several important regulatory steps of the inflammatory response (FIG. 2). MIF transcriptionally suppresses glucocorticoid induction of the nuclear factor-κB (NF-κB) inhibitor IκB

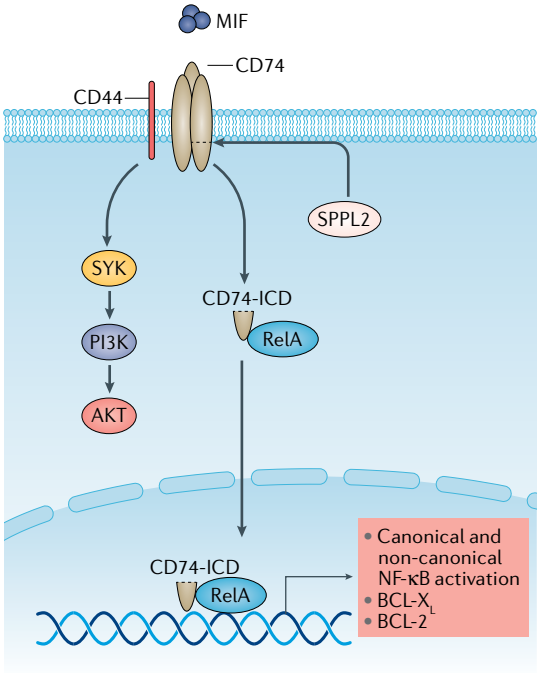

Fig. 3 | **The MIF–CD74–CD44 signal transduction cascade mediates activation and survival signalling in B cells.** Engagement with macrophage migration inhibitory factor (MIF) initiates the intramembrane cleavage of CD74 via the SPPL2A protease to generate a 42 amino acid intracytoplasmic domain (CD74-ICD). This cleavage requires the co-receptor CD44, which activates SYK, to phosphorylate AKT in a phosphoinositide 3-kinase (PI3K)-dependent manner. CD74-ICD interacts in the cytoplasm with the nuclear factor-κB (NF-κB) family member RelA to enter the nucleus and regulate transcription of NF-κB target genes. Adapted with permission from Shachar, I. et al. CD74, the natural receptor for MIF, regulates cell survival in health and disease, in *The MIF Handbook*, 1st edn (ed. Bucala, R.) 55–76. Copyright 2012, World Scientific (REF.[156]).

in vitro[45,46] and the mitogen-activated protein kinase (MAPK) inactivator MAPK phosphatase-1, which dephosphorylates extracellular-signal-regulated kinase 1/2 (ERK1/2), JUN N-terminal kinase (JNK) and p38 MAPKs[47,48]. MIF also reverses glucocorticoid-mediated suppression of cytokine-induced cytoplasmic phospholipase A_2 ($cPLA_2$) activity and arachidonic acid release by upregulating ERK1/2 MAPK activation[49]. Intracellular arachidonate is also important post-transcriptionally, to enhance the translation of numerous pro-inflammatory cytokine mRNAs, such as those encoding TNF and IL-6 (REF.[50]). The ability of MIF to antagonize glucocorticoid-mediated immune suppression is consistent with observed clinical correlations between MIF expression and glucocorticoid dose[51] and with the increased expression of IκB reported in glucocorticoid-resistant patients with SLE[46].

The observations that low, physiological concentrations of glucocorticoids stimulate MIF release from monocytes, macrophages and T cells indicate further homeostatic relationships between these mediators.[35,52] The glucocorticoid-induced secretion of MIF is tightly regulated and follows a bell-shaped dose–response curve: at high, anti-inflammatory concentrations of glucocorticoids (>10^{-8} M), MIF secretion is prevented[53]. The fact that MIF is neither induced nor has a counter-regulatory effect on immunosuppression at high glucocorticoid concentrations suggests a default mechanism to protect the host from overwhelming inflammatory reactions[53]. Inhibition of the immunosuppressive action of glucocorticoids appears to be a critical mechanism for the inflammatory action of MIF, and the position of MIF within the host response is consistent with its control of both the magnitude and the set-point of inflammatory activation response[53]. Thus, MIF inhibition has been proposed as a potentially useful pharmacological strategy for the treatment of inflammatory and autoimmune diseases, especially conditions characterized by resistance to glucocorticoid therapy or by glucocorticoid dependence[54].

MIF signalling

The cognate MIF receptor is a two-component signalling complex comprising the ligand-binding protein CD74 and the signal transducer CD44 (REFS[55,56]). Notably, CD74 is the cell-surface-expressed form of the MHC class II invariant chain, which functions intracellularly to facilitate MHC II peptide loading in antigen-presenting cells. However, CD74 is also expressed by multiple cell types independently of MHC II[57]. Upon MIF engagement, CD74 initiates the horizontal membrane recruitment of CD44; both proteins then undergo phosphorylation of their cytosolic domains to initiate downstream signal transduction[58] (FIG. 2). In monocytes and stromal cells, signalling proceeds by activation of lymphocyte-specific protein tyrosine kinase (Lck), a CD44-associating non-receptor tyrosine kinase and Src family member; Lck activation is followed by activation of MAPK kinase (MEK), leading to sustained ERK1/2 MAPK phosphorylation, upregulation of $cPLA_2$ and nuclear translocation of p53, thus inhibiting activation-induced apoptosis and prolonging cell survival and inflammatory activation[49,56,59]. MIF has an important function in the upregulation of pattern recognition receptors, such as Toll-like receptor 4 (TLR4) and dectin-1, with an accompanying increase in innate signalling[5,60]. Further signalling pathways, including the NF-κB, protein kinase B (also known as AKT) and phosphoinositide 3-kinase (PI3K) pathways, and AMP-activated protein kinase (AMPK) can be upregulated by MIF in different cell types to produce additional pro-activation and pro-survival functions[61,62]. Notably, the chemokine receptors CXCR2 and CXCR4 associate with CD74 at the cell surface and mediate non-cognate interactions with the chemokine-like, pseudo(E)-LR domain of MIF[63]. CXCR2 activation mediates cell migration and, by receptor de-sensitization, accounts for the eponymous effect of MIF on macrophage retention within inflammatory sites[63].

Engagement with MIF initiates the intramembrane cleavage of CD74 via the SPPL2A protease to generate a 42 amino acid intracytoplasmic domain (CD74-ICD) in B cells and possibly other cell types[57,64,65] (FIG. 3). CD74-ICD interacts in the cytoplasm with p65 (also known as

the NF-κB family member RelA) to enter the nucleus and regulate transcription of NF-κB target genes, including in mouse cells the gene encoding the protein p53-related TAp63, which transactivates *Bcl2* (REF.[65]). CD74-ICD also forms complexes with Runt-related transcription factor 1 (RUNX1) and RUNX3, which bind to chromatin and regulate gene transcription[66]. The co-receptor CD44 is necessary for CD74 intramembrane cleavage and participates in this process by activating the Src family kinase SYK to phosphorylate AKT in a PI3K-dependent manner[67]. The tyrosine kinase receptor MET also engages with CD74 and CD44 on the B cell surface after MIF stimulation, which renders the cell sensitive to autocrine hepatocyte growth factor/scatter factor (HGF), further increasing proliferation and cell survival signalling[68].

Notably, the MIF family member D-DT[32] also activates CD74 (REF.[69]). The gene encoding D-DT is adjacent to *MIF* on human chromosome 22q11.23 and was most likely created by chromosomal duplication. Similar to MIF, D-DT is expressed by many tissues and cell types, circulates in healthy individuals and is expressed in vitro and in vivo in many of the same inflammatory circumstances as MIF[70]. Distinct structural features of D-DT, such as the absence of a CXCR2-binding pseudo(E)-LR domain or common genetic polymorphisms, together with data from experimental models of ischaemic tissue injury suggest that this MIF family member might selectively function in cell survival rather than inflammatory activation[71].

Notably, a circulating truncated form of CD74, termed soluble CD74 (sCD74), exists and is most likely produced as the ectodomain product of the SPPL2A cleavage pathway[72]. sCD74 binds to plasma MIF, thereby reducing MIF signal transduction activity on target cells. Current data are limited, but circulating levels of sCD74 appear to be increased in some inflammatory disorders and presumably downregulate MIF activity in settings of high systemic MIF production, CD74 activation and intramembrane cleavage[72,73].

MIF can also be involved in intracellular protein–protein interactions. For example, a major regulatory action of MIF involves binding JUN activation domain-binding protein 1 (JAB1; also known as the COP9 signalosome subunit 5 (CSN5)), resulting in inhibition of JAB1-induced JNK activity and AP-1 transcriptional activation, thus reducing proliferative activation[74].

MIF and the NLRP3 inflammasome

Studies published in the past few years have provided an insight into the upstream functions of MIF in arming monocyte and macrophage effector functions in rheumatic diseases. MIF controls the expression of *NLRP3*, an essential step for activation of the NLRP3 inflammasome, which is necessary for the rate-limiting proteolytic cleavage of IL-1 family member precursors to produce active cytokines (for example, IL-1, IL-18 and IL-33)[75,76]. These interleukins regulate the activation and differentiation of innate and adaptive immune cells into distinct cell subsets; for example, IL-1β produced by innate immune cells promotes T helper 17 cell responses[77,78]. This pathway is particularly important in crystal-induced arthritis (for example, gout and pseudogout), in which crystals activate the inflammasome intracellularly[79], as well as in SLE, in which monocytes respond to the small nuclear ribonucleoprotein (snRNP) immune complex, a combination of the lupus target antigen U1 snRNP (U1-snRNP) and anti-U1-snRNP autoantibody[78,80]. The translocation of U1-snRNP from the nucleus to the cell membrane occurs in apoptotic keratinocytes damaged by ultraviolet light, a known trigger for SLE, suggesting a potential role for this molecule in initiating and/or propagating autoimmunity[81]. IL-1β production is markedly increased in snRNP immune complex-stimulated human monocytes, and blockade of MIF–CD74 signalling with a small-molecule MIF antagonist decreased caspase-1 activation and IL-1β production[75,78]. Monosodium urate crystals induce IL-1β production via the NLRP3 inflammasome and genetic deletion or blockade of MIF reduces IL-1β production in a model of gout[82]. These studies collectively support a regulatory effect of MIF on the NLRP3 inflammasome.

Genetics of MIF in rheumatic disease

In an investigation in the USA of 184 patients with RA stratified by clinical severity, direct sequencing of the *MIF* locus revealed a variant microsatellite in the promoter region[12]. This microsatellite comprises a 4-nucleotide CATT repeat that is present in 5–8 copies (−794 $CATT_{5–8}$, rs5844572) (FIG. 4), with a higher CATT number associated with greater baseline and stimulus-activated *MIF* transcription. Patients with RA who had the genotype conferring the lowest MIF expression (−794 $CATT_{5,5}$) were protected from severe, erosive disease, as defined by measures of disease activity such as the Health Assessment Questionnaire (HAQ) and Larsen score (OR 8.2)[12]. The association of the *MIF* locus with RA severity was confirmed in a larger European study (n = 554), in which the −794 $CATT_7$ allele, which was present in 13% of participants, correlated with high MIF production and severity of radiological joint damage[13]. A second promoter polymorphism at MIF −173 (G/C, rs755662) probably lacks functionality but is often detected in association studies because the less frequent −173*C allele is in linkage disequilibrium with the high-expression −794 $CATT_7$ microsatellite[1]. Reduced locus heterogeneity, which increases the statistical power to detect associations, also favours associations at the −173 site over the −794 site. The *MIF* −173*C allele and the haplotype comprising −173*C and the −794 $CATT_7$ allele have been associated with disease susceptibility in JIA[16,83] and in a cohort of patients with inflammatory polyarthritis (defined as swelling of two or more joints lasting for 4 weeks or more)[84]. Evidence of an association between the −173*C allele and increased disease severity, as assessed by the number of involved joints and a reduced response to glucocorticoids, was also established in an analysis of 136 patients with systemic-onset JIA[85].

The transcription factor ICBP90 (inverted CCAAT box-binding protein of 90 kDa, encoded by the gene *UHRF1*) binds to the *MIF* promoter microsatellite and regulates −794 $CATT_{5–8}$ length-dependent transcription in monocytes, B cells, T cells and synovial

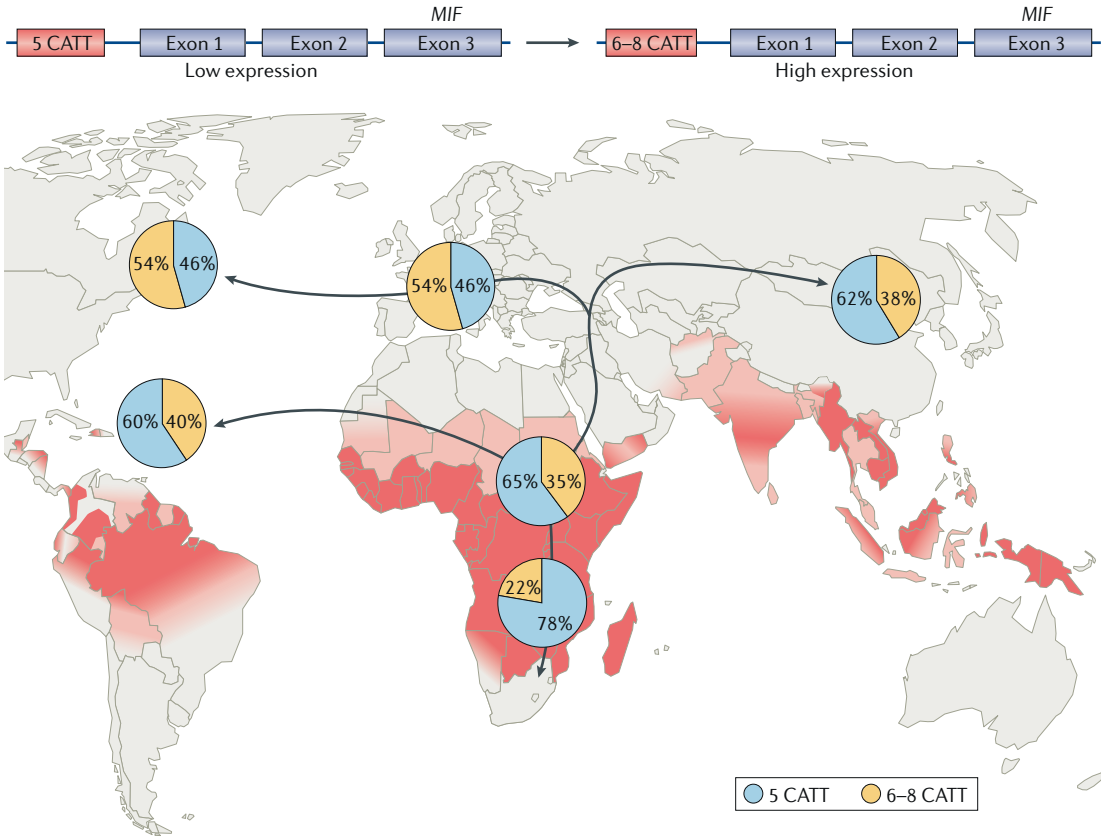

Fig. 4 | **Diagram of the human *MIF* gene showing its exonic structure and the variant microsatellites in the promoter region.** The −794 microsatellite comprises a 4-nucleotide CATT repeat that is present in 5–8 copies, with a higher number of repeats associated with greater *MIF* transcription. The global map illustrates the population stratification of these alleles and the expansion of the microsatellite overlaid on historic human migration patterns and current malaria prevalence. Adapted from REF.[1], CC-BY.

fibroblasts[86]. Whole-genome transcription analysis of synovia from patients with RA shows a strong correlation between *UHRF1* and *MIF* expression, and between genes in *UHRF1* short hairpin RNA-treated and MIF short hairpin RNA-treated RA synovial fibroblasts, consistent with ICBP90 being an important regulator of *MIF* transcription at the polymorphic promoter microsatellite[86].

Additional studies have reported the *MIF* −173*C allele to be associated with high concentrations of circulating MIF and increased disease susceptibility and severity in patients with RA[14], as well as in patients with psoriatic arthritis[87,88] and in clinically defined subsets of patients with sarcoidosis[89]. Patients with diffuse cutaneous SSc also have a higher prevalence of the high-expression −794 $CATT_7$–*MIF* −173*C haplotype than patients with limited cutaneous SSc[17]. Fibroblasts from patients with this risk haplotype produce more MIF, suggesting a pathogenic function[17]. This genetic association was replicated in a study of 3,800 individuals with SSc[18], and a follow-up investigation reported an association between the −173*C *MIF* allele and pulmonary arterial hypertension in patients with diffuse cutaneous SSc[90]. Circulating MIF has been suggested as a biomarker of pulmonary arterial hypertension independent of SSc and is considered a tractable therapeutic target in this condition[91]. It should be noted that neither the −173 G/C nor the −794 $CATT_{5–8}$ *MIF* allele is detected by the DNA chips used in genome-wide association studies and the microsatellite must be assessed using specialized methodology (BOX 1).

The possibility that MIF has a role in SLE follows from pathological findings that infiltrating macrophages are major constituents of crescentic lesions in lupus nephritis and their presence is associated with severe glomerular injury[92]. Evidence of MIF as a disease modifier in SLE was first obtained by clinical observations that circulating MIF concentrations correlate with the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) index score[51]. In the largest study of *MIF* genotype in patients with SLE to date (n = 1,369), *MIF* genotype was assessed in relation to disease incidence, clinical manifestations and circulating MIF levels[20]. Among patients with established SLE, those with nephritis, serositis or central nervous system involvement had a high frequency of high-expression alleles (that is, >5 CATT repeats or $CATT_{6–8}$) compared with patients without those sequelae. These high-expression *MIF* alleles also correlated with circulating MIF and TNF levels, as well as with *MIF* expression by monocytes in response to nucleic acid-responsive TLR ligation, which contributes to signals that break immune tolerance and sustain activation of autoimmune responses[93]. Importantly, these findings were similar in both white and black patients,

which is notable as these two groups may have distinct genetic predisposition to autoimmunity. An association between high-expression *MIF* alleles, SLE susceptibility and circulating TNF levels has also been observed in smaller studies of patients with SLE[19,21].

Notably, in a large cohort of patients with SLE stratified by ethnicity, the high-expression *MIF* haplotype (−173*C and −794 $CATT_7$) occurred more frequently in healthy individuals than in the cohorts of white or black patients with SLE, suggesting a potential protective effect of high-expression *MIF* alleles for the development of disease[20]. The incidence of a positive anti-nuclear antibody (ANA) test[20], which can occur in as many as 20% of healthy individuals (depending on the assay used) and is associated with a predisposition to developing SLE[94], was also lower in those with the high-expression *MIF* haplotype. MIF may provide protection from the development of ANAs by facilitating pathogen clearance, particularly in the case of infections that, when not cleared, prompt B cell expansion and persistent autoantibody production. This scenario is supported by data showing that high-expression *MIF* alleles are associated with improved clearance of pathogens such as *Streptococcus pneumoniae* or meningococcus, which can persist in a subclinical state[3,95]. The defective clearance of apoptotic products has been proposed to be important for SLE pathogenesis as apoptotic nuclei might overwhelm the reticuloendothelial system, break immune tolerance and induce autoantibody production against nuclear components[96]. As MIF inhibits activation-induced apoptosis by promoting ERK1/2, p53 and AKT-mediated survival pathways[59,97], high-expression *MIF* alleles might reduce the apoptotic response during inflammation and decrease the likelihood of persistent B cell activation and an ANA response progressing to SLE.

In systemic vasculitis, the presence of >5 CATT repeats is more common in patients with granulomatosis with polyangiitis than in healthy individuals[22]. The importance of this association is supported by the proportional relationship between −794 $CATT_{5\text{-}8}$ microsatellite length and *MIF* gene expression in response to granulomatous stimulation in vitro, such as induced by mycobacteria[98] or β-glucan[22], and by evidence that transgenic mice that overexpress *Mif* in lung epithelium have increased numbers of lung granulomas and higher mortality than wild-type mice in a model of granulomatous disease[22]. Children with Kawasaki disease, a form of juvenile vasculitis, are susceptible to coronary artery aneurysm and those patients who carry the *MIF* −173*C allele are at an increased risk of this complication[99]. In accordance with the action of MIF in counter-regulating glucocorticoid immunosuppression[35,36,44–46], high-expression *MIF* alleles were more frequent in patients with clinically defined glucocorticoid resistance than in patients defined as glucocorticoid-responsive, among patients with JIA, nephrotic syndrome or inflammatory bowel disease[100–102].

A remarkable feature of the functional *MIF* promoter microsatellite is its population stratification, with the highest prevalence of the low-expression −794 $CATT_5$ allele found in sub-Saharan Africa[1] (FIG. 4). Given the strong selective pressure of endemic malaria, which has coexisted with ancestral human populations in this region, low-expression *MIF* alleles have been hypothesized to provide some protection from *Plasmodium* infection[103]. Genetic epidemiology studies support this concept and show a relationship between longer repeats of the −794 CATT microsatellite and the development of severe malarial anaemia, which is a leading cause of death in children with malaria[4,104,105].

Box 1 | *MIF* alleles in autoimmune diseases

Rheumatoid arthritis
- Susceptibility: OR 1.30, CI 0.88–1.93 (REF.[14]); OR 1.59, CI 1.03–2.46 (REF.[13])
- Severity: OR 8.2, CI 1.03–65.6 (REF.[12]); OR 2.89, CI 1.05–7.93 (REF.[14])

Juvenile idiopathic arthritis
- Susceptibility: OR 1.9, CI 1.4–2.7 (REF.[15])

Systemic sclerosis
- Susceptibility: OR 1.10, CI 1.00–1.19 (REF.[18])
- Severity: OR 1.94, CI 1.14–3.32 (REF.[17]); OR 1.21, CI 1.07–1.38 (REF.[90])

Systemic lupus erythematosus
- Susceptibility: OR 1.84, CI 1.35–2.79 (REF.[19]); OR 1.86, CI 1.22–2.84 (REF.[21]); white individuals OR 0.63, CI 0.53–0.89; black individuals OR 0.46, CI 0.23–0.95 (REF.[20])
- Severity: 11–42% versus 58–89%, $P < 0.04$ (for serositis, nephritis and CNS involvement)[20]

Granulomatosis with polyangiitis
- Susceptibility: OR 1.4, CI 1.08– 1.76 (REF.[22])

Severity determined in cohorts with clinically defined severe versus mild disease. CI, 95% confidence interval; CNS, central nervous system.

MIF function in rheumatic diseases

Rheumatoid arthritis. The experimental finding that immunoneutralization or genetic deletion of MIF reduces disease in mouse models of inflammatory arthritis provided impetus for exploring the mechanistic function of MIF in RA, and was initially supported by data showing that MIF deficiency reduces the expression of multiple cytokines downstream of MIF, such as TNF, IL-1, IL-6 and IL-17 (REFS[39,106–109]). MIF is highly expressed in the plasma and synovium of patients with RA, and expression levels correlate with high-expression *MIF* genotypes[13,15,110–112]. Cultured human synovial fibroblasts spontaneously produce MIF, which induces CD74-dependent proliferative signalling, inflammatory prostaglandin (PGE_2) production and resistance to apoptosis[113,114]. MIF also increases the expression of matrix-degrading metalloproteinases[115,116] and promotes angiogenic responses necessary for expansion of destructive rheumatoid pannus[117–119]. In mononuclear cells, MIF signals through CD74 to phosphorylate ERK1/2 and inhibit activation-induced, p53-dependent apoptosis, thereby sustaining high levels of inflammatory cytokine production[59]. Increased NF-κB signalling and enhanced vascular cell adhesion molecule 1 (VCAM1) and intercellular adhesion molecule 1 (ICAM1) expression also

occur as a consequence of MIF stimulation[61,120]. T cells in patients with RA produce MIF, and in vitro data suggest its importance in promoting joint erosion by upregulating expression of the osteoclastogenic TNF family member receptor activator of NF-κB ligand (RANKL)[121]. Rheumatoid synovial fibroblasts have a distinct pathogenic phenotype, with higher levels of COX2 expression and PGE_2 production, and increased invasiveness[119,122]. Synovial fibroblasts isolated from the joints of patients with RA produce MIF at concentrations that reflect their *MIF* genotype, with the highest levels of PGE_2 production, Rho GTPase activation, and migratory and invasive activity observed in cells with high-expression *MIF* promoter microsatellites (>5 CATT repeats)[58].

The gene encoding the MIF signalling co-receptor CD44 comprises 19 exons, of which 10 participate in alternative splicing to produce variants with an extended ectodomain structure[123]. Rheumatoid synovial fibroblasts with high-expression *MIF* promoter variants produce excess MIF and have increased expression and recruitment of CD44 into a functional signal transduction complex[58]. MIF signalling increases the production of the CD44v3–CD44v6 splice variants by upregulating expression of the Tra2α RNA splicing factor[58]. These extended CD44 isoforms mediate cellular migration, adhesion and invasion by mechanisms that involve increased matrix interaction and the creation of neodomains for growth factors and matrix metalloproteinases[123]. CD44 forms a co-complex with αvβ3 integrin[124] and the MIF–CD74–CD44 signalling axis induces αvβ3 integrin expression and matrix adhesion; this effect is also associated with enhanced RhoA GTPase activation, which is necessary for cell motility[58]. These cumulative actions on the rheumatoid synovium, which exhibits features of a locally invasive tumour, have led to the notion that MIF signalling recapitulates important features of malignant transformation[119,125,126], including resistance to apoptosis[59,97,106], proliferative signalling[114], evasion of growth suppressors[127,128] and angiogenesis[117].

Systemic lupus erythematosus. Interest in the function of MIF in the pathogenesis of SLE developed initially from its function in tissue macrophage retention[26,129] and data showing that renal expression of MIF is increased in glomerulonephritis and correlates with leukocyte infiltration, glomerular injury and impaired kidney function[130,131]. MIF is overexpressed in lupus-prone mice and $Mif^{-/-}$ MRL/*lpr* mice are protected from renal and skin disease with reduced renal macrophage recruitment, glomerular IgG deposition and urinary CCL2 excretion[132]. Pharmacological MIF antagonism also reduces glomerular injury, interstitial inflammation and $CD74^+$ leukocyte recruitment into kidneys in genetically distinct NZB/NZW F1 lupus-prone mice, with decreased plasma levels of TNF and CCL2 and reduced intrarenal levels of mRNA encoding TNF, IL-1β and CCL2 (REF.[133]). Circulating levels of autoantibodies against double-stranded DNA and splenic T cell and B cell surface activation indices were not affected by anti-MIF or small-molecule MIF antagonism, although fewer B cells were evident in the secondary lymphoid tissue[133]. The latter finding is consistent with the requirement for MIF in the survival of recirculating B cells, which is a consequence of MIF downregulating the anti-apoptotic factor BCL-2 (REF.[134]). The reduction in the expression of inflammatory cytokines and decrease in intrarenal leukocyte content after MIF blockade were further supported by a microarray-based analysis of gene expression, which showed a generalized downregulation of pro-inflammatory cytokines, chemokines and MIF-dependent signalling intermediates[133]. These studies[132,133] provided scientific support for the further clinical development of anti-MIF (imalumab) and anti-MIF receptor (milatuzumab) antibodies. Both antibodies have shown favourable safety profiles in phase I studies, and milatuzumab has since advanced into phase II testing for lupus nephritis[135–137].

MIF-dependent pathways have been investigated in clinically defined cohorts of steroid-sensitive and steroid-resistant patients with SLE[46]. Increased MIF levels were detected in patients who were unresponsive to glucocorticoid treatment, which is in accordance with a previous study showing a correlation between circulating MIF concentration and glucocorticoid treatment[51]. Closer investigation revealed higher cytosolic MIF content and higher nuclear levels of NF-κB in the peripheral blood monocytes of glucocorticoid-resistant patients, consistent with mechanistic studies showing that MIF antagonizes glucocorticoid-induced IκB expression[45]. *MIF* gene silencing increases IκB levels in cultured monocytes from glucocorticoid-resistant patients with SLE, whereas the addition of MIF decreased IκB expression and increased NF-κB expression in cells from glucocorticoid-sensitive patients[46]. A composite score of type I interferon-regulated chemokines is a validated biomarker of SLE disease activity[138]; notably, circulating MIF levels have also been shown to be more highly correlated with persistent active disease than this type I interferon-regulated chemokine score[139].

Spondyloarthritis. SpA is a chronic inflammatory disease that predominantly involves the axial joints and the entheses, and shows a strong association with the *HLA-B27* class I locus. Serum MIF levels are substantially higher in patients with SpA than in healthy individuals and independently predict SpA disease progression[140]. MIF levels are also higher in the synovial fluid and the number of MIF-producing macrophages and Paneth cells is increased in the gastrointestinal tracts of patients with SpA compared with healthy individuals[140]. Monocytes from these patients have low expression of CD74-ICD[140], which would be consistent with increased intramembrane proteolysis of CD74 from sustained MIF signalling[57]. MIF also activates β-catenin in osteoblasts and promotes the mineralization of osteoblasts[140], supporting a mechanistic role in the reactive bone formation that characterizes SpA osteopathology. Intriguingly, a high prevalence of anti-MIF receptor (anti-CD74) autoantibodies has been reported in patients with SpA, with evidence that the presence of autoantibodies of the IgA subclass closely correlates with early disease[141]. Whether these autoantibodies mediate blocking or stimulatory effects through CD74 remains to be examined.

Prospects for precision medicine

Both imalumab (anti-MIF) and milatuzumab (anti-CD74) have reached clinical application, with efficacy data reported in early studies[136,137,142,143]. Imalumab has a favourable safety profile[136] and milatuzumab has received orphan drug designation for multiple myeloma, a malignancy of B cells where MIF–CD74 survival pathways are highly active[67]. Milatuzumab has shown efficacy in lupus nephritis in a phase IB study[137].

The pharmacological targeting of MIF by small-molecule antagonists has been facilitated by the vestigial tautomerase active site of MIF, which overlaps structurally with the CD74 binding domain[144,145]. This feature has been exploited in multiple efforts to design small-molecule MIF tautomerase inhibitors that target this site[146–149] and a subset of such inhibitors block MIF binding to CD74 and show therapeutic activity in mouse models of SLE[133]. The small-molecule MIF antagonist that is furthest advanced in clinical development is ibudilast, which was originally developed as a phosphodiesterase inhibitor but was discovered to inhibit MIF allosterically[150]. Remarkably, ibudilast binds to a dynamic site of MIF that is not present in the (apo) crystal form of MIF; that is, this site is only revealed when ibudilast binds to MIF. Once bound, the ensuing conformational changes eliminate MIF activity[150]. Ibudilast has shown efficacy in a phase II study of multiple sclerosis, an autoimmune disease in which the high-expression *MIF* genotype confers risk for progressive disease[151,152]. The singular dynamic properties of MIF, which enable its ready transition among multiple conformations[153], have also made possible the discovery of MIF agonists that might be applied therapeutically in circumstances in which low-expression *MIF* alleles are deleterious[5,154,155].

Conclusions

MIF occupies an apex position in the regulation of the immune response, with evolutionary pressure evidently leading to the persistence of commonly occurring functional promoter polymorphisms in the human population[1]. Polymorphic genes are an important basis for variation in host immunity and the *MIF* allelic system probably arose as a population response to lethal infection, and its presence clearly affects the clinical expression of autoimmunity. The precise interplay between *MIF* and other genes in the pathogenesis and clinical expression of different autoimmune diseases is not fully understood. Nevertheless, clinical correlations from human genetic studies seem to be consistent with the known actions of MIF in inflammatory activation, glucocorticoid responsiveness, and cell survival and apoptosis. *MIF* genotyping might offer prognostic information and might be used to improve clinical management of rheumatic diseases. The possibility that some patients have a predilection for disease sequelae based on their *MIF* genotype would support a pharmacogenomic approach to therapeutic intervention. The association between high-expression *MIF* alleles and disease severity also supports the possibility that anti-MIF therapies might be most effectively used to treat patients who have the high-expression *MIF* genotype.

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Publisher's note

OPINION

Establishing outcome measures in early knee osteoarthritis

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Abstract | The classification and monitoring of individuals with early knee osteoarthritis (OA) are important considerations for the design and evaluation of therapeutic interventions and require the identification of appropriate outcome measures. Potential outcome domains to assess for early OA include patient-reported outcomes (such as pain, function and quality of life), features of clinical examination (such as joint line tenderness and crepitus), objective measures of physical function, levels of physical activity, features of imaging modalities (such as of magnetic resonance imaging) and biochemical markers in body fluid. Patient characteristics such as adiposity and biomechanics of the knee could also have relevance to the assessment of early OA. Importantly, research is needed to enable the selection of outcome measures that are feasible, reliable and validated in individuals at risk of knee OA or with early knee OA. In this Perspectives article, potential outcome measures for early symptomatic knee OA are discussed, including those measures that could be of use in clinical practice and/or the research setting.

Osteoarthritis (OA) is a leading cause of chronic pain, disability and health-care utilization, with knee OA contributing the greatest burden[1–4]. OA is associated with increased rates of comorbidity (for example, obesity and heart disease)[1] and is one of the most burdensome disabilities worldwide[2]. The incidence, burden and socioeconomic impact of OA is considerable and growing[3,5]. Therefore, a shift in the approach to the management of patients with OA is needed, from treating patients with established OA to a proactive approach that focuses on mitigating risk factors. The classification and monitoring of early OA, on a trajectory from normal to symptomatic and/or radiographic OA, would allow the development and evaluation of interventions in clinical and research settings to prevent or slow down the disease process at a time the disease is probably more amenable to modification.

Although the definition of early OA and appropriate outcomes are under development, OA is probably heterogeneous in terms of its presentation and progression. Knee OA might progress slowly over a period of 10 or more years, rapidly or not at all[6]. Predicting the development and progression of the disease through identifying risk factors and mechanisms of OA is important in the management of chronic disease to inform targeted prevention and treatment strategies. Such prediction is difficult because of the heterogeneous presentation of OA; however, the availability of increasingly sophisticated statistical and computational methods, microsimulation modelling and large population-based cohort studies make this approach increasingly viable. For example, widely used online prediction tools are now available for evaluating the future risk of osteoporotic fractures and for guiding clinicians in preventive management of osteoporosis[7–9]. Comparable reliable and validated outcome measures for early knee OA will inform the evaluation of risk factors for the progression of early OA. More than one set of risk factors and models will probably be needed to predict early OA in the future.

In the Rotterdam and Chingford studies (two prospective population-based studies), researchers were able to predict incident radiographic knee OA using a combination of clinical, genetic and radiographic factors[10]. When performing risk assessment and creating a predictive model for early knee OA, many aspects need to be considered: the definitions of the outcome and prognostic factors; the duration of the clinically relevant prediction period; and the setting in which the risk prediction tool will be used (for example, primary care, secondary care or the research setting). For instance, expensive and intensive predictive tools such as MRI scans and biochemical markers might be restricted to secondary care and/or the research setting.

In this Perspectives article, we highlight considerations for best practice in the selection of outcome measures for use in clinical practice and the research setting to evaluate patients at initial presentation of early knee OA across different outcome domains: patient-reported outcomes, clinical features, physical function outcomes, modifiable lifestyle-related outcomes (such as adiposity, physical activity and nutrition), biomechanical outcomes, imaging features and biochemical markers[11]. We suggest outcome measures that could be considered for use in individuals with early knee OA in clinical care and the research setting using published evidence (primarily from populations with post-traumatic and established OA), emerging evidence (ongoing studies) and clinical expertise (BOX 1). The outcome measures highlighted are relevant to individuals who are at risk of OA or fit the provisional criteria for early knee OA based on patient-reported outcomes of pain and function, together with clinical signs (joint line tenderness or crepitus (that is, grating

Box 1 | **Proposed outcomes for the assessment of early pre-radiographic OA**

Below, we provide suggestions for outcome measures that could be used to assess individuals with early pre-radiographic osteoarthritis (OA) in clinical practice and in the research setting. Many of these measures have been evaluated primarily in established OA[42,43,46–49,56–58,65,152]; thus, further research is needed to evaluate the validity of each outcome measure in early OA and to investigate how outcomes change with progression of OA.

In clinical practice and research settings:

- Patient-reported outcomes

The Knee Injury and Osteoarthritis Outcome Score (KOOS) can be used to measure pain during activity, other symptoms (for example, stiffness, grinding, catching, swelling, knee flexion and extension), function in daily life and during sport and recreational activities, and quality of life across different age and treatment groups. The Intermittent and Constant Assessment of Pain (ICOAP) questionnaire can be used to evaluate constant and intermittent pain.

- Clinical features

A clinical assessment including joint line tenderness should be performed in individuals with new-onset symptoms of knee pain, stiffness, crepitus or a feeling of 'giving way'.

- Physical function outcomes

Three measures seem promising for use in the clinical setting on the basis of their reproducibility, patient acceptability and the equipment[152] and expertise required: the single leg hop test[42,43,46–49], the 30-second chair sit-to-stand test[56–58], the star excursion balance test[43,50–55] and measures of quadriceps strength[43,46,47,51,65]. Multiple additional functional measures have been validated for use in the research setting.

- Modifiable lifestyle-related outcomes

Adiposity can be assessed by measuring body fat percentage or fat mass index (fat mass in kilograms/height in metres squared) using dual-energy X-ray absorptiometry or bioelectrical impedance analysis if available. BMI is more feasible in the clinical setting, although it has limitations for use in athletes. Levels of physical activity can be assessed using a validated physical activity monitor or a validated questionnaire if objective methods are not available. Nutrition outcomes are not currently suggested for use in routine clinical care; however, the 3-day dietary record provides reliable estimates of nutrient intake.

In research setting only:

- Biomechanical outcomes

Measures of biomechanical outcomes require further research and are not currently suggested for use in routine clinical care. However, such outcomes are ideal for informing the underlying mechanisms of OA progression and informing treatment interventions in the research setting.

- Imaging features

The utility of plain radiography in early OA is limited. Although MRI has superior sensitivity to change, has validity in the context of early OA[152] and is hence ideal in the research setting, MRI is not thought appropriate for the routine clinical care setting because of its high cost and potential risk of over-diagnosis.

- Biomarkers

No biomarkers are currently of use in routine clinical care; however, further validation of proteomic, lipidomic and metabolomic tools in the research setting could lead to informative cartilage and synovial fluid profiles and provide important insights into OA progression.

and crackling sounds)) and a radiographic Kellgren–Lawrence (KL) grade of 0 or 1 (REF.[12]). Although proposed as important evidence-informed clinical outcome measures, these outcome measures will require additional validation and possible modification to suit local primary-care and other health-care settings, as well as periodical updates.

Patient-reported outcomes

A patient-reported outcome is any patient health status that is reported directly by the patient without interpretation by others (for example, the clinician). Measures of these outcomes commonly take the form of a questionnaire. Most relevant patient-reported outcome measures have been developed to assess individuals with either a knee injury (for example, International Knee Documentation Committee 2000 (IKDC2000)) or established OA (for example, the Western Ontario and McMaster Osteoarthritis Index (WOMAC)), although one questionnaire has been developed to cover the full spectrum of OA from injury to established OA (the Knee Injury and Osteoarthritis Outcome Score (KOOS)). The relative merits of these and other available instruments that measure self-reported pain, function and quality of life have been the subject of previous reviews[13,14]. Other patient-reported outcome measures, such as the Patient-Reported Outcomes Measurement Information System (PROMIS), have been developed using computer-adaptive strategies and might also prove relevant for use in people with early knee OA[15]. Many of the considerations that influence the choice of measure in established OA (for example, respondent burden, cost or availability) apply also in early OA.

Ultra-brief (1 or 2 domains) unidimensional generic measures, such as the 11-point Numerical Rating Scale (NRS) and the 36-item Short Form Health Survey (SF-36) bodily pain scale, have been previously recommended for established OA[16] and are probably also applicable in early OA. However, the disadvantage of unidimensional measures is that they provide a restricted view of the character and intensity of the pain[16,17], which is probably inappropriate based on emerging evidence from qualitative studies in patients with early knee OA[18–20]. For instance, these patients report that their initial symptoms can be experienced as an 'awareness' of the knee, loss of confidence or the need to 'be careful', as opposed to 'pain'. Furthermore, reporting OA pain as 'constant' or 'present on most days' might lead to floor effects (that is, most individuals might have scores at the lower end of the scale) in early OA, as these patients often report episodic and intermittent pain with certain activities. For example, data from the Osteoarthritis Initiative indicate that pain during ascending or descending stairs is most likely to be the earliest reported functional difficulty[21]. Accordingly, the knee-related quality of life subscale of KOOS considers various pain-related aspects of early OA (such as awareness of a knee problem or loss of confidence in the knee)[14,15], and the Intermittent and Constant Assessment of Pain (ICOAP) questionnaire includes a subscale for intermittent pain symptoms[22].

The ICOAP questionnaire was designed to evaluate the pain experience of individuals with OA, and there is an increasing amount of evidence supporting its reliability and validity[22]. This questionnaire considers pain intensity, frequency and the impact of pain on mood, sleep and quality of life, and is intended to be used alongside a measure of physical function[22]. By contrast, the KOOS was developed for self-reporting of patient-relevant outcomes across the lifespan, from the time of knee injury and potential knee OA onset to severe OA[23–26]. In five separate subscales, this tool assesses perceived pain and other symptoms (for example, stiffness, grinding and catching), perceived difficulty with function during daily life, sport and recreational activities,

and knee-related quality of life. The measurement properties of KOOS have been reported in studies of young, middle-aged and elderly groups with knee injury or OA, and across a spectrum of treatments[14]. A comprehensive literature review, which included 37 studies evaluating KOOS measurement properties in participants with knee injuries and/or OA, showed that KOOS has adequate content validity, internal consistency, test–retest reliability, construct validity and responsiveness for age-relevant and condition-relevant subscales[14]. The KOOS is feasible to administer electronically and in paper form, and KOOS scoring instructions and population-based KOOS reference data are available. In addition, longitudinal KOOS data have been collected from >100,000 patients in surgical registries of anterior cruciate ligament (ACL) reconstruction and knee replacement, facilitating comparison with many different populations[27,28]. Furthermore, KOOS data from the Osteoarthritis Initiative cohort, which consists of individuals who are at increased risk of OA or who have established OA, are freely available (see Related links). The Osteoarthritis Initiative also collects a wide range of other self-reported, clinical and imaging data.

Another important consideration for outcome measures of OA is that the early phase of knee OA is often associated with the emergence of adaptive behaviour. Symptom frequency and intensity might be minimized through the selection of behaviours (for example, performing some activities less often), optimization of behaviours (for example, advanced planning of activities, including anticipatory analgesic use) and compensatory adaptations (for example, modifying the way activities are performed)[29]. Therefore, adaptive behaviour is an important consideration for outcome measures in early OA[30]. For example, the Questionnaire to Identify Knee Symptoms (QuIKS) includes questions such as "I am considering stopping a favourite activity due to my knees" and "I am considering changing my exercise routine due to my knee problems"[31].

OA-specific measures developed for more advanced OA cannot be assumed to have adequate psychometric performance when applied to early OA. Yet, the requirement for adequate performance in early OA must be balanced against the benefits for a coherent evidence base that comes from using common measures across the spectrum from early to advanced OA. Of the existing measures, the KOOS and ICOAP questionnaire seem to best strike this balance and are therefore strong candidates for evaluating early knee OA (BOX 1), particularly as these instruments focus on different aspects, and both have the advantage of being freely available. Published reviews of the psychometric properties of these two measures require systematic updating with specific attention to their performance in early OA.

Clinical features

Clinical features of early OA, such as joint tenderness and crepitus, are easy to examine in the primary-care setting, and their assessment is also relevant in the research setting. These measures might be associated with the development of OA in the future, even in the absence of radiological findings of OA (BOX 1). For example, joint line tenderness (of the tibiofemoral and/or patellofemoral joint lines) at baseline was found to be a strong predictor of pain progression at 5 years (moderate progression adjusted OR = 3.9, 95% CI 2.3–6.6)[32] in the CHECK cohort, which included patients with new-onset knee pain or stiffness[33]. Several studies have evaluated the ability of physical signs to predict the clinical onset of structural radiographic OA in patients with an increased risk of OA[32–36]. Data from the HONEUR study, which included 549 participants who were recruited at the first presentation of knee pain in primary care, suggest that joint line tenderness, crepitus, pain with passive flexion and a self-reported swollen knee predict incident radiographic tibiofemoral knee OA after 6 years[34]. Using MRI features of knee OA as an outcome, data from the Rotterdam Study show that joint line tenderness together with the 'feeling of giving way' are associated with the incidence of tibiofemoral knee OA, but identify crepitus as a good predictor of patellofemoral OA[35,36]. Clinical examination of the knee (including joint line tenderness and crepitus) has good inter-observer reliability in patients with evident knee OA if a standardized approach to the assessment is used[37]. However, the various components of such examinations require further assessment for their reliability and validity in the research setting and standardization for use in the clinical setting for early knee OA.

Physical function outcomes

Given that the early pre-radiographic stage of OA is associated with intermittent symptoms and adaptive physical behaviour, the clinical evaluation of patients with, or at risk of, early knee OA should incorporate robust outcome measures of physical function[38]. Currently, no consensus exists regarding which outcome measures are most relevant for use in this population. For the purposes of this Perspectives article, physical function is operationally defined as 'physiological functions' or 'the ability to move around and to perform daily activities' that can be classified as 'body functions and structure' or 'activities and participation', respectively, using the WHO International Classification of Functioning, Disability and Health (ICF) model[39]. As physical function is multidimensional, both performance-based and physical impairment measures (which might require specialized equipment and raters) are discussed in this section. Emerging evidence suggests that some of these outcome measures might be suitable for the evaluation of patients with early OA and individuals at risk of OA[40–45] (TABLE 1).

A range of performance-based measures are available, although the degree to which their measurement properties (such as their reliability and validity) have been tested and the range of populations they have been tested in vary (TABLE 1). Performance-based measures that have undergone fairly extensive investigation include the single leg hop for distance test[42,43,46–49], the cross hop for distance test[42,46–49], the 6-metre timed hop test[42,46–49], the star excursion test (and the similar Y-balance test)[43,50–55], the 30-second chair sit-to-stand test[56–58] and the 6-minute walk test[40,41]. However, some data are now also available for the vertical drop jump test[43,59], the single leg squat test[43,60–62], the unipedal dynamic balance test[43,63] and the 20-metre shuttle run test[43,64]. The most commonly reported outcome of physical impairment is quadriceps muscle strength[43,46,47,51,65], but there might also be value in considering the strength of other muscles of the lower extremities, including the hamstring, hip abductor and hip adductor muscles[66]. However, insufficient information is available to advocate a specific mode of contraction (that is, isotonic, isokinetic or isometric) or type of contraction (that is, concentric or eccentric) to assess.

Because of floor and ceiling effects, separate measures are required to cover the wide range of ages and abilities of patients with early knee OA in both the clinical and the research settings. Functional outcome measures that should be considered for use in research and in clinical physical and exercise therapy practice, on the basis of their measurement properties and ability to span the full spectrum of patient ages and abilities, include the single leg hop test for distance, the 30-second chair sit-to-stand test, the 6-minute walk test, the star excursion balance test and a quadriceps strength measure. Performance-based

outcome measures should be performed in a standardized, validated and reproducible manner to enable detection of change over time. Video demonstrations and detailed instructions for standardized testing are available online (see Related links). Further research validating functional outcome measures in 'at-risk' populations (such as individuals with obesity, an intra-articular knee injury or a varus or valgus malalignment) and 'early-OA' populations is required, and should inform the periodic updating of these suggested functional outcome measures.

Modifiable lifestyle-related outcomes

Modifiable risk factors related to lifestyle, such as obesity, dietary inadequacies and physical inactivity, might accelerate disease onset and progression through a combination of mechanical and systemic mechanisms[67]. Identifying these modifiable risk factors in individuals with early knee OA is important for the prevention of OA.

Several measures of adiposity or weight have been studied in established OA, but less so in early OA. These measures include BMI, waist-to-height ratio and waist circumference[68–72]. In addition to contributing to an increased mechanical load, adiposity is thought to have a metabolic and pro-inflammatory function in OA; therefore, a direct measure of adiposity, such as fat mass, percentage of fat mass (percentage of total mass) and fat mass index (fat mass in kilograms/height in metres squared), for example, through the use of dual-energy X-ray absorptiometry or bioelectrical impedance analysis[73], might be useful in the assessment of early OA[74–77]. The location of fat deposits influences their metabolic and inflammatory

Table 1 | **Important physical function outcomes**

Outcome measure	Test measure	Equipment required	Reliability			Error	Validity		Responsiveness and interpretability	Appropriate risk group (age)	Refs
			Intra-test	Inter-test	Retest		Structural	Hypothesis testing			
Single leg hop for distance	Length (cm)	Measuring tape	+	–	–	–	–	+/–	–	Post-trauma (≤45 years)	42,43, 46–49
Cross hop for distance	Length (cm)	Measuring tape	+	–	–	–	–	+/–	–	Post-trauma (≤45 years)	42,46–49
6-Metre timed hop test	Time (s)	Measuring tape	+	–	–	–	–	+	–	Post-trauma (≤45 years)	42,46–49
Star excursion balance test	Length (% leg length)	Measuring mat, tape and skilled rater (leg length)	+	+	+	+	–	+	–	Post-trauma or obese (all ages)	43,50–55
30-Second chair sit-to-stand test	Count (number of repetitions)	Chair and timer	+	+	–	–	–	–	–	Post-trauma or obese (all ages)	56–58
6-Minute walk test	Length (m)	Flat 20-Metre walking area, timer and chair	–	–	–	–	–	–	–	Obese (all ages)	40,41
Vertical drop jump	Risk rating	31-Centimetre high box	+	+	–	–	–	+/–	–	Post-trauma (≤45 years)	43,59
Single leg squat	Risk rating	None	+	+	–	–	+/–	+/–	–	Post-trauma or obese (all ages)	43,60–62
Unipedal dynamic balance	Time (s)	Balance pad and timer	–	+	+	–	+	+	–	Post-trauma or obese (all ages)	43,63
20-Metre shuttle run	Stage	Coloured tape and instructions	–	–	+	+	+/–	+	–	Post-trauma (≤45 years)	43,64
Quadriceps strength	Force (Nm/kg)	Hand-held or isokinetic dynamometer and skilled rater	+	+	+	+	+	+	+	Post-trauma or obese (all ages)	43,46, 47,51,65
Hamstring strength	Force (Nm/Kg)	Hand-held or isokinetic dynamometer	+	+	+	+	+/–	+/–	+/–	Post-trauma or obese (all ages)	40,42,66
Hip adductor or hip abductor strength	Force (Nm/kg)	Hand-held or isokinetic dynamometer	+	+	+	+	–	+/–	–	Post-trauma or obese (all ages)	40,42,66

+, supporting evidence; –, no supporting evidence; +/–, conflicting evidence.

potential, and therefore might be important considerations[78].

A systematic review has shown a moderate level of evidence supporting a relationship between obesity (that is, increasing weight, BMI or total body fat mass) and the presence of bone marrow lesions (BMLs) in the knee in individuals with OA[71]. Total fat mass is positively associated with an increased risk of knee cartilage defects, the presence of BMLs in healthy individuals aged 25–60 years[79] and medial tibiofemoral cartilage volume loss over 2–10 years in adults aged 51–81 years[80,81]. A high waist-to-height ratio or waist circumference (indicative of abdominal adiposity) is associated with an increased risk of OA progression in the hand[82]; however, neither outcome is associated with the longitudinal loss of tibial or patellar cartilage volume or defects in adults (aged 50–79 years) in the community[74,83]. To better understand this relationship, a distinction between subcutaneous and visceral adiposity using valid assessment techniques, such as MRI or CT, is probably needed.

Physical activity is a modifiable outcome that might delay the onset of functional limitation and prevent obesity, and is essential for normal joint health[84]. In addition, physical activity can reduce pain and disability among individuals with OA and increase their physical performance and self-efficacy[85–87]. Low-intensity or moderate-intensity physical activity might protect against the onset of disability related to symptomatic OA, whereas a sedentary lifestyle or high levels of strenuous physical activity are considered risk factors[88–90]. Many variations of self-reported measures of physical activity exist, including global or short recall questionnaires, although most measures have limited accuracy[88–90]. Wearable monitors that measure body motion can be used to assess physical activity and energy expenditure. The most commonly used sensor, validated across multiple populations, is an accelerometer (for example, the ActiGraph accelerometer GT3X)[91], which captures frequency, intensity and duration of physical activity in a time-stamped manner. By comparison, the large selection of off-the-shelf accelerometers, often contained in smart phones, might be more suitable for measuring physical activity in the primary-care setting as they are less expensive, easier to use and widely available[92,93]. However, most accelerometers are not validated for measuring cycling or swimming. In general, objective measures of physical activity such as measures captured by an accelerometer have stronger relationships with function in OA than self-reported outcomes[94] and provide a more accurate assessment of physical activity and sedentary lifestyle.

Nutrition interventions, such as weight loss[95,96], are lifestyle-related changes that can potentially improve OA symptoms. Beyond the link between obesity and knee OA (and therefore the important contribution of weight loss)[97,98], the contribution of nutritional factors is an emerging and important area of research, although limited clinical evidence is available to date. For example, low dietary intake of fibre[99] or omega-3 polyunsaturated fatty acids[100] and high-fat diets[101] are risk factors for OA and/or worsening of pain in OA and might therefore warrant monitoring in early OA. Many of the nutrients or dietary patterns tested to date probably contribute to pathology via alterations in body weight or inflammation, although the direct effects of these factors requires further investigation. The tools to monitor dietary intake are numerous, and their appropriateness for each clinical or research setting needs to be assessed. They include the Food Frequency Questionnaire, the 24-hour dietary recall assessment tools (using either paper-based or web-based automated self-administered 24-hour dietary recall)[102] and the 3-day or 7-day weighed food record. The use of tools that assess adherence to diets that reduce inflammation, such as the Mediterranean Diet Adherence Screener[103], might also be warranted in the future.

Hence, objective measures of adiposity are desirable. BMI is a useful outcome measure for assessing adiposity in the primary-clinical setting because of its familiarity, validity and reference ranges. However, BMI has limitations for use in young athletes. Although weight loss can improve OA symptoms, further research is needed to identify a means of assessing important OA-related nutritional factors. Assessment of physical activity using a validated accelerometer, to accurately capture activity through each domain and intensity, is a promising area that requires further study.

Biomechanical outcomes

Biomechanical outcomes are readouts of joint mechanics (such as joint movement, loading or muscle activation patterns) that are typically measured in the research setting but can sometimes also be measured in the primary-care setting.

Measures of joint mechanics can be employed to assess OA severity, but also to investigate the causes of OA onset and progression. For example, altered joint mechanics following knee injury might contribute to the onset and development of post-traumatic OA[38]. Indirect evidence to support this concept comes from observations of altered joint movement, loading and muscle activation patterns following injury[104–109], with radiographic knee OA (KL grade of ≥2)[110–112], with ageing[113,114] and following joint arthroplasty[115–117]. Abnormal joint alignment[118,119], alteration in the external knee adduction moment and increased varus alignment are often regarded as indicators of altered joint mechanics associated with increased OA severity[112]. Additional risk factors for the development of OA include aberrant dynamic joint stability[120,121], muscle atrophy[122], neuromuscular inhibition[123], muscle weakness[124–126] and compensatory muscle activation mechanisms[110,111,116]. These changes might alter cartilage loading and contact mechanics. Indeed, some studies have indicated that changes in tibiofemoral cartilage contact locations[38,127], elongated path lengths[128], force magnitudes[105,129,130] and deformations[127,128] are associated with OA onset and progression. In turn, OA progression might be caused by progressive degradation of cartilage through interactions of articular movement and cartilage loading abnormalities, chronic inflammation, resultant tissue remodelling and other OA risk factors by increasing the susceptibility of cartilage and subchondral bone to damage and degradation in regions inadequately adapted to these altered loads[127,131–135]. Over time, this process might result in altered cartilage thickness and clinically relevant cartilage thinning in different regions of the articular cartilage surfaces. To verify this mechanism, longitudinal data on joint mechanics, cartilage thickness, and cartilage structure and integrity in OA are needed[136,137]. Integration of this information with other risk factors for OA-related changes might inform the development of novel patient-specific, diagnostic or predictive models to aid in early patient screening, intervention efficacy monitoring and the development of new therapeutics[129,130,132,138,139]. Armed with these data and models, new wearable monitors might enable biomechanical outcome assessment in the clinic and community[133–135,140,141], and might provide the possibility of developing and monitoring personalized treatment plans.

Measuring the range of motion of a joint might help in the assessment of OA severity in the primary-care setting. Although other biomechanical outcome measures (such as

Box 2 | **MRI-defined OA**

Tibiofemoral OA:
A definition of tibiofemoral osteoarthritis (OA) on MRI would be the presence of both group [A] features or one group [A] feature and two or more group [B] features.

Group [A], after exclusion of joint trauma within the past 6 months (by history) and exclusion of inflammatory arthritis (by radiographs, history and laboratory parameters):

- Definite osteophyte formation[a]
- Full thickness cartilage loss

Group [B]:

- Subchondral bone marrow lesion or cyst not associated with meniscal or ligamentous attachments
- Meniscal subluxation, maceration or degenerative (horizontal) tear
- Partial thickness cartilage loss (where full thickness loss is not present)
- Bone attrition

Patellofemoral OA:
A definition of patellofemoral OA requires both of the following involving the patella and/or anterior femur:

- A definite osteophyte[a]
- Partial or full thickness cartilage loss

[a]The definition of a 'definite osteophyte' was not delineated in the Delphi process and requires further validation. This box has been adapted from Hunter et al.[160].

measures of knee adduction moment, joint kinematics and cartilage loading) are currently not feasible to collect in most clinical settings, they can be employed to help understand the mechanisms of OA progression and are an important consideration in the research setting to inform the design of orthotics and exercise, bracing and surgical interventions. In the future, validated wearable monitors might help to assess biomechanical outcomes of early interventions in the clinic and community. Evidence suggests that variation in one outcome measure (for example, a biomechanical outcome) is not independent but rather can influence variations in the quantitative state of other measures (for example, biochemical markers or imaging features)[142–146]. Thus, future research should consider the interaction between different outcome measures to potentially increase the sensitivity of detecting early OA[131,143].

Imaging features

OA is a complex syndrome that at the local level is best characterized as a whole-joint disease involving multiple tissue pathologies. To characterize and monitor the various structural components involved in OA, a number of different imaging modalities have been used, the most common being radiography, ultrasonography and MRI. This section predominantly focuses on plain radiography and MRI, as ultrasonography has several limitations that have constrained its development and validity in this area, including observer dependency and its inability to assess BMLs and to adequately image deep articular joint structures (including the meniscus and cartilage)[147].

MRI has an important function in the OA research setting, with compositional MRI techniques becoming increasingly important owing to their capacity to assess 'pre-morphological' biochemical compositional changes of articular and periarticular tissues. Although radiography remains the primary imaging modality in OA clinical trials and in daily medical practice, known limitations for visualizing OA features notably limit the utility of radiography both clinically and in the research arena. Ultrasonography can be a useful adjunct to radiography and MRI, particularly for the evaluation of synovitis. Emerging hybrid imaging techniques, including PET–MRI and PET–CT, enable simultaneous evaluation of the joint and assessment of morphological changes and metabolic activities; these hybrid systems might have an increasing role in OA research and clinical practice in the future[148].

Radiographic features of OA are generally classified using the KL grading system[149] and include joint space narrowing, osteophyte formation, sclerosis and deformity of bony contours[150]. Minimum radiographic joint space width is the gold standard recommended by the FDA for detecting structural changes in patients with knee OA in clinical trials. However, standardized measures of radiographic positioning and fixed location joint space width does not reach the same degree of responsiveness (sensitivity to change) in knee OA as quantitative measures of cartilage thickness on MRI[151]. Moreover, radiographic features such as loss of joint space, sclerosis and deformity of bone are associated with late-stage OA and are detected earlier and with greater sensitivity by MRI[152].

Conventional MRI enables the evaluation of morphological changes related to early OA, including but not limited to cartilage damage, meniscal damage, synovitis, the presence of BMLs and ligamentous damage. In one study of 255 patients with knee pain (aged 40–79 years), BMLs were present in 11% of individuals without radiographic OA (KL grade 0), 38% of individuals with pre-radiographic OA (KL grade 1) and 71% of individuals with radiographic OA (KL grade of ≥2)[152,153]. Similarly, 42% of patients with a diagnosis of symptomatic OA without radiographic features (KL grade of <2) had BMLs and 57% had cartilage loss[154]. Although there is a paucity of data regarding the timeline of structural changes in the period between a joint injury sustained in youth and the onset of clinical post-traumatic OA, advanced MRI techniques have been used to detect subtle cartilage damage at the time of ACL injury[155]. Furthermore, macroscopic cartilaginous changes, the presence of BMLs and bone morphological changes might be detectable by conventional MRI techniques as early as 2 years after ACL reconstruction or other intra-articular knee injury, and potentially before the development of radiographic OA[6,156–159].

In 2011, a definition of the criteria for the identification of OA on MRI was proposed to facilitate earlier detection of OA[160,161] (BOX 2). In one study of patients who had undergone ACL reconstruction, 19% and 17% of the patients met the MRI criteria for tibiofemoral and patellofemoral OA, respectively, at 1 year[162]. Using the same criteria for MRI-defined OA in patients who participated in a clinical trial of ACL reconstruction, 31% of the patients had tibiofemoral OA and 9% had patellofemoral OA at 5 years[163]. Importantly, some of the changes included in these criteria are undetectable by radiography (such as cartilage thickness and BMLs). Different methodologies can be used to measure structural changes in the knee by MRI including the use of semi-quantitative measures (using scoring systems such as the MRI Osteoarthritis Knee Score (MOAKS) and the Anterior Cruciate Ligament Osteoarthritis Score (ACLOAS)[153,164]), quantitative measures (including measures of cartilage thickness, BML volume, effusion-synovitis volume and meniscal extrusion) and measures obtained using compositional imaging modalities (including T2 mapping, T1ρ mapping, delayed gadolinium-enhanced MRI of cartilage (dGEMRIC), sodium MRI and glycosaminoglycan chemical exchange saturation transfer (gagCEST)), which measure cartilage composition and quality[165]. For synovitis assessment, contrast-enhanced MRI and semi-quantitative scoring systems based on contrast-enhanced MRI are available that enable clear delineation of the synovium from effusion[166].

In population-based studies, a high proportion of radiographically normal knees have osteophytes and cartilage damage detectable by MRI, suggesting that MRI has

a higher sensitivity for detecting potential pathological changes than radiography[152]. However, defining what changes are pathological and what changes are part of a normally ageing joint remains a challenge[167]. The link between anatomical evidence of OA and patient symptoms and function is still rather weak[168,169]. Ultimately, the association between the presence of these findings on MRI and subsequent illness related to OA (alteration in patient function and symptoms) needs to be identified in longitudinal follow-up studies[170] to avoid over-diagnosis because of incidental MRI findings[152,153,171–173]. Notably, the distinction between pathology and normal features of the ageing joint is unclear, and further research to elucidate the clinical relevance of MRI findings in early knee OA is warranted.

Hence, the utility of plain radiography in early OA is limited, as only relatively late OA changes are detectable. As technology improves, assessing changes in bone shape or trabecular bone texture of subchondral bone might be of use. MRI has superior validity and sensitivity to change in the context of early OA[152]. Although not appropriate for all primary-care settings because of its high cost and risk of over-diagnosis, MRI is a critical component of ongoing outcome validation research in early knee OA.

Biochemical marker outcomes

Biochemical markers of joint tissue turnover can reflect disease-relevant biological activity that might precede structural changes detectable on plain radiographs or even on MRI scans. Some biochemical markers detected in the blood, urine or synovial fluid might be associated with, or predictive of, incident radiographic OA. Ideally, biochemical markers of early OA must clearly differentiate between normal (physiological) and pathological tissue turnover, as well as between the early stages of the disease and more advanced joint destruction. For clinical utility, biochemical markers must also be unaffected by other disorders and be easily and consistently measurable in the clinical setting[174]. Biochemical markers of early OA might be used to identify pre-radiographic changes at the molecular level, facilitate OA drug discovery and potentially enable a more rational and personalized approach to health-care-related OA management by prompting earlier and more targeted treatments and interventions[175].

Studies of incident OA have identified some of the earliest molecular abnormalities associated with OA and have therefore provided biochemical marker candidates for early OA identification. For example, in a retrospective nested case–control study, the serum concentrations of four proteins (matrix metalloproteinase 7, IL-15, plasminogen activator inhibitor 1 and soluble vascular adhesion protein 1) were altered in individuals who later developed OA compared with individuals who remained healthy[176]. Similarly, in other studies, high serum concentrations of cartilage oligomeric matrix protein (COMP) and hyaluronan predicted incident knee joint space narrowing and osteophyte formation (COMP only) 7 years later[177], whereas high serum concentrations of COMP or low serum concentrations of aggrecan predicted incident radiographic knee OA over 10 years[178]. By contrast, the concentrations of other molecular biomarkers of inflammation (including IL-6, IL-8, IL-10, TNF and IFNγ in the serum and synovial fluid) in individuals 2 years after an acute ACL injury did not predict the development of structural knee OA at 5 years[163]. Furthermore, mean baseline serum concentrations of osteocalcin were associated with 3-year incident radiographic hand OA (KL grade of ≥2) but not with knee OA in premenopausal and perimenopausal women[179]. Other potential biochemical markers include bioactive lipids (such as low-density lipoprotein, cholesterol and eicosanoids, including hydroxyeicosatetraenoic acid and its derivatives) as markers of pain and inflammation[180], and metabolic profiles, which have been shown to differ in the synovium between patients with OA and healthy individuals[181].

In 2006, the US NIH-funded OA Biomarkers Network and the OARSI Clinical Trials Biomarkers Working Group proposed a new classification system for OA biochemical markers termed BIPED (burden of disease, investigative, prognostic, efficacy of intervention and diagnostic)[182,183]. The purpose of this classification was to clarify the intended primary use of a biochemical marker[182,183]. However, a systematic review performed in 2010 concluded that individual biochemical markers and categories of biochemical markers for knee and hip OA, including their nature, origin and metabolism, need further investigation and validation[184]. In 2016, the BEST glossary designed by the FDA–NIH Biomarker Working Group to harmonize and clarify terms used in translational research was published[185]. Harmonization of these terms is important as their inconsistent use can hinder the evaluation and interpretation of scientific evidence. One of the aims of the BEST resource is to distinguish between biochemical markers and clinical assessments and to describe the distinct functions of biochemical markers in biomedical research, clinical practice and medical product development. This glossary should facilitate all aspects of biochemical marker research, including the testing, validation and commercialization of biochemical markers of early OA.

Biochemical and molecular profiling of biological fluids and joint tissues can provide a global view of the physiological state of an OA joint. Refinements in omics approaches and advances in analytical platforms and technologies should enable improved profiling of different stages of disease. To be clinically useful, biochemical markers need to be properly qualified (that is, the biochemical marker should be linked with a biomechanical and/or clinical outcome) for early OA, and they must adhere to the BEST guidelines to be effectively used in the clinical setting rather than in an exploratory and hypothesis-testing research setting. Soluble biochemical markers require further study, validation and qualification as markers of susceptibility to, or risk of, early OA before being adopted for widespread use in the clinical care setting.

Conclusions

There are various outcome domains that can be assessed in patients with early knee OA in research and/or clinical settings, including patient-reported outcomes, clinical features, physical function outcomes, modifiable lifestyle-related outcomes (such as adiposity, physical activity and nutrition), biomechanical outcomes, imaging features and biochemical markers. Promising patient-reported outcome measures for this purpose include the KOOS and the ICOAP questionnaire. Physical function outcome measures (for example, the single leg hop test and measures of quadriceps strength) and the fat mass index are also valid and reliable. With increasing popularity worldwide, validated wearable physical activity monitors for quantifying levels of physical activity and a 3-day weighed food record for nutritional intake (for example, calorie intake) have potential. MRI-defined OA and biochemical markers, although promising, require specific health-care and research facilities where the assessment of these outcomes is possible and body fluids can be collected, stored and measured according to standard operating procedures. Patient preferences and psychosocial outcomes are also important considerations in future research examining early knee OA

outcome measures[186]. In this regard, further patient-engaged research is recommended.

Importantly, multiple factors must be considered to facilitate risk assessment and the development of predictive models for early knee OA. Furthermore, definitions are needed for the potential outcomes, exposures, confounding and effect-modifying variables, duration of the clinically relevant prediction period and the setting in which the risk prediction tool will be used. As such, further research validating outcomes in individuals 'at risk' of early OA progression (for example, individuals with an intra-articular knee injury and/or who are obese) and in those with early OA is required.

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Author contributions
C.A.E., J.L.W., N.K.A., A. Ma., L.S.L. and F.P.L. wrote the article. All authors researched data for the article, provided substantial contributions to discussion of content and reviewed and/or edited the manuscript before submission.

Competing interests
C.A.E., J.L.W., A. Ma., N.K.A., K.L.B., C.M.T., R.A.R., D.T., J.L.R., G.K., D.G.L., T.A., M.E., V.B.K., E.L., S.B.-Z., J.R., G.P., F.P.L., L.S.-M., M.A.R. and A. Mo. declare no competing interests. E.M.R. and L.S.L. declare that they contributed to the development of the KOOS. L.S.L. also declares that he contributed to the development of the ICOAP and the Anterior Cruciate Ligament Osteoarthritis Score (ACLOAS). A.G. is a consultant to AstraZeneca, Merck Serono, TissueGene and Pfizer, and he is a shareholder of Boston Imaging Core Lab, LCC. D.J.H. is a consultant to Merck Serono, Pfizer, TissueGene and TLCBio, and contributed to the development of the MRI Osteoarthritis Knee Score (MOAKS).

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