BONE

Osteoclastogenic T_{reg} cells promote bone loss in inflammatory arthritis

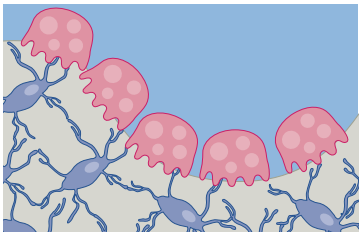
Regulatory T (T_{reg}) cells are critical to maintenance of immune tolerance, but an inflammatory milieu can promote their conversion to a pro-inflammatory phenotype marked by loss of FOXP3 expression (so-called exFOXP3 cells). New research now reveals that IL-1β induces a population of FOXP3$^+$ T_{reg} cells that contribute to bone erosion in mice with inflammatory arthritis, providing new insights into the mechanisms of bone loss in autoimmune arthritis and the contribution of IL-1 in particular.

The researchers first observed that, in mice lacking the endogenous IL-1 receptor antagonist (IL-1Ra), which develop T cell-mediated arthritis, anti-IL-1β treatment improved arthritis more effectively when administered early in the disease course than when arthritis was established. In particular, early treatment reduced bone erosion substantially more than late treatment.

"Looking for the reason for this difference, we observed that early treatment forestalled the development of a population of FOXP3$^+$ T_{reg} cells that expressed RANKL and that were capable of driving osteoclast formation," notes corresponding author Peter Nigrovic. Further investigations established that this osteoclastogenic T_{reg} cell phenotype could be induced in both mouse and human cells when naive CD4$^+$ T cells were cultured under T_{reg} cell-polarizing conditions with supplemental IL-1β, and that RANKL expression was required to confer osteoclastogenic capacity to the induced T_{reg} cells.

In mice with antibody-mediated arthritis (the K/B×N serum transfer model), adoptive transfer of CD4$^+$FOXP3$^+$ T_{reg} cells from IL-1Ra-deficient mice accelerated bone erosion. Moreover, a population of T_{reg} cells with features of the osteoclastogenic T_{reg} cells that contributed to bone erosion in mice were identified in synovial tissue samples from patients with rheumatoid arthritis.

> "IL-1β induces a population of FOXP3$^+$ T_{reg} cells that contribute to bone erosion"



Credit: Springer Nature Limited

"These studies extend the understanding of how T_{reg} cells become skewed by an inflammatory environment to become pathogenic, without losing their identity as T_{reg} cells," explains Nigrovic. "Furthermore, the findings define a new pathway by which IL-1 works within adaptive immunity to promote inflammatory joint disease."

Sarah Onuora

ORIGINAL ARTICLE Levescot, A. et al. IL-1β-driven osteoclastogenic T regulatory cells accelerate bone erosion in arthritis. *J. Clin. Invest.* https://doi.org/10.1172/JCI141008 (2021)

SYSTEMIC LUPUS ERYTHEMATOSUS

CAR T cells induce remission in a patient with refractory SLE

Chimeric antigen receptor (CAR) T cell therapy has been used to treat a patient with systemic lupus erythematosus (SLE) for the first time. The immunotherapy approach, which is already approved for use in the treatment of some types of cancer, induced rapid clinical remission of severe and refractory disease with no notable adverse effects.

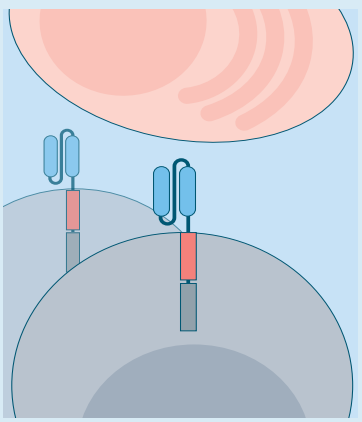
This clinical breakthrough builds on preclinical work that showed the potential of CAR T cells to ablate autoantibodies and CD19$^+$ B cells and to improve disease manifestations in mouse models of lupus. Autoreactive B cells have long been a target for SLE therapy, but the efficacy of existing B-cell-depleting drugs has been disappointing; the hope is that the CAR T cell approach will achieve more complete B cell depletion.

The 20-year-old female patient underwent leukapheresis and preparatory lymphodepleting chemotherapy before receiving an infusion of autologous CAR T cells that had been genetically engineered to recognize the B cell surface antigen CD19. Following the infusion, the CD19 CAR T cells expanded in vivo, increasing from 0.31% of total circulating T cells at day 3 to 27.69% at day 9, and remained detectable during the subsequent 7 weeks.

> "cessation of the patient's symptoms was accompanied by sustained depletion of circulating B cells"

Complete cessation of the patient's symptoms was accompanied by sustained depletion of circulating B cells and the rapid disappearance of anti-double-stranded DNA antibodies. "We suspect that a large part of the autoimmunity in this patient came from B cells and plasmablasts (bearing CD19) but not long-lived plasma cells, which are CD19 negative and hence would not be killed by the CAR T cells," reports corresponding author Georg Schett.

Whether the B cells will return — and with them autoimmunity — without further treatment remains to be seen, but the demonstration of the safety and feasibility of the treatment is encouraging. "If the patient remains free of symptoms, autoimmunity and treatment, CAR T cell treatment may indeed be a breakthrough into a new era of immunotherapy of rheumatic diseases," says Schett. The approach will now be used in additional cases of severe SLE and also move into clinical studies.



Credit: Springer Nature Limited

Sarah Onuora

ORIGINAL ARTICLE Mougiakakos, D. et al. CD19-targeted CAR T cells in refractory systemic lupus erythematosus. *N. Engl. J. Med.* **385**, 567–569 (2021)
RELATED ARTICLE Kansal, R. et al. Sustained B cell depletion by CD19-targeted CAR T cells is a highly effective treatment for murine lupus. *Sci. Transl Med.* **11**, eaav1648 (2019)

BONE

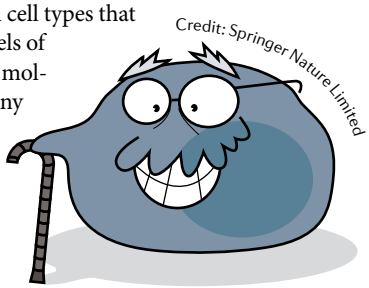
Ageing stem cells hold the key to age-related bone degeneration

The effects of ageing on the skeleton are intrinsically linked to changes in immune cell production and bone turnover that can result in osteoporosis and in a reduced ability to repair fractures. A study published in *Nature* has revealed how skeletal stem cells (SSCs) change with age and the effects these changes have on bone turnover and fracture repair, as well as a method to rejuvenate bone healing in old mice.

"We have been very interested in understanding how ageing affects stem cell function, particularly those stem cells that give rise to bones and the immune system," explains co-corresponding author Charles Chan. "We reasoned that understanding the identity of the origin of the bone-forming and niche-forming cell types could guide us to new ways to understand age-related degeneration of skeletal tissue, potentially revealing new therapeutic approaches to reverse skeletal ageing."

The researchers began by investigating SSCs in young (2-month-old) and old (24-month-old) mice and discovered that SSCs from old mice were reduced in both number and functional capacity compared with those from young mice. "We also found that aged SSCs produced fewer cells of the bone and cartilage lineages and were shifted towards generating stromal cell types that expressed high levels of pro-inflammatory molecules such as colony stimulating factor 1 (CSF1)," states first author Thomas Ambrosi. "These molecules in turn increased formation of bone-resorbing osteoclasts and other inflammatory myeloid cell types that are commonly known to be a source for systemic 'inflammaging'."

> "SSCs from old mice were reduced in both number and functional capacity"



Intriguingly, the poor fracture healing capacity of old mice could be restored to a level similar to that of young mice by treating old mice with femoral fractures with hydrogels containing the growth factor BMP2 and a low dose of a CSF1 antagonist. This treatment combination improved fracture healing and restored bone strength compared with untreated old mice, suggesting potential as a treatment for osteoporotic fractures.

"Regenerating tissue by activating endogenous stem cells locally, rather than growing them ex vivo, presents a much safer and more cost-effective strategy for repairing skeletal tissues," suggests co-corresponding author Michael Longaker.

Joanna Clarke

ORIGINAL ARTICLE Ambrosi, T. H. et al. Aged skeletal stem cells generate an inflammatory degenerative niche. *Nature* https://doi.org/10.1038/s41586-021-03795-7 (2021)

SYSTEMIC LUPUS ERYTHEMATOSUS

Metabolic reinvigoration of immune tolerance

Immune therapies that induce immune tolerance are important in preventing transplant rejection but can be ineffective in autoimmunity. A new study in *JCI Insight* reveals that metabolic modulation restores immune tolerance induction in B6.*Sle1.Sle2.Sle3* lupus-prone mice.

Treatment with anti-CD45RB antibodies blocked T cell activation in healthy mice but not in lupus-prone mice. As immune cell metabolism is altered in systemic lupus erythematosus (SLE), the researchers undertook transcriptomic and metabolic analysis of $CD4^+$ T cells from wild-type B6 and lupus-prone mice. "We determined that, in healthy mice, the induction of durable immune tolerance was associated with a specific change in $CD4^+$ T cell metabolism during the induction period, which contrasted with lupus-prone mice in which metabolism was resistant to the effect of immune therapy," explains corresponding author Daniel Moore. Both glucose metabolism and oxidative phosphorylation were abnormal in $CD4^+$ T cells from lupus-prone mice.

> "metabolic modulation restores immune tolerance induction in … lupus-prone mice"

The altered metabolism in $CD4^+$ T cells from lupus-prone mice led to reduced cell surface levels of CD45RB, the target of the therapeutic antibodies. Furthermore, the glycosylation pattern on CD45RB was altered, which resulted in reduced binding of anti-CD45RB antibodies to $CD4^+$ T cells. These data show that abnormal metabolism in $CD4^+$ T cells from lupus-prone mice leads to changes in CD45RB expression and glycosylation that thwart anti-CD45RB antibody therapy.

To test whether modulating metabolism could restore immune tolerance induction, the researchers administered the glycolysis inhibitor 2-deoxyglucose and the oxidative phosphorylation inhibitor metformin to lupus-prone mice, which restored antibody binding to $CD4^+$ T cells. Triple therapy (two metabolic inhibitors and anti-CD45RB antibodies) ameliorated SLE-like pathology in these mice, including preventing expansion of pathological immune cells (such as T follicular helper cells and germinal centre B cells) and reducing IgG deposition in the kidneys and circulating levels of anti-double-stranded DNA antibodies, even 6 months after treatment.

"Our studies point to a new mechanism for resistance to immune therapy by demonstrating that metabolic changes that may be associated with immune cell activation can lead to differential glycosylation of cell surface proteins that may be targets of immune therapy," concludes Moore.

Grant Otto

ORIGINAL ARTICLE Wilson, C. S. et al. Metabolic pre-conditioning in CD4 T cells restores inducible immune tolerance in lupus prone mice. *JCI Insight* https://doi.org/10.1172/jci.insight.143245 (2021)
RELATED ARTICLE Sharabi, A. & Tsokos, G. C. T cell metabolism: new insights in systemic lupus erythematosus pathogenesis and therapy. *Nat. Rev. Rheumatol.* **16**, 100–112 (2020)

SYSTEMIC SCLEROSIS

Promise and challenge of systemic sclerosis therapies

Yumeko Kawano and Lorinda Chung

The development of treatments for systemic sclerosis has historically been hampered by the clinical heterogeneity of the disease and limited understanding of its pathogenesis. Encouragingly, advances including the identification of important molecular targets and improvements in clinical trial design have now greatly increased the number of investigative therapies.

Refers to Campochiaro, C. & Allanore, Y. An update on targeted therapies in systemic sclerosis based on a systematic review from the last 3 years. *Arthritis Res. Ther.* **23**, 155 (2021).

Systemic sclerosis (SSc) is a heterogeneous, multisystem connective tissue disease marked by fibrosis of the skin and internal organs, immune dysregulation and microvascular disease. SSc is classified into diffuse cutaneous (dcSSc) or limited cutaneous (lcSSc) subtypes on the basis of the extent of skin involvement. Beyond skin thickening, patients with SSc can also develop serious internal organ involvement, including cardiac, pulmonary, renal and gastrointestinal disease, but the clinical manifestations and natural course of the disease can vary widely. Historically, there have been no effective disease-modifying therapies for SSc and mortality has been high, particularly for patients with dcSSc[1]. However, over the past decade we have made substantial strides in our understanding of the aberrant immune activation, signalling pathways and cytokines involved in SSc fibrosis[2]. In turn, these discoveries have spurred a number of clinical trials investigating novel therapeutic targets in SSc[3]. A new review by Campochiaro and Allanore provides a useful update on the growing field of novel potential SSc therapeutics[2].

Campochiaro and Allanore undertook a systematic review of clinical trials of targeted therapies for SSc published between 2016 and 2020, focusing on the treatment of skin and lung disease. The studies included in the review ranged from small pilot trials to large multicentre studies, and evaluated therapies targeting various molecules and mechanisms involved in the inflammatory and fibrotic pathways of SSc including IL-6, the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway, T cell and B cell activation, cannabinoid receptors and transforming growth factor-β, among others. Twenty studies met the inclusion criteria including two phase III randomized controlled trials (RCTs): the SENSCIS trial of nintedanib and the focuSSced trial of tocilizumab[4,5]. Both phase III studies demonstrated a slower decline in forced vital capacity (FVC) in patients with SSc-associated interstitial lung disease (SSc-ILD) who received the active treatment compared with placebo, and nintedanib and tocilizumab have since been approved by the FDA for use in SSc-ILD. Importantly, these two trials show that successful RCTs in SSc can be accomplished through multicentre, international collaborations despite the rarity of the disease. By contrast, the results of many of the phase I and phase II trials included in the review were negative, although the findings are still instructive. Specifically, the selection of the appropriate target patient population, selection of the most robust primary outcome measure and the use of background and/or combination therapy are key considerations for future clinical trials design in SSc (FIG. 1).

Because SSc is a heterogeneous disease, we need to take into account the mechanism of action of the particular drug under investigation before selecting the target patient population for enrolment into clinical trials. For instance in the focuSSced study for tocilizumab, elevated serum concentration of C-reactive protein was used to select for patients with upregulation of the IL-6 pathway[5]. In a phase II trial of abatacept, skin biopsy samples of the patients revealed that those with inflammatory or normal-like gene signatures tended to respond to the T cell co-stimulation blocker, whereas those with fibroproliferative gene signatures did not[6]. The natural history of dcSSc, with fluctuations and individual variation in skin tightening over time, can also complicate efforts to determine true treatment effects in a clinical trial focused on skin outcome measures using the modified Rodnan skin score (mRSS). Strategies to enrich study populations for patients whose skin disease is likely to progress include enrolment of patients with very early dcSSc or low baseline mRSS[7]. However, the use of such enrichment strategies needs to be balanced against the feasibility of enroling enough patients, as investigations of promising treatments can be derailed by insufficient enrolment.

The choice of outcome measures is also crucial to ensuring the success of a clinical trial in SSc. The review by Campochiaro and Allanore listed the various outcome measures used in each study but did not comment on their respective merits or limitations. Traditionally, mRSS has been used as the primary end point in clinical trials of treatments for dcSSc. mRSS seemed to be a good marker for disease activity, as progressive skin thickening not only affects patients' quality of life and functional status, but also predicts mortality and internal organ involvement[8]. However, as we mentioned above, skin thickening can vary markedly among patients with dcSSc, with some patients showing spontaneous improvement over time, making it difficult to demonstrate statistically significant treatment effects in a clinical trial. Several promising trials included in the review article such as riociguat, abatacept, and tocilizumab failed to demonstrate statistically significant improvements in mRSS despite enriching for patients at high risk of skin fibrosis progression. At this juncture, we must start to look beyond skin outcomes to measure success in SSc clinical trials. Many current and upcoming clinical trials are instead focusing on lung function or using composite outcome measures.

As a major contributor to mortality in SSc, ILD is an important indication to study. It can also be easier to study than skin disease, as FVC is a more robust and less variable outcome measure than mRSS. In fact, the focuSSced trial of tocilizumab failed to meet its primary end point of change from baseline in mRSS, but did demonstrate stability in % predicted FVC over 48 weeks; this protective effect was seen across the spectrum of ILD severity, including in patients with mild, moderate or severe disease[9]. Another outcome measure that is starting to be used in clinical trials is the ACR Composite Response Index in Systemic Sclerosis (CRISS), which is a composite outcome measure that aims to capture the multifaceted nature of SSc, including cardiac, pulmonary, renal and skin end points as well as patient-reported outcomes and patient and physician global assessments. A new definition of low disease activity (LDA) has also been proposed for each major organ system — a state in which patients are likely to have lower risk of adverse outcomes and higher health-related quality of life. These criteria for LDA have yet to be tested but serve as a useful framework for refining composite outcome measures in the future[10]. Further studies are necessary to determine if these new outcome measures will perform well enough to be used in the evaluation of new therapies for regulatory approval in SSc.

Furthermore, future trials must take into account that mycophenolate mofetil is now considered standard of care for SSc and a large proportion of patients will be taking this background therapy, which will affect not only the measured efficacy but also the tolerability of the investigational drug. Nevertheless, the outlook for future therapies is promising, given the multiple targetable pathways involved in SSc pathogenesis. Combination therapy, whether used in a step-up or upfront approach, will need to be evaluated in RCTs but could be a potential strategy for SSc-ILD. Indeed, an ongoing phase II RCT (NCT03221257) is investigating the upfront combination of mycophenolate mofetil and pirfenidone, an antifibrotic agent approved for the treatment of idiopathic pulmonary fibrosis. The main limiting factor for such combination therapies is likely to be tolerability, particularly with respect to gastrointestinal adverse effects as the vast majority of patients with SSc have gastrointestinal symptoms related to their underlying disease.

The review by Campochiaro and Allanore provides a snapshot of the rapidly evolving landscape of SSc therapeutics, highlighting that this is truly an exciting time for clinical trials for this rare disease. An unprecedented number of new therapies are now under investigation for SSc. The goal of developing personalized medicine for SSc — driven by each patient's clinical characteristics and biomarkers — could be within reach in the near future.

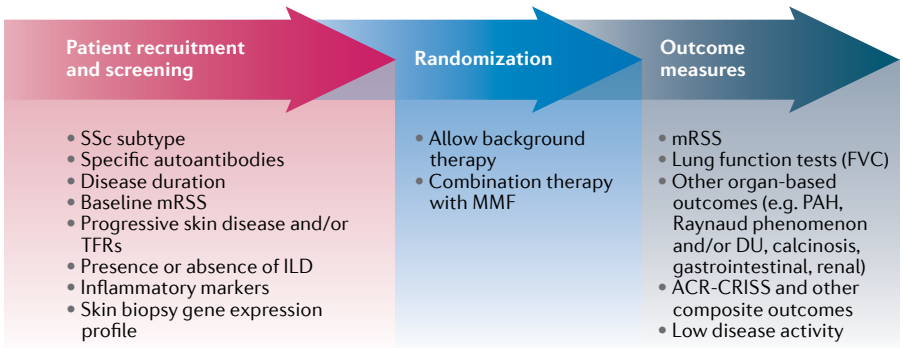


Fig. 1 | **Schematic for improving SSc clinical trial design.** Important considerations for the design of future clinical trials in systemic sclerosis (SSc) include the selection of the appropriate target patient population, the use of background and/or combination therapy and selection of the most robust outcome measures. ACR-CRISS, ACR Composite Response Index in Systemic Sclerosis; DU, digital ulcer; FVC, forced vital capacity; ILD, interstitial lung disease; MMF, mycophenolate mofetil; mRSS, modified Rodnan skin score; PAH, pulmonary arterial hypertension; TFR, tendon friction rub.

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Competing interests

L.C. declares that she has served as a consultant and/or advisor and has received grant funding from Boerhinger Ingelheim, has served on the Steering Committee and acted as a consultant and/or advisor for Eicos Sciences, has served as a consultant and/or advisor for Mitsubishi Tanabe and Genentech and was a member of the Data Safety Monitoring Board for Reata. Y.K. declares no competing interests.

RISK FACTORS

Is air pollution linked with poor response to biologics?

Naizhuo Zhao and Sasha Bernatsky

Limited data suggest associations between air pollution and rheumatic disease risk and outcomes. More sophisticated research is needed to clarify the conditions under which air pollution might influence the health of people with rheumatic disease, including their response to biologic drugs.

Refers to Adami, G. et al. Environmental air pollution is a predictor of poor response to biological drugs in chronic inflammatory arthritides. *ACR Open Rheumatol.* https://doi.org/10.1002/acr2.11270 (2021).

Few would debate that air pollution poses serious risks to health. Though inhaled air pollutants lead to local airway inflammation (and thus might worsen respiratory conditions such as asthma), they also trigger systemic autoimmune responses[1]. A few studies have demonstrated associations between air pollution exposures and risk for rheumatic diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus[2,3]. A group from Verona, Italy noted multiple air pollutants were correlated with high C-reactive protein (CRP) levels, and potentially with disease flares in patients with RA[4]. Adami et al.[5] now suggest that ambient air pollution exposures might be associated with poor response to biologic DMARD (bDMARD) treatment in patients with chronic inflammatory arthritis.

In their current study, Adami et al.[5] identified 1,257 patients with RA, ankylosing spondylitis or psoriatic arthritis from the University of Verona registry of biological therapies (presumably pooled for better power) who had a mean of 4.8 clinic visits per patient from 2013–2018. By averaging air pollutant concentrations in the 60 days preceding each visit, they observed an exposure-dependent relationship between particulate matter with a diameter of ≤10 μm (PM_{10}; inhalable particles commonly found in smoke and dust) and CRP levels. Patients exposed to >50 μg/m^3 PM_{10} had a 150% higher risk of elevated CRP (>5 mg/l), and patients exposed to >40 μg/m^3 PM_{10} had a 65% higher risk of elevated CRP, compared with patients with lower PM_{10} exposures. Even with the PM_{10} threshold set at 30 μg/m^3 (below the safety level defined by the EU Air Quality Directive), exposed individuals had a 38% higher probability of having an elevated CRP level compared with those who were unexposed.

Adami et al.[5] further conducted a case–crossover study by identifying 280 patients with at least one bDMARD switch due to drug ineffectiveness and at least one visit at which their treatment had been stable for at least 6 months. Mean concentrations of many air pollutants (including carbon monoxide, nitric oxide (NO), nitric dioxide, oxides of nitrogen, ozone, particulate matter with diameter of ≤2.5 μm ($PM_{2.5}$) and PM_{10}) in the 60 days before a visit at which a switch took place were higher than in the 60 days before a visit at which treatment was stable. The authors concluded that the higher air pollutant levels were associated with therapy ineffectiveness.

Potential limitations of this study include the choice of design. Case–crossover is most useful for studying brief exposures that cause transient changes in the risk of an event; whether it is the best design to evaluate air pollution and drug switching in chronic arthritis is debatable. Selection bias and generalizability should also be considered, as the case–crossover analyses performed by Adami et al. were limited to a select group of registry patients[5]. Moreover, as Verona is a region with a population that is relatively homogeneous for race/ethnicity (almost 90% Italian), it is unknown if the findings would be similar in more diverse populations. In addition, many factors (including education, smoking, occupational exposures and income) correlate with regional differences in air pollution that might also be related to poor outcomes in arthritis. The choice of a case–crossover design might represent an attempt to prevent these factors from being overt confounders (as at least some of these factors would presumably be constant over time in an individual) but, as noted, this design is not ideal for investigating chronic disease outcomes.

Another potentially important issue is the inability to control for calendar effects in the case–crossover analyses by Adami et al.[5]. Over time, more bDMARDs have become available, and thus drug switching might have become more common. Any changes in air pollution that were correlated with bDMARD switching during the same time period could therefore represent a potential source of bias or confounding (although the study by Adami et al. was completed over a relatively short period of time, during which air pollution levels in the Verona area were relatively stable).

In the study by Adami et al.[5], exposure data were retrieved from five air-quality monitoring stations. Air pollution concentrations measured by one of the five monitoring stations were assigned to all patients living within 10 km of the station, which could generate exposure misclassification. At present, remote sensing data in conjunction with an atmospheric chemical transport model or a geostatistical model is usually used to improve

spatial resolution of air pollution estimates[6] and reduce the exposure misclassification. It would be interesting to see if the findings from Adami et al.[5] could be replicated in other jurisdictions where the concentrations of air pollutants are lower (such as other parts of Europe) or higher (such as India and China) than Northern Italy. It can be expected that, in extending the study area to a larger geographic territory, the range and variation of air pollutant exposures could be greater, and the distortions of air pollution exposures on the effectiveness of bDMARDs might be more problematic.

> “Reports of associations between air pollution and rheumatic disease outcomes … are on the rise”

Among the seven air pollutants studied by Adami et al.[5], the largest difference (between switching versus stable treatment observations) was related to NO. However, the results do not necessarily prove that NO has a stronger adverse effect on bDMARD outcomes than the other air pollutants. Concentrations of different air pollutants are usually correlated in space, as they share common sources (such as road traffic)[7], which could lead to confounding. Most studies do not properly account for the fact that, at any given time, people are exposed to multiple (not single) air pollutants. Thus, discerning which air pollutant is really associated with the observed health outcomes is difficult. Novel statistical methods (such as Bayesian kernel machine regression[8] and quantile-based g-computation[9]) developed specifically for analysing the effects of exposure mixtures on health outcomes would be helpful to assess associations between air pollutant exposures and rheumatic disease outcomes in the future.

Reports of associations between air pollution and rheumatic disease outcomes (including disease activity) and/or characteristic biomarkers (such as anti-citrullinated protein antibodies) are on the rise[1,2,10]. Adami et al.[5] now suggest that environmental air pollution might be associated with poor drug therapy response. If these effects can be replicated in other datasets (with more appropriate designs and modelling), it could help us to better understand which ambient air pollutants are most harmful, and how we might most efficiently reduce some of the rheumatic disease burden that is potentially attributable to environmental factors.

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Competing interests
The authors declare no competing interests.

Autoinflammation and autoimmunity across rheumatic and musculoskeletal diseases

Zoltán Szekanecz[1] ✉, *Iain B. McInnes*[2], *Georg Schett*[3,4], *Szilvia Szamosi*[1], *Szilvia Benkő*[5] *and Gabriella Szűcs*[1]

Abstract | Most rheumatic and musculoskeletal diseases (RMDs) can be placed along a spectrum of disorders, with autoinflammatory diseases (including monogenic systemic autoinflammatory diseases) and autoimmune diseases (such as systemic lupus erythematosus and antiphospholipid syndrome) representing the two ends of this spectrum. However, although most autoinflammatory diseases are characterized by the activation of innate immunity and inflammasomes and classical autoimmunity typically involves adaptive immune responses, there is some overlap in the features of autoimmunity and autoinflammation in RMDs. Indeed, some 'mixed-pattern' diseases such as spondyloarthritis and some forms of rheumatoid arthritis can also be delineated. A better understanding of the pathogenic pathways of autoinflammation and autoimmunity in RMDs, as well as the preferential cytokine patterns observed in these diseases, could help us to design targeted treatment strategies.

When discussing rheumatic and musculoskeletal diseases (RMDs), it is not always clear whether the disease is strictly an autoimmune disease or is an autoinflammatory disease with unchecked inflammation but without autoimmunity[1–4]. Therefore, it is important to revisit the classification used to describe RMDs[1–4].

When considering whether a disease is an autoimmune disease versus an autoinflammatory disease, systemic lupus erythematosus (SLE) and monogenic systemic autoinflammatory diseases (SAIDs) can be considered as prototypes of autoimmune and autoinflammatory diseases, respectively[3,4]. Autoimmune diseases are characterized by the loss of immune tolerance, the recognition of self-antigens and the activation of T cells and B cells, followed by the production of specific autoantibodies and the damage of multiple organs owing to a dysregulated adaptive immune response[1,3,5]. Autoinflammatory diseases are not directed by specific antigens, and they harbour systemic chronic inflammation without a break in immune tolerance or the generation of specific autoantibodies[4,6]. External environmental factors such as infections, temperature changes or mechanical stress can also lead to the development of inflammation and provoke flare in certain genetic backgrounds, expanding the definition of autoinflammation[4,6].

RMDs are distributed along a spectrum based on the involvement of autoimmunity and autoinflammation in them (FIG. 1). Monogenic SAIDs are at the autoinflammatory end of the spectrum, and SLE and antiphospholipid syndrome (APS) are at the autoimmune end. Rare monogenic autoimmune diseases such as autoimmune polyendocrine syndrome 1, immune dysregulation, polyendocrinopathy, enteropathy, X-linked and autoimmune lymphoproliferative syndrome will not be discussed in this Review as they are not classical RMDs[7]. Diseases related to autoimmunity that are discussed here include SLE, rheumatoid arthritis (RA), polyarticular juvenile idiopathic arthritis (pJIA), systemic sclerosis (SSc), APS, primary Sjögren syndrome (pSS), idiopathic inflammatory myopathies (IIMs), mixed connective tissue disease and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV)[3,4,8–10] (FIG. 1). As discussed later, a mechanistic immunological classification of RA has been proposed based on the heterogeneity of disease subtypes[8,9]. In addition to monogenic SAIDs, diseases related to autoinflammation and discussed in this Review include gout, spondyloarthritis (SpA), systemic juvenile idiopathic arthritis (sJIA), oligoarticular juvenile idiopathic arthritis, adult-onset Still disease (AOSD), Behçet disease and Schnitzler syndrome[3,4] (FIG. 1). As described previously, most of these autoimmune and autoinflammatory diseases can also be considered to be 'mixed-pattern' conditions[4]. Indeed, there is no strict divide between autoimmune and autoinflammatory diseases as some RMDs comprise elements of autoimmunity and autoinflammation. In such mixed-pattern RMDs,

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Key points

- Rheumatic and musculoskeletal diseases (RMDs) form a continuum between classical autoimmune and autoinflammatory conditions.
- Classical autoinflammatory and autoimmune diseases are associated with the activation of innate immunity and adaptive immune responses, respectively.
- There are some 'mixed-pattern' disorders that carry the features of both autoimmune and autoinflammatory conditions, and one disorder might have autoimmune and autoinflammatory characteristics at different stages of disease development.
- The autoimmune, autoinflammatory or mixed phenotype of RMDs might help us to develop and administer therapies targeted to specific disease phenotypes.

autoantibody-mediated pathology has been observed alongside activation of the innate immune system, including of Toll-like receptors (TLRs) and of the inflammasome. Moreover, immune cells and mediators characteristic of both autoimmunity and autoinflammation can be involved in these diseases[1,3,5,11] (FIG. 1).

Indeed, in terms of immunity, autoimmune and autoinflammatory conditions can have an innate or adaptive immunological background[2,3] (FIG. 2). Innate immunity delivers non-specific cellular and humoral immune responses and confers the first defensive responses against pathogens. Innate immune responses are usually directed against pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). Several molecular systems, including TLRs, NOD-like receptors (NLRs), the caspase recruitment domain (CARD) receptor family, proteins of the complement system, cytoplasmic DNA-sensing molecules and inflammatory multimolecular complexes such as inflammasomes, have evolved to permit diverse recognition and activation and effector function within innate immunity. Immune cells activated during innate immune responses include macrophages, natural killer cells, neutrophils and mast cells (FIG. 2). In addition, other cell types, such as epithelial and endothelial cells, are also induced to express molecules recognizing DAMPs and PAMPs and are classed as 'innate responders'. Epithelial barriers and their dysfunction, partially through alterations in the microbiome, might also play a crucial role in RMDs. The activation of innate immune responses is primarily characteristic of autoinflammation and the development of autoinflammatory diseases (FIG. 1). Within the cytokine superfamilies, the IL-1 family, TNF superfamily members, IL-6 and the type I interferons are particularly implicated in innate immune responses[1,4,12–14].

Adaptive immunity is teleologically younger than innate immunity and exists only in vertebrates. As it enables an immunological memory to form in response to the first encounter with a pathogen, a prompt immune response can develop after consecutive contacts with the same external stimulus. Adaptive immunity is pathogen-specific and driven by T lymphocytes and B lymphocytes, and long-term defence can develop. Temporal and spatial regulation of such a response, as well as its attenuation, is needed to prevent tissue and organ damage. The sustained activation of adaptive immune responses and immunoregulatory defects can lead to the development of classical autoimmune diseases[1–3,5] (FIG. 1).

During the past decade, multiple efforts have been made to better understand the nature of autoimmunity and autoinflammation[1,4], including those using genome-wide association studies, mRNA sequencing, molecular imaging and the study of tissue-specific antigen and gene expression patterns[1,3,4]. In this Review, we first discuss the key features of diseases that are predominantly autoimmune or predominantly autoinflammatory, before describing the overlap between autoimmunity and autoinflammation in RMDs. We also underscore mechanisms shared by autoimmunity and autoinflammation, such as the involvement of pathogenic pathways that are characteristic of autoinflammation in autoimmune conditions (and vice versa), and we highlight how understanding these shared mechanisms might enable us to enhance the efficacy of therapeutics and realize the potential of personalized medicine in rheumatology.

Major features of autoimmune RMDs

SLE, a prototype of systemic autoimmunity, produces more than 100 autoantibody specificities and manifests in various systemic organs (FIG. 1). SLE is based on robust T cell and B cell activation and the formation of immune complexes, whereas cells and mediators that are characteristic of autoinflammation, such as inflammasome activation and the production of IL-1, do not seem to have a major role in this disease[15]. Nonetheless, innate immunity still has an important role in SLE. Indeed, single-nucleotide polymorphisms associated with SLE include those in the genes encoding TLRs (*TLR7* and *TLR9*), complement receptors (*C3*, *C4* and *C1Q*) and Fc receptors (*FCGR2A* and *FCGR3B*), all of which are components of the innate immune response (TABLE 1). The accumulation of 'cellular debris' in tissues and blood in patients with SLE, including as a result of secondary necrosis and the formation of neutrophil extracellular traps (NETs), leads to a breach in immune tolerance and the formation of immune complexes, which triggers the release of inflammatory mediators and organ damage[15,16]. This cell debris-induced breach in immune tolerance is closely linked to dysfunction in complement receptors and Fc receptors. Indeed, mutations in genes encoding proteins of the complement system and the activation of a type I interferon (that is, IFNα and IFNβ) signature, which is also a feature of an innate immune response, are central features of SLE[14,15,17]. The complement genes responsible for susceptibility to SLE are *C1Q*, *C2* and *C4* (REF.[15]). Partial or complete deficiency in C1, C2 or C4 disrupts early steps of the complement cascade, resulting in inadequate clearance of immune complexes. In addition, the Fc receptors FcγRIIIA and FcγRIIIB have anti-inflammatory activity as they clear immune complexes, and mutations in genes encoding these proteins impair this clearance function. In carriers of single-nucleotide polymorphisms associated with SLE, environmental factors that induce cell death, such as ultraviolet light, are necessary for development of the disease[15,18–20]. In SLE, extracellular DNA triggers an *IFN* gene response associated with the production of IFNα and IFNβ. DNA activates *IFN* genes (for example,

IFNA) via the stimulator of interferon genes (STING)–IRF3 pathway and TLR7 and TLR9 (REFS[15,19]). Eventually, the persistence of an interferon signature contributes to disease progression[15,18,21].

The importance of the type I interferon signature and that of other risk alleles associated with components of the innate immune response has also been described in the predominantly autoimmune diseases SSc, IIMs and pSS. For example, in SSc, the type I interferon signature appears early in disease, before the onset of fibrosis, and correlates with an increase in the expression of B cell-activating factor (*BAFF*) mRNA (the protein product of which promotes B cell activation) and an increase in collagen synthesis[22,23]. In the IIMs polymyositis and dermatomyositis, the expression of type I interferon-regulated genes has also been associated with disease activity[24]. Furthermore, high expression of interferon-induced genes has been observed in the skin of patients with dermatomyositis[25]. In pSS, clinical symptoms, disease activity and B cell activation are also associated with the type I interferon signature[26,27]. Finally, certain subsets of RA presumably show a type I interferon signature that promotes the production of autoantibodies such as anti-citrullinated protein antibody (ACPA), anti-carbamylated protein (anti-CarP) and rheumatoid factor[17,28–30], and RA also carries other autoinflammatory features (see below)[8,31,32].

Features of autoinflammatory RMDs

SAIDs comprise an expanding group of diseases, including monogenic diseases caused by inborn errors (also known as periodic fever syndromes) and adult-onset SAIDs such as AOSD, Schnitzler syndrome and idiopathic recurrent autoimmune pericarditis (IRAP)[33–36].

Monogenic autoinflammatory RMDs. In contrast to autoimmune RMDs, monogenic SAIDs are exclusively autoinflammatory conditions[37] (FIG. 1; TABLE 1). A common feature of these diseases, which include both sporadic and monogenic inherited diseases with an overactive innate immune system, is recurrent febrile episodes in the absence of infectious agents. The best described diseases in this group include familial Mediterranean fever (FMF), periodic fever, aphthosis, pharyngitis and adenitis syndrome, hyper-IgD and periodic fever syndrome (also known as mevalonate kinase deficiency), TNF receptor-associated periodic syndrome (TRAPS), Blau syndrome and cryopyrin-associated periodic syndromes (CAPS). CAPS include three diseases caused by mutations in *NLRP3*, the gene encoding the

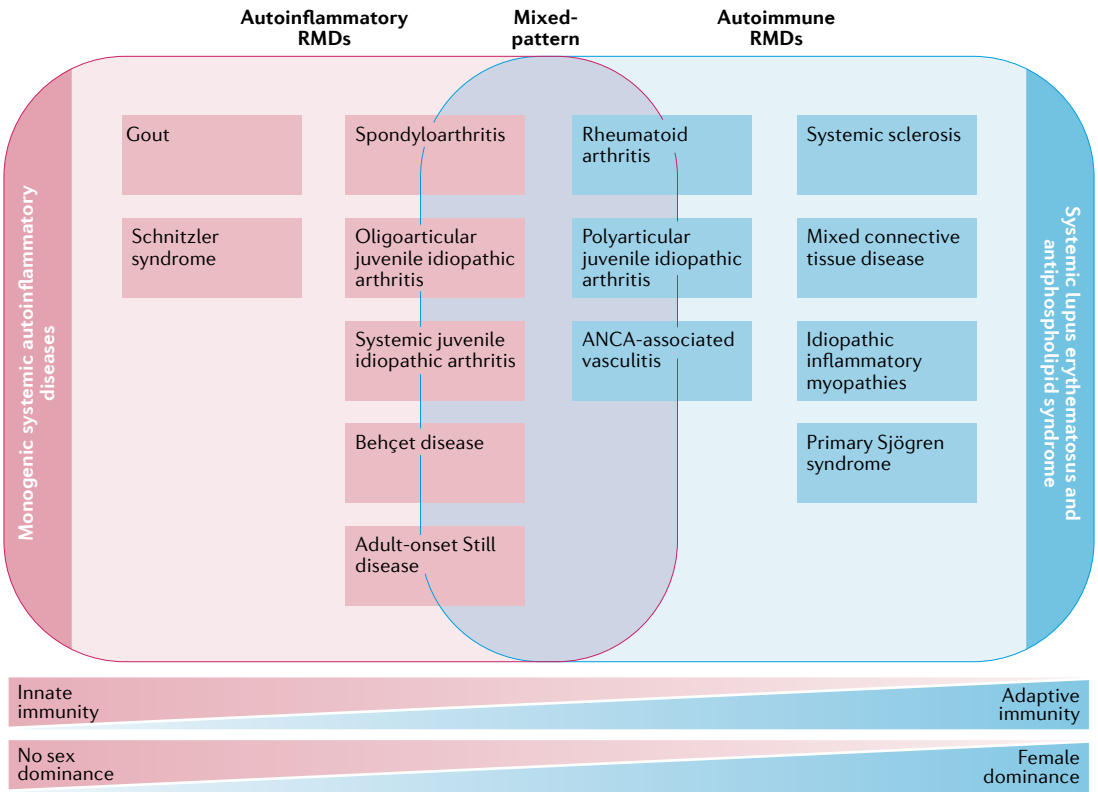

Fig. 1 | **Spectrum of autoinflammatory, mixed-pattern and autoimmune diseases.** Prototypes of a classical autoinflammatory disease are the group of monogenic systemic autoinflammatory diseases known as periodic fever syndromes (pink). Prototypes of classical autoimmune disease are systemic lupus erythematosus and antiphospholipid syndrome (blue). Diseases in the middle of the spectrum might be considered mixed-pattern rheumatic and musculoskeletal diseases (RMDs; mixed colour). Indicated by the spectra at the bottom of the figure, classical autoinflammatory conditions are characterized by a predominance of innate immunity and have no sex dominance. By contrast, classical autoimmune conditions are associated with more prominent adaptive immune responses and female dominance. ANCA, antineutrophil cytoplasmic antibody.

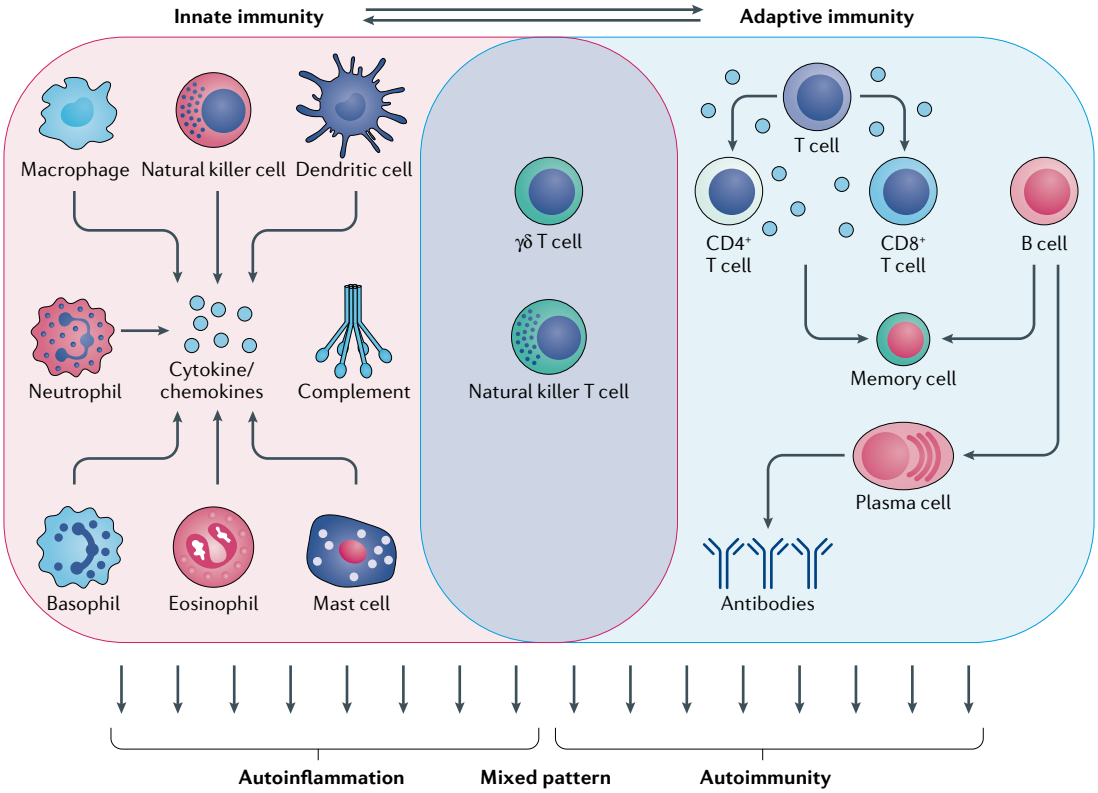

Fig. 2 | **Cellular mediators of autoimmunity and autoinflammation.** Cells of the innate immune system, including macrophages, natural killer cells, dendritic cells, mast cells and different granulocyte subsets, and the complement system promote autoinflammation. Cells of the adaptive immune system, including different T lymphocyte subsets, B cells and plasma cells, as well as T memory cells and B memory cells, are primarily involved in the development of autoimmunity. Natural killer T cells and γδ T cells are at the crossroads of autoinflammation and autoimmunity and promote the development of mixed-pattern immune-mediated inflammatory diseases. Most of the cells involved in the development of autoinflammation and autoimmunity produce cytokines and chemokines (as indicated by the blue circles), whereas plasma cells release antibodies.

NLRP3 protein, namely familial cold autoinflammatory syndrome, Muckle–Wells syndrome and chronic infantile neurologic cutaneous and articular syndrome[38,39]. The clinical features of these monogenic SAIDs have been discussed elsewhere[37–39]. Most of these diseases are caused by inborn errors, although some such as FMF, TRAPS, CAPS, hyper-IgD and periodic fever syndrome, deficiency of adenosine deiminase 2 (ADA2), periodic fever, aphthosis, pharyngitis and adenitis syndrome, and type I interferonopathies can also have adult onset[33,34]. Monogenic SAIDs are mostly associated with mutations in *MEFV*, the gene encoding pyrin, *NLRP3*, or other genes encoding proteins that regulate inflammation, metabolism and body temperature (for example, *NOD2*; also known as *CARD15*)[37,39–41] (TABLE 1). Currently, our understanding of monogenic SAIDs is moving from a gene-centric view towards a systems-based view, and various convergent pathways — such as pyrin and the actin cytoskeleton, protein misfolding and cellular stress, NF-κB dysregulation and interferon activation — have been associated with autoinflammation in SAIDs[42].

Molecular pathways underlying autoinflammation. Activation of the NLRP3 inflammasome and the IL-1β pathway are key events in the pathogenesis of most monogenic SAIDs and polygenic SAIDs (introduced below)[12,43,44]. In the presence of a characteristic genetic mutation, certain external environmental factors (for example, infection, smoking or hormonal factors) can cause uncontrolled activation of the inflammasome, resulting in the development of a cytokine-mediated systemic inflammatory condition[12,43,44]. DAMPs and PAMPs are involved in the initiation of inflammasome activation. Activation of the NLRP3 inflammasome is mediated by the NLR family protein NLRP3 and leads to the activation of caspase 1, which cleaves the cytokine precursors pro-IL-1β and pro-IL-18 to produce the biologically active forms of IL-1β and IL-18, respectively[12,40,41,43]. In response to increased production of IL-1β and IL-18, the endogenous cytokine antagonists IL-1 receptor antagonist (IL-1Ra) and IL-18 binding protein (IL-18bp) restore the balance of these cytokines in the body[12,40,41,43]. Loss of function mutation in genes encoding cytokine antagonists also leads to increased activation of IL-1α and IL-1β (REFS[40,41]).

Activation of NF-κB signalling contributes to the development of certain autoinflammatory diseases, and NOD2, a NLR family protein in addition to NLRP3 that recognizes bacterial dipeptides, is an important regulator of NF-κB signalling. *NOD2* mutation has a role

in the pathogenesis of Blau syndrome and in Crohn's disease[40].

Monogenic SAIDs associated with IL-1β family activation include FMF, familial cold autoinflammatory syndrome, chronic infantile neurologic cutaneous and articular syndrome, hyper-IgD and periodic fever syndrome, Muckle–Wells syndrome and pyogenic arthritis, pyoderma gangrenosum and acne[40,41]. The different gene mutations present in each disease result in activation of the NLRP3 inflammasome and uncontrolled secretion of IL-1β (REFS[40,41]). In addition to IL-1β and IL-18, TNF is also involved in the pathogenesis of some monogenic autoinflammatory disorders[40,41]. Other pathogenetic mechanisms that affect innate immunity and have been implicated in the pathogenesis of SIADs include NF-κB activation, endoplasmic reticulum stress, mutations in genes encoding endogenous cytokine antagonists, dysregulation of actin filament formation (in actinopathies), enhanced expression of *IFN* (in interferonopathies) or a reduction in the enzymatic activity of ADA2 (REFS[33,34]). TRAPS, which is one of the most prevalent monogenic SAIDs, is associated with heterozygous variants in *TNFRSF1A*, the gene encoding TNF receptor 1 (REFS[33,45,46]). Possible pathogenic mechanisms of TRAPS include enhanced NF-κB and NLRP3 activation through increased endoplasmic reticulum stress, defective autophagy or defective receptor shedding leading to TNF-induced cell death and, eventually, autoinflammation[33,45,46].

Polygenic autoinflammatory RMDs. Among polygenic autoinflammatory conditions we will discuss sJIA and gout, two well-known prototypes. sJIA is a typical autoinflammatory disease associated with fever, rash, hepatosplenomegaly and lymphadenopathy, especially in the early, acute phase[47]. Genetic and epigenetic changes are associated with this disease but, although mutations have been described in several genes, unlike in periodic fever syndromes, none of these mutations alone results in sJIA[47]. Gene mutations characteristic of monogenic diseases (for example, mutations in *NLRP3*, *NOD2* and *MEFV*) are not observed in sJIA[47]. sJIA has, rather, been associated with genes encoding pro-inflammatory cytokines (such as *IL1*, *IL1R*, *IL6*, *IL10* and *IL20*) and other mediators of inflammation (such as *IL8* and *MIF*; *MIF* encodes macrophage migration inhibitory factor)[47] (TABLE 1). The proteins encoded by these genes are involved in the innate immune response and, ultimately, create an inflammatory microenvironment; the activation of effector T cells only occurs as a consequence of autoinflammation[3,47]. In the more advanced stage of sJIA, activation of the adaptive immune system and joint tissue destruction can be observed, suggesting that sJIA is associated with the activation of innate and (to a lesser extent) adaptive immunity at different stages of the disease[48,49]. Nonetheless, B cell-mediated autoimmunity is absent in sJIA. Important questions are how and when spurious inflammation in sJIA switches to chronic inflammation[1,49], and whether this switch can be prevented or delayed by early intervention with anti-IL-1 or anti-IL-6 strategies[50].

Autoinflammation is also essential in the development of gout and the central event of gouty inflammation is the activation of white blood cells by monosodium urate (MSU) crystals[12,51,52]. Cell membrane damage by activated leukocytes and their mediators results in the activation of pattern recognition receptors, inducing a response against cellular debris to try to minimize the damage. MSU crystals act as DAMPs and are phagocytosed through TLR2 and TLR4 to form a phagolysosome. Phagolysosome formation is followed by activation of the NLRP3 inflammasome, which leads to the activation of caspase 1 and to the release of IL-1β and IL-18 (REFS[12,51,52]). The production and release of the pro-inflammatory cytokines IL-1, IL-6 and TNF from cells of the innate immune system, independent of inflammasome activation, initiate an inflammatory cascade in which additional mediators of inflammation, such as matrix metalloproteinases, prostaglandins, leukotrienes and reactive oxygen species, also play a role[12,51].

Although monogenic SAIDs, sJIA and gout are the prototypes of autoinflammatory RMDs, AOSD, Behçet disease, IRAP, synovitis, acne, pustulosis, hyperostosis, osteitis syndrome and Schnitzler syndrome can also be classified as adult-onset SAIDs[33–35,38] (FIG. 1). AOSD is an acquired fever syndrome characterized by well-defined clinical (intermittent fever, typical rash and arthritis) and laboratory (hyperferritinaemia, leucocytosis, neutrophilia and abnormal transaminase levels) features. AOSD has been associated with an increased production of cytokines, including of IL-1, IL-6, IL-18 and TNF[53]. Activation of the NLRP3 inflammasome and pathological IL-1 signalling have also been observed in patients with AOSD[53]. Mutations in *MEFV* and *TNFRSF1A* (the gene encoding TNF receptor 1) have been described in patients with AOSD, linking AOSD to monogenic SAIDs[54] (TABLE 1). Behçet disease is a systemic vasculitis affecting the small vessels, and most commonly manifests as mucosal and genital ulcers and uveitis.

Table 1 | **Genes associated with common autoimmune and autoinflammatory disorders**

Classification	Disease	Associated genes
Autoimmune diseases	Systemic lupus erythematosus	*TLR7*, *TLR9*, *C3*, *C4*, *C1Q*, *FCGR2A*, *FCGR3B*, *IFNA*
	Systemic sclerosis	*IFN* signature genes
	Idiopathic inflammatory myopathy	*IFN* signature genes
Autoinflammatory diseases	Monogenic systemic autoinflammatory diseases	*NLRP3*, *NOD2*, *MEFV*, *TNFRSF1A*, *MVK*, *TNFAIP3*, *ADA2*, *TREX1*, *UBA1*
	Systemic juvenile idiopathic arthritis[a]	*IL1*, *IL1R*, *IL6*, *IL10*, *IL20*, *IL8*, *MIF*
	Adult-onset Still disease[a]	*MEFV*, *TNFRSF1A*, *NLRP3*
	Behçet disease[a]	*MEFV*, *TNFRSF1A*, *NLRP3*, *HLAB51*
Mixed-pattern diseases[a]	Ankylosing spondylitis	*HLAB27*, *ERAP1* (also known as *ARTS1*)
	Rheumatoid arthritis	*HLADRB1*, *PTPN22*, *NLRP3*, *MEFV*, *NOD2*

This table is not comprehensive and shows only the most common diseases and their genetic associations. [a]Diseases that can also be mixed-pattern diseases.

In addition to other cytokines, the NLRP3–IL-1 system is important in the development of Behçet disease, meaning that this is a predominantly autoinflammatory condition that can also have mixed-pattern features (see below)[55–57]. Again, mutations in *MEFV* and *TNFRSF1A* are more common in this disease compared with other autoinflammatory conditions[4]. Schnitzler syndrome is also an acquired fever syndrome and is characterized by chronic urticaria associated with monoclonal gammopathy, recurrent fever, bone pain and arthralgia. It is considered to be a neutrophil dermatosis with notable involvement of neutrophils, cells that are involved in innate immunity[58]. Hereditary factors are unlikely to play a role in the pathogenesis of this disease based on its late onset in patients[33,36,59,60].

Mixed-pattern RMDs

Diseases with features of both autoinflammatory and autoimmune RMDs include SpA and some forms of RA. These disorders have also been termed mixed-pattern RMDs[4] (FIG. 1).

As well as ankylosing spondylitis (AS) and psoriatic arthritis (PsA), forms of SpA include enteropathic arthritis (also known as inflammatory bowel disease-associated arthritis), reactive arthritis and undifferentiated SpA[61,62]. In contrast to classical autoimmune diseases, SpA is associated with HLA-B but not with HLA-DR, which is characteristic of polygenic autoimmune diseases[61,63–65]. Moreover, unlike other autoimmune diseases, there is no female dominance in SpA. Furthermore, SpA has been associated with autoantibodies; some patients with AS and PsA have autoantibodies to mutated citrullinated vimentin, CarP, sclerostin, heat shock proteins or β_2-microglobulin[61,63–65]. CD74 is the invariable γ-chain of MHC class II, and anti-CD74 antibodies are considered to be specific for SpA in European but not Asian cohorts[65]. Among cytokines, in addition to TNF, IL-17 and IL-23 seem to have a predominant role in mixed-pattern RMDs[61,66]. Associations of SpA with mutations in *ERAP1* (also known as *ARTS1*, encoding endoplasmic reticulum aminopeptidase 1) and with MHC class I suggest that T cells interact with cytokine pathways, including the IL-23–IL-17 axis but not the IL-1 pathway, in patients with this disease[56,57,67] (TABLE 1). In terms of autoinflammation, NLRP3 and caspase 1 are upregulated in AS, suggesting that autoinflammation is involved in the pathogenesis of this disease[68]. In short, features of both autoimmunity (such as autoantibodies) and autoinflammation (such as gender balance and natural immune responses to microbial pathogens) have been identified in SpA[61].

RA generally has autoimmune features in the early phase of the disease but has a macrophage and fibroblast-dominated pathogenesis in the chronic phase. Thus, RA is an example of a condition in which the phase of the disease relates to its autoimmune or autoinflammatory nature[4,10,30,69]. Five patients with seropositive RA had HLA-DRB1*01 and/or HLA-DRB1*04 shared epitopes as well as mutations in *NLRP3*, *MEFV* or *NOD2* (REF.[9]) (TABLE 1). These patients showed features of autoinflammation and responded to colchicine[9]. Based on the findings of this study, the authors proposed an immunology-based reclassification of RA that includes classical seropositive autoimmune RA, autoinflammatory seronegative forms of RA and mixed forms of RA that are seronegative[8,9]. This proposed reclassification reflects the commonly accepted idea that RA is a syndrome based on different pathophysiologic events rather than a single disease.

Juvenile idiopathic arthritis can also be a mixed-pattern disease with both autoinflammatory and autoimmune features. For example, pJIA shares many of the features of adult RA described above[47,70]. Also, although sJIA is largely considered to be a SAID dominated by innate immunity-driven inflammation, in later stages it can progress towards an adaptive immunity-dependent arthritis[47–49].

Among diseases primarily considered to be autoinflammatory, AOSD and Behçet disease have also been associated with adaptive immunity and T cell responses and thus can also be considered mixed-pattern conditions[4,56,57]. AOSD can be systemic with predominantly autoinflammatory features or have a chronic articular pattern resembling classical RA, which could have relevance for therapy. For example, different phenotypes of AOSD respond to different biologics[4,71]. Moreover, genetic analysis has confirmed that sJIA and AOSD might form a continuum of a single disease. Specifically, sJIA and AOSD can share common genes, and the differentiation between these two diseases is mainly based on the age of onset[35]. Behçet disease, a primarily autoinflammatory condition, is also associated with the MHC class I molecule HLA-B51, notable T cell responses and the production of IL-23 and IL-17 (REFS[56,57]), highlighting that it also has features of autoimmune conditions.

Finally, among monogenic SAIDs, haploinsufficiency of A20 — which is caused by mutations in *TNFAIP3*, the gene encoding the NF-κB regulatory protein A20 (REFS[33,72]) — is a good example of a condition with autoimmune and autoinflammatory features that result from the same pathogenetic pathways. This disease carries characteristics of RA, gout, Behçet disease, AOSD, SLE, periodic fever, aphthosis, pharyngitis and adenitis syndrome, as well as skin, ocular and gastrointestinal symptoms. Therefore, diagnosis and differential diagnosis of haploinsufficiency of A20 is difficult[72].

In conclusion, mixed-pattern RMDs carry both classical autoimmune and autoinflammatory features and are often associated with non-rheumatic conditions[1,3,4,8].

Innate immunity in autoimmune RMDs

Having discussed the main features of autoimmune, autoinflammatory and mixed-pattern RMDs, it is important to consider the innate immune mechanisms that most commonly occur in both autoinflammatory and autoimmune diseases.

We have already discussed activation of the NLRP inflammasome and the consequent production of IL-1β and IL-18 in autoinflammation[12,44]. However, these features have also been demonstrated in autoimmune and mixed-pattern conditions. NLRP3 activation and the consequent production of cytokines, as well as relevant genetic polymorphisms (for example, in *NLRP3* and

NOD2), have been associated with RA[30,73–76], SpA[77,78], pJIA and oligoarticular juvenile idiopathic arthritis[70]. NLRP3 is also activated, with inflammasome activation leading to tissue injury, in autoimmune RMDs such as RA[79,80], SLE[76,81,82], SSc[83,84], pSS[85] and IIMs[86]. TLR-dependent pathways and abnormal TLR signalling are also characteristic for SLE, RA and other autoimmune RMDs[82].

Type I interferon is upregulated in genetically based interferonopathies, which are not always linked to autoimmunity. STING is a DNA sensor, and a mutation in the gene encoding this protein can lead to the induction of genes involved in IFNα and INFβ-mediated responses and thus, indirectly, the synthesis of numerous pro-inflammatory cytokines[14,40,87]. Rare examples of these interferonopathies also include STING-associated vasculopathy with onset in infancy as well as Aicardi–Goutiéres syndrome[14,40,87]. As discussed above, type I interferon signatures play a key role in autoimmune diseases such as SLE and can also be involved in RA and SSc[87].

NETs are web-like structures of decondensed chromatin, histones and antimicrobial peptides that are involved in the defence against pathogens[58,88–90] and, primarily, have a role in autoinflammatory conditions such as gout[91,92] or Schnitzler syndrome[58]. In gout, the formation of NETs might also be a counter-regulatory mechanism aimed at resolving inflammation[91,92]. Specifically, NETs can stop gout episodes by inducing neutrophil death, encapsulating MSU crystals and inactivating cytokines[91,92]. However, neutrophil activation and NET formation contribute to autoimmune-mediated inflammation in SLE[90,93], RA[90,92] and AAV[90,92].

Prolonged innate immunity-based inflammation can induce adaptive immune responses, as described above for sJIA[48]. However, this phenomenon can also be observed in other RMDs. In monogenic SAIDs and other autoinflammatory diseases, an acute 'hyper-inflammatory state' leading to the resolution of inflammation within days and a prolonged 'autonomous inflammatory state' have been proposed to occur[49,94]. In the latter state, prolonged IL-1β and IL-18 production, in part in synergy with IL-6 and IL-23 activation, can promote T cell differentiation, the induction of T helper 17 cells (T_H17 cells) and the production of IL-17 (REFS[49,95]). Moreover, IL-18 can induce adaptive T_H1 cells and B cells[49]. Thus, innate immunity is involved in some autoimmune RMDs. Finally, a sustained innate immune response can induce trained immunity in autoimmune RMDs, which can contribute to the activation of adaptive immune pathways[49,96].

Comorbidities associated with RMDs

Comorbidities are associated with many RMDs and determine their outcome. The most relevant comorbidities are cardiopulmonary disease (including cardiovascular disease, IRAP and interstitial lung disease (ILD)), osteoporotic fractures, neuropsychiatric manifestations, diabetes mellitus and malignancies[97,98].

The inflammatory condition accelerated atherosclerosis and the consequent cardiovascular disease can carry both autoimmune and autoinflammatory features[99–101]. The autoantibodies ACPA[102,103] and anti-carP[104] might be involved in the development of atherosclerosis in RA. Citrullinated proteins have been detected in the atherosclerotic plaque, suggesting a possible target for ACPA in RA[103]. With respect to autoinflammation, in one large study *NLRP3* gene polymorphisms were not associated with cardiovascular disease in RA[105], whereas in another cohort the presence of the *NLRP3*^{Q705K} minor allele doubled the risk of stroke (also known as transient ischaemic attack) but did not increase the risk of myocardial infarction in RA[106]. In patients without rheumatic disease, NLRP3 and caspase 1 transcripts are abundantly expressed in atherosclerotic plaques[107]. Polymorphisms in CARD-containing protein 8 were not associated with any type of cardiovascular event in RA[106]. With respect to pro-inflammatory cytokines, inflammatory atherosclerosis associated with RMDs has been characterized by the increased production of TNF and IL-6 (REFS[99,100]). In addition, both IL-1 and IL-18 are abundantly produced in the atherosclerotic plaques[107,108], and IL-18 is a predictor of mortality in patients with cardiovascular disease[109]. In patients with SLE, IL-18 production has also been associated with kidney damage and cardiovascular disease[82].

The comorbidity IRAP should also be considered when monitoring and treating RMDs. Recurrent pericarditis can occur in viral infections but can also be associated with various autoimmune RMDs (for example, SLE, SSc, IIMs, pSS and RA) and autoinflammatory RMDs (for example, FMF, TRAPS and Behçet disease)[110,111]. IRAP can carry some autoimmune features as it has been linked to the production of anti-heart and anti-intercalated disk autoantibodies, as well as to autoreactive T cells[110]. However, IRAP has also been associated with notable NLRP3 activation, and cases resistant to NSAIDs, corticosteroids and/or colchicine might respond well to the inhibition of IL-1 (REFS[110,111]). Based on these observations, IRAP can also be considered an autoinflammatory disease[110–112].

ILD is mostly associated with autoimmune conditions such as SSc or IIMs, and the presence of specific autoantibodies, such as anti-Scl70, anti-PLββ-7 and anti-PL-12, correlates with an increased risk of developing ILD in these diseases[113,114]. By contrast, there is limited information on the possible involvement of autoinflammation in ILD. One study investigated the role of NLRP3 inflammasomes in patients with idiopathic pulmonary fibrosis and in patients with RA and usual interstitial pneumonia. IL-1β and IL-18 levels were elevated in bronchoalveolar lavage fluid and bronchoalveolar lavage fluid macrophage cultures from patients with RA and usual interstitial pneumonia compared with healthy individuals[115]. However, the role of autoinflammation in ILD has not been confirmed.

A great number of autoimmune (for example, SLE), autoinflammatory (for example, TRAPS and FMF) and mixed-pattern (for example, Behçet disease) diseases also have neuropsychiatric comorbidities. Based on the nature of these manifestations, these comorbidities might not have the same pathogenesis; however, neuroinflammation could be a common link between these disorders[4,57,116,117].

Finally, most RMDs have been associated with generalized bone loss leading to osteoporosis and fragility fractures[68,97,118]. Proinflammatory cytokines, such as TNF, IL-1, IL-6 and IL-17 (REF.[118]), as well as various DAMPs, including purine metabolites and fatty acids, have been implicated in inflammatory bone disorder[68]. Cytokines and DAMPs both stimulate NLRP3 and NLRC4 inflammasomes, and NLRP3-deficient mice are protected from bone loss[68]. Thus, autoinflammation is implicated in osteoporosis that occurs secondary to RMDs.

Treating RMDs across the spectrum

The pathogenesis of autoimmunity and autoinflammation, especially the cytokine networks characteristic of these conditions, might enable effective targeting strategies[43,66,119].

Treating autoinflammatory diseases. Autoinflammation often responds well to recombinant IL-1RA (anakinra), anti-IL-1β antibody (canakinumab) or recombinant IL-1R fusion protein (rilonacept)[119–121]. Canakinumab has been registered for the treatment of CAPS, TRAPS, FMF, AOSD, sJIA and refractory gouty flares[122,123]. In addition, rilonacept[124,125] and anakinra[126] are also effective in treating monogenic SAIDs. Among the less common monogenic SAIDs, recombinant IL-18bp can be administered in NLRC4 inflammasome-associated diseases caused by the overproduction of IL-18 (REF.[41]). In autoinflammatory diseases associated with NF-κB activation, such as TRAPS, IL-1 inhibitors are the first-choice treatment; however, TRAPS also responds well to TNF inhibitor therapy as the TNF receptor activates the NF-κB pathway[41]. With respect to gout, IL-1 inhibitors are effective in treating refractory flares, with most data available for canakinumab[12,127], although rilonacept[128] and anakinra[129,130] are also effective in treating gouty flares. For patients with sJIA, canakinumab[131,132], the anti-IL-6 receptor antibody tocilizumab[133] and anakinra[134] are registered for treatment, and rilonacept[135] is also effective in treating this disease. Canakinumab[136] and anakinra[126,137] are effective in, and registered for, treating patients with AOSD. Rilonacept can be administered off-label to patients with AOSD[137], and TNF and IL-6 inhibitors are also effective in treating patients with AOSD[32,138]. IL-1 inhibitors, such as canakinumab and anakinra, also showed efficacy in treating patients with Behçet disease[139]. All IL-1 inhibitors are also effective in patients with Schnitzler syndrome[36,140].

Treating autoimmune diseases. In autoimmune diseases, T cells, B cells and their cytokines play a notable role in disease pathogenesis, and the B cell inhibitor rituximab can be used off-label for treating most autoimmune diseases, including SLE[141], SSc[142], dermatomyositis[143] and pSS[144]. Belimumab, an anti-BAFF antibody, has been approved for the treatment of SLE[145], and the CTLA4–Ig fusion protein abatacept can also be administered to inhibit T cells in selected cases of SLE[146], SSc[147] and pSS[148]. It is also possible that cytokines that activate T_H17 cells (such as IL-17 and IL-23) and are used to treat RMDs with a mixed innate (neutrophil activation) and adaptive (T cell activation) background (such as AS and PsA) might also effectively treat classical autoimmune diseases. By contrast, cytokine inhibitors such as those that block IL-1 and TNF, which are effective in autoinflammatory diseases and in diseases such as RA with both autoinflammatory and autoimmune features, show limited efficacy in these autoimmune diseases. However, the IL-6 inhibitor tocilizumab gave promising results in SSc[149] and might be tried in the treatment of other autoimmune diseases[150,151].

TNF appears to be an excellent target in many inflammatory diseases, such as RA, AS, PsA and pJIA[66]. However, it might not be the optimal target in classical autoimmune disorders, such as SLE, SSc, AAV or pSS[66].

Treating mixed-pattern diseases. JAK inhibitors have been approved for treating RMDs with a mixed innate and adaptive immune activation, such as RA and SpA, and preliminary data suggest that they show promise for the treatment of patients with SLE, IIM, pSS, type I interferonopathies, sJIA, AOSD, Behçet disease and monogenic SAIDs[152]. Mixed-pattern diseases could also be treated with a combination of therapeutic strategies. For example, haploinsufficiency of A20, AOSD, Behçet disease or sJIA can be treated with TNF, IL-1 or IL-6 inhibitors based on the dominance of autoinflammatory versus autoimmune features in the patient[66,71,72].

Finally, trials to inhibit common molecular mechanisms of autoinflammation and autoimmunity, such as inflammasomes or NETs, have been carried out[89]. Several inflammasome inhibitors that target components of the NLRP3 cascade are under investigation for the treatment of autoinflammatory conditions[12,44,153]. Among currently used anti-rheumatic drugs, antimalarials and JAK inhibitors also inhibit NETs[89]. Some inhibitors of the protein arginine deiminase enzyme involved in protein citrullination might also block NET formation[89].

Conclusions

Autoimmune and autoinflammatory RMDs can be considered to be a spectrum of disorders. Monogenic SAIDs, and SLE and APS, are likely to represent the two ends of this spectrum of RMDs. Autoinflammatory diseases such as gout, sJIA, Behçet disease, AOSD or Schnitzler syndrome are characterized by the activation of innate immunity, whereas classical autoimmune diseases such as SSc, IIM, pSS, mixed connective tissue disease or seropositive RA are associated with adaptive immune responses and the production of autoantibodies. In addition to the fact that both autoinflammatory and autoimmune diseases can carry some features of the other disease type, there are mixed-pattern diseases that include SpA, AAV, pJIA, oligoarticular juvenile idiopathic arthritis and some forms of RA. The involvement of characteristic pathogenic proteins or pathways, such as of PAMPs, DAMPs, pattern recognition receptors, complement or inflammasome activation in autoinflammation, or of type I interferon signatures and the production of autoantibodies in autoimmunity, along with preferential cytokine patterns, might help inform the design of directed treatment strategies.

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Advances in epigenetics in systemic sclerosis: molecular mechanisms and therapeutic potential

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Abstract | Systemic sclerosis (SSc) is a prototypical inflammatory fibrotic disease involving inflammation, vascular abnormalities and fibrosis that primarily affect the skin and lungs. The aetiology of SSc is unknown and its pathogenesis is only partially understood. Of all the rheumatic diseases, SSc carries the highest all-cause mortality rate and represents an unmet medical need. A growing body of evidence implicates epigenetic aberrations in this intractable disease, including specific modifications affecting the three main cell types involved in SSc pathogenesis: immune cells, endothelial cells and fibroblasts. In this Review, we discuss the latest insights into the role of DNA methylation, histone modifications and non-coding RNAs in SSc and how these epigenetic alterations affect disease features. In particular, histone modifications have a role in the regulation of gene expression pertinent to activation of fibroblasts to myofibroblasts, governing their fate. DNA methyltransferases are crucial in disease pathogenesis by mediating methylation of DNA in specific promoters, regulating expression of specific pathways. We discuss targeting of these enzymes for therapeutic gain. Innovative epigenetic therapy could be targeted to treat the disease in a precision epigenetics approach.

Systemic sclerosis (SSc) is an idiopathic autoimmune rheumatic disease with three cardinal features: inflammation, vascular abnormalities and fibrosis, primarily affecting the skin and lungs[1,2]. The interlinking among this triad is not clear and the pathogenesis of the disease is not well defined. In keeping with almost all autoimmune diseases, SSc is generally more common in women. Primarily, three cell types are involved in the disease: immune cells, endothelial cells and fibroblasts. On the basis of the extent of skin involvement, SSc falls into one of two subtypes: limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc). In lcSSc, the skin fibrosis is limited to the hands, face, feet and forearms, whereas in dcSSc the fibrosis can be more widespread, affecting the trunk and other extremities. Interstitial lung disease is much more common in dcSSc than in lcSSc[3] and is a major contributor to morbidity and mortality[4]. Raynaud phenomenon is also considered part of the clinical spectrum of SSc. Although SSc is relatively rare, it is the most deadly among all the autoimmune rheumatic diseases[5]. Currently, no specific therapy is available that modifies the fibrotic element of the disease, most likely a reflection of the complex interplay between the various factors, including genetic and epigenetic factors, contributing to the disease pathogenesis. SSc is also notoriously clinically heterogeneous, which adds even more complexity, especially in regard to clinical trials[6]. In the past 10 years, epigenetic aberrations have been uncovered in SSc that could affect the triad of cardinal SSc features and both pathogenesis and biomarkers. In this Review, we examine the latest discoveries relating to epigenetics in SSc and discuss possible targeting of these for therapeutic gain.

The incidence of SSc is ~1.5–1.7% in families with a history of SSc compared with 0.026% in the general population[7], suggesting the disease risk has a genetic component. Although some genome-wide association studies have identified specific loci for SSc risk, the effects of these loci are relatively modest and the genes are mainly involved in general immunity that is shared with other autoimmune disorders, such as MHC-related genes[8,9]. It is much more likely that the disease is underpinned by epigenetic mechanisms, initiated by an environmental trigger (or triggers). An environmental trigger for initiating disease has been suggested for some time; however, the precise trigger has not been clearly defined. Occupational exposure to silica in industrial workers has been linked to SSc[10]; although the mechanisms are unclear, the immune system seems to be involved[11]. Various other environmental factors have been suggested, including infection, diet and radiation.

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Key points

- In systemic sclerosis (SSc), epigenetic aberrations are prominent in the main cell types involved in the disease pathogenesis.
- DNA in SSc fibroblasts seems to be hypermethylated, leading to repression of gene expression of negative regulators such as SOCS3.
- Studies of open regions of chromatin using ATAC sequencing have identified multiple regions of transcriptionally active genes, although their function (or functions) needs further investigation in understanding the role in SSc pathogenesis.
- Non-coding RNAs, including long non-coding RNAs and microRNAs, have been linked to SSc in the past few years and might be targets for anti-fibrotic therapy through alteration of their levels.
- Epigenetic drugs already in use for other indications, such as decitabine, could be repurposed for SSc.

An overview of epigenetics

Today epigenetics is defined as the study of heritable changes in gene expression that are not caused by changes in the DNA sequence. Indeed, 'epi' means 'above', so the term literally means 'above the genetics'. Multiple types of epigenetic change influence gene expression, but are chiefly from a few categories: DNA methylation, non-coding RNAs and histone modifications. These epigenetic changes can exert a profound influence on cell function; notably, they can be modified rapidly and reversibly. The genome of every cell in the body is identical, but the epigenome specifies its distinct phenotype. The epigenetic mechanisms known to be critical in promoting disease phenotype in SSc cells are delineated in FIG. 1.

DNA methylation

Of the epigenetic mechanisms, DNA methylation was the first to be recognized and most extensively characterized. Methylation of DNA was first discovered around the same time as DNA, but it was not until many years later that its biological role became appreciated. Methylation of DNA is characterized by the addition of a methyl group on the fifth carbon of cytosine, thus forming 5-methylcytosine. This addition occurs mainly in CpG dinucleotides, which is a cytosine followed by a guanine nucleotide. This modification enables binding of DNA methylation proteins such as methyl binding domain proteins, which, in turn, recruit histone-modifying and chromatin-remodelling enzymes, resulting in a chromatin-repressing structure and repressed gene expression[12].

The enzymes that are responsible for catalysing the addition of methyl groups onto DNA are called DNA methyltransferases, of which in mammals there are three: DNMT1, DNMT3A and DNMT3B[13]. DNMT1 maintains DNA methylation that requires UHRF1 protein[14], whereas DNMT3A and DNMT3B catalyse de novo DNA methylation and can be induced by various factors. Until recently, DNA methylation was thought to be an irreversible event. This view changed with the discovery that the *MLL* partner ten–eleven translocase 1 (TET1) converts 5-methylcytosine into 5-hydroxymethycytosine in a 2-oxyglutarate-dependent manner[15]. 5-hydroxymethycytosine can be further oxidized to 5-formylcytosine and 5-carboxylcytosine, which can be recognized and excised by a DNA glycosylase[16,17]. These findings suggest that both active and passive demethylation are involved in regulating gene expression. Notably, these processes can be influenced by multiple factors, such as cytokines and chemokines[18]. DNA methylation changes in several cell types have been implicated in SSc[19,20].

Methyl binding domain
A family of methyl-CpG-binding domain proteins that translate the DNA methylation signal and that work in concert with other proteins such as histone deacetyl transferases to facilitate gene repression.

Non-coding RNAs

MicroRNAs. MicroRNAs (miRNAs) are small, non-coding, single-stranded RNA molecules ~22 nucleotides in length that are involved in post-transcriptional regulation[21]. MicroRNAs negatively regulate gene expression by imperfectly binding to the 3′ untranslated region (UTR) of target mRNAs, resulting in their repression[21,22].

MicroRNAs are found throughout the genome. They have their own promoters and can be transcribed independently. Alternatively, they can share promoters with host genes, or can be co-transcribed as a single transcript[23]. Mechanistically, microRNAs are initially transcribed as primary miRNA molecules that are folded in a stem loop structure. These molecules are then processed by the microprocessor complex, which is formed by the RNase III family member ribonuclease 3 (also known as Drosha) binding to microprocessor complex subunit DGCR8 (REFS[24,25]). This complex cleaves the primary miRNA, resulting in pre-miRNA, which is subsequently exported to the cytoplasm via exportin-5r[26], where endoribonuclease Dicer cleaves the pre-miRNA to generate double-stranded miRNA duplexes[27]. One strand of the mature miRNA then binds argonaute proteins to form the RNA-induced silencing complex, which ultimately leads to repression of target gene and target protein output. The target specificity of the RNA-induced silencing complex is the result of its interaction with complementary sequences on the target mRNA, termed the miRNA response elements. The degree of complementarity determines the mechanism of silencing: direct slicing of target mRNA, translational inhibition or mRNA decay[21]. It is now known that miRNAs can affect virtually every function of cellular life and thus have substantial importance in various diseases, including SSc. We discuss discoveries of miRNAs in SSc, primarily in the past 2 years, including miR-27a-3p and its regulation of Wnt signalling — a critical regulator of fibrosis.

Long non-coding RNAs. Rapid advances in deep-sequencing technologies have identified many long non-coding RNAs (lncRNAs) in the entire human genome. These lcnRNAs account for a huge proportion of the total genome. lncRNAs comprise multiple species of RNA >200 nucleotides in length and are transcribed by RNA polymerase II. The GENCODE project suggests that ~16,000 human lncRNA genes exist[28]. Although they are pervasive, ascribing functions to the lncRNAs has been difficult. In general, they function to regulate gene expression by distinct mechanisms. One mechanism is through chromatin regulation: at the chromatin, lncRNAs interact with a variety of proteins that either facilitate or inhibit their binding at target DNA regions, thus ultimately altering gene expression[29,30]. The best studied example of chromatin regulation by lncRNA is X chromosome inactivation by *XIST* to ensure appropriate X chromosome dosing[31]. Another method by which this type of RNA regulates gene expression is through

Histone tails
Flexible regions that flank both ends of the histone fold and that can be modified by a plethora of modifications that impact chromatin dynamics and gene expression.

Lactylation
An epigenetic modification whereby the metabolite lactate is deposited on histone lysine residues.

direct binding to DNA, forming an RNA–DNA–DNA triplex that is either repressive or activating[32]. Indeed, the lncRNA *MEG3* forms a triplex that regulates transforming growth factor-β (TGFβ), which is critical in fibrosis[33]. A final mechanism is to act as a decoy that can bind and sequester transcription factors or microRNAs, thereby inhibiting their binding[34,35] and derepressing their mRNA targets. Although these mechanisms are the generally accepted functions of lncRNAs, it is likely that other, as yet undescribed, mechanisms exist. In a later section, lncRNAs relevant to SSc, such as HOTAIR, are discussed.

Histone modifications

DNA wraps around histone proteins to form nucleosomes (the fundamental unit of chromatin, with 150 bp of DNA around core histone proteins), the core of which comprises two copies of histones H2A, H2B, H3 and H4 assembled into an octamer. Here, the histone tails can be chemically modified to modulate gene expression by altering DNA accessibility to binding. Histone modifications can take a variety of forms depending on the moiety added to the histones. Many studies have identified the numerous chemical moieties that can be covalently attached to and removed from histones. These post-translational modifications include acetylation, methylation, ubiquitylation, sumoylation and lactylation[36,37].

Lysine acetylation is the histone modification most studied to date. This histone mark leads to a loosening of chromatin and a permissive gene expression state. By contrast, histone methylation can lead to either gene expression or repression, depending on which lysine is modified and how many methyl groups are deposited.

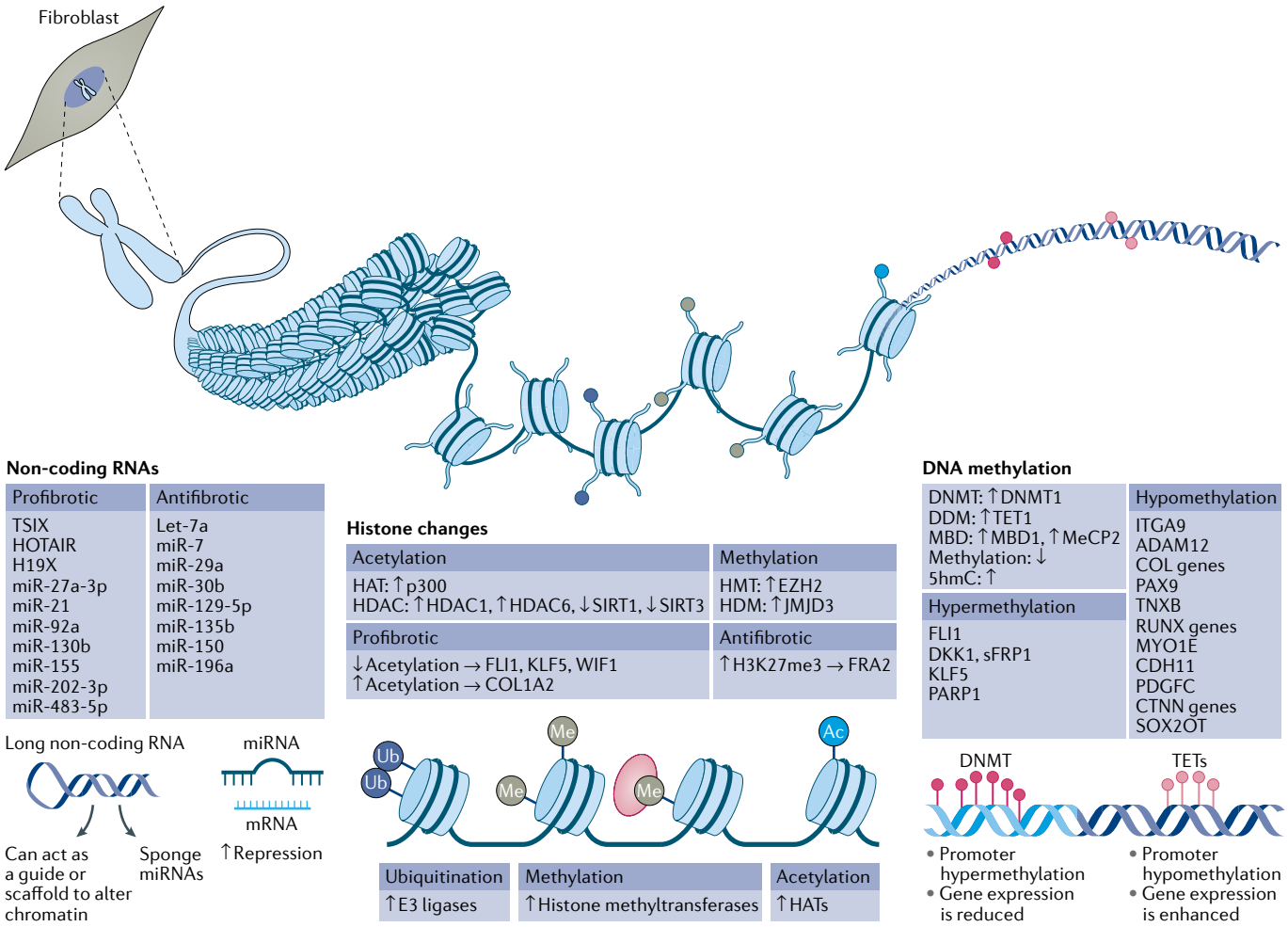

Fig. 1 | **Epigenetic mechanisms.** The three epigenetic mechanisms of DNA methylation, histone modifications and non-coding RNAs are critical in all cell types pertinent in systemic sclerosis (SSc) pathogenesis, including fibroblasts. The main findings related to these epigenetic mechanisms in SSc fibroblasts are summarized in the figure. DNA methylation in promoter regions, mediated by DNA methyltransferase (DNMT) enzymes, represses gene expression. Demethylation, through ten–eleven translocation (TET) enzymes, leads to enhanced gene expression. Histone modifications include acetylation, mediated via histone acetyl transferases (HATs) that add an acetyl group onto the histone tails, methylation, which is mediated via specific methyltransferases, and ubiquitylation, mediated via E3 ligases; removal of ubiquitylation is mediated via deubiquitinases. Histones can also be sumoylated and lactylated (not shown). Long non-coding RNAs (lncRNAs) are ≥200 nt in length and affect gene expression by acting as scaffolding or guiding other binding proteins, or by sponging and sequestering microRNAs (miRNAs). miRNAs work by binding to the 3′ untranslated region of their target mRNAs, culminating in translational inhibition or mRNA decay, and are thus negative regulators of gene expression. EZH2, enhancer of zeste homologue 2; HDAC, histone deacetyl transferase; HDM, histone demethylase; MBD, methyl-CpG-binding domain protein; MeCP2, methyl-CpG-binding protein 2; sFRP1, secreted frizzled-related protein 1.

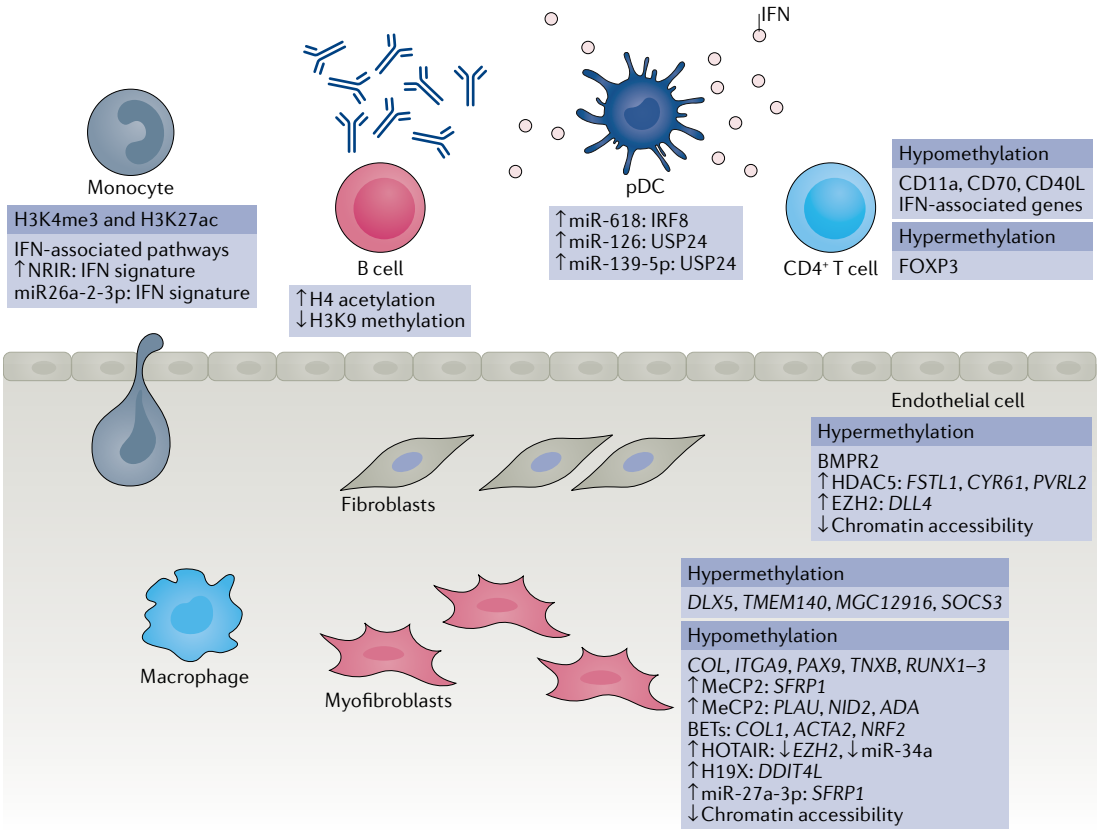

Fig. 2 | **Cell type-specific epigenetic aberrations in systemic sclerosis.** Cell types associated with altered epigenetic marks in systemic sclerosis include monocytes, macrophages, dendritic cells, endothelial cells and fibroblasts. Hypermethylated and hypomethylated genes, dysregulated microRNAs and long non-coding RNAs are indicated within the figure. BET, bromodomain and extra terminal; EZH2, enhancer of zeste homologue 2; HDAC5, histone deacetyl transferase; IFN, interferon; MeCP2, methyl-CpG-binding protein 2; pDC, plasmacytoid dendritic cell.

Histone ubiquitylation is less well understood but can regulate other histone modifications in epigenetic crosstalk. Sumoylation is the addition of a sumo group to lysine residues in specific proteins, resulting in either positive or negative regulation of expression. In a 2019 study, the addition of lactate to histone tails has been found to alter gene expression and affect the differentiation of macrophages to the M2 phenotype, mediated primarily by metabolic polarization[38]. This phenomenon has not been examined in SSc, but it is well described that M2 cells are elevated in SSc.

A variety of enzymes termed 'writers' facilitate the addition of specific modifications to specific residues on the histone tails and enzymes termed 'erasers' remove them. For instance, acetyl groups are deposited by the enzymes histone acetyl transferases (HATs) and these marks are erased by the family of enzymes called histone deacetyl transferases (HDACs), of which there are four classes, HDAC class I, II, III (also called sirtuins) and IV. Histone methyltransferases add a methyl group onto either lysine or arginine residues, much as HATs acetylate lysine residues. Examples of specific methyltransferases include histone-lysine *N*-methyltransferase EZH2 (enhancer of zeste homologue 2), which trimethylates at lysine residue 27, and histone-lysine *N*-methyltransferase EHMT2 (also known as protein G9a), which monomethylates at lysine. Histone methylation marks are associated with either repressive or active chromatin states. The removal of histone methylation marks is undertaken by enzymes called histone demethylases (HDMs). The first histone demethylase identified was protein-arginine deiminase type-4 (PAD4; also known as peptidylarginine deiminase 4), which removes arginine methyl groups[39]. Lysine demethylation is facilitated by lysine-specific histone demethylase 1A (LSD1) and Jumonji domain-containing demethylases (JMJDs)[40]. LSD1 can only remove monomethyl and dimethyl marks on histones, whereas JMJDs can remove all three marks (that is, monomethyl, dimethyl and trimethyl marks)[41]. Of course, there is huge complexity within the histone modification system, as histones are able to carry multiple marks of different substrates and can be monomethylated, dimethylated or trimethylated, and one epigenetic modification can affect another in a cell-dependent and context-dependent manner, adding to the complexity of regulation. Specific histone modifications such as trimethylation have been associated with SSc and are discussed below.

Histone acetyl transferases (HATs). A group of enzymes that mediate the addition of an acetyl group onto lysine residues on histones to modulate gene expression.

Histone deacetyl transferases (HDACs). A group of enzymes that mediate the removal of acetyl groups from lysine residues on histones, positively regulating gene expression.

Epigenetic dysregulation of SSc cells

Multiple cell types are associated with SSc pathogenesis. In this section, we examine each cell type associated with the disease and their epigenetic alteration in SSc pathogenesis (FIG. 2).

Immune cells

Although fibrosis is the common end point in SSc, with excessive extracellular matrix (ECM) deposition in target organs due to activation of fibroblasts, inflammation is a common feature. It could be the initial insult that sets in motion a chain of events leading to fibrosis[42].

Immune cell aberrations identified in SSc include activation of both the innate and adaptive immune system and crosstalk with stromal cells[42]. Indeed, highly specific and mutually exclusive autoantibodies against Scl-70 (topoisomerase I), centromeres or RNA polymerase III, to name a few, are diagnostic of SSc and indicative of the underlying immune dysregulation. T cells are important cells in autoimmune diseases and are activated in response to antigen as part of the adaptive immune system. A study found global hypomethylation in isolated $CD4^+$ T helper cells from patients with SSc, with reduced DNMT1 levels, relative to cells from healthy individuals[43]. Because this was a global analysis, the results of this study are difficult to interpret as gene-specific alterations of methylation that can impinge on cell function are not identified. A more informative study, which looked at methylation on a specific gene, *CD40L*, found it to be hypomethylated in $CD4^+$ T cells from women with SSc compared with healthy women, coincident with increased expression of CD40L at the protein level[44]. This increased protein expression is important, as CD40L on T cells binds its receptor CD40 to dendritic cells promoting maturation, cytokine production in DCs and effectively promotes T cell activation and maturation[45]. It is notable that this study was performed in T cells from female patients, as CD40L is encoded on the X chromosome, which could explain the preponderance of SSc in women. In a separate study, demethylation of *CD70* was found to be associated with enhanced expression of CD70 in isolated $CD4^+$ cells from patients with SSc[46]. CD70 is part of the TNF superfamily and is associated with inflammation, suggesting enhanced inflammation in elevated CD70 $CD4^+$ T cells in SSc. Hypomethylation of *ITGAL* (which encodes CD11a) has also been demonstrated in $CD4^+$ T cells from patients with SSc[47]. This hypomethylation would result in increased CD11a expression, which would mediate enhanced migration of T cells to the site of fibrosis.

Whole-genome bisulphite sequencing in isolated $CD4^+$ T cells from patients with SSc and healthy individuals was reported in a 2019 study[48]. Differentially methylated regions were observed across 340 genes[48]. Pathways differentially methylated included the Wnt and Hippo pathways, which are already known to be involved in fibrosis[49]. Hypomethylation of multiple genes associated with the type I interferon pathway was found in both $CD4^+$ and $CD8^+$ cytotoxic T cells from patients with SSc, along with elevated interferon protein levels[50]. These findings suggest that in SSc, upregulation of type I interferon is mediated by DNA demethylation. Dysregulated interferon expression has been recognized in SSc, but the cell types responsible remain to be determined. An important study published in 2020 used a combination of both epigenomics and transcriptomics in $CD4^+$ T cells to identify differentially methylated regions associated with T cell activation. The results indicated that DNA methylation influenced $CD4^+$ T cell gene expression through long-distance DNA interactions via CCC-TC binding factor (CTCF)[51]. This study provides the first description in SSc of long-range enhancer interactions through CTCF. CTCF is now recognized as a master regulator of genome organization, which can act as an enhancer insulator depending on where it is placed[52]. In light of its potential importance as a regulator of gene expression in SSc, presumably through its role as an enhancer, CTCF merits in-depth investigation in this context.

As part of the innate immune system, monocytes and macrophages are important in SSc pathogenesis[53]. A 2019 study employed chromatin immunoprecipitation with sequencing, alongside RNA sequencing, in isolated monocytes from patients with SSc and healthy individuals. This approach identified 1,046 and 534 genomic loci that had aberrant H3K4me3 and H3K27ac marks, respectively. The genes correlated with these histone marks were enriched for immune, interferon and anti-viral pathways[54]. Functionally, interferon stimulation led to increased binding of signal transducer and activator of transcription 1 (STAT1) and STAT3 at relevant promoters, which could be blocked by an inhibitor of bromodomain histone readers. Bromodomain histone readers are the proteins that 'read' the acetylated protein and regulate gene expression through the recruitment of other factors, suggesting that an inhibitor of bromodomain could restore 'primed' monocytes[54] to a non-primed state. We had previously demonstrated that monocytes stimulated with Toll-like receptor 8 (TLR8) used histone modifications to drive pro-fibrotic molecule release from SSc monocytes[55]. Results from these studies imply that histone modifications are operative in the pro-fibrotic phenotype of SSc monocytes. The lncRNA *NRIR* (negative regulator of the interferon response) was found on RNA sequencing analysis to be upregulated in SSc monocytes, strongly correlating with the interferon gene signature in patients with SSc[56]. Functionally, knockdown of *NRIR* in $CD14^+$ monocytes by small-interfering RNA reduced TLR-mediated upregulation of pro-inflammatory genes[56]. Although these findings suggest that *NRIR* regulates inflammation, the mechanism was not identified. As mentioned, miRNAs are negative regulators of gene expression by binding to 3′ UTRs of mRNAs. miR-26a-2-3p in SSc monocytes was found to negatively correlate with interferon signatures in blood from patients with SSc, and exogenous delivery to monocytes of miR-26a-2-3p mimics negatively regulated TLR-mediated upregulation of interferon genes[57], suggesting that this microRNA has functional anti-inflammatory effects.

Plasmacytoid dendritic cells (pDCs) are a small but significant subset of dendritic cells that, unlike conventional dendritic cells, are in the circulation. pDCs are important interferon-producing cells and might have a role in SSc pathogenesis through the production of interferons, proteins key to promoting adaptive immunity and antigen presentation. These cells were found in higher frequency in the blood of patients with SSc than in healthy individuals and miR-618 expression was found to be elevated in these pDCs[58]. Interestingly, the expression

of miR-618 was elevated in patients with early disease without overt fibrosis. Functionally, the researchers confirmed that miR-618 targets interferon regulatory factor 8 (IRF8), which modulates the development of pDCs[58]. In a 2021 study, again of pDCs, miR-126 and miR-139-5p were significantly upregulated in patients with SSc compared with healthy individuals[59], underscoring the role of miRs in pDCs in SSc. Furthermore, a classic TLR9 agonist upregulated the expression of these two microRNAs and a proteomic screen suggested that ubiquitin carboxyl-terminal hydrolase 24 (USP24) is a target protein of both[59]. The target protein is not confirmed fully in this study, but USP24 is an ubiquitin-specific peptidase that regulates protein turnover and reduction of this activity could prolong interferon release.

The critical involvement of DCs in SSc was demonstrated in a 2020 study[60] that surveyed genome-wide chromatin accessibility in eight types of primary skin cells from patients with SSc, thus creating comprehensive epigenetic regulomes of these cells. Through this analysis, skin-resident DCs showed the greatest disease-associated changes in chromatin accessibility. In addition, these cells seem to facilitate the most upregulated cell–cell receptor–ligand interactions with other cell types; they also show the strongest correlation with skin fibrosis, and are found to be increased in affected skin compared with normal skin. Other cells, such as $CD4^+$ T cells and $CD8^+$ T cells, also showed altered chromatin accessibility, especially between affected and non-affected paired skin samples.

Endothelial cells

In light of the prominent microvascular injury that is the hallmark of SSc, endothelial cell regulation and dysfunction are of great interest. Dermal microvascular endothelial cells isolated from SSc skin retain their abnormal phenotype, including impaired angiogenesis and barrier dysfunction, during ex vivo passage[61,62]. Epigenetic changes, specifically DNA methylation and histone changes, have been reported in SSc endothelial cells. Downregulation of the gene encoding bone morphogenetic protein receptor type 2 (BMPR2), which is implicated in TGFß signalling, was seen in SSc endothelial cells, and attributed to hypermethylation at its promoter region[63]. Two histone-modifying enzymes, HDAC5 and EZH2, were upregulated in endothelial cells in skin from patients with dcSSc compared with cells from healthy individuals[64,65]. The elevated expression of these enzymes contributed to the anti-angiogenic state of the dcSSc endothelial cells, though through different mechanisms. By utilizing assay for transposase-accessible chromatin using sequencing in dcSSc endothelial cells with knockdown of HDAC5, *FSTL1*, *CYR61* and *PVRL2* were identified to play functional roles in angiogenesis[66]. Upregulation of EZH2, and hence an increase in H3K27me3 marks in dcSSc endothelial cells, inhibited angiogenesis[65]. Follow-up functional studies showed that the anti-angiogenic effect of EZH2 was mediated by the Notch pathway, specifically via Notch ligand delta-like protein 4 (DLL4).

A comprehensive analysis of chromatin accessibility in dcSSc endothelial cells with the assay for transposase-accessible chromatin using sequencing published in 2021 (REF.[66]) found a global reduction in chromatin accessibility in dcSSc endothelial cells compared with cells from healthy individuals. Pathway enrichment and gene ontology analysis of the genes annotated in differentially accessible regions revealed enrichment in genes involved in nitric oxide–guanylate cyclase, cilium, ECM and the nervous system. Among the neuronal genes, downregulation of *NRXN1* in dcSSc endothelial cells could contribute to impaired angiogenesis. In addition to chromatin accessibility, 24 putative transcription factors were enriched in dcSSc endothelial cells. Among them, ETV2, SNAI2 and ELF1 were found to bind more in dcSSc endothelial cells than in healthy endothelial cells. The transcription factors differentially recruited in dcSSc and healthy endothelial cells were enriched in pathways including telomerase regulation, nerve growth factor-stimulated transcription, p53 effectors and TGFβ-related pathways, to name a few. The study further highlighted the critical role of ETV2, which could be responsible for the significant enrichment of genes involved in the nervous system identified in the differential chromatin accessibility analysis. In addition, ETV2 could affect angiogenesis in dcSSc endothelial cells, although that hypothesis requires further analysis.

Fibroblasts and myofibroblasts

Fibroblasts and myofibroblasts are the effector cells responsible for SSc fibrosis. Upon tissue injury or inflammatory activation, the plasticity of quiescent fibroblasts enables them to transform into myofibroblasts, which are characterized by accumulation of stress fibres, expression of α-smooth muscle actin (αSMA), and increased matrix protein secretion, increased contractility and enhanced interaction with ECM[67]. The perpetual stimuli (such as numerous cytokines, TGFβ, injury, chemokines, mechanical stress and reactive oxygen species) lead to activation of the fibroblast from a biosynthetically quiescent cell to a metabolically active wound-healing cell with distinct transcriptomic profiles and functions.

Genome-wide DNA methylation analysis of fibroblasts explanted from patients with dcSSc or lcSSc patients and healthy individuals revealed distinct DNA methylation patterns in the two disease subtypes[68]. In a 2021 study, genome-wide differential DNA methylation analysis of primary dermal fibroblasts from 15 patients with SSc and 15 healthy individuals, all of African ancestry[69], revealed that 17 genes and 11 promoters were differentially methylated. One gene, *DLX5*, was elevated in dermal fibroblasts from patients with SSc compared with those from healthy individuals[69]; no functional analysis was undertaken in this study, but in a kidney fibrosis model *DLX5* was shown to promote fibrosis via regulation of Notch signalling[70], suggesting that *DLX5* promotes fibrosis. In a 2019 study we demonstrated a pro-fibrotic role for methyl-CpG-binding protein 2 (MeCP2), which is a methylated DNA binding protein that leads to transcriptional repression[71]. Mechanistically, MeCP2 led to enhanced Wnt signalling by binding to the hypermethylated promoter of the Wnt inhibitor secreted frizzled-related protein 1 (sFRP1)[71]. In a separate study, MeCP2 overexpression in dermal fibroblasts

Stress fibres
Contractile actin bundles found in non-muscle cells, composed of actin and non-muscle myosin II.

inhibited myofibroblast differentiation, proliferation and migration, as well as decreased the cells' contractile properties[72]. Through RNA sequencing and functional validation studies, *PLAU*, *NID2* and *ADA* were identified as MeCP2-target genes[72]. In lung fibrosis models, another methylated DNA binding protein, methyl-CpG-binding domain protein 2 (MBD2), was found to mediate fibrosis via polarization of M2 macrophages and deficiency of MBD2-attenuated fibrosis[73].

It is now well-established that IL-6 concentrations are elevated in SSc[74] and that this cytokine is pro-fibrotic via what is termed IL-6 'trans signalling', whereby cells use a soluble form of the IL-6 receptor instead of the membrane-bound form[75]. However, although STAT3 is known to be important in the IL-6 trans signalling pathway, the precise mechanism of JAK–STAT signalling has only been recently demonstrated. A study published in 2020 demonstrated that TGFβ upregulates the expression of DNA methyltransferases to increase hypermethylation of the promoter of *SOCS3* (encoding suppressor of cytokine signalling 3, an inhibitor of STAT3), leading to its repression[19]. Mice with fibroblast-specific deletion of *Socs3* exposed to bleomycin had exacerbated fibrosis compared with $Socs3^{fl/fl}$ mice. Remarkably, this phenotype could be rescued by treatment with the global demethylator 5-aza[19]. The epigenetically mediated reduction of SOCS3 expression lowers the threshold for activation of STAT3 and thus pro-fibrotic transcriptional programmes, and this effect is maintained ex vivo even after multiple passages. This mechanism could explain how cells can maintain their pro-fibrotic phenotype in culture, as they are epigenetically 'locked'.

Bromodomain and extra terminal (BET) proteins are epigenetic readers that regulate gene expression by binding to acetylated lysine residues on histones or transcription factors. They thus serve a crucial role in regulating gene expression. The anti-fibrotic potential of BET inhibition was shown in in vitro, ex vivo and in vivo systems of SSc[76,77]. In SSc lung fibroblasts treated with JQ1, a BET inhibitor, mRNA expression of αSMA, was reduced and expression of the anti-oxidant transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) was increased, and the BET protein bromodomain-containing protein 4 (BRD4) was enriched at the *NOX2* promoter, suggesting that BRD4 regulates redox pathways[78]. Indeed, JQ1 reduced aged-related lung fibrosis in mice[79].

In a 2020 study in SSc fibroblasts, the lncRNA HOTAIR was associated with αSMA^{+} cells[80]. Levels of HOTAIR were upregulated in these cells compared with cells from healthy donors, and forced overexpression of HOTAIR activated healthy dermal fibroblasts to differentiate into myofibroblasts. Mechanistically, HOTAIR increased EZH2 expression and H3K27me3, which suppressed miR-34a expression and ultimately led to enhanced Notch activity, culminating in fibrosis[80]. A follow-up study demonstrated that downstream of Notch signalling was GLI2, and that Notch-mediated GLI2 expression elicited myofibroblast activation[81]. GLI2 is an important transcription factor of the Hedgehog signalling pathway, and is known to have an important influence on fibrosis[82,83].

Another lncRNA with relevance to SSc identified in the past few years is the paternally imprinted, maternally expressed lncRNA H19X. RNA sequencing of SSc skin revealed that H19X is upregulated in SSc[84]. In isolated fibroblasts stimulated with TGFβ1, H19X was induced in a dose-dependent manner, and knockdown of H19X reduced ECM synthesis in SSc fibroblasts, implicating H19X as an epigenetic regulator of ECM production. Of note, silencing of H19X also caused fibroblast apoptosis. Because H19X regulates miR-424 and miR-503 expression, this regulation was thought to be a mechanism of the TGFβ-mediated effects of H19X, although this proved not to be the case; rather, the mechanism seems to be genomic conformation that alters the expression of *DDIT4L*, among other genes[84]. *DDIT4L* expression is reduced by H19X, and siRNA knockdown of *DDIT4L* increased collagen production[84]. Few studies on DDIT4L exist, but one study showed alterations of expression of *DDIT4L* in radiation-induced fibrosis[85]. These studies suggest that lncRNAs are viable therapeutic targets, although in at least one animal model it appears that H19X was not critical in fibrosis.

We recently described levels of miR-27a-3p to be elevated in dermal fibroblasts from patients with SSc in association with reduced serum concentrations of sFRP1 (REF.[86]). Overexpression of miR-27a-3p led to reduced sFRP1 in dermal fibroblasts, with increased ECM deposition and reduced concentrations of MMP1. We demonstrated that the 3′ UTR of *SFRP1* has a binding site for miR-27a-3p and that it is a bona fide target of the miRNA[86]. This finding suggests that strategies that modulate this miRNA could restore Wnt inhibition and fibrosis. A 2020 publication described the novel role of IL-31 in fibrosis in SSc, and showed that the IL-31 receptor was regulated by miR-326, which was significantly decreased in SSc lung fibroblasts compared with those from healthy individuals[87]. Studies have also shown that miR-16-5p is downregulated and that its target, NOTCH2, is upregulated in SSc fibroblasts; blocking miR-16-5p led to elevated NOTCH2 expression and increased ECM deposition[88].

In addition to studies examining specific epigenetic marks in SSc fibroblasts, another study described the chromatin landscape and transcription factor footprints in fibroblasts from patients with dcSSc and healthy individuals[66]. Similar to dcSSc endothelial cells, chromatin accessibility in dcSSc fibroblasts was reduced overall. Genes located in differential chromatin accessibility regions were enriched in pathways related to the nervous system. Among the genes in these pathways, *ENTPD1*, a neuronal gene that was downregulated in dcSSc fibroblasts, showed pro-fibrotic properties upon overexpression in dcSSc fibroblasts. HINT-ATAC analysis identified 24 transcription factors with differential activity in dcSSc and normal fibroblasts, among which only two, RUNX1 and RUNX2, were significantly enriched in dcSSc fibroblasts compared with healthy cells.

Targeting epigenetic aberrations

The dynamic and reversible nature of epigenetic modifications makes them highly attractive targets for drug development. Indeed, many so-called epi-drugs have

already been developed and several have been evaluated in clinical trials[89]. In fact, the DNMT inhibitor azacitidine and the HDAC inhibitor suberanilohydroxamic acid (also known as vorinostat) are already approved in the USA for treating various forms of cancer. As detailed above, in SSc, many potential epigenetic targets have been identified from in vitro, ex vivo or in vivo experiments; these targets are summarized below and in TABLE 1.

Drugs targeting DNA methylation

The DNMT inhibitors azacitidine and decitabine have been tested in animal models of skin fibrosis. They show potent anti-fibrotic effects in vitro and in vivo[90,91].

Table 1 | **Effects of epigenetic modifiers in experimental models of SSc**

Drug	Epigenetic targets	Targets	Model	Phenotypic effects	Ref.
Drugs targeting DNA methylation					
Azacitidine	DNMTs	CD11a/ITGAL	Human SSc $CD4^+$ T cells	Increased proliferation, production of IgG by co-cultured B cells, and induced expression of collagen by co-cultured fibroblasts	47
Azacitidine	DNMTs	FOXP3	Human SSc $CD4^+$ T cells	Increased T_{reg} cell generation	92
Azacitidine	DNMTs	DKK1; sFRP1	Human SSc dermal fibroblasts; murine bleomycin-induced skin fibrosis	Reduction of fibrosis in mice	91
Azacitidine	DNMTs	KLF5	Human dermal fibroblasts	NA	93
Azacitidine	DNMTs	PARP1	Human dermal fibroblasts	NA	94
Drugs targeting histone modifications					
Trichostatin A	HDAC I and HDAC II	WIF1	Human SSc dermal fibroblasts; murine bleomycin-induced skin fibrosis	Reduction of collagen in human cells and in mice	98
MC1568	HDAC Iia	NR4A1ctcf	Human dermal fibroblasts	Reduction of collagen	99
SIRT1720	SIRT1	NA	Human dermal fibroblasts	Reduction of collagen and αSMA	101
Resveratrol	SIRT1	TGFβ–p300 pathway	Human dermal fibroblasts; human SSc dermal fibroblasts; murine bleomycin-induced skin fibrosis	Reduction of collagen, αSMA, cell migration and contraction in cells	101
Resveratrol	SIRT1	mTOR pathway	Human SSc dermal fibroblasts; murine bleomycin-induced skin fibrosis	Reduction of collagen in cells and skin fibrosis in mice	102
Hexafluoro	SIRT3	SMAD3 activation	Human lung fibroblasts; murine bleomycin-induced skin and lung fibrosis	Reduction of collagen, αSMA, cell migration and contraction, reactive oxygen species in cells; reduction of lung and skin fibrosis in mice	103
DZNep	EZH2	DLL4	Human SSc endothelial cells	Restoration of angiogenic potential in cells	65
DZNep	EZH2	H3K27me3; LRRC16A	Human SSc dermal fibroblasts; murine bleomycin-induced skin fibrosis	Reduction of collagen and cell migration in cells, and reduction in skin fibrosis in mice	65
GSK126	EZH2	H3K27me3	Human SSc dermal fibroblasts; murine bleomycin-induced skin fibrosis	Reduction of cell contraction in cells and skin fibrosis in mice	65
GSK126	EZH2	GLI2	Human SSc dermal fibroblasts	NA	80
GSKJ4	JMJD3	H3K27me3; FRA2	Human SSc dermal fibroblasts; murine bleomycin-induced skin fibrosis; murine topol-induced skin fibrosis	Reduction of collagen in cells and skin thickness in mice	109
BIX01294	G9a	PGC1α	Human IPF fibroblasts	Reduction of collagen and αSMA	111
JQ1	BET	TGFβ2	Human SSc dermal fibroblasts; ex vivo skin explants	Reduction of collagen in skin explants	76
JQ1	BET	NA	Human SSc dermal fibroblasts; murine bleomycin-induced skin fibrosis	Reduction of fibrotic-related genes, migration, proliferation, and cell contraction in cells; reduction of skin fibrosis in mice	77
Drugs targeting non-coding RNAs					
Let7a	Let-7a	NA	Murine bleomycin-induced skin fibrosis	Reduction of skin thickness	122
Remlarsen	miR-29	NA	Human skin	Reduction of collagen expression and fibroplasia development in skin wounds	116
AntagomiR-155	miR-155	Wnt–β-catenin pathway, AKT pathway	Murine bleomycin-induced skin fibrosis	Reduction of skin thickness, collagen expression, and αSMA^+ fibroblasts	114

αSMA, α-smooth muscle actin; αBET, bromodomain and extra terminal; DLL4, delta-like protein 4; DNMT, DNA methyltransferase; EZH2, enhancer of zeste homologue 2; HDAC, histone acetyl transferase; IPF, idiopathic pulmonary fibrosis; NA, not available; sFRP1, secreted frizzled-related protein 1; SSc, systemic sclerosis; TGFβ, transforming growth factor-β; T_{reg} cell, regulatory T cell.

Because DNA methylation is dysregulated in immune cells, fibroblasts and endothelial cells in SSc, it is not surprising that inhibition of DNMT affects multiple pathways in these cells. For instance, in $CD4^+$ T cells, azacitidine treatment enhanced *FOXP3* expression[92]. In dermal fibroblasts, DNMT inhibition led to upregulation of transcription factors FLI1 and KLF5, the SMAD3 modulator PARP1 and the WNT antagonists sFRP1 and DKK1, all of which blocked fibrosis[90,93,94]. DNMT and HDAC inhibition in SSc endothelial cells restored the expression of bone morphogenetic protein receptor II expression[63]. Notably, decitabine is licensed for use in the treatment of acute myeloid leukaemia and seems tolerable[95]. Of course, because these inhibitors globally demethylate DNA, they could have unacceptable off-target effects. An ideal drug would demethylate a densely methylated locus to restore gene expression, thus limiting off-target effects. One way this goal has been achieved is through the use of the CRISPR–Cas9 gene editing system to tether the TET1 catalytic domain[96]. This innovative approach seems to target gene-specific promoters; whether this approach could be used in vivo for long-term alterations remains unknown.

Drugs targeting histone modifications

Trichostatin A (TSA) is an inhibitor of HDAC class I and II enzymes that has been extensively studied in SSc. TSA had potent anti-fibrotic properties in explanted SSc fibroblasts and in animal models of SSc, by downregulating genes associated with ECM and Wnt pathways[97,98]. Selective blockade of class II HDACs with MC1568 showed more potent anti-fibrotic effects than with the class I HDAC inhibitor PD106, suggesting that class II HDACs are more critical in SSc fibrosis[99]. The BET bromodomain inhibitor JQ1 has received considerable attention as a potential novel therapeutic. JQ1 effectively blocked fibrosis in SSc fibroblasts and bleomycin-induced skin fibrosis, as well as in SSc skin explants, by downregulating fibrotic genes[76,77]. Of note, inhibitors of class I and class II HDACs block inflammatory responses in macrophages, by increasing mRNA decay[100]; although this effect might be beneficial in the context of SSc, it could increase the risk of infection.

In addition to class I and II HDACs, class III HDACs (sirtuins) are promising therapeutic targets in SSc. Class III HDACs, in contrast to the other HDACs, seem to protect against fibrosis, as demonstrated by the anti-fibrotic effect of SIRT1 activators, resveratrol and SIRT1720, in both SSc fibroblasts and bleomycin-induced fibrosis in mice[101,102]. Similarly, activation of the mitochondrial sirtuin SIRT3 by hexafluoro mitigated both lung and skin fibrosis in mice[103]. These results were further supported by reports of prominent anti-fibrotic properties of sirtuins in lung fibrosis[104–107] and liver fibrosis[108]. These studies suggest that pharmacological interventions to selectively enhance the expression or function of specific sirtuins might represent a potential therapeutic approach in SSc.

Histone methylation is dynamically regulated by HTMs and HDMs. In SSc, the histone mark H3K27me3 seems to be associated with fibrosis. Selective inhibition of its demethylase JMJD3 using GSKJ4 attenuated fibroblast activation and fibrosis in mice[109]. In addition, blockade of its methyltransferase EZH2 by DZNep or GSK126 not only alleviated SSc fibrosis, but also improved the angiogenic activity of SSc endothelial cells[65]. DZNep has also been found to be anti-fibrotic in liver fibrosis models[110].

The histone methyltransferase G9a, which deposits H3K9me marks on chromatin, is an important novel factor in fibrosis. Although it has not yet been examined in the context of SSc, G9a has been found to be elevated in fibrotic mouse models[111]. Specific inhibition of G9a with the inhibitor BIX01294 attenuated bleomycin-induced fibrosis with derepression of PGC1α[111]. Given that TGFβ seemed to upregulate G9a, it could be that G9a is also operative in SSc.

p300 is an acetyltransferase that deposits an acetyl group onto a lysine residue in histones and other cellular proteins. It has been implicated in fibrosis in various organs and is notably upregulated in SSc fibroblasts[112]. Mechanistically, p300 is upregulated by TGFβ in fibroblasts, causing acetylation of *COL1A1* gene and transcriptional activation by SMAD2 and SMAD3 (REF.[112]). Inhibition of p300 reduces fibrosis. CCS1477 is the first small molecule inhibitor of p300 clinically available. CCS1477 is currently in phase I clinical trials for drug-resistant prostate cancer, multiple myeloma and tumours with specific driver mutations[113] (NCT04068597, clinicaltrials.gov). Although not specifically tested for SSc, it is possible that this could be used in SSc. Further evidence of the role of CCS1477 or other p300 inhibitors in clinical trials will be useful.

Drugs targeting non-coding RNAs

A few studies have proposed approaches to targeting certain miRNAs in SSc. One such example is antagomiR-155, which targets miR-155. Topical application of antagomiR-155 effectively ameliorated bleomycin-induced skin fibrosis in mice[114]. Remlarsen, a mimic of miR-29 (which is known to be remarkably downregulated in SSc[115]), was shown to be safe and tolerable in healthy individuals and effectively reduced ECM and fibroplasia in incisional skin wounds, demonstrating its anti-fibrotic effects[116]. Let7a has been found to be significantly reduced in SSc skin and also reduced by TGFβ1 stimulation in vitro, and in vivo administration of Let7a mimics retarded fibrosis in mice with bleomycin-induced fibrosis compared with controls[117]. RXI-109 is a miRNA therapeutic that targets connective tissue growth factor (CTGF) to reduce fibrosis and is being evaluated in a clinical trial of age-related macular degeneration (NCT02599064). One issue with therapy to replace or inhibit miRNAs is the RNA is rather unstable and RNAses are present in blood at relatively high concentrations. Some authors have conjugated miRNAs to cholesterol to stabilize them in vivo, thus increasing their efficacy[118]. Getting the treatment to the relevant tissue is another issue. Ideally, one would want the miRNA to target only a specific cell type, in much the same way that miravirsen (an experimental drug for hepatitis C) blocks miR-122 with great efficacy,

as miR-122 is liver-specific. Currently, the pharmacodynamics of miRNAs are unclear in vivo, as is the best dosing schedule. At present, no clinical inhibitor of lncRNAs exists.

Considerations for therapy

Although it is now well-recognized that epigenetics is a critical contributor to SSc pathogenesis, and that epi-drugs are potential therapeutics for this disease, there are many hurdles to overcome. Inconsistent results regarding the specific effects of epigenetic modifications have been reported; examples include SIRT1 and EZH2, which have reportedly produced opposite experimental results regarding fibrosis[119,120]. Perhaps more specific inhibitors or activators targeting epigenetics should be utilized for future development. The potential of combinatorial epi-drug therapy with existing regimens should be explored, so that the potential toxicities and/or adverse effects of current drug options can be minimized while therapeutic efficacy is maximized. In the cancer field, combination therapy seems to induce robust, durable therapeutic responses. Last, with the advance of precision medicine, patient stratification to account for SSc heterogeneity should be taken into consideration in treatment decisions. This caveat holds equally true for drugs aimed at modifying epigenetic aberrations.

Conclusions

Studies of epigenetics in SSc published in the past 5 years, enabled by powerful new methodologies and computational tools, have uncovered multiple epigenetic aberrations in different cell types that affect the disease. The three main cell types that we have detailed in this review are markedly affected by these epigenetic modifications. The reversibility of epigenetic aberrations makes them highly amenable to modification, and thus attractive therapeutic targets. Although multiple epi-drugs exist, the heterogeneity of SSc and its unpredictable clinical course might mean that the appropriate drugs (or, more likely, combinations of drugs) must be linked to the aberration; in essence, a precision epigenetic medicine approach. We shall end this Review with two questions: are the epigenetic marks found in SSc stable over time, and if so, can their alteration be a marker of response to treatment? For instance, in liver fibrosis, cell-free plasma DNA methylation of specific CpGs in the PPARγ gene promoter could stratify patients according to fibrosis severity, regardless of aetiology[121]. Indeed, a 2015 study identified differentially methylated regions in cell-free plasma that could discriminate between lung cancer and interstitial lung disease[122]. Such a liquid biopsy in SSc would be extremely useful and less invasive than a skin biopsy.

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The endothelium–bone axis in development, homeostasis and bone and joint disease

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Abstract | Blood vessels form a versatile transport network that is best known for its critical roles in processes such as tissue oxygenation, metabolism and immune surveillance. The vasculature also provides local, often organ-specific, molecular signals that control the behaviour of other cell types in their vicinity during development, homeostasis and regeneration, and also in disease processes. In the skeletal system, the local vasculature is actively involved in both bone formation and resorption. In addition, blood vessels participate in inflammatory processes and contribute to the pathogenesis of diseases that affect the joints, such as rheumatoid arthritis and osteoarthritis. This Review summarizes the current understanding of the architecture, angiogenic growth and functional properties of the bone vasculature. The effects of ageing and pathological conditions, including arthritis and osteoporosis, are also discussed.

Gorham–Stout disease
A rare osteolytic disease that is causally linked to the overgrowth and invasion of lymphatic vessels.

Blood vessels are an extensively branched, tree-like system of endothelial tubules, which, with a few exceptions such as cartilage and the lens of the eye, extends into every tissue in the body. Circulating blood is carried from the heart into the periphery by arteries, which can resist high blood pressure and are able to modulate blood flow owing to their coverage by contractile vascular smooth muscle cells. Arterioles, the smallest arteries, feed into highly branched capillary beds, which lack smooth muscle cell coverage and, instead, are associated with support cells called pericytes. Capillaries are drained by small venules, which feed into larger, smooth muscle cell-covered veins. The main function of this hierarchically organized vascular network is the transport of a wide range of different cargoes, including hormones, gases, nutrients, waste products and circulating cells[1,2]. Meeting the physiological demands of the majority of organs requires cooperation with a second endothelial system, the lymphatic vasculature, which mediates liquid homeostasis, nutrient uptake and immune surveillance[3,4].

In the skeletal system, lymphatic vessels are normally absent, and the emergence of ectopic lymphatics is associated with osteolysis and progressive bone loss in human disorders such as Gorham–Stout disease[5,6]. Likewise, changes affecting the blood vessel network, which is the focus of this Review, are associated with the progression of bone diseases including cancer and osteoporosis[7,8]. Disruption of the vascular supply to bone, which can be caused by a variety of conditions including bone fracture and joint dislocation, as well as accidentally during surgery or by high-dose glucocorticoid treatment, triggers osteonecrosis and substantial local cell death[9–11]. Conversely, ectopic angiogenic blood vessel growth and inflammation of the synovial membrane are closely integrated processes in the pathogenesis of joint diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA)[12,13]. These examples illustrate why it is critical to understand fundamental features of the vasculature in the skeletal system, crosstalk between endothelial cells and other cell types, and the molecular signals controlling bone homeostasis, repair and pathobiological processes.

Endothelial cell networks in different organs exhibit specialized morphological features and gene expression profiles, which reflect different functional roles[14–17]. For example, whereas the vasculature in the lung is specialized for gas exchange, local blood vessels participate in blood ultrafiltration in the kidney, support metabolic processes in the liver and are part of the blood–brain barrier that protects the central nervous system against potentially toxic substances and immune cells from the circulation. In addition, endothelial cells are often a source of paracrine molecular signals (termed 'angiocrine'), which control the behaviour of other cell types in the surrounding tissue[18,19]. Endothelial cell-derived instructive signals regulate endodermal cells during liver and pancreas development in the early mouse embryo[20,21]. Angiocrine signals also control the regeneration of the liver and lung after tissue injury in addition to crucial roles in development[22–24]. Moreover, vascular endothelium provides protective and nurturing niches

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Key points

- The vascular system is essential for bone development and growth.
- Capillary endothelial cells consist of multiple subpopulations with distinct molecular and functional properties.
- The type H endothelial subpopulation communicates with chondrocytes and perivascular osteoblast lineage cells during development and fracture repair, and type H capillaries are reduced in ageing and osteoporosis.
- Blood vessels influence the behaviour of fibroblast-like synoviocytes and macrophages in the arthritic joint.
- Pre-osteoclasts secrete factors that affect bone angiogenesis and the abundance of type H endothelial cells.
- Interdependent crosstalk between endothelial cells and other cell populations in bone might provide novel entry points for anti-osteoporotic therapy.

for multiple adult stem cell populations, such as neural stem cells[25,26], spermatogonial stem cells[27], muscle stem cells[28] and hepatic progenitor cells[29].

In the skeletal system, endothelial cells and vessel-associated reticular cells provide niche microenvironments for haematopoietic stem cells that are important for lifelong blood formation in the healthy organism, but also have implications for haematological diseases such as leukaemia[30–33]. Similarly, endothelial cells communicate with osteoprogenitor cells during bone development and fracture healing[34–36]. The identification of different capillary subtypes with distinct locations and functional roles in long bone[34] has further enhanced our understanding of the heterogeneity and specialization of the bone vasculature. These findings shed new light on bone development and homeostasis, but also on the role of skeletal blood vessels in osteoporosis, arthritis, ageing and fracture healing, as discussed in this Review.

Vasculature in skeletal development

The generation of skeletal elements during development involves two distinct modes of ossification. Flat bones such as the cranium and ilium are generated through the direct conversion of mesenchymal cells into bone-forming cells (osteoblasts) in a process known as intramembranous ossification. By contrast, endochondral ossification, which involves the formation of an intermediate cartilage template that is subsequently converted into calcified tissue, is used to generate the majority of the skeletal structures, including the appendicular skeleton and vertebrae[37,38] (FIG. 1). These processes have been predominantly studied in animal models, and this Review refers to findings in mice unless mentioned otherwise.

Blood vessels in endochondral ossification. The invasion of growing blood vessels is an important step in all modes of osteogenesis and is triggered by extracellular matrix and growth factor signals such as vascular endothelial growth factor A (VEGF-A). VEGF-A is a known master regulator of angiogenesis that signals through VEGF receptor 2 (VEGFR2), a receptor tyrosine kinase expressed by endothelial cells, osteoprogenitor cells and other cell populations[39–41]. Hypertrophic chondrocytes and osteogenic progenitor cells are the main sources of VEGF-A, thereby regulating angiogenesis in bone[40,42–44]. Angiogenesis involves endothelial cell proliferation and, in most developing and regenerating organs, the emergence of endothelial sprouts from pre-existing vessels[45]. Pointed, filopodia-extending endothelial protrusions reach out from the periosteal vasculature during the vascularization of the femoral cartilage shaft in the embryo and lead to the formation of a first vessel plexus. This process is coupled to ossification, the formation of the primary ossification centre[40,42,46,47] and, later, the secondary ossification centre in the epiphysis (FIG. 1a). By contrast, extension of the primary ossification centre in the postnatal femur or tibia involves a different mode of angiogenesis, namely the extension of blunt vessel buds from vessel loops (arches) in close proximity to hypertrophic chondrocytes in the growth plate[35,48] (FIG. 1b). Early experiments using ink injection or corrosion casting in combination with electron microscopy had already indicated the existence of bulb-shaped terminal vessel structures near the growth plate, but lacked insight into the organization and behaviour of the endothelial cells surrounding the vessel lumen[49–51]. Modern static and dynamic microscopic imaging studies have confirmed that distal vessel buds are fully lumenized and show that they are formed from multiple endothelial cells that interact with the surrounding chondrocyte matrix through short filopodia[48]. Vessel buds protrude into the space created by the apoptosis of growth plate chondrocytes, and new vessel arches are generated by the anastomosis of two adjoining buds[48] (FIG. 1b). At their proximal end, the distal arches are connected to relatively straight, column-shaped capillaries that are strongly associated with perivascular bone mesenchymal stromal cells (BMSCs) and osteoprogenitor cells. The heterogeneity and functional properties of BMSCs in osteogenesis and haematopoiesis represent a large and complex topic that goes beyond the scope of this Review and has been covered elsewhere[32,52–55].

Endothelial cell subpopulations in the skeletal system. The endothelial cells of all three substructures (buds, arches and columns) share a high expression of the cell adhesion molecule CD31 (also known as platelet and endothelial cell adhesion molecule 1) and the sialoglycoprotein endomucin (Emcn). High expression of these two markers and association with osteoprogenitor cells are also defining features of capillaries in the endosteum that line the inner surface of compact bone. Accordingly, these capillaries and their endothelial cells are known as $CD31^{hi}Emcn^{hi}$ or type H[34,35]. Endosteal type H vessels connect to the highly branched and relatively irregular sinusoidal vasculature of the bone marrow cavity, which is formed of endothelial cells that express comparably low amounts of CD31 and Emcn ($CD31^{lo}Emcn^{lo}$ or type L)[34] (FIG. 1a). The base of the type H capillary columns in the metaphysis is also connected to the bone marrow vasculature at the metaphyseal–diaphyseal interface, which connects the metaphysis to the diaphysis (FIG. 1c). In line with their presence at a few distinct locations, but also owing to the large size of the bone marrow cavity, type H endothelial cells are much less abundant than their type L counterpart[34]. In addition

Epiphysis
Rounded portion at the end of a long bone that ossifies separately and is typically part of a joint.

Metaphysis
The section of the bone that mediates growth (length extension) and the connection between the diaphysis and epiphysis.

Diaphysis
The midsection (shaft) of long bone, which is enclosed by cortical bone and harbours bone marrow.

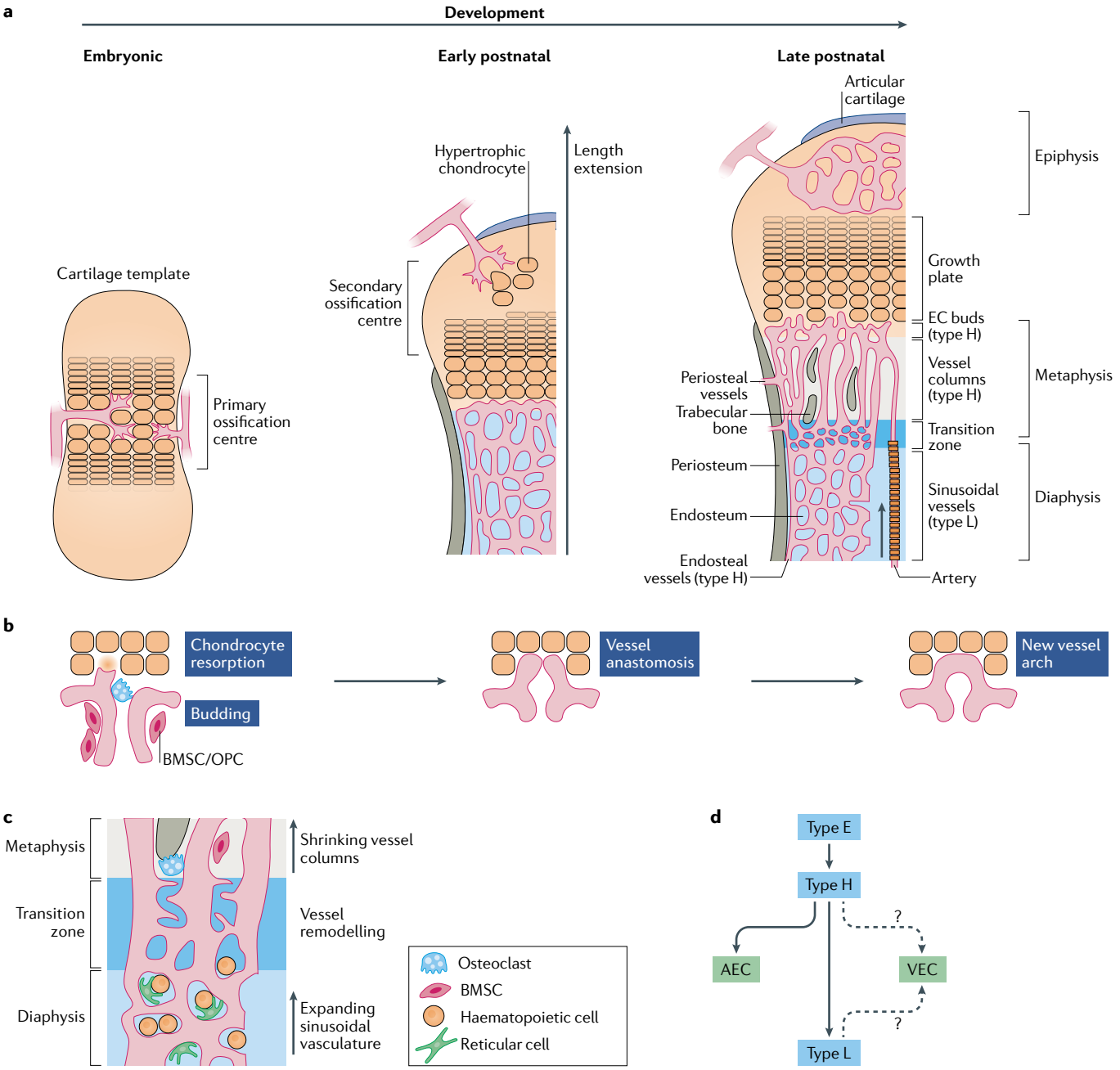

Fig. 1 | **Organization of the bone vasculature during development.** **a** | During endochondral osteogenesis in the developing embryo, signals provided by hypertrophic chondrocytes trigger the invasion of blood vessels into an initially avascular cartilage template (left). This process coincides with the onset of osteogenesis and the formation of the primary ossification centre. Similarly, vessel ingrowth into hypertrophic cartilage of the distal ends of long bone, which occurs postnatally in mice, triggers secondary ossification centre formation (centre). Postnatal growth and bone length extension in late postnatal and adolescent mice is accompanied by the establishment of morphologically and molecularly distinct capillary subpopulations (right). $CD31^{hi}Emcn^{hi}$ (type H) endothelial cells include vessel buds (EC buds) in direct proximity to the growth plate, metaphyseal vessel columns and endosteal capillaries, whereas sinusoidal $CD31^{lo}Emcn^{lo}$ (type L) endothelial cells are found in the bone marrow. Arrow indicates perfusion through the artery. **b** | Resorption of hypertrophic chondrocytes in the growth plate enables the invasion of type H vessel buds, which emerge from distal vessel arches (left). Anastomotic fusion of contiguous buds (centre) leads to the formation of new arch-shaped vessels (right), from which new buds can subsequently emerge. Osteoclasts, bone mesenchymal stromal cells (BMSCs) and osteoprogenitor cells (OPCs) are associated with metaphyseal type H vessels. **c** | Reduction of the metaphysis after the decline of developmental growth is accompanied by expansion of the bone marrow cavity at the transition zone. Bone marrow contains haematopoietic cells and reticular cells, whereas immature BMSCs are mostly confined to the metaphysis and endosteum[156]. Bone marrow expansion involves remodelling of type H vessel columns into sinusoidal (type L) vessels and the removal of trabecular bone by osteoclasts at the metaphyseal–diaphyseal interface through endothelial sprouting. **d** | Type E endothelial cells are abundant in embryonic long bone and give rise to type H cells, which can subsequently generate arterial endothelial cells (AECs) and venous endothelial cells (VECs), but also type L sinusoidal endothelial cells. Although sinusoidal vessels connect directly to the large central vein, the lineage relationship between VECs and other endothelial cell populations remains to be demonstrated (dashed arrows).

to their high expression of CD31 and Emcn at both the protein and the transcript level, type H endothelial cells also have high expression of certain growth factors, including platelet-derived growth factor A (PDGF-A), PDGF-B and fibroblast growth factor 1 (FGF1), which might explain the presence of osteoprogenitor cells around type H capillaries[34,56]. Type H vessels, and particularly the buds in proximity to the growth plate, also have high expression of the Notch ligand DLL4, which is an important regulator of angiogenesis[35,56]. High expression of transcripts for the bone morphogenetic protein (BMP) family members BMP1, BMP4 and BMP6 (factors known to promote bone formation) in freshly isolated $CD31^{hi}Emcn^{hi}$ endothelial cells relative to $CD31^{lo}Emcn^{lo}$ endothelial cells might contribute to the coupling of angiogenesis and osteogenesis in bone development[56].

Another, transiently existing endothelial cell population that is strongly associated with osteoprogenitor cells and has a high expression of CD31 and Emcn, termed type E (for embryonic), was discovered in embryonic and early postnatal long bones[56]. Type E endothelial cells have a particularly high expression of angiogenic and pro-osteogenic genes, induce osteogenic differentiation of mesenchymal cells in 3D spheroid cultures, and, as shown by genetic lineage tracing, give rise to type H and type L endothelial cells in postnatal life[56] (FIG. 1d). Although the precise function of type E endothelial cells requires further investigation, this endothelial subpopulation might enable the rapid bone growth that occurs during late embryonic and early postnatal development.

Arterial endothelial cells in bone express markers that are also typical of arteries in other organ systems, such as DLL4, the transmembrane ligand ephrin B2 and the transcription factor Sox17 (REFS[35,57–59]). Likewise, arterial endothelial cells in many organs including bone express the kinase BMX and can be genetically targeted in mice carrying the tamoxifen-inducible *Bmx-CreERT2* transgenic allele[60,61]. Arteries in bone are also associated with high expression of the chemokine CXCL12 and the cytokine stem cell factor[61,62], which are important regulators of haematopoiesis. Haematopoiesis is an extensive topic, so the function of the bone endothelium and vessel-associated cells in blood formation is not covered in this Review. However, it is important to mention that sinusoidal (type L) endothelial cells of the bone marrow have important roles in the trafficking of haematopoietic cells[63–65] and include specialized vessels that serve as vascular niches for myelopoiesis[66]. Expression of the glycoprotein podoplanin, as well as the adhesion molecules intercellular adhesion molecule 1 and E-selectin, can also be used to distinguish between sinusoidal and arterial endothelial cells by flow cytometry[61].

Molecular pathways underlying bone angiogenesis. In addition to VEGF signalling, several other pathways, including the Notch, hypoxia-inducible factor (HIF), BMP, Slit–Roundabout (Robo) and Hippo signalling pathways, control bone angiogenesis and type H vessel formation (BOX 1) with strong implications for osteogenesis. Several reviews have addressed the role of signalling pathways in bone endothelium in great detail[12,67,68], so we only mention a few critical interactors and regulators in this section.

Endothelial Notch signalling inhibits endothelial cell proliferation, sprouting and vessel growth in many different organs and experimental conditions. However, in postnatal bone, Notch activation in endothelial cells promotes angiogenesis, type H vessel formation and osteogenesis[35]. The basis for these organ-specific differences in endothelial Notch function remains unknown. HIF signalling upregulates VEGF-A expression in many different cell types that are exposed to hypoxia, including chondrocytes. In bone endothelial cells, HIF1α controls type H vessel formation and increases endochondral angiogenesis and osteogenesis[34,57]. Similarly, BMPs are well known for their ability to promote osteogenesis; however, some of the ligands can also activate endothelial cells and stimulate blood vessel growth, providing another molecular link between angiogenesis and osteogenesis[69,70]. Furthermore, osteoblasts regulate

Box 1 | **Signalling pathways that control bone angiogenesis**

Vascular endothelial growth factor pathway
Vascular endothelial growth factor A (VEGF-A) is a member of the VEGF family that is generated in different isoforms, some of which lack critical sequence motifs required for retention at the cell surface and for extracellular matrix binding. Several cell surface receptors and co-receptors for VEGF-A are known, including VEGF receptor 1 (VEGFR1), VEGFR2 and neuropilin 1. VEGF-A is also an important regulator of vascular permeability[157]. In addition to its important function in endothelial cells, VEGF-A is also involved in controlling osteoblast lineage cells and inflammation[41,158].

Hypoxia-inducible factor pathway
Hypoxia-inducible factor (HIF) heterodimers are transcription factors composed of one α-subunit (HIF1α, HIF2α or HIF3α) and a common β-subunit (HIF1β). HIF proteins are unstable at high oxygen concentrations, leading to HIF hydroxylation by prolyl hydroxylases, ubiquitylation by von Hippel–Lindau ubiquitin ligase and proteasomal degradation[159].

Notch pathway
Endothelial cells predominantly express the Notch receptors 1 and 4, as well as the Notch ligand DLL4. Concentrations of DLL4 are increased by Notch signalling and in response to VEGF-A, which generates feedback loops, because Notch also suppresses VEGFR2 signalling. Notch has important cell-autonomous roles in many different cell types and is also critically required for the maintenance of osteoprogenitor cells[160,161].

Slit–Robo pathway
Slit ligands are secreted proteins that were originally identified as regulators of axon guidance in invertebrates. In mammals, three known family members (SLIT1, SLIT2 and SLIT3) and their Roundabout (Robo) transmembrane receptors (Robo1–4) are involved in numerous processes, including neuronal wiring and angiogenesis[162,163].

Hippo pathway
YAP1 and TAZ (also known as WWTR1) are transcriptional co-activators that promote gene expression and growth through interactions with DNA-binding transcriptional enhanced associate domain transcription factors (TEAD1-4), but also through other transcriptional regulators. In response to phosphorylation by an upstream signalling cascade involving the serine–threonine-protein kinases STK3 and STK4 as well as LATS1 and LATS2, YAP1 and TAZ are retained in the cytoplasm and subjected to proteasomal degradation[164].

Bone morphogenetic protein pathway
The bone morphogenetic protein (BMP) pathway involves a large family of ligands belonging to the transforming growth factor-β (TGFβ) superfamily. BMP signalling involves binding to type I and type II heterotetrameric TGFβ family serine–threonine kinase receptor complexes, followed by the phosphorylation of mothers against decapentaplegic homologue (SMAD) proteins 1, 5 and 8, which are transferred from the cytoplasm into the nucleus to control gene expression[165,166].

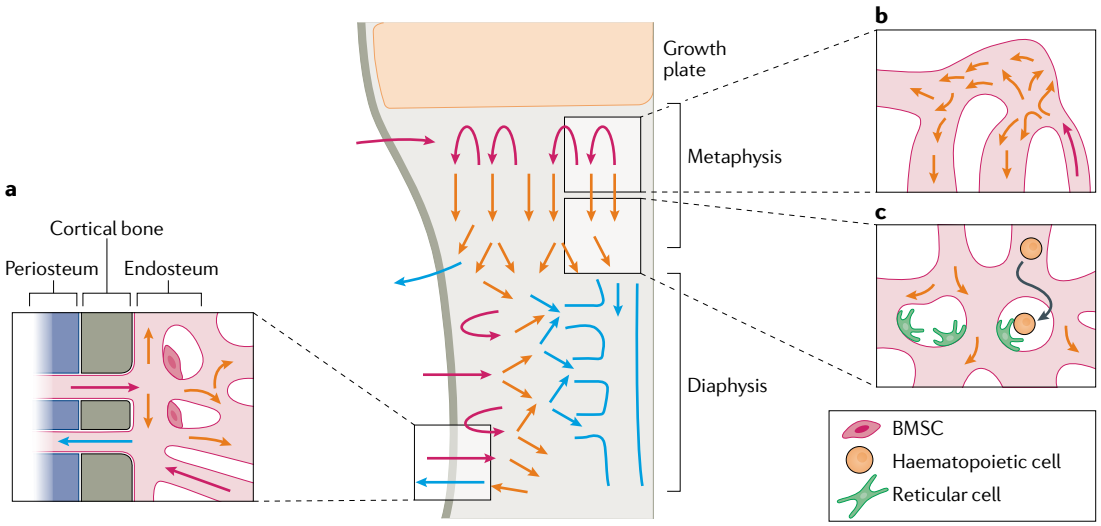

Fig. 2 | **Blood flow in long bones.** Perfused blood flows at a low speed through capillaries and sinusoidal vessels (orange arrows) before entering draining veins (blue arrows). Arterial flow in the metaphysis and endosteum is shown as red arrows. **a** | Periosteal vessels penetrate through cortical bone and supply the endosteum, which harbours a fraction of the immature bone mesenchymal stromal cells (BMSCs). **b** | Although the relatively narrow arteries and arterioles in bone permit laminar perfusion, flow slows down substantially and becomes turbulent after entry into capillaries. **c** | Flow is very slow in the sinusoidal vasculature, which facilitates transendothelial migration of homing leukocytes.

angiogenesis and type H vessels in a paracrine fashion through the secretion of soluble Slit homologue 3 protein (SLIT3) and activation of Robo receptors on endothelial cells[71–73].

The transcriptional co-regulators YAP1 and TAZ (also known as WWTR1), components of the Hippo signalling pathway, suppress angiogenesis in postnatal bone. Endothelial cell-specific loss-of-function mutant mice have augmented angiogenesis, an increased expression of HIF pathway target genes and increased bone formation[74]. Given that expression of YAP1 and TAZ in endothelial cells positively regulates blood vessel growth in the postnatal retina and other developing tissues[74–79], Hippo signalling is another example of a signalling pathway that affects angiogenesis in bone in the opposite fashion to other organs. Although further work is required to uncover the underlying mechanisms involved in this discrepancy, such as potential differences in endothelial chromatin organization or the organ-specific expression or activity of certain transcriptional (co-)regulators, these findings might have relevance for therapeutic approaches that target vascular growth.

Bone vasculature in homeostasis

Morphologically, the vasculature of long bone displays the classical hierarchical arrangement of arteries carrying afferent blood flow, draining veins and interconnecting capillaries (FIG. 2). In the femur, which has been the most extensively studied in animal models, multiple different sources of blood supply have been described[80–82]. A so-called nutrient artery enters the diaphysis through the cortex, extends over a considerable distance through the marrow cavity, and branches out in the metaphysis. The epiphysis and associated cartilage are supplied by epiphyseal arteries and vessels of the ring of La Croix[83–86]. Small periosteal vessels, recently re-described as transcortical vessels, cross the cortex at numerous locations along the bone shaft and contribute substantially to both afferent and efferent blood flow[82,87,88] (FIG. 2a). Arteries and periosteal vessels also provide afferent blood flow into the skull. Periosteal vessels might facilitate the direct access of cells from the bone marrow to nearby tissues, as has been shown for the migration of skull bone marrow-derived myeloid cells towards the surface of the adjacent brain[89].

In long bone, both periosteal vessels and arteriolar branches emerging from the nutrient artery feed into the type H capillaries of the endosteum, which, in turn, drain into the sinusoidal vasculature of the bone marrow[90]. Similarly, the distal arterioles in the metaphysis connect to type H capillaries near the growth plate and thereby provide flow that will reach the bone marrow through the metaphyseal–diaphyseal interface. The type L sinusoidal capillaries in the femoral bone marrow drain into a large central vein, which is a major route for outbound flow. Whereas arterioles have few side branches and are relatively narrow, with a diameter of ~10 µm or less, capillaries in the metaphysis and diaphysis are much wider and have numerous interconnections. Accordingly, arterial laminar flow becomes turbulent and slows down rapidly after entry into the capillary network[48,63] (FIG. 2b). These features, together with the spatial distribution of arterial–capillary connections, also generate distinct metabolic zones characterized by low levels of oxygenation in the bone marrow and higher levels of oxygenation in the metaphysis and endosteum[34,48,90]. Slow flow in sinusoidal vessels might also facilitate the transendothelial migration of blood cells (FIG. 2c); as mentioned previously, leukocyte trafficking seems to occur predominantly at sinusoidal vessels in adult mice[63–65].

Ring of La Croix
A perichondral structure that surrounds the growth plate laterally.

Endothelial cell populations. Insight into the heterogeneity of endothelial cells in the skeletal system is critical for understanding the functional roles and regional specialization of the vasculature. In the past few years, single-cell RNA sequencing data have been used to establish cell atlases for many different species, organs and conditions. The analysis of adult bone stromal cells, however, has so far uncovered a surprisingly limited number of endothelial cell subpopulations, which correspond to arterial and arteriolar, sinusoidal and mitotic cells[53–55]. An independent approach, namely single-cell protein expression mapping by cytometry by time of flight (CyTOF), uncovered 28 distinct stromal cell subsets, including three endothelial populations, two of which were arterial and sinusoidal (type L) endothelial cells[91]. The third CD31$^+$ population was found in the bone fraction and might represent type H endothelial cells or arterioles located in the proximity of osteoblast lineage cells. These studies[53–55,91] did not investigate developing bone, which might explain why type E endothelial cells are not represented in the data. Likewise, the absence of bud endothelial cells near the growth plate and the lack of a distinct endosteal endothelial cell population might reflect the fact that these cells are comparatively rare compared with other endothelial subpopulations. Alternatively, it is feasible that these cells are molecularly similar to other metaphyseal (type H) endothelial cells. Moreover, given that bone is heavily calcified and extracellular matrix-rich, the recovery of different endothelial subsets might depend on the method used for cell isolation. One frequently used method, flushing of the marrow cavity, does not extract endosteal vessels, which remain attached to the bone shaft[34]. Mechanical crushing of bone samples, a different approach to cell isolation, is likely to avoid this issue but might increase the fraction of damaged and therefore discarded cells. Taken together, it is not unlikely that future approaches will provide a more comprehensive insight into bone endothelial cell subpopulations and their molecular heterogeneity.

Role of mechanical forces. Mechanical forces, particularly increased loading, can promote bone formation in the adult organism. Besides chondrocytes, fully differentiated osteoblast lineage cells that are embedded in calcified bone, namely osteocytes, have important roles in mechanosensing[92,93]. The osteocyte lacuno–canalicular network is connected to adjacent blood vessels and, similar to osteoblasts, osteocytes might be a source of VEGF, thereby controlling bone angiogenesis and endothelial cell behaviour[94,95]. On the basis of in vitro experiments, osteocyte apoptosis, which is associated with reduced interstitial fluid flow, has been proposed to increase the release of VEGF-A and thereby promote the angiogenic activity of endothelial cells[96]. Sclerostin, a potent negative regulator of bone formation, is another osteocyte-derived molecule that promotes angiogenesis in endothelial cells in culture[97]. Sclerostin is downregulated at the transcript and protein levels in response to mechanical loading in mice, whereas unloading has the opposite effect[98,99]. Interestingly, hindlimb unloading-induced bone loss is accompanied by a reduction of type H capillaries, whereas mechanical loading stimulates bone angiogenesis and type H vessel formation[100,101]. Despite these interesting insights, the precise interactions between osteocytes and the vasculature under different physiological conditions is far from being understood, and further research is required.

Bone and joint vasculature in disease

Bone is a surprisingly dynamic tissue that undergoes lifelong renewal and remodelling, which involve the balanced activity of bone-forming osteoblasts and bone-degrading osteoclasts. Impairment of this balance results in reduced bone mineral density (osteopenia) or osteoporosis, a disease characterized by bone weakness, increased risk of fracturing, loss of mobility and chronic pain. Osteoporosis is very common in people over the age of 50 years, and one in three women and one in five men will experience osteoporotic fractures in their lifetime[102]. Bone loss also occurs in RA and OA, where it is based, in part, on higher activity of bone-resorbing osteoclasts[103]. Interactions between bone-forming osteoblasts and the vasculature during age-related bone loss is discussed in the section on Bone vasculature during ageing. In this section, we focus on the unique role of bone-resorbing osteoclasts in vascular growth and its implication in bone remodelling, osteoporosis and OA. We also look at interactions between synovial cells and vasculature in inflammatory joint disease.

Interactions between blood vessels and osteoclast lineage cells. Osteoclasts are unique myeloid cells derived from monocytic precursor cells. Upon exposure to the rate-limiting factor receptor activator of NF-κB ligand (RANKL), which is released by osteoblasts, osteocytes and other cells, and in the presence of simultaneous low levels of the antagonist osteoprotegerin, monocytic cells fuse and thereby generate polynuclear, strongly polarized cells (FIG. 3). Cytoskeletal protrusions enable osteoclasts to build a sealing zone and generate an acidic compartment for bone resorption[104]. A landmark study from 2019 revealed that osteoclasts in the bone emerge from tissue-resident erythro-myeloid progenitor cells that undergo fusion with circulating monocytes throughout life and in response to pathological challenges[105] (FIG. 3). In addition to monocytes, dendritic cells can also fuse with osteoclasts, as becomes evident in inflammatory conditions[106].

In a steady state, osteoclasts have a so-called immunological tolerogenic phenotype, which means that they stimulate tolerogenic CD4$^+$ and CD8$^+$ T cells that do not become activated by antigens and thus dampen inflammation[107,108]. However, osteoclasts derived from dendritic cells or Ly6C^{hi} monocytes[107] are so-called inflammatory osteoclasts, which can trigger the participation of TNF-generating CD4$^+$ T cells in the inflammatory response[107]. Inflammatory osteoclasts are heterogeneous and can be subdivided into CX_3CR1^+ and CX_3CR1^- subpopulations[109]. Osteoclasts that lack the chemokine receptor CX_3CR1 are highly inflammatory and resorptive, whereas CX_3CR1^+ cells are less resorptive and, at least in vitro, seem to modulate the inflammatory response[109]. These results indicate that

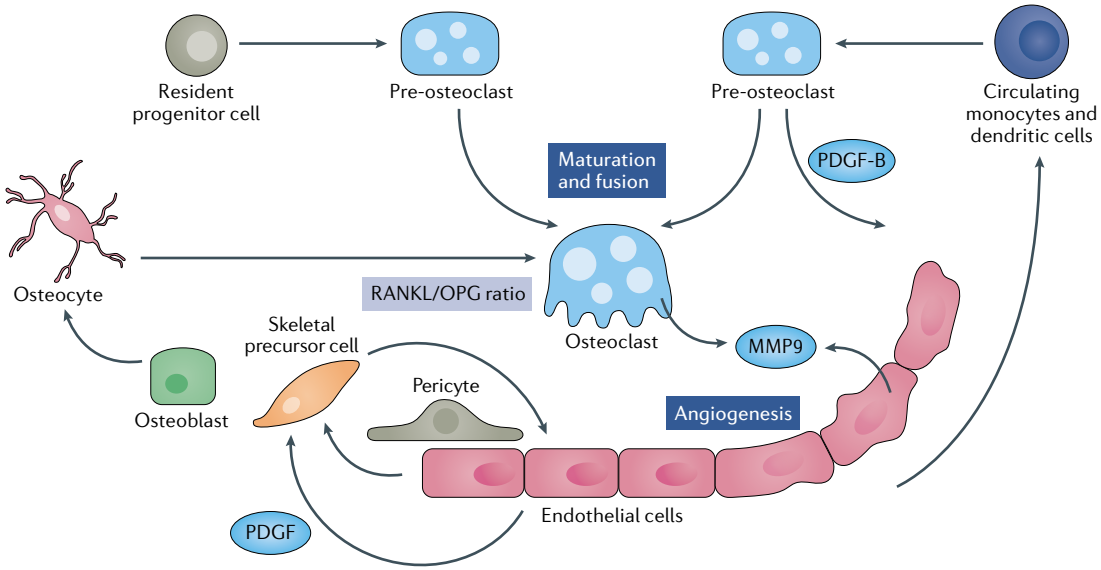

Fig. 3 | **Osteoclast–endothelial cell crosstalk.** Osteoclasts are generated by the fusion of myeloid resident progenitor cells and circulating precursor cells. The rate-limiting signalling molecule for fusion is receptor activator of NF-κB ligand (RANKL), which has to exceed the antagonistic action of osteoprotegerin (OPG) to induce osteoclastogenesis. RANKL is mainly released by osteocytes and osteoblasts that are derived from skeletal precursor cells, which are often perivascular. Skeletal precursor cells can be recruited close to the vasculature by platelet-derived growth factor (PDGF)–PDGF receptor-β signalling, which is triggered by endothelial cells. Non-fused pre-osteoclasts promote angiogenesis by producing PDGF-B, whereas mature osteoclasts were thought to facilitate angiogenesis by producing matrix metalloproteinase 9 (MMP9), which degrades the extracellular matrix. Data from the past couple of years have challenged this concept by demonstrating that endothelial cell-derived MMP9 is required for angiogenic processes in areas with non-degradative osteoclasts. Future research will clarify the exact contribution of endothelial cells and osteoclasts to matrix remodelling necessary for angiogenesis.

the heterogeneity and functional specialization of osteoclasts is just starting to be unravelled.

Osteoclasts and their progenitor cells have been implicated in the regulation of vascular growth in bone as providers of various pro-angiogenic factors[110]. In addition, osteoclasts have been identified as a source of matrix metalloproteinase 9 (MMP9), which is important for angiogenesis both in bone explants and in vivo[111]. However, the conclusion that osteoclasts stimulate angiogenesis through MMP9 was challenged by another study that described a subgroup of vessel-associated osteoclasts[112]. Vessel-associated osteoclasts are reportedly involved in the anastomoses of type H vessels, but not in the resorption of hypertrophic cartilage. The same study used genetic experiments to show that MMP9 produced by endothelial cells, but not by osteoclasts, is essential for cartilage resorption and directional bone growth[112] (FIG. 3).

Pre-osteoclasts, but not monocytes or mature osteoclasts, were found to induce angiogenesis, type H vessel formation and osteogenesis via the secretion of PDGF-B[113]. Ovariectomy-induced osteoporosis in mice led to a reduction in serum and bone marrow concentrations of PDGF-B and a concomitant decrease in type H vessels in long bone[113]. Treatment with exogenous PDGF-B or administration of cathepsin K, which increases the number of pre-osteoclasts and thereby the endogenous concentrations of PDGF-B, stimulated type H vessel formation and osteogenesis in ovariectomized mice[113]. The decrease in type H vessels in ovariectomy-induced osteoporosis, together with the strongly increased osteoclast activity, might challenge bone integrity by diminishing interactions with osteoblasts and osteocytes, leading to further reductions in bone quality. $CD31^{hi}Emcn^{hi}$ (type H) vessels have also been implicated in OA development in several studies[114–116]. Pre-osteoclast-mediated release of PDGF-B contributed to OA pathogenesis and the induction of $CD31^{hi}Emcn^{hi}$ vessels in subchondral bone that started to invade the joint cartilage in the destabilization of the medial meniscus model of OA[116]. Conditional ablation of PDGF-B expression in pre-osteoclasts attenuated aberrant subchondral bone angiogenesis and joint damage, whereas transgenic overexpression of PDGF-B in pre-osteoclasts resulted in spontaneous OA[116]. The exact mechanism of PDGF-B function remains to be elucidated. Expression of the corresponding receptor, PDGF receptor-β (PDGFRβ), is absent in endothelial cells but is present in various mesenchymal stromal cell populations, including skeletal stem and progenitor cells, as well as committed osteoblast lineage cells and synoviocytes[117–119]. Bulk and single-cell transcriptomic analysis has identified a subset of Osterix-positive skeletal stem and progenitor cells that express PDGFRβ, which responds to endothelial cell-derived PDGF[117] (FIG. 3). PDGF–PDGFRβ signalling maintains a proliferative, immature and migratory cell pool with a high affinity for blood vessels[117]. The migratory phenotype of these cells involves the upregulation of MMP9 downstream of PDGFRβ. Whether the crosstalk between

endothelial cells, stromal cells and osteoclasts also participates in arthritis needs further investigation, which is much needed as therapeutic approaches that interfere with the degradation of cartilage and bone remain insufficient for many patients.

Whereas numerous studies have linked pre-osteoclasts to the regulation of blood vessels, mature resorbing osteoclasts seem to be less important in this context. In the tail vertebrae of mice treated with clodronate, a bisphosphonate, blood vessels are present despite osteoclast-mediated bone resorption being blocked[120]. Similarly, the lack of osteoclasts in osteopetrotic Fos-knockout mice does not lead to the absence of blood vessels[120]. In fact, improved bone sample processing and imaging have revealed that the treatment of mice with the bisphosphonate alendronate leads to an increase in type H vessels in long bone[48]. Further studies are needed to understand how blocking osteoclast function, a mainstay of osteoporosis therapy, affects the vasculature in bone, as well as the downstream effects on bone formation and osteocyte survival, and the consequences for bone quality.

Interactions between blood vessels and synovial cells. In inflammatory joint diseases such as RA, bone destruction occurs as a consequence of chronic inflammation. In this chronic inflammatory process, the crosstalk among blood vessels, leukocytes and stromal cells are all important (FIG. 4). Chronic inflammation, synovial swelling and pannus formation with subsequent bone and cartilage degradation are hallmarks of RA. Aberrant angiogenesis is observed in the subchondral area and in the pannus itself[121]. Tissue-resident macrophages and fibroblast-like synoviocytes (FLSs) are presumably the first trigger of inflammation-induced angiogenesis, which is likely to occur in concert with hypoxia-controlled signalling

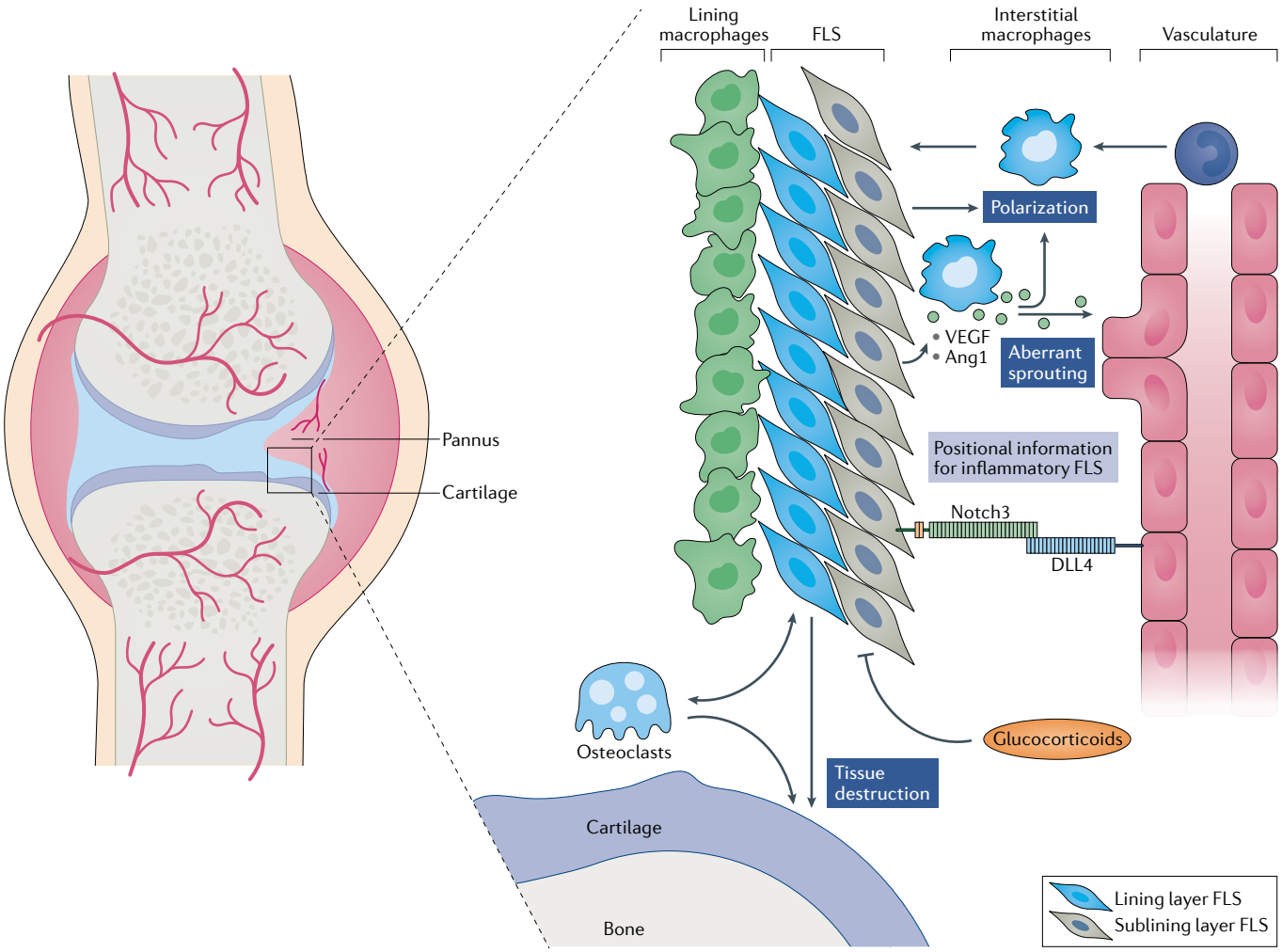

Fig. 4 | **Vasculature, fibroblast-like synoviocyte and macrophage interactions in RA.** In rheumatoid arthritis (RA), the pannus consists of lining macrophages, lining layer fibroblast-like synoviocytes (FLSs) located at a distance from the vasculature, sublining layer FLSs that are close to the vasculature, interstitial resident and monocyte-derived macrophages, and other recruited immune cells (not shown). Tissue-resident macrophages and FLSs trigger aberrant angiogenesis via vascular endothelial growth factor (VEGF), angiopoietin 1 (Ang1) and other factors. The vasculature itself provides positional information for FLSs about whether they belong to the pro-inflammatory active sublining layer FLSs or the more tissue-destructive lining layer FLSs via the Notch ligand DLL4, which signals through Notch3. Lining layer FLSs promote tissue destruction, presumably by inducing osteoclastogenesis and degradative enzyme release. Sublining layer FLSs promote pro-inflammatory macrophage polarization, which in turn triggers angiogenesis. Anti-inflammatory glucocorticoids suppress inflammation by affecting FLSs in RA.

Secondary spongiosa
The region where newly formed bony trabeculae are remodelled into mature trabeculae.

Primary spongiosa
The site near the growth plate where trabecular bone formation is initiated.

pathways (via HIF), as well as pro-inflammatory mediators and pro-angiogenic factors (such as VEGF-A or angiopoietin 1) that induce vessel sprouting[121]. The presence of blood vessels also seems to define the degree of inflammatory potential of FLSs by inducing a position-dependent gene expression programme[122] that spans from FLSs lining the synovial membrane to those that are in close proximity to blood vessels. The closer FLSs are to blood vessels, the more they express the pro-inflammatory marker Thy1 (also known as CD90) and resemble active pro-inflammatory cells. The lining layer Thy1$^-$ FLSs are supposed to have a larger part in tissue destruction than Thy1$^+$ cells, as revealed by cell ablation and transplantation experiments[123]. Instructive signals, such as expression of the Notch ligand DLL4 by endothelial cells, which leads to the activation of Notch3 on FLSs, promotes the Thy1$^+$ pro-inflammatory signature[122]. Thus, vessels support the pro-inflammatory phenotype of FLSs during the arthritic process and, accordingly, genetic and pharmacological inhibition of Notch signalling ameliorates inflammation.

In addition to the interactions between FLSs and the endothelium in RA, there are also important roles for macrophages. In the context of joint inflammation, macrophages can be subdivided by their functional phenotype. Subsets of resident macrophages provide a barrier in the synovium that protects against excessive inflammation, whereas recruited monocyte-derived macrophages in the synovial cavity actively contribute to joint inflammation[124]. Epithelial cell-like macrophages at the synovial lining might be derived from self-renewing resident macrophages located in the synovial tissue. During inflammation, this barrier becomes disrupted and allows the infiltration of pro-inflammatory cells, including polymorphonuclear granulocytes and monocyte-derived macrophages, from the circulation via transendothelial migration. In addition, it is very likely that blood vessels communicate with macrophages indirectly in RA. As previously stated, blood vessels polarize FLSs towards the Thy1$^+$ pro-inflammatory phenotype via DLL4–Notch signalling[122]. In turn, these pro-inflammatory FLSs could trigger pro-inflammatory polarization of macrophages. This scenario is supported by a study demonstrating that immune-suppressive glucocorticoids decrease inflammation by acting on FLS and leading to anti-inflammatory polarization of macrophages[125]. Thus, an endothelial cell–FLS–macrophage interaction axis can be thought of as controlling inflammation in RA, with different FLS subpopulations promoting inflammation and bone destruction, respectively. This axis might also promote the resolution of inflammation and could therefore provide an unexploited therapeutic target.

Effects of glucocorticoids on blood vessels. Glucocorticoids are used for the treatment of inflammatory diseases such as RA, asthma or skin conditions, but adverse effects include glucocorticoid-induced osteoporosis, which occurs frequently owing to the abundant use of steroids[126–128]. Conditional mutations that impair glucocorticoid signalling have revealed a pivotal role for glucocorticoids in osteoblast[129], osteoclast[130–132] and osteocyte[133,134] function. The effects of glucocorticoids on the bone vasculature have only recently been considered. In the femoral head, but less so in the distal femur, glucocorticoid administration decreased local vascularization, which was accompanied by decreased expression of HIF1α and VEGF[135]. In juvenile mice, bone angiogenesis and type H vessel formation were disrupted by glucocorticoid administration, which was linked to reduced PDGF-B expression in pre-osteoclasts[136]. This effect of glucocorticoids relies, in part, on cathepsin K, a protease involved in bone resorption. Inhibition of cathepsin K blocks the effects of the glucocorticoid prednisolone in the secondary spongiosa, and even enhances H type vessel formation in the primary spongiosa[137]. This decrease in type H vessels could contribute to the deleterious effects of glucocorticoids, and might be relevant for several hallmarks of glucocorticoid-induced osteoporosis, such as the attenuation of bone growth, the inhibition of osteoblast differentiation and osteocyte apoptosis. Direct regulation of *PDGFB* transcription via transrepression of p65–NF-κB has been suggested, but effects were only observed at very high doses and the putative NF-κB binding site was not functionally evaluated[136]. Whether this is the primary mechanism by which glucocorticoids affect *PDGFB* expression remains to be proven, and contributions of other glucocorticoid-regulated factors cannot be excluded.

Bone vasculature during ageing

Ageing is associated with a loss of mineralized bone and increased fracture risk[138] (FIG. 5), which are further enhanced by osteoporosis. These conditions are associated with reduced skeletal blood flow both in humans as they age[139,140] and in animal models of ageing[48,141,142].

Role of blood vessels in regenerative osteogenesis. Bone development and fracture repair share many features, and both processes rely on angiogenesis[42,143]. The entry of osteoblast precursor cells correlates with blood vessel ingrowth into cartilage during the developmental formation of the primary ossification centre, but the simultaneous entry of vessels and osteoblastic cells is also observed during fracture healing[36]. Treatment of mice with a soluble, neutralizing VEGF receptor not only decreased angiogenesis during the repair of femoral fractures but also impaired osteogenesis, callus mineralization and bone healing[144]. Conversely, exogenous VEGF-A enhanced blood vessel formation, ossification and callus remodelling. Osteoblast lineage cells are an important source of VEGF-A and thereby contribute to different phases of bone repair. During the repair of drilled lesions in tibias, VEGF produced by osteoblasts promoted macrophage recruitment and angiogenesis in the inflammatory phase, which initiates the repair process[41]. Later in the regeneration process, during the endochondral ossification stage, VEGF-A produced by osteoblasts and hypertrophic chondrocytes stimulates vessel growth, osteoclast recruitment and cartilage resorption at the repair site. The role of osteoblast-derived VEGF extends into the final remodelling phase of the repair process[41].

Perivascular BMSCs expressing glioma-associated oncogene homologue 1 (GLI1) interact with type H

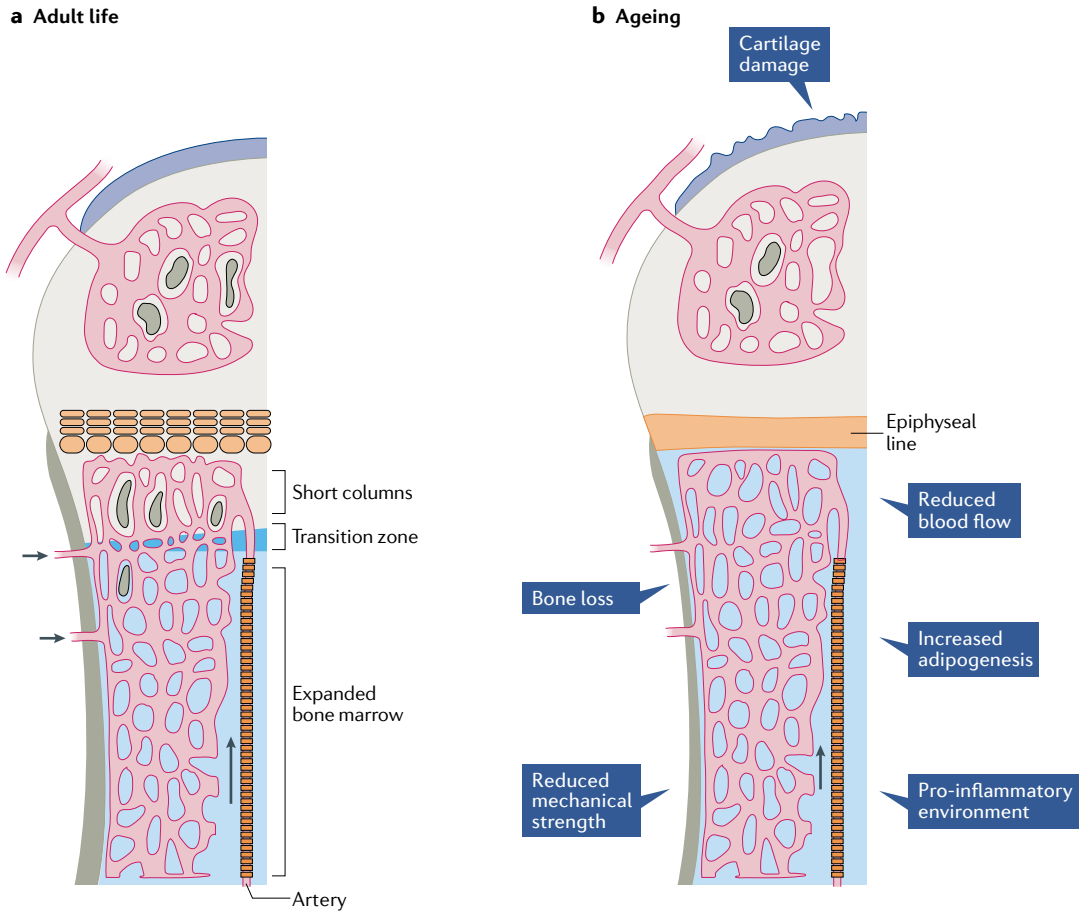

Fig. 5 | **Remodelling of the bone vasculature in adult life and ageing. a** | Owing to the progressive remodelling of type H columns into sinusoidal vessels in the transition zone at the metaphyseal–diaphyseal interface during development, the bone marrow cavity is substantially enlarged in adult mice, whereas the growth plate and metaphysis are substantially smaller than in young animals. Accordingly, the abundance of type H endothelial cells and the length of vessel columns declines in adult and ageing mice. **b** | With increasing age, type H endothelial cells become scarce and the bone shaft is largely remodelled into a large marrow cavity. The growth plate is converted into an epiphyseal line, which is largely devoid of chondrocytes. Articular cartilage is affected by the loss of cartilage matrix and cellularity with age, which is accompanied by increased oxidative stress and apoptosis. Other features of bone ageing include the gradual loss of mineralized bone, an increase in inflammatory processes and increased adipogenesis, which lead to reduced mechanical strength. The number of arteries and amount of blood flow are also reduced, but the underlying reasons are not well understood.

capillaries during bone development and defect healing. Defect healing involves the expansion of type H endothelial cells, and this increase and bone repair are both impaired by genetic ablation of GLI1$^+$ cells[145]. In addition, both osteoprogenitor cells and macrophages express VEGF-A and are closely associated with type H vessels in the forming and maturing callus in a mouse osteotomy model[146]. Other molecular signals can also mediate the crosstalk between different cell populations in growing and regenerating bone. In a mouse model of augmented postnatal bone formation, an increase in type H vessels preceded the appearance of the high bone mass phenotype[71]. Effects on the vasculature were mediated by osteoblast-derived SLIT3, which activated the receptor Robo1 on endothelial cells. Remarkably, administration of recombinant SLIT3 improved bone fracture healing and suppressed ovariectomy-induced bone loss[71]. These and other results indicate that the crosstalk between endothelial cells and bone-forming cells might represent a potential therapeutic target for the improvement of bone mass and prevention of osteoporosis.

Blood vessels as a target for anti-osteoporotic treatments. The reduced skeletal blood flow that occurs in osteoporosis might affect a range of physiological features, including nutrient delivery, tissue metabolism and the influx of calcium and phosphate[147,148]. Surgical or pharmacological interference with normal blood flow alters endothelial cell behaviour and reduces the abundance of type H vessels in murine femurs[48]. As in the ovariectomy-induced model of osteoporosis, normal ageing results in a profound diminishment of not only type H vessels and associated osteoprogenitor cells but also of arteries and arterioles in the femur (FIG. 5), which is likely to contribute to reduced perfusion and impaired bone homeostasis[34,48,57]. In mice, enhanced HIF activity in endothelial cells via tissue-specific inactivation of von Hippel–Lindau ubiquitin ligase leads to

increases in type H vasculature and perivascular osteoprogenitor cells, resulting in augmented trabecular bone formation[34]. Treatment of 60–65-week-old mice with deferoxamine mesylate, which enhances HIF1α stability and activity, also increases type H vasculature and mineralized trabecular bone[34]. Likewise, endothelial cell-specific genetic approaches to enhancing Notch signalling lead to the growth of type H vessels, increases in osteoprogenitor cells and trabecular bone formation in ageing animals[48,57]. These proof-of-principle experiments indicate that skeletal blood vessels are not only responding to ageing processes in the surrounding tissue but might also represent a therapeutic target for the treatment of osteoporosis either alone or in combination with anabolic or anti-resorptive drugs.

Translating from mouse to human

Early studies indicated substantial similarities in the organization of the bone vasculature in different mammalian species, and the involvement of blood vessels in fetal bone development and in fracture healing in the adult[49,51,84,85,149,150]. Although our understanding of the cellular and molecular processes in the human skeletal system are currently very limited, several reports already suggest that certain important findings from animal models might be relevant for humans. For example, human bone endothelial cells that express the cell surface protein CD105, which are associated with skeletal development and regeneration, share critical features with murine type H endothelial cells[151]. Human type H vessels are a sensitive biomarker of bone mass in ageing individuals and in those with osteoporosis[152]. Likewise, $CD31^{hi}Emcn^{hi}$ endothelial cell abundance is positively associated with bone mineral density in human femur neck and with total hip bone mineral density, but not with bone mineral density in the lumbar vertebra[153]. Moreover, the percentage of $CD31^{hi}Emcn^{hi}$ endothelial cells in postmenopausal women was substantially lower than that in premenopausal women[153]. Taken together, the existing evidence is encouraging and indicates that at least some of the fundamental findings made in mice are of broader relevance and might be translatable to humans. At the same time, it should be noted that the number of published reports is still rather limited and that more research is needed to get a better understanding of the processes that occur in the healthy and diseased human skeletal system.

Conclusions

Even though it is unquestionable that future work is required to provide more insight into the role of the vasculature and capillary subpopulations in bone development, homeostasis, regeneration, healthy ageing and disease, it is increasingly evident that vascular cells are not just building blocks of a transport network and, instead, actively control critical processes through communication with a variety of other cell types. Such interactions include crosstalk with chondrocytes and perivascular osteoblast lineage cells that is bidirectional and results in a coupling of angiogenesis and osteogenesis. Osteoclasts are also associated with bone vessels, and the abundance of type H endothelial cells and bone angiogenesis are controlled by signals provided by pre-osteoclasts. By contrast, there is relatively limited evidence of a potential direct regulation of osteoclasts by endothelial cell-derived signals[112,154,155]. Thus, it remains to be addressed whether bone endothelial cells directly control osteoclastogenesis and thereby bone turnover, fracture healing and conditions such as osteopenia and osteoporosis. Furthermore, blood vessels are likely to have important roles in OA and other joint diseases, not just through their role in immune cell migration, but also through interaction with synoviocytes and other cell populations in the joint. The identification of relevant molecular signals and potential therapeutic relevance requires further investigation.

Taken together, it is clear that the vasculature in the skeletal system is much more than a passive conduit system and that learning more about its function, dynamic modulation and molecular crosstalk with other cell types in the local microenvironment offers great opportunities. So far, the treatment of diseases affecting bone has almost exclusively focused on addressing the balance between osteoblasts and osteoclasts to enhance bone formation or inhibit bone resorption. The latter is currently the main strategy used in anti-osteoporotic therapy, but the quality of bone is not improved, despite attenuated bone loss. We consider the versatile function of the bone vasculature an important factor in balancing the right dose of bone turnover to improve bone quality for the treatment of diseases such as osteoporosis or OA that currently impose huge burdens on our ageing population.

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Early-stage symptomatic osteoarthritis of the knee — time for action

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Abstract | Osteoarthritis (OA) remains the most challenging arthritic disorder, with a high burden of disease and no available disease-modifying treatments. Symptomatic early-stage OA of the knee (the focus of this Review) urgently needs to be identified and defined, as efficient early-stage case finding and diagnosis in primary care would enable health-care providers to proactively and substantially reduce the burden of disease through proper management including structured education, exercise and weight management (when needed) and addressing lifestyle-related risk factors for disease progression. Efforts to define patient populations with symptomatic early-stage knee OA on the basis of validated classification criteria are ongoing. Such criteria, as well as the identification of molecular and imaging biomarkers of disease risk and/or progression, would enable well-designed clinical studies, facilitate interventional trials, and aid the discovery and validation of cellular and molecular targets for novel therapies. Treatment strategies, relevant outcomes and ethical issues also need to be considered in the context of the cost-effective management of symptomatic early-stage knee OA. To move forwards, a multidisciplinary and sustained international effort involving all major stakeholders is required.

Osteoarthritis (OA) is the most prevalent joint disease, affecting over 500 million individuals globally, of whom more than 260 million have knee OA[1], representing a 9.3% increase from 1990 to 2017 (REF.[2]). In view of its major contribution to disease burden, we here focus on knee OA and the opportunities provided by defining and identifying persons with symptomatic early-stage OA of the knee. The term 'symptomatic' signifies the group of individuals seeking health care for their symptoms and who thereby differ from persons with risk factors for OA but without symptoms.

Management of OA should preferably aim to reduce the burden of the disease by changing its course to prevent long-term disability, but so far the efforts to do so have typically targeted patients in relatively late stages of the disease. Routine OA management is too often reactive rather than being proactive in identifying and treating patients in the early stages of the disease. Intervening early might stand a better chance of success, before the advent of chronic pain, severe joint destruction with biomechanical derangement, reduced function, disability and development of comorbidities[3–6]. Although the concept of early-stage disease is now embraced in many other chronic conditions such as diabetes mellitus, cardiovascular disease and Alzheimer disease[7–9], it also seems to be relevant for chronic arthritic diseases such as rheumatoid arthritis (RA)[10–12] and psoriatic arthritis[13]. A systematic review and meta-analysis of cohort studies and randomized controlled trials (RCTs) reporting outcome data of early RA supported the presence of a therapeutic 'window of opportunity', even when the heterogeneity of patients in the studies was accounted for[14].

The diagnosis and classification of symptomatic early-stage knee OA has been insufficiently explored and is yet to be agreed upon. Whether a window of opportunity exists for early-stage OA remains to be shown but, at least from the patient's perspective, early detection and intervention are relevant. The concept of early detection as a window of opportunity is supported by studies involving young patients undergoing surgical interventions for knee joint surface repair, such as autologous chondrocyte transplantation or implantation of an osteochondral scaffold, and early physical therapy interventions[3–5,15,16], as well as by the success of comprehensive management programmes in primary care such as Good Life with Osteoarthritis in Denmark (GLA:D®)[17]. To improve the chance of success in clinical studies that aim to slow disease progression and, importantly, ensure cost-effectiveness, stratification of the population with early-stage knee OA to identify those with an increased risk of disease progression will be required. Identification of people at the early stages of OA could also be helpful in limiting the long-term effects of the disease. As an example, a study in the Osteoarthritis

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Key points

- Early-stage knee osteoarthritis (OA) could present a 'window of opportunity' in which to arrest the disease process at the early stages and restore joint homeostasis.
- The initiating cellular and molecular cascade of events in early disease need to be studied in more detail and connected to triggering events and the patient profile.
- The goal of classification criteria for early-stage knee OA is to enable discrimination of patient populations with early-stage symptomatic knee OA, who are at increased risk of structural progression, from patients with knee symptoms due to other reasons.
- Final classification criteria for early-stage knee OA should be validated by a multidisciplinary panel of experts in the field with involvement of all relevant stakeholders.
- Early diagnosis in clinical practice enables proper disease management and reduction of the burden of disease.

Initiative (OAI) cohort suggested that the risks of experiencing a fall or fracture are higher (>50% and 85% greater, respectively) in people newly diagnosed with OA of the hip or knee compared with people of a similar age and characteristics without hip or knee OA[18].

The development of OA represents a continuum from health to the first presence of OA biomarkers — detected in body fluids or by non-invasive imaging, in the absence of clinically relevant symptoms/signs — then to symptomatic early-stage OA, established OA and finally end-stage OA (FIG. 1). While we acknowledge the process as a continuum, staging of it enables proper clinical management and research. In the 'at risk' stage and in the absence of clinical symptoms/signs, local or systemic molecular biomarkers or imaging biomarkers (for example, detected by MRI) could help identify patients at increased risk of developing full-blown knee OA. Patients with symptomatic early-stage knee OA typically present in primary health care with intermittent, activity-induced knee pain and/or discomfort[19], with limited or no radiographic changes. As the disease progresses, structural changes (such as osteophyte formation, joint space narrowing and subchondral bone sclerosis) become apparent and detectable on standard radiographs. At this stage, joint homeostasis has been lost, biomechanical derangement has occurred, and no approved treatment exists that can slow or reverse the disease process. Along with structural changes, the clinical symptoms typically worsen, with accompanying pain sensitization, and the disease manifestations become chronic. The presence of predisposing factors including family history of OA, previous knee injury and obesity can accelerate both symptoms and structural progression towards the later stages of the disease, which are defined by evident structural damage, pain and functional limitations, and other clinical complications[20].

The course of the disease is typically diverse and in an individual patient with OA is largely unpredictable. A substantial group of the OA population can follow a pattern of disease inertia; others worsen slowly whereas some follow an accelerated track[21,22]. Only a minority of all patients diagnosed with knee OA will ultimately undergo joint replacement surgery, but they nonetheless represent a sizeable and costly minority[23,24]. In the USA alone, the predicted annual count of total knee replacement procedures in 2020 is >1 million, and is predicted to increase by 400% by 2040 (REF.[25]).

Diagnosis of symptomatic early-stage knee OA provides the opportunity to manage the disease at an earlier stage with currently recommended first-line programmes. Indeed, despite the common perception of limitations in dealing effectively with knee OA[26], tools are now available to manage patients with knee OA, in particular in the early stages, and to reduce the disease burden[27,28]. These tools include, but are not restricted to, educational and exercise programmes, prevention of abnormal load or injury, approaches to enhancing coping strategies and managing expectations and, when needed, the addition of appropriate pain relief by use of local or systemic medication. Early-stage intervention also provides the opportunity to address the need for personalized lifestyle changes, including the promotion of exercise and weight control.

The accurate definition of early-stage knee OA by use of validated classification criteria would result in more homogeneous patient populations that would also enable better understanding of the mechanisms that drive the development of the disease. It would also facilitate interventional trials to validate therapeutic targets in the proper disease context and hopefully lead to therapies that can slow down joint destruction and even restore joint homeostasis. The development of the classification criteria discussed here are intended to serve as a 'first filter' to enrich for the patient population of interest; namely, those with early-stage knee OA. We expect that continued work by specialist groups will identify molecular and imaging biomarkers to further refine these classification criteria and/or enrich for patients at high risk of disease progression.

The purpose of this Review article is to assess the current best understanding of symptomatic early-stage knee OA and to highlight key knowledge gaps. These gaps most critically include optimized case finding, thus early diagnosis, in primary care, as well as defining symptomatic early-stage disease by use of classification criteria. We discuss treatment strategies and suggested outcome measures, and the ethics and risks associated with changing disease criteria as well as the need to align all stakeholders in this endeavour, including patients, health-care professionals, researchers, regulators and industry partners.

Diagnosis of early-stage knee OA

Diagnosis of early-stage disease focuses on case finding primarily in primary care, and proper management of the individual patient in clinical practice; this goal sits in contrast to that of classification criteria, which is to define early-stage disease with the aim of specifying homogeneous patient groups for clinical studies[29]. The diagnosis of early-stage knee OA is typically suspected when a chronic pattern of knee pain or discomfort develops over weeks to months, with periods of worse pain, stiffness and functional limitations for a week or more, interspersed with periods of little or no pain[30] (BOX 1). Clinical examination mostly reveals pain upon mobilization, joint-line tenderness, crepitus or mild joint effusion. Radiographic findings are of limited value in early-stage disease, as one of the typical features of OA — joint space narrowing — might not appear for many years[31].

The presence of Heberden's nodes, the bony swellings of the joint closest to the fingertips, is suggestive of generalized poly-articular disease[32] and has been associated with knee OA progression[32]. The diagnosis of early-stage knee OA can be further supported by the presence of risk factors such as older age, high body mass index (BMI), history of knee trauma or a family history of OA such as a history of joint replacement in first-degree relatives. Importantly, the absence of other differential diagnoses (for example, other arthritic diseases such as psoriatic arthritis and reactive arthritis) further supports the diagnosis of symptomatic early-stage knee OA. To date, to the best of our knowledge, no validated diagnostic criteria are available for early-stage knee OA. The Italian Society for Rheumatology has proposed a set of criteria for early symptomatic knee OA for the purpose of referral to rheumatologists, developed through a three-phase process comprising focus groups (including expert clinicians, researchers and patients), a systematic literature review and group discussions followed by a Delphi survey[33]; these criteria are yet to be validated. The CRiteria for the Early Diagnosis of Osteoarthritis (CREDO) group is working to develop a set of diagnostic criteria with relevance to primary care, using the Cohort Hip and Cohort Knee (CHECK) study cohort[34]. The latest report from this group, published in 2020, proposed three predictive models for the development of clinically relevant knee OA, as defined by experts; the first of these models was based on factors obtained from questionnaires and physical examination, the second model added radiographic factors and the third also included high-sensitivity C-reactive protein test. The predictive performance of these models was tested against experts' diagnosis of clinically relevant knee OA 5–10 years later, with the results indicating that the performance of all three models was 'fair' in making the distinction between cases with and without clinically relevant knee OA[34].

Classification criteria for early-stage knee OA

Classification criteria for symptomatic early-stage knee OA are needed in order to define more homogeneous patient populations for studies of epidemiology, natural

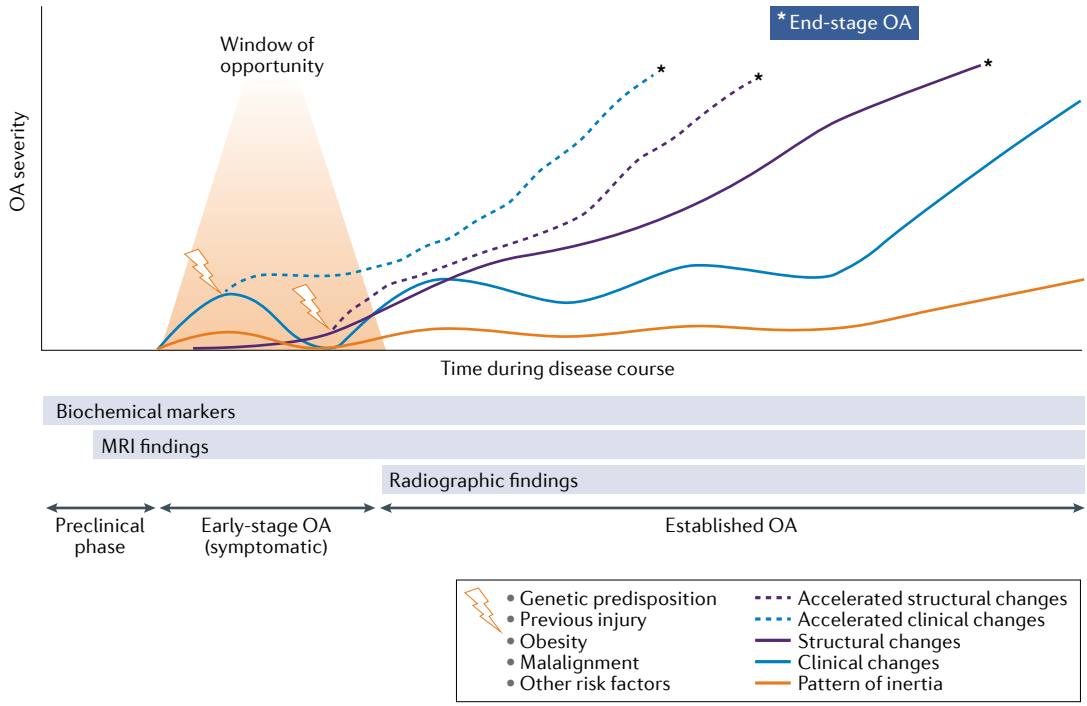

Fig. 1 | **The natural course of knee osteoarthritis.** This schematic presents the natural course of knee osteoarthritis (OA) from both clinical (blue line) and structural (black line) perspectives. The dashed lines signify the acceleration of clinical (blue) and structural (black) course of knee OA in the presence of predisposing (risk) factors for progression. The orange line presents the pattern of inertia. At the preclinical stage, some biomarkers (biochemical and MRI biomarkers) might be useful in identifying patients at increased risk of knee OA incidence. At the symptomatic stage of the disease, there are no or only limited radiographically detectable structural changes (Kellgren & Lawrence grade 0–1). People with early-stage knee OA typically present in health care with intermittent, activity-induced knee pain; this stage could serve as a 'window of opportunity' to arrest the OA disease process and restore joint homeostasis. As the disease progresses, structural changes (such as osteophyte formation, joint space narrowing, subchondral bone sclerosis and others) also become apparent and detectable on radiographs. At this stage, joint homeostasis is lost, biomechanical derangement often occurs, and the disease process thus becomes largely irreversible. Along with structural changes, the clinical symptoms also typically worsen with accompanying pain sensitization and develop towards chronicity. The presence of predisposing factors such as family history, previous knee injury and obesity, among others, could accelerate both clinical and structural progression towards end-stage disease, defined as evident structural damage, pain and functional limitations and/or other clinical complications. A minority of all patients diagnosed with knee OA will undergo joint replacement surgery, whereas a considerable part of the population of patients with OA follows a pattern of inertia.

Box 1 | **Diagnosis of symptomatic early-stage knee OA**

Pain pattern
- Chronic knee pain pattern developing over weeks to months
- Mechanical in nature increasing with loading and (over)use

Clinical features
- Joint line tenderness
- Crepitus or patellar grinding
- Mild joint effusion
- (Almost) normal range of motion

Radiographic findings
- Limited relevance in early-stage disease other than discrete bone remodelling or early osteophyte formation

Further supporting evidence
- Older age
- High body mass index
- History of knee trauma
- Family history of osteoarthritis (OA) (e.g. history of joint replacement surgery)
- Absence of other differential diagnoses

history and disease mechanisms and, importantly, for interventional studies. In contrast to diagnostic criteria, classification criteria typically aim to achieve high specificity and allow for lower sensitivity, and are thus less inclusive but more sharply defined[29]. The classification criteria should be reliable, universally applicable, clinically sensible and as precise as possible. The goal is to enable the discrimination of patients with early-stage symptomatic knee OA from patients with knee symptoms arising owing to other reasons, including acute knee injuries or other arthritic diseases. Further stratification of the subset of patients with early-stage symptomatic knee OA, for example by adding certain risk factors or by more comprehensive phenotyping, could enable enrichment of populations for patients whose knee OA will progress. This is a daunting task as no gold-standard definition of symptomatic early-stage knee OA exists, and even the existing classification criteria for established knee OA vary considerably[35,36]. The 1986 classification criteria[35] issued by the ACR (then known as the American Rheumatism Association) are the most frequently used. However, patients fulfilling the clinical and radiological ACR criteria for knee OA will already have considerable joint damage involving several tissues, such as cartilage, meniscus, underlying bone and synovium. A set of criteria for early-stage knee OA proposed in 2012 by the European Society for Sports Traumatology, Knee Surgery and Arthroscopy[37] bases classification on the presence of knee pain associated with degenerative changes detected by MRI or arthroscopy and is thus more targeted towards second-line health-care providers, typically orthopaedic surgeons and rheumatologists. A more recent set of criteria[38], proposed in 2018 by an international consortium, was designed to identify symptomatic patients with early-stage knee OA with a focus on primary care, as the majority of these patients are first seen by a general practitioner. These criteria rely on broadly applicable, simple, patient-based assessments and clinical examination in the absence (or near-absence) of radiological abnormalities (FIG. 2). The performance of the 2018 criteria in predicting structural and clinical progression of knee OA in the OAI population was encouraging, and the inclusion of additional clinical findings, such as presence of knee effusion and Heberden's nodes, improved the predictive performance of the originally proposed criteria[39]. This set of classification criteria has also been applied in other populations, for example, in the Iwaki Health Promotion Project cohort to investigate the prevalence and risk factors of early-stage knee OA in the Japanese general population[40–42]. Importantly, the use of validated classification criteria in future studies exploring biomarkers and other risk factors for early-stage knee OA would enable cross-study comparisons and meta-analyses. However, none of the proposed classification criteria sets have yet been validated. In order to do so, further comprehensive efforts are ongoing through a well-accepted four-phase process, which has previously been used in the development of classification criteria for other rheumatic diseases (FIG. 3). Thus, both data-driven and consensus-based, decision-science-informed approaches are being used to develop and validate a scoring system for symptomatic early-stage knee OA classification.

One of the challenges in developing classification criteria for early-stage knee OA is to exclude patients with knee pain due to other causes, in particular patients with chronic pain syndrome or related widespread pain syndromes such as fibromyalgia. Specific exclusion criteria, based on clinical expertise and/or validated questionnaires, are warranted.

Final classification criteria for early-stage knee OA should be proposed by a multidisciplinary panel of experts in the field, should include patients, and should result in validated criteria with the greatest content validity and construct validity. Such an ambitious project is ongoing and is guided by a multidisciplinary working group with observers from several relevant professional societies.

Risk factors and early-stage symptomatic knee OA

There is no indication that the risk factors for symptomatic early-stage knee OA would be much different than those for established knee OA.

The major risk factors for OA have been reviewed elsewhere[43], and include age (or years of exposure to any risk factor), overweight and obesity, joint trauma, high occupational joint loading, genetic susceptibility and, for women, menopause. On the basis of this list of factors, the presentation of a women who is postmenopausal, has overweight, has knee pain most days of the preceding month and has a history of repetitive knee (over) loading in the context of professional or recreational activities will prompt a clinician to suspect early-stage knee OA. If some additional non-modifiable risk factors are detected, such as a family history of knee replacement surgery, the clinician should assign this patient to a well-defined care trajectory.

ACR classification criteria for knee OA

Clinical and laboratory criteria	Clinical and radiographic criteria	Clinical criteria
• Knee pain At least five of the following: • Age > 50 years • Stiffness < 30 min • Crepitus • Bony tenderness • Bony enlargement • No palpable warmth • ESR < 40 mm/h • RF < 1:40 • Synovial fluid analysis indicative of OA	• Knee pain • Osteophytes At least one of the following: • Age > 50 years • Stiffness < 30 min • Crepitus	• Knee pain At least three of the following: • Age > 50 years • Stiffness < 30 min • Crepitus • Bony tenderness • Bony enlargement • No palpable warmth

Proposed classification criteria for early-stage knee OA

Using MRI or arthroscopic findings	Without MRI data

• Knee pain: at least two episodes of pain for >10 days in the past year
• Standard radiography: KL grade 0 or 1 or 2 (osteophytes only)
At least one of the following:
• Arthroscopy: ICRS grade I–IV in at least two compartments or grade II–IV in one compartment with surrounding softening and swelling
• MRI: at least two of the following:
  • At least grade 2 BLOKS for size of cartilage loss
  • At least grade 2 BLOKS for percentage full-thickness cartilage loss
  • Signs of meniscal degeneration
  • At least grade 2 BLOKS for size of bone marrow lesions
	• Patient-based questionnaires (KOOS): 2 out of the 4 KOOS subscales need to score 'positive' (≤85%)
	• Clinical examination: at least one of the following needs to be present:
	  • Joint line tenderness
	  • Crepitus
	• Radiography: KL grade 0–1 standing, weight bearing (at least two projections: posteroanterior fixed-flexion and skyline for patellofemoral OA)

Fig. 2 | **Comparison of newly proposed classification criteria for early-stage knee OA and the ACR classification criteria for knee OA.** Criteria for diagnosis and classification are related and could overlap in the features included, but diagnostic criteria focus on case finding and management of the individual patient in clinical practice whereas classification criteria aim to define disease with the goal of specifying homogeneous patient groups for studies of epidemiology, natural history and disease mechanisms and, importantly, for interventional studies. In contrast to diagnostic criteria, classification criteria typically aim to achieve high specificity and allow for lower sensitivity, and are thus less inclusive but more sharply defined. BLOKS, Boston Leeds Osteoarthritis Knee Score; ESR, erythrocyte sedimentation rate; ICRS, International Cartilage Repair Society; KL, Kellgren & Lawrence; KOOS, Knee Injury and Osteoarthritis Outcome Score; OA, osteoarthritis; RF, rheumatoid factor.

As yet, it is not possible to firmly establish the relative weight and/or ranking of OA risk factors, although overweight and obesity is reported to account for the single largest population-attributable risk[44,45]. More data are needed to construct a risk assessment tool such as those available for prevention and treatment of cardiovascular disease[46] and osteoporosis[47]. Risk assessment is also directly related to which outcomes are taken into account. For example, for reimbursement agencies, restoring function and returning to work might be the top priority, whereas from the patient's perspective, reducing pain and/or symptoms and maintaining function might be most important.

Although not intended for diagnosis, classification criteria for early-stage knee OA could also help with the identification of subjects at the early-stages of the disease, while detecting additional relevant risk factors could enable further stratification of early-stage knee OA patient subgroups with respect to specific phenotypes and management. For instance, OA in a relatively young (35–45 years old) man with a history of knee trauma and preceding surgical intervention, such as partial meniscectomy, is probably mechanistically distinct from that in a woman who is postmenopausal and has overweight aged 55–65 years old with a strong family history of bilateral total knee replacement. Therefore, these patients might be expected to respond differently to different treatments. For clinical trials aiming to slow or stop disease progression, criteria that enrich for those at highest risk of disease progression within the early-stage knee OA population are of importance. In that context, specialist groups are working to identify molecular and imaging biomarkers that could further refine the aforementioned classification criteria[48,49]. Such patient stratification could be a way of getting new drugs to market quickly as the intervention would reach the right patient in the right 'window' of the disease process, thereby increasing the chance of developing cost-effective treatments — an important requirement in the real world of limited health-care resources. These concepts have been proposed for other chronic diseases such as Parkinson disease[50].

Fig. 3 | **Development of validated classification criteria for rheumatic diseases.** The flow chart depicts the major steps in the ongoing process of developing and validating classification criteria for early-stage knee osteoarthritis. Such criteria sets have been developed for rheumatic diseases including rheumatoid arthritis[121,123,124], systemic sclerosis[125–128], systemic lupus erythematosus[129,130], gout[131–133], IgG4-related disease[134] and Sjögren syndrome[135].

Biomarkers in early-stage knee OA

In order to assess early-stage pathogenic events and develop appropriate biomarkers that reflect the early-stage processes in knee OA, it is critical to properly define early-stage disease and develop validated classification criteria widely accepted by the global community, so that all studies reflect a similar patient population and are comparable. Once early-stage disease has been well-defined, a better understanding of the cellular and molecular basis of the early disease processes in OA is crucial as this knowledge could enable us to identify the transition from a 'merely painful knee' to a knee with symptomatic early-stage OA disease.

No single unifying cellular or molecular cascade has been associated with the early disease processes, probably owing to OA typically being heterogeneous, also in its early stages[51]. The cellular and molecular events associated with early disease are diverse and are dependent on a number of factors, including those that initiate the disease, such as a single major trauma or a series of repetitive micro-traumata[52], inflammation[53] or infection. The initial disease processes act in a specific context that is influenced by patient characteristics such as unfavourable biomechanics[54], advanced age, genetic background and/or sex, and are modulated by comorbidities such as obesity and metabolic syndrome[55] (FIG. 4). Some of the molecular processes are catabolic and contribute to disease progression, whereas others are anabolic, which are of particular relevance in early disease and represent attempted or failed repair. As for the mechanisms driving early disease, a distinction has to be made between different patient populations and how their disease is initiated, as mentioned above. As an example, in post-traumatic knee OA events such as cell apoptosis, necrosis and premature senescence together with biomechanical overload can dominate[56–60]. In other patients with early-stage knee OA, angiogenic and metabolic changes with inflammation and involvement of the innate immune system could be of more relevance, and could be modified by age, sex and genetic background[61]. As another example, evidence is mounting that metabolic changes promoted by poor diet, obesity, ageing and comorbidities such as type 2 diabetes mellitus drive disease processes in chronic low-grade inflammatory diseases such as OA[55].

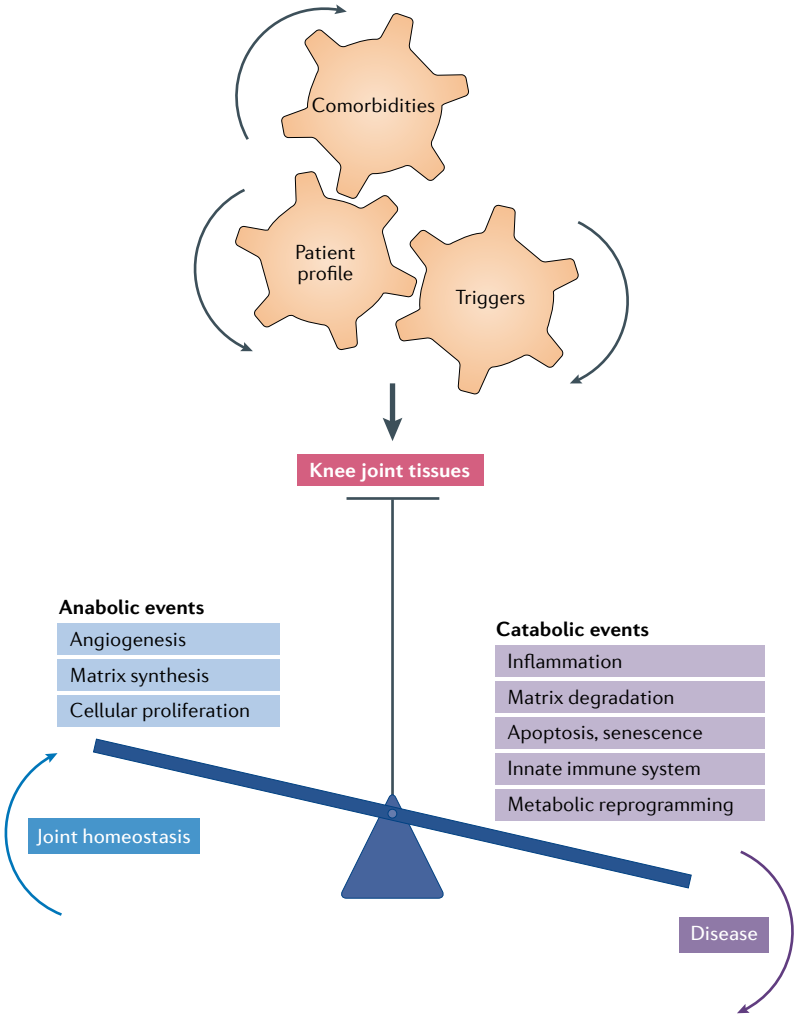

Fig. 4 | **Cellular and molecular events of early OA.** Triggering factors (for example, major trauma, repetitive minor trauma, inflammation, infection or altered biomechanics), patient profile (including characteristics such as sex, genetics, age, anatomy and history) and comorbidities (such as metabolic syndrome, obesity or diabetes mellitus) interact to affect all joint tissues in the knee, including the osteochondral unit, synovium, meniscus, infrapatellar pad and ligaments, resulting in activation of specific molecular cascades that lead to catabolic and anabolic events. Catabolic events include inflammation induced by several mediators such as damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs); matrix degradation by matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5); activation of the innate immune system mediated by macrophages, Toll-like receptors and complement activation; metabolic reprogramming; and senescence. Enhanced anabolism is mediated through the activation of mostly developmental pathways, such as transforming growth factor β (TGFβ)–bone morphogenetic protein (BMP) and fibroblast growth factor 2 (FGF2) signalling. When anabolic events are successful, joint homeostasis is restored; when catabolism is overwhelming, the disease process becomes chronic and probably irreversible. OA, osteoarthritis.

Further stratification of early-stage knee OA might require the use of biomarkers based on aetiopathogenic insights. Besides contributing to defining the stage of the disease process, biomarkers can serve additional purposes, such as identifying patients at increased risk of progression from an early-stage disease process to established OA[62].

Biomarkers can be divided into molecular and imaging biomarkers[63], the latter mainly related to MRI. A detailed discussion of potential biomarkers is outside the scope of this Review, but a brief view is provided below; the reader is referred to several reviews published in the past few years for further discussion of this topic[48,49,64,65].

Molecular biomarkers are typically measured in body fluids such as serum, urine or synovial fluid, and can reflect systemic processes or local, joint-specific processes. For early-stage knee OA, we would anticipate that synovial fluid is probably the body fluid most reflective of the local processes in the joint, as any systemic effect of the OA disease process might not yet be detectable. Considerable efforts to identify molecular biomarkers by the 'candidate protein' approach have had limited success in identifying and qualifying biomarkers that are useful in the clinical or trial setting.

Renewed efforts using a genome–proteome–metabolome-wide-association approach are ongoing[66]. Both approaches are handicapped by our limited understanding of how best to identify OA subpopulations with regard to genotype, phenotype, risk factors and more. Following the discovery of a promising biomarker comes assay validation and then qualification to confirm the clinical utility of the biomarker using retrospective then prospective human cohorts. One of the major challenges with the use of molecular biomarkers is to ensure their robustness and reproducibility at a relevant scale in different populations, different settings and different laboratories. Unfortunately, many candidate biomarkers seem not to provide much additional value beyond that of the known risk factors[67,68].

Imaging biomarkers include features of MRI and ultrasonography for diagnosing or classifying early-stage OA[69].

Box 2 | **Core outcomes for early-stage knee OA**

Currently available clinically relevant outcomes
- Patient-reported outcomes (e.g. KOOS, ICOAP questionnaire)
- Clinical features (e.g. joint line tenderness, crepitus, effusion)
- Lifestyle-related features (e.g. BMI)
- Structural features (e.g. knee radiograph features)

Potential clinically relevant outcomes
- Physical activity monitored using wearable devices

Outcomes of use for research purposes
- Imaging biomarkers (e.g. ultrasonography and MRI biomarkers)
- Molecular biomarkers

BMI, body mass index; KOOS, Knee Injury and Osteoarthritis Outcome Score; ICOAP, Intermittent and Constant Osteoarthritis Pain; OA, osteoarthritis.

Ultrasonography lacks clear findings in early-stage OA but has some potential to non-invasively detect aspects of the joint tissues that can indicate active disease, such as the presence of effusion or synovitis. However, results of a 2017 study indicate that examination by ultrasonography is no more sensitive than clinical examination by appropriately trained clinicians[70]. Meniscal pathology can be partially detected, specifically meniscal extrusion, but meniscal abnormalities can be much better defined and detected on MRI.

MRI has great potential with respect to imaging biomarkers for early-stage knee OA and has been discussed in detail elsewhere[71]. However, MRI still has limitations, including a lack of understanding of what particular MRI findings represent at the tissue, cellular and molecular level, for instance, the processes underlying subchondral bone abnormalities[72]. Another hurdle is the great sensitivity of MRI and the difficulty of distinguishing pathological and clinically relevant findings from what can be regarded as normal joint tissue remodelling and ageing[73,74]. Suffice it to say, this technology could help to detect the effect of an intervention at the tissue level, but the clinical relevance, or how it affects the course of the disease process and its progression, is still to be investigated in detail and agreed upon.

Outcomes of early-stage knee OA

When defining a patient population with early-stage OA, and thus intrinsically creating a new class of patients, it is essential to define appropriate outcomes for these patients. A 2019 review presented and discussed an extensive list of potentially relevant outcomes[65]. With respect to early-stage OA, an ambitious outcome would be full 'remission' or 'minimal disease activity', outcomes also used for other arthritic diseases such as RA[75]. This state can be defined in several ways, for instance as restoration of joint homeostasis at the molecular level with the disappearance of abnormalities detected by imaging with sensitive tools such as MRI and, from the patient perspective, as the absence of pain, discomfort, symptoms or signs and restoration of function. That outcome is the ideal, of course, but by creating a category of patients with early disease, we should aim for that. Alternatively, as has already been done for inflammatory arthritis[76], we could define 'very low disease activity' for patients with OA, potentially measured by use of outcome tools such as the Knee Injury and Osteoarthritis Outcome Score (KOOS) or the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Objective assessment of joint function also seems appropriate and methods to do so have been presented[65]. Also of interest are dynamic functional assessments using wearable monitors, as these devices can document real-life performance in activities of daily living as well as in professional and recreational life[65,77].

For interventional trials, it is important to define the changes required to claim that a drug has disease-modifying activity and could thus be considered a disease-modifying osteoarthritis drug (DMOAD)[78,79]. In early-stage knee OA, radiographic findings on plain radiographs are minimal, and demonstrating disease progression in this regard requires large numbers of patients over long time periods, typically 3–4 years or more. Other imaging technologies such as MRI and refinements thereof could be valuable in this context[71]. Adaptive trial designs that enable patient subgroup enrichment can be considered[80]. As an example, subgroups of patients with early-stage knee OA who are at a high risk of structural progression can be selected on the basis of having Kellgren & Lawrence grade I on radiographs, as these patients will have a much higher risk of developing Kellgren & Lawrence grade II with osteophytes and joint space narrowing (a sign of established OA) than patients who have no radiological abnormalities[39,81] Using an adaptive trial design, initial treatment of different subgroups of patients with early-stage knee OA can be followed by interim analysis, after which only subgroups that benefit from the treatment are randomly allocated to receive further treatment or placebo and the other groups are dropped or re-assigned to alternative trial arms. Investigations using machine learning and MRI-detected bone-shape changes found that this measure is far more sensitive to change than plain radiograph scores[82], suggesting that novel DMOAD trial designs could become feasible with the use of more advanced imaging strategies. Demonstration that an intervention has positive effects on symptoms and functional outcomes superior to those of placebo for the entire study population, in combination with showing a structural benefit for a high-risk subgroup, could justify the highly sought after 'DMOAD' label. Stakeholders in the field need to reach consensus and set the standards for outcomes of early-stage knee OA (BOX 2).

Management strategies

Knee pain is common among those aged over 50 years, and a very variable proportion of these individuals are assigned a diagnosis of knee OA when seeking health care or being examined in population-based studies[83,84]. On continued follow-up, one-quarter to one-third of those originally assigned as having 'only' knee pain received a diagnosis of knee OA[84,85]. There is thus a clear need to reduce variation in diagnosing both knee OA

and early-stage knee OA, and to understand the consequences for the patient who has symptoms but does not receive a diagnosis, in terms of missed early opportunities for best management of OA. For the symptomatic patient, a missed diagnosis represents a missed opportunity.

In the initial attention to early-stage disease, the focus should be on reducing the burden of disease through identifying in the routine clinical setting persons with knee symptoms and increased likelihood of early-stage OA, and to treat them with the tools available now[28]. If implemented and applied to the right patient, best practice disease management can reduce the burden of the disease, and might even affect disease progression[15,27,28,86].

No evidence has been presented on which to base best management specifically for patients with symptomatic early-stage OA. Logic suggests that the preferred treatment should be the first-line management approach recommended for almost any patient with knee pain and OA: a structured programme of education, information and exercise, and weight loss when needed[87,88]. Published results show that this management approach is beneficial both for those with mild symptoms and for those with more severe symptoms, and is associated with reduced pain and consumption of analgesics, less sick leave, better quality of life and function and increased physical activity[89,90]. Although evidence of the effects of exercise and lifestyle modification on OA-related joint structural integrity remains limited[91,92], the reductions in pain, consumption of analgesics and sick leave at the population level has the potential to importantly reduce the OA burden[15,17,86,90–92]. The societal advantage gained might be as important as that from decreasing the need for joint replacement in more severe stages of OA.

Exercise might potentially affect the disease process, including in early-stage OA[15,86], but limited implementation and patient compliance remain major hurdles[93–96]. Initiatives have been developed to overcome some of these hurdles, such as the Better Management of Patients with OA programme[91] and the GLA:D® programme for people with knee and hip pain[92]. The GLA:D® project is an outstanding example of how to successfully implement evidence-based clinical guidelines in primary health-care practice. Its underlying principles focus on patient education, patient empowerment, exercises and self-management and routine documentation of outcomes. This project now serves as a template for establishing similar initiatives in other countries including Australia, Canada, China, New Zealand and Switzerland[17]. To expand the reach, implementation and cost-effectiveness of these first-line management principles for OA, digital tools have been introduced[90]. A low-cost, low-tech, low-risk early intervention strategy such as this requires the identification of patients with early-stage disease and proper patient stratification. Tools are available to the caregivers to do so, but so far are poorly implemented[26,93,95–102]. From the patient's perspective, quality of life remains affected and more attention by caregivers is required[103]. Establishing a proactive case-finding strategy for symptomatic early-stage knee OA, identifying those patients at risk of disease progression and designing a care trajectory supported within primary care represents a major opportunity to reduce the globally heavy disease burden of OA. The unfortunate defeatist perception exists that OA is a disease for which nothing can be done other than alleviating the symptoms. This bias arises from our often drug-driven health-care system and the fact that no DMOADs are yet available. The past failure to develop DMOADs is due to many factors, including the complexities of the disease process and disease stages, the heterogeneity of the patient population and that translational animal models seem to incompletely predict the outcome of treatments in humans. A better understanding of the cellular and molecular processes of the distinct disease stages in the OA patient, patient stratification based on scientific insights and the use of model systems that reliably predict outcomes in human patients could help us move forwards in the quest to affect disease development and delay progression (BOX 3). The design of interventional trials in early-stage disease will be no less challenging than in previous (failed) trials with disease-modifying ambition[78,79]. The continued development, validation and qualification of biomarkers will be critical to identify and monitor early-stage OA in clinical trials.

Stratification of the OA population to identify persons with early-stage symptomatic disease combined with a high risk of disease progression could present an attractive target to influence disease development before the advent of chronic pain, secondary processes and severe joint destruction. Drugs are in development that have articular

Box 3 | **Research gaps and proposed agenda**

Existing gap
To date, no validated diagnostic criteria are available for early-stage knee osteoarthritis (OA).

Proposed research agenda

- Develop diagnostic criteria or validated tools for the diagnosis of early-stage knee OA with the aim to properly manage the individual patient, in particular in primary care.

Existing gap
The definition of symptomatic early-stage knee OA has been insufficiently explored and is yet to be agreed upon. The 1986 classification criteria by the ACR are the most frequently used, but patients fulfilling the clinical and radiological ACR criteria for knee OA will already have significant joint damage.

Proposed research agenda

- Develop and validate classification criteria for early-stage OA to define homogeneous patient groups for clinical studies.

Existing gap
By defining early-stage knee OA, a new class of patients are being created; it is essential to define the most appropriate outcomes for these patients.

Proposed research agenda

- Identify and validate outcome measures associated with early-stage OA.

Existing gap
Insufficient understanding of the early-stage disease processes.

Proposed research agenda

- Investigate underlying cellular and molecular mechanisms driving the development of early-stage OA and OA progression.
- Identify potential biomarkers (wet or dry) of early-stage disease and associated with disease progression.

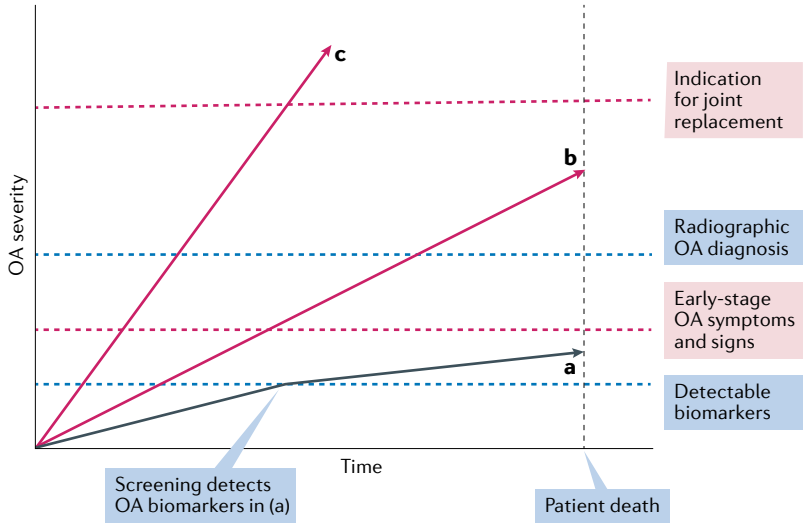

Fig. 5 | **The potential for overdiagnosis and overtreatment of OA.** In this schematic, the vertical axis represents 'global' osteoarthritis (OA) disease severity and the horizontal axis represents time (and patient age). The two horizontal dashed red lines represent the degree of severity (disease stage) at which symptoms begin, or become so severe that joint replacement surgery may be indicated. The lower horizontal dashed blue line represents the disease stage at which biomarkers (MRI or molecular biomarkers) might first detect an increased risk for disease, and the upper horizontal dashed blue line when a diagnosis of radiographic OA can be made using plain radiography. The vertical dashed line represents the time at which death could take place. The arrows labelled a, b and c represent OA disease trajectories with different rates of disease development. In this schematic, individuals along trajectory a, whose disease progresses slowly or not at all, would be over-diagnosed by biomarkers; they would never experience symptoms and any treatment would be overtreatment. Individuals along trajectories b and c could benefit from both having their symptoms recognized as early-stage OA and receiving beneficial symptomatic treatment before the point at which a radiographic diagnosis would be made. Future research could show whether OA treatment at this early stage can modify the development of further symptoms and structural joint changes for individuals along trajectories b or c, thus providing OA disease modification.

cartilage or other joint tissues as primary targets[104–106]. Published results at the time of writing this Review suggest that some of the novel compounds in development might influence joint structure, but this effect has not yet been shown to translate into a proven effect on patient symptoms. A comprehensive review of new drug studies is outside of the scope of this review but has been discussed elsewhere[107].

An alternative strategy to prevent disease progression in early-stage OA involves regenerative medicine. There is interest in the intra-articular injection of stem cells for the treatment of knee OA, and some interesting results have been described but there is a clear lack of pivotal, high-quality clinical studies[108,109]. Other approaches involve the use of gene therapy or a combination of cell therapy and gene therapy by intra-articular injection into the knee joint of engineered cells that carry viral and non-viral vectors expressing growth factors[110–113]. Tissue engineering solutions are attracting attention; deep osteochondral lesions are a clear risk factor for developing OA, and some promising approaches to osteochondral repair have been reported[114]. However, better evidence is needed from more rigorous RCTs, preferably using placebo surgery controls[115].

For the patient with knee pain suggestive of early-stage OA seeking primary care, of central importance is clear communication with the patient about what is causing the symptoms, what to expect and what can be done and, importantly, what can prevent progression to chronic pain and sensitization[6,116].

Overdiagnosis, overtreatment and ethics

By introducing the concept of symptomatic early-stage OA for patients with knee symptoms and signs — akin to 'proper, established' OA but without most of the classical radiographic changes — the current disease definition for OA is expanded. With redefining the disease comes the risk of overdiagnosis and overtreatment, and of being seen as acting in self-interest and the interests of industry[117] (FIG. 5). Concern might also be raised about the creation of a new disease that lacks an effective treatment. However, as discussed in this Review and other efforts for early case-finding, the focus should be on people who seek health care because of knee symptoms and who, in a clinical consultation, can be diagnosed with high likelihood as having early-stage knee OA. The majority of these patients can be successfully managed with existing first-line treatments including education, structured exercise programmes, weight loss (when needed) and add-on pain relief, for example with intermittent, low-dose NSAID, when indicated[87,92,118].

Continued research on genetics, biomarkers, and cell and tissue processes active in the earliest stages of OA could lead to the identification of individuals at-risk of OA before they develop any symptoms. However, identifying and possibly treating those at risk but without symptoms raises broad concerns of overdiagnosis, overtreatment, the number needed to treat to prevent one case of symptomatic disease, and cost (FIG. 5).

Conclusions

Management of the high burden of knee OA remains a major public health challenge, with no disease-modifying drug treatments available. Efforts in the field should focus on future opportunities, drawing on past experience and on strategies that have already been successfully pursued and implemented for the management of other chronic diseases incorporating new and innovative technologies. This entails basic research to identify new mechanisms of disease as potential treatment targets. Noting that past bench-to-bedside translational research with the aim of bringing DMOADs to the market has met with limited success, an increased focus on the human clinical disease and its subtypes and adapting management approaches and trial designs seem paramount.

OA is a heterogeneous disease and in a minority of patients leads to joint failure that requires joint replacement, a procedure still considered the most important 'breakthrough' treatment in OA. The disease heterogeneity slows progress in research and treatment. Compounding this problem is the unpredictable course of the disease, elegantly described as a state of inertia[21], and the challenge of identifying factors that trigger the transition from stable disease to disease progression.

In view of these challenges, disease stratification seems to be a prime goal[119]. Among stratification strategies,

detecting and defining early-stage disease could present a 'window of opportunity' as already illustrated with notable effects in patients with RA[120,121].

As well as ongoing research to diagnose early-stage disease in primary care, efforts to develop and validate classification criteria for symptomatic early-stage knee OA[38] are summarized in this Review. Such criteria will serve to define more homogeneous patient populations for clinical studies, and will help us to better understand the mechanisms of disease and provide a sound scientific basis for the development of new disease-modifying therapies. The corresponding clinical diagnosis helps to identify the patient in primary care, thus providing them with access to appropriate, proactive disease management. However, caution is also advised, as overdiagnosis and overtreatment is a risk[122], in particular when new drugs or other interventional or surgical therapies enter the market.

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Author contributions
A.Ma., L.S.L., A.Mo. and F.P.L. researched data for the article. All of the authors made substantial contributions to discussions of the content, writing the article and reviewing and/or editing of the manuscript before submission.

Competing interests
L.S.L declares that he serves as member of an AstraZeneca Data and Safety Monitoring Board, has acted as a consultant for the planning of phase II and III clinical trials for Paradigm Biopharmaceuticals Australia & Ireland, is a member of an expert group for assessing research proposals on musculoskeletal pain for Pfizer/Lilly USA, acts as a consultant for the scientific evaluation and publication of outcomes of an eHealth app for hip and knee osteoarthritis (Arthro Therapeutics Sweden), and was a member of an expert group for National Guidelines Osteoarthritis Care 2020 for the National Board of Health and Welfare Sweden. A.Mo. declares that he has acted as a consultant for Abbvie, AlphaSights, Artialis SA, Atheneum Partners, Flexion Therapeutics, Galapagos, GSK Consumer Healthcare, Guidepoint Global, Image Analysis Group, Kolon TissueGene, Novartis, Pacira Biosciences Inc, Pfizer Consumer Healthcare, Servier, Sterifarma, and Science Branding Communications; has received research funding from the European Commission (FP7, IMI, Marie Skłodowska-Curie, ES Struktūrinės Paramos), Versus Arthritis (Arthritis Research UK) and initiated research contracts with Merck KGaA and Kolon TissueGene; he has received speaker payments from Achē Laboratórios Farmacêuticos, the American College of Rheumatology, Bioiberica SA, the Korean Society for Osteoarthritis and Cartilage Repair, Laboratoires Expanscience, the Spanish Society of Rheumatology, Sanofi, the Heilongjiang Rheumatology Association and the Zhujiang Hospital of Southern Medical University; he currently serves as President of the Osteoarthritis Research Society International (OARSI), a member of the Advisory Board of Research Square and he is a member of the Scientific Advisory Board of Kolon TissueGene; however, none of the organizations listed above was involved in the conceptualization, design, data collection, analysis, decision to publish, or preparation of this manuscript. M.E. declares that he has received an honorarium for serving on a 1-day advisery board for Pfizer; he also serves as an Executive Board Member (Treasurer) for OARSI. A.Ma. and F.P.L. declare no competing interests.

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Management of gout in chronic kidney disease: a G-CAN Consensus Statement on the research priorities

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Abstract | Gout and chronic kidney disease (CKD) frequently coexist, but quality evidence to guide gout management in people with CKD is lacking. Use of urate-lowering therapy (ULT) in the context of advanced CKD varies greatly, and professional bodies have issued conflicting recommendations regarding the treatment of gout in people with concomitant CKD. As a result, confusion exists among medical professionals about the appropriate management of people with gout and CKD. This Consensus Statement from the Gout, Hyperuricemia and Crystal-Associated Disease Network (G-CAN) discusses the evidence and/or lack thereof for the management of gout in people with CKD and identifies key areas for research to address the challenges faced in the management of gout and CKD. These discussions, which address areas for research both in general as well as related to specific medications used to treat gout flares or as ULT, are supported by separately published G-CAN systematic literature reviews. This Consensus Statement is not intended as a guideline for the management of gout in CKD; rather, it analyses the available literature on the safety and efficacy of drugs used in gout management to identify important gaps in knowledge and associated areas for research.

Gout is the most common form of inflammatory arthritis in men over the age of 40 years. The prevalence of gout has been reported to range from 0.1% to 10%[1,2]. The prevalence of gout is generally higher in men (5.2%) than in women (2.7%) according to the most recent data from the US National Health and Nutrition Examination Survey (NHANES)[2]. Kidney impairment is common in people with gout: as many as ~70% of adults with gout have an estimated glomerular filtration rate (eGFR) of <60 ml/min/1.73 m^2, and 20–24% have an eGFR of <30 ml/min/1.73 m^2 (TABLE 1)[3,4]. Reduced GFR is a risk factor for the early development of tophi, suggesting that renal function might modulate the severity of gout[5,6]. The reverse is also true, as the prevalence of gout is higher in people with chronic kidney disease (CKD): 24% of adults with an eGFR of <60 ml/min/1.73 m^2 have gout compared with 2.9% of adults with an eGFR of >90 ml/min/1.73 m^2 (REF.[7]). The prevalence of gout is higher in men with CKD than in women with CKD[7]. Hyperuricaemia (defined as a serum urate level of >6.8 mg/dl in men and >6.0 mg/dl in women) is also common in the context of advanced CKD, with a prevalence of 64% in people with stage 3 CKD and 50% in those with stage 4 or 5 CKD[7].

Monosodium urate (MSU) crystals, which form in the presence of hyperuricaemia, cause gout flares in large part by activating monocytes and macrophages, with resultant NLRP3 inflammasome-mediated IL-1β release, many other local and systemic high-grade pro-inflammatory responses, and articular neutrophil influx and activation[8]. Hyperuricaemia is an amplifying factor for MSU crystal-induced inflammation, priming certain monocyte–macrophage pro-inflammatory responses in humans and mice[9]. In this context, evidence from multiple studies supports a low-grade inflammatory phenotype in CKD, which is linked with increased serum concentrations of C-reactive protein, many pro-inflammatory cytokines, prostaglandins and leukotrienes, and with intestinal dysbiosis[10]. The cross-talk between the systemic inflammatory states of CKD and gout, as well as common comorbidities of both diseases that are modulated by low-grade inflammation, is likely to have clinical consequences. The same is the case for the inflammation-modulating effects in CKD and gout of obesity, type 2 diabetes mellitus and renin–angiotensin system activation in the pathophysiology of hypertension, and for the use of statins (which modulate trained immunity in monocytes and macrophages)

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Table 1 | **Stages of CKD**

Stage	Description	eGFR (ml/min/1.73 m^2)
1	Normal or high GFR	≥90
2	Mild CKD	60–89
3A	Mild to moderate CKD	45–59
3B	Moderate to severe CKD	30–44
4	Severe CKD	15–29
5	End-stage CKD	<15

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

and metformin and ω-3 fatty acids (which inhibit MSU crystal-induced inflammation)[11–13]. Additionally, ultrasonography studies have demonstrated renal medullary echogenicity in patients with severe gout[14], potentially attributable to MSU crystalluria and the development of tophi within the renal medulla[15]. MSU crystal-driven inflammation might thus directly affect renal structure and function in patients with gout.

There is a paucity of data on the natural history of gout, which has several stages of development (FIG. 1). The Gout, Hyperuricemia and Crystal-Associated Disease Network (G-CAN) has previously endorsed a definition of the disease state 'gout' that requires current or prior clinically evident symptoms or signs resulting from MSU crystal deposition[16]. Gout is generally considered a chronic disease, with episodic highly symptomatic flares. Poorly controlled gout can have a substantial impact on an affected individual and the individual's family. Inadequately treated gout leads to recurrent gout flares, the formation of tophi (which contain aggregated masses of MSU crystals in joints and certain soft tissues), chronic gouty arthritis and joint erosion. Ulceration and infection associated with tophi occurs frequently, and surgical interventions for these sequelae have a high rate of complications[17]. Substantial time off work, poor health-related quality of life and disability are common in those with poorly controlled gout[18–20]. Gout is associated with frequent hospital admissions, particularly in patients with hyperuricaemia and inadequate allopurinol use and/or dose[21,22]. However, not all individuals with gout develop severe disease, and whether everyone diagnosed with gout requires long-term urate-lowering therapy (ULT) has been questioned[23].

Care for people with gout and CKD presents important challenges. For instance, the clinical presentation of gout in this high-risk, comorbid population is variable, with a higher frequency of atypical presentations than in those without CKD[24]. However, quality evidence to guide the management of gout in people with CKD is lacking, owing at least in part to the exclusion of people with CKD from trials of gout therapies, failure to report results stratified by renal function and inconsistencies in the outcome measures used and reported[25,26]. The resultant knowledge gaps have contributed to concerns regarding gout treatment efficacy and safety, some of which are legitimate and others questionable[27,28]. The use of ULT in the context of advanced CKD varies greatly among rheumatologists, nephrologists and generalists[29], and professional bodies have issued conflicting recommendations regarding the treatment of gout in people with concomitant CKD[27,28]. These inconsistencies frequently result in confusion and, consequently, suboptimal gout management with failure to achieve recommended target urate levels[30,31]. Moreover, pharmacological options for treating gout flares and lowering urate concentrations are often restricted by physicians, other health-care professionals (such as pharmacists) and patients who have appropriate concerns and/or misconceptions about drug toxicity or the need to adjust medication doses. As a result, outcomes in people with gout and CKD are commonly poor[32] (FIG. 2).

This Consensus Statement from G-CAN aims to discuss the evidence (or lack of) for the management of gout in people with CKD and to identify key research questions that will address challenges faced in managing gout and CKD. We focus on CKD stages 3–5 (TABLE 1), for which there remain the most debate and concern about appropriate therapy for coexistent gout. This Consensus Statement is not intended as a guideline for the management of gout in CKD; rather, it analyses the available literature on the safety and efficacy of drugs used in gout management to identify important gaps in knowledge and associated areas for research. We do not analyse the role of ULT in people with asymptomatic hyperuricaemia and CKD, as treatment of asymptomatic hyperuricaemia with ULT is not currently recommended or approved in most areas of the world[28]. We do not consider non-pharmacological interventions for gout in CKD, such as dietary interventions or weight loss. Finally, this Consensus Statement does not discuss the particularly complex management and frequently severe clinical course of patients with hyperuricaemia and gout after transplantation of a kidney or other major organ.

Methods

G-CAN comprises a group of individuals with expertise and an interest in gout and other crystal deposition-associated arthritic diseases as well as hyperuricaemia. G-CAN was formed to foster collaboration and research in these disease areas. During the G-CAN symposium in 2016, management of gout in the context of CKD was identified as an area of high interest, with critical gaps

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in knowledge about the efficacy and safety of drugs used for management of gout flares, gout flare prophylaxis and long-term ULT. G-CAN therefore endorsed systematic reviews of the evidence for the use of medications to manage gout flares as well as ULTs. These reviews[33,34] form the basis for this Consensus Statement and provide the evidence to support key areas for research in each section. People with gout were not directly involved in this Consensus Statement. The work was led by C.L.H., L.K.S. and A.L.G. in collaboration with the G-CAN Directors (R.T. and H.K.C.) and Board. H.F., H.L.P., M.F. and A.B.V.-S. were selected as fellows with an interest in gout to assist with the work. The G-CAN Board, which includes the authors R.T., D.B.M. and H.K.C., approved the final manuscript.

Literature review methods

As mentioned above, systematic literature reviews on the safety and efficacy of pharmacological therapies for gout in people with CKD were conducted to identify knowledge gaps and research priorities. The systematic literature review of ULTs[33] was led by L.K.S. in conjunction with the fellows H.F. and A.B.V.-S., and the systematic literature review of therapies for gout flares and prophylaxis[34] was led by A.L.G. and C.L.H. in conjunction with the fellows H.L.P. and M.F. We focused on medications currently approved for or commonly used for gout, including those used in the management of gout flares and flare prophylaxis when starting ULT (colchicine, NSAIDs, corticosteroids and IL-1 inhibitors) and those used as ULT (allopurinol, febuxostat, probenecid, benzbromarone, lesinurad and pegloticase).

Briefly, the search captured articles in PubMed, The Cochrane Library and EMBASE published from 1 January 1959 to 31 January 2018. Studies were included if they enrolled people with gout, an eGFR of <60 ml/min/1.73 m^2 or a creatinine clearance of <60 ml/min and exposure to the medications of interest. Studies were excluded if they were not available in English, primarily included people without gout, did not report information on eGFR or creatinine clearance, were letters, opinion articles or review articles, or were animal studies, basic science or purely laboratory-based studies. For assessing efficacy of a ULT of interest, the main outcome was the proportion of study participants who achieved the target serum urate concentration of <6.0 mg/dl, stratified by renal function. Two reviewers independently screened the full texts to identify eligible studies for data extraction; any discrepancy identified during the screening phase was discussed by the two reviewers to reach consensus. Detailed methods and results of the literature reviews have been previously reported[33,34].

Identification of research areas

Data from the literature were thematically analysed by the leaders and fellows of each literature review team to identify general issues with the currently available data with regard to gout and gout studies in people with concomitant CKD as well as specific issues with individual medications.

The research areas and general requirements for studies identified were agreed on by the leaders and fellows of each literature review team and then circulated to all authors of this Consensus Statement. Agreement was reached by consensus of all authors via e-mail, and final approval was granted by the G-CAN Board. No effort was made to prioritize the research areas.

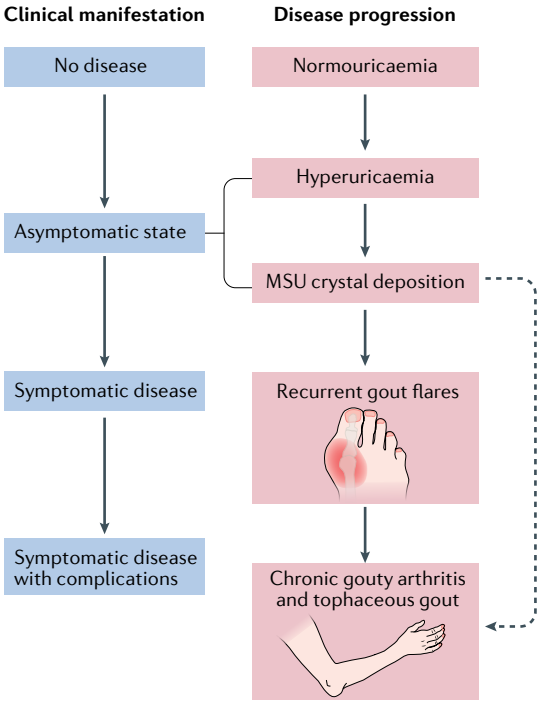

Fig. 1 | **Stages of gout.** Gout progresses through several classic disease stages and corresponding clinical manifestations. In some cases, advanced disease stages and complications can appear prematurely, without earlier disease stages or clinical manifestations being apparent (for example, tophaceous gout without prior gout flares), although this pattern is uncommon. MSU, monosodium urate. Adapted from REF.[80], Springer Nature Limited.

Issues with studies of gout and CKD

Two important areas of concern were identified with respect to studies of gout and CKD: issues related to the natural history of gout in people with CKD and generic study-related issues.

General research areas

Several issues related to the natural history of gout in people with CKD were identified as general areas for research (summarized in BOX 1). As mentioned above, data on the natural history of gout (FIG. 1) are scarce but the application of modern imaging techniques, such as dual-energy CT, has led to the recognition of a pre-symptomatic phase of gout in some individuals in whom MSU crystal deposition occurs in joints, soft tissues and vascular sites before the first gout flare (asymptomatic MSU crystal deposition)[35,36]. Whether this pre-symptomatic phase is more common in people with CKD in whom the inflammatory response to crystals might be suppressed remains unknown, as does the timing of progression from asymptomatic to symptomatic gout in people with CKD.

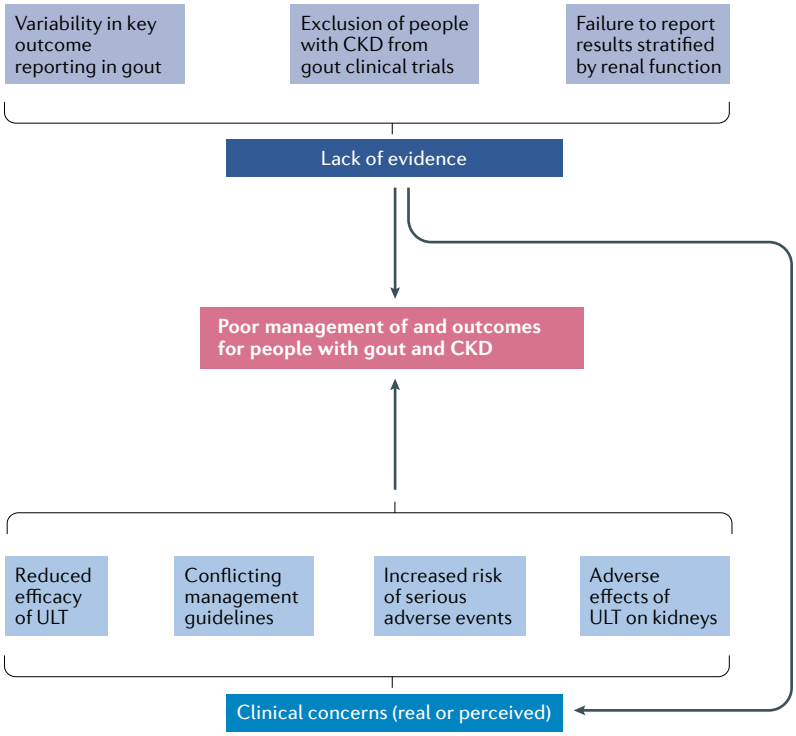

Fig. 2 | **Reasons for poor outcomes in people with CKD and gout.** This schematic provides a conceptual framework to explain poor management and outcomes in people with gout and chronic kidney disease (CKD). No good-quality evidence is available to guide treatment decisions because clinical trials have traditionally excluded participants with advanced CKD or, when these participants are enrolled, the trials have failed to report outcomes stratified by renal function. In addition, comparing and contrasting studies is difficult because of variability in reporting of outcomes for both urate-lowering therapy (ULT) and gout flare studies (this problem is not unique to gout in the context of CKD). In addition, many health-care team members involved in the management of people with gout and CKD have valid concerns about confusing guidance (conflicting recommendations among treatment guidelines from prominent societies), and harbour misconceptions (including that ULTs will have an adverse effect on renal function (and the ULT dose should therefore be adjusted), the risk of adverse effects (mainly allopurinol hypersensitivity) and that ULT will have reduced efficacy). These factors lead to excessively conservative approaches to the treatment of gout in people with CKD, which often does not achieve optimal treatment outcomes.

Other questions to be addressed concern prediction of the disease course and the need for ULT in those with gout and CKD. The question of whether ULT is required could be an even more important issue in individuals potentially at increased risk of adverse events associated with therapy, such as those with CKD. An increased risk of mortality in people with gout, typically from cardiovascular and cerebrovascular disease, has been reported in association with the presence of subcutaneous tophi and high serum urate concentrations, but not with renal insufficiency[37]. Whether urate lowering in people with gout and CKD alters mortality was not considered as part of the literature reviews and this consensus statement.

Research is also needed to assess the effects of gout treatment on CKD and renal function. ULT in people with gout was reported in one study to lead to an improvement in renal function, although how much of this improvement related to a reduction in NSAID use and how much related specifically to the effects of urate lowering is not clear[38]. In addition, the results of this study were stratified only by baseline creatinine clearance of <80 ml/min versus ≥80 ml/min. A post hoc analysis of an allopurinol dose-escalation study suggested that changes in creatinine clearance did not differ when stratified by baseline renal function[39]. Stratifying the efficacy and safety of gout flare treatment and ULT by renal function should be emphasized in all gout studies because gout and CKD frequently coexist. An example of a trial designed to incorporate such stratification is the Veterans Affairs (VA) Stop GOUT study (NCT02579096), which is designed to evaluate the 'treat-to-target' dose escalation of allopurinol versus febuxostat in people with gout[40]. Although this study excluded individuals with stage 4 or 5 CKD, it included a pre-planned analysis of those with stage 3 CKD.

Study-related issues

The main generic study-related issues that contribute to a paucity of data on how to safely and effectively use medications for gout in people with CKD are discussed below, and G-CAN-proposed requirements for gout studies are summarized in BOX 2.

Study populations. In general, people with substantial kidney impairment have been excluded from clinical trials, greatly limiting the data on which to base decisions regarding how to best treat gout in this population. Most pharmaceutical trials of newer therapies, such as febuxostat, excluded individuals with an eGFR of <30 ml/min/1.73 m^2 (REFS[41,42]) and in some cases excluded those with an eGFR of <60 ml/min/1.73 m^2 (REF.[43]). Therefore, many data are derived from small case series, cohort studies and retrospective studies. Many studies with larger numbers of people with CKD included a mixture of those with asymptomatic hyperuricaemia and those with gout, but outcomes were not analysed separately.

Study reporting. Even when people with CKD were enrolled in clinical trials, few studies reported the outcomes stratified by kidney function. For example, only 12 of 96 articles reporting 91 original studies of allopurinol and 20 of 41 articles reporting 34 original studies with febuxostat in people with CKD and gout reported data according to renal function[33]. As a consequence, it is not possible to draw specific conclusions about the efficacy and safety of drugs used for gout in CKD. Furthermore, there are differences in the way outcome measures are reported. For example, serum urate was reported differently in these studies, as percentage reduction in serum urate, percentage of participants who achieved a target serum urate concentration, absolute reduction in serum urate and mean serum urate concentration at study end[25,44]. Likewise, gout flare was also inconsistently reported[25,26,34]. Such variability in study outcome reporting precludes meta-analysis, owing to difficulty comparing different studies.

Drugs used for flares and prophylaxis

For drugs used in the management of gout flares or flare prophylaxis when starting ULT (namely NSAIDs, colchicine, corticosteroids and IL-1 inhibitors), the efficacy outcomes of interest are resolution or prevention

Box 1 | **G-CAN-proposed general research priorities for people with gout and CKD**

- Is the natural history of gout, including the transition from asymptomatic hyperuricaemia to symptomatic gout, the same in people with and without chronic kidney disease (CKD)?
- In people with CKD and gout, can we predict who will develop tophaceous or erosive disease (and thus require more intensive urate-lowering therapy) and who will have a benign course?
- Does the treatment of gout and treat-to-target management of gout reduce progression of CKD and/or improve renal function?

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of gout flares, respectively. For this G-CAN Consensus Statement, safety outcomes for each drug were individualized. The specific issues identified with medications used for the management of gout flares and prophylaxis in people with CKD are discussed below, and the G-CAN-proposed research priorities are outlined in BOX 3.

Drugs used to manage gout flares

NSAIDs are generally contra-indicated in people with CKD, and the published literature in gout generally aimed to show the potential for renal-related adverse effects in people with CKD[34]. Although NSAIDs have well-established adverse effects, there has been some suggestion that these drugs could be used in those with end-stage renal disease for short periods of time[45].

There are a small number of randomized controlled trials (RCTs) of colchicine for treatment of gout flares, and none of these reported outcomes stratified by renal function[34]. Pharmacokinetic studies have indicated that clearance of colchicine is decreased in those with severe kidney impairment (eGFR 15–29 ml/min/1.73 m^2) and that there is minimal clearance of colchicine by haemodialysis[46]. Thus, the recommendations for use of colchicine in CKD remain largely empirical.

Corticosteroids have been generally accepted as the safest option in most people with gout flares and concomitant CKD. The newer IL-1 antagonist therapies, such as canakinumab and anakinra, are not widely available, and there are no RCTs investigating their use in people with gout and CKD for which results are presented according to kidney function. Data from case series and case reports are reassuring[34]. Essentially, these agents are widely used for a variety of conditions, but there is a relative paucity of data on their use in people with gout and CKD. In people without gout, the clearance of anakinra has been shown to be directly related to renal function and the drug is not cleared by dialysis[47]. It has therefore been suggested that in patients with an eGFR of <30 ml/min/1.73 m^2, anakinra should be administered every other day[47]. By comparison, canakinumab is a human IgG with a large molecular size (~150 kDa), so not much renal excretion is expected[48].

Box 2 | **G-CAN-proposed requirements for pharmacological gout studies**

- Whenever possible, people with all stages of chronic kidney disease (CKD) should be included in clinical trials of medications used in the management of gout.
- Pre-specified secondary analyses stratified by CKD stage should be reported for all clinical trials, cohort studies and observational studies.
- Pre-specified secondary analyses stratified by CKD stage for study participants with gout, independently of those with asymptomatic hyperuricaemia, should be reported for all clinical trials, cohort studies and observational studies.
- Standardized reporting of outcome measures, particularly serum urate concentrations and gout flares, are required to ensure that data can be compared across studies and meta-analyses can be undertaken.

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Drugs used for flare prophylaxis

Although the medications used for flare prophylaxis are the same as those used to treat flares, they are generally used at lower doses and for longer periods of time (months rather than days or weeks). A post hoc analysis of three phase III RCTs in people starting febuxostat who also received prophylaxis with colchicine included participants with an eGFR of <30 ml/min/1.73 m^2 but again the results were not stratified by renal function[49]. Long-term use of colchicine in the general population has been associated with bone marrow suppression and neuromyotoxicity[50], but whether these effects are increased in those with gout and CKD is unknown. Whereas short-term courses of glucocorticoids can be considered to have an acceptable risk–benefit profile, long-term use of glucocorticoids for flare prophylaxis can be associated with an increased risk of glucocorticoid-related adverse events, particularly infections, as seen in other rheumatic diseases[51,52]. This risk could be particularly concerning in a population that is already at high risk of severe infections, such as those with CKD. Whether the gout flare rate when starting ULT is the same in those with CKD as in those without, and whether prophylaxis is always required, are unknown, although a recent study of incremental use of febuxostat suggested that prophylaxis might not be required when a dose-escalation approach is used[53].

Urate-lowering therapies

The appropriate use of ULT in people with gout and CKD is one of the most controversial areas of gout management. For example, the latest guidelines issued by the ACR, EULAR and the British Society for Rheumatology differ in important areas, such as allopurinol dosing in people with CKD[27,28,54]. For drugs used as ULT (allopurinol, febuxostat, probenecid, benzbromarone, lesinurad and pegloticase), efficacy outcomes, as endorsed by most rheumatology professional society management guidelines, include achieving a target serum urate concentration (that is, <6 mg/dl or <5 mg/dl), resolution of tophi, reduction or elimination of gout flares over time, improvement in quality of life indicators, and radiographic changes[27,28,54]. For this G-CAN Consensus Statement, safety outcomes for each drug were individualized. The general issues identified as well as specific issues with individual drugs are discussed below and the G-CAN proposed research priorities are outlined in BOX 4.

Level of renal function precluding ULT

Because most large RCTs have excluded people with substantial renal impairment, there are few data from RCTs to inform decisions about when specific ULTs should

not be used on the basis of kidney function. No studies have specifically examined the risks and benefits of not treating gout in people with CKD with ULT, and all current guidelines recommend ULT treatment in this population. In many patients, but not all, untreated gout causes considerable morbidity in its own right, and in those with CKD the only option for treating flares might be long-term corticosteroids, which is associated with further morbidity.

In general, two main reasons are given for avoiding ULT in people with CKD: lack of efficacy and an increased risk of adverse events. There is a general reluctance to use allopurinol in those with an eGFR of <30 ml/min/1.73 m^2 owing to concerns about the risk of allopurinol hypersensitivity syndrome (AHS) and poor outcomes in those with substantial renal impairment who develop AHS[55]. Despite fewer data for febuxostat than for allopurinol, there has been more acceptance of using febuxostat in people with CKD, on the basis of the knowledge that febuxostat is mainly metabolized in the liver and is not dependent on renal function for excretion. One popular school of thinking is that probenecid is ineffective in patients with an eGFR of <50 ml/min/1.73 m^2 and therefore should generally be avoided in this setting, but data suggest otherwise[56]. Benzbromarone is effective even in those with eGFR as low as 20 ml/min/1.73 m^2, but it is not available in many countries owing to the risk of hepatotoxicity. Lesinurad was rapidly determined to be contraindicated in those with an eGFR of <30 ml/min/1.73 m^2, given an increased risk of worsening kidney function, and the drug is no longer marketed. Whether the combination of xanthine oxidase inhibitors (XOIs; allopurinol or febuxostat) with uricosurics (such as probenecid) is a viable strategy in people with gout and CKD is unknown as these combinations share the same limitations of uricosurics by themselves, and evidence that is even more limited. Pegloticase is largely under-studied although the available data suggest it has similar efficacy and safety in those with impaired kidney function and those with normal kidney function[33]. As there are some data indicating that the frequency of gout flares decreases with advancing CKD and after dialysis, it is plausible that some patients with mild hyperuricaemia or normouricaemia and no flares will not require ULT[57].

Box 3 | **G-CAN-proposed research priorities for gout drugs in CKD**

Colchicine

Treatment of gout flares

- Safe and effective dosing of colchicine in chronic kidney disease (CKD): how should the AGREE trial[a] colchicine dose be modified in different stages of CKD?
- How should colchicine be used in people with end-stage renal disease (ESRD) on dialysis?
- Is the risk of drug interactions with colchicine greater in patients with CKD?
- Whether the dose of colchicine should be altered when used in combination with atorvastatin in people with CKD.

Gout flare prophylaxis

- Can low-dose colchicine be used in people with ESRD on dialysis?
- Is there an increased risk of adverse effects with low-dose, longer-term colchicine use in people with CKD?

NSAIDS

Treatment of gout flares

- Are short-term NSAIDs safe in the context of ESRD?
- Are longer-term NSAIDs safe in the context of ESRD?

Gout flare prophylaxis

- Are some NSAIDs safer than others for longer-term prophylactic use?

Glucocorticoids

Treatment of gout flares

- What is the most appropriate duration of oral prednisone use for gout flares?

Gout flare prophylaxis

- Is there an increased risk of tophi in people receiving corticosteroids for gout flare prophylaxis?
- Is there a minimum safe dose or treatment duration in people in whom glucocorticoids need to be used for prophylaxis?

IL-1 inhibitors

Treatment of gout flares

- Is IL-1β inhibition a safe option in CKD?
- Are infection considerations of concern in people with gout?
- Should the dose of anakinra/canakinumab be adjusted based on kidney impairment?

Gout flare prophylaxis

- Is the use or dosing the same for flares as for gout flare prophylaxis?

General

Gout flare prophylaxis

- Is gout flare prophylaxis always required for people with gout and CKD starting urate-lowering therapy?

G-CAN, Gout, Hyperuricemia and Crystal-Associated Disease Network. [a]The AGREE trial was a randomized, controlled trial of high-dose versus low-dose colchicine for managing gout flares[81].

ULT with renal replacement therapy

There is a paucity of data on the safety and efficacy of ULT in people on haemodialysis, and even less in those on peritoneal dialysis. It has been suggested that haemodialysis should reduce serum urate concentration such that specific ULT is no longer required[58,59]. However, this is not a universal finding[60]. It has also been reported that serum urate is at the target concentration less often in those on haemodialysis than in those on peritoneal dialysis, perhaps due to the intermittent rather than continuous removal of urate through dialysis[60]. The data for use of allopurinol and febuxostat in patients undergoing haemodialysis are predominantly limited to case reports and case series[61–65]. For allopurinol, detailed information about the effect of haemodialysis on plasma concentrations of oxypurinol (the active metabolite of allopurinol) indicates that it is effectively dialysed[66] and suggests that allopurinol should be given after haemodialysis.

Appropriate dosing of XOIs

As mentioned above, allopurinol dosing in CKD is one of the most controversial areas in gout management owing to the risk of AHS in people with CKD. On the basis of primarily case series and a retrospective case–control study[67], there is general agreement that the starting dose of allopurinol should be low and increased slowly, although no prospective trial data are available to prove or disprove the rationale that such an approach will reduce the risk of AHS. Use of allopurinol is further complicated by the large inter-individual variability

Box 4 | **G-CAN-proposed research priorities for ULT in CKD**

Allopurinol

- Does commencing allopurinol at a lower dose reduce the risk of allopurinol hypersensitivity syndrome (AHS)?
- How quickly can allopurinol dose be escalated while avoiding AHS and/or severe cutaneous adverse drug reactions?
- Which are the most important risk factors for AHS, and can we more accurately predict who will get AHS-based risk factors?
- Can dialysis improve outcomes in people with AHS?
- Does starting allopurinol at a low dose and gradually increasing the dose reduce the risk of flares and thus alleviate the need for flare prophylaxis?
- Can we predict the dose of allopurinol required to achieve the target urate concentration?
- Does allopurinol provide protection for the heart or kidneys in people with gout, chronic kidney disease (CKD) and cardiovascular disease?
- Is there a differential between peritoneal and haemodialysis with regard to urate lowering?

Febuxostat

- Is febuxostat neutral or associated with an increased risk of cardiovascular death in people with gout, CKD and cardiovascular disease?
- Is febuxostat safer than allopurinol in CKD?
- Are lower starting doses of febuxostat (10–20 mg) less likely than higher doses to cause flares in those who have no good options for prophylaxis?

Probenecid

- At what level of kidney function is probenecid ineffective?
- Is combination therapy with xanthine oxidase inhibitors safer or more effective than probenecid monotherapy?

Benzbromarone

- Is the risk of hepatotoxicity lower in those receiving benzbromarone 100 mg daily compared with higher doses?
- Is there a level of CKD at which benzbromarone should not be used?

Pegloticase

- Is there any difference in risk of immunogenicity with pegloticase in CKD?
- What is the role of concomitant immunosuppression to avoid anti-drug antibodies in those with CKD?
- Does CKD alter the indications for debulking of palpable and erosive tophaceous disease in CKD using recombinant PEGylate uricase therapy?
- Is earlier tophaceous disease debulking in CKD, using recombinant PEGylate uricase therapy, a better approach than initial conventional oral urate-lowering therapy (ULT)?
- Is the effect of rebound flares as severe in advanced CKD?

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in the dose required to achieve the target serum urate concentration (100–900 mg daily). Despite data suggesting that allopurinol dose escalation can achieve target serum urate concentrations even in those with kidney impairment[39,68,69], the belief that the allopurinol dose should be reduced in people with CKD ('renally dosed') remains pervasive. In comparison to allopurinol, febuxostat has a narrower dose range (40–120 mg daily) and there has been more willingness to use febuxostat in people with CKD. In the largest study of febuxostat in CKD, which enrolled 96 people with an eGFR in the range 15–50 ml/min/1.73 m^2, febuxostat 60–80 mg daily was associated with a reduction in serum urate concentration (compared with placebo) with no decline in renal function[70].

Hypersensitivity reactions to XOIs

Both allopurinol and febuxostat have been associated with hypersensitivity reactions, which can be severe with either drug[71,72]. For allopurinol-related reactions, a number of risk factors in addition to kidney impairment have been identified, including the allopurinol starting dose, the presence of HLA-B*58:01 and concomitant use of diuretics[73]. The interaction between the identified risk factors, particularly allopurinol starting dose, renal impairment and HLA-B*58:01, seems to be especially important. As might be expected with any life-threatening reaction, mortality is higher in those with pre-existing CKD[55]. Oxypurinol concentration, which is influenced by allopurinol dose, the presence of diuretics and renal function, might have a role in the pathophysiology of AHS. In vitro studies have shown allopurinol hypersensitivity to be mediated by an oxypurinol-specific T cell response, and drug concentration is an important factor in T cell sensitization[74,75]. However, there is no evidence that a specific oxypurinol concentration precipitates AHS, as many individuals tolerate high concentrations and AHS has been reported in some with low concentrations, indicating that other factors must be involved. The critical combination of risk factors in HLA-B*58:01-negative individuals remains to be determined. Currently, treatment of AHS is supportive. The combined evidence that those with CKD and high oxypurinol concentrations have a poorer outcome and that oxypurinol is readily dialysed begs the question as to whether early dialysis can improve outcomes in people with AHS. There are no data about CKD or renal function and the risk of drug reaction with eosinophilia and systemic symptoms (DRESS) associated with febuxostat treatment.

Cardiovascular risk with XOIs

In the general population, CKD is known to be associated with an increased risk of cardiovascular disease (CVD)[76]. There has been debate about the use of febuxostat in people with CVD given the results of the Cardiovascular Safety of Febuxostat and Allopurinol in Patients with Gout and Cardiovascular Morbidities (CARES) trial[77] and the Febuxostat versus Allopurinol Streamlined Trial (FAST)[78]. CARES was a large RCT in people with gout and pre-existing CVD conducted in the USA that found no increased risk related to treatment with febuxostat compared with allopurinol for the primary end point, which was a composite of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke or unstable angina with urgent revascularization (HR 1.03, 95% CI 0.87–1.23). People with an eGFR of <30 ml/min/1.73 m^2 were excluded from the study but the risk of these events did not differ in those with normal, mild or moderate kidney impairment. However, pre-specified secondary analyses revealed an increased risk of cardiovascular-related death (HR 1.34, 95% CI 1.03–1.73) and death from any cause (HR 1.22, 95% CI 1.01–1.47) in those receiving febuxostat compared with allopurinol; unfortunately, there was no stratification by renal function in the secondary analysis[77]. Although there are a number of issues with the CARES study[79], it raised issues about the relative safety of allopurinol

and febuxostat in people with gout and CVD and led to a black box warning for febuxostat use. The results of the CARES study have been challenged by FAST, another large RCT conducted in European countries, which also compared the cardiovascular safety of febuxostat and allopurinol in patients with gout[78]. Enrollees in FAST had at least one additional cardiovascular risk factor, but patients with advanced CKD were excluded. Febuxostat was non-inferior to allopurinol for the primary end point (a composite of hospitalization for non-fatal myocardial infarction or biomarker-positive acute coronary syndrome; non-fatal stroke; or cardiovascular death). In contrast to the CARES trial, FAST found that treatment with febuxostat was not associated with an increase in cardiovascular death or all-cause death. Overall, fewer deaths occurred in the febuxostat group than in the allopurinol group[78]. When comparing the CARES trial and FAST, FAST had more complete follow-up and better event adjudication (linked to national databases), which provides reassurance about the use of febuxostat, although the findings cannot be directly extrapolated to patients with advanced CKD. Whether CKD modulates this risk or whether febuxostat has a better cardiovascular safety profile than allopurinol in this population remains to be determined.

Role of combination ULT

Combination therapy with a XOI and a uricosuric can be very effective, and if uricosuric toxicity is a consequence of urate concentration within renal tubules then combination therapy could theoretically ameliorate such toxicity. However, as uricosuric treatment is usually not considered for patients with advanced CKD this approach is largely untested.

Conclusions

This Consensus Statement highlights where knowledge regarding the management of gout in people with CKD remains incomplete, and proposes a research agenda to address the most important areas of uncertainty, which includes a better understanding of the natural history of gout in people with CKD. A greater knowledge of the safety of treatments used for the management of gout flares, as well as the requirement for flare prophylaxis in this population, is also needed. Additional investigation is required to determine the safe dosing of allopurinol in people with CKD, as well the prediction and management of AHS. Further research is also required to help determine whether febuxostat is associated with increased cardiovascular risk or is in fact risk-neutral, and whether febuxostat is safer to use than allopurinol in people with gout and CKD. The safety and efficacy of uricosuric medications at different levels of renal function is another area where further research would be of benefit. Evidence regarding the use of pegloticase in people with gout and CKD is limited. From the standpoint of treating or preventing gout flares, the knowledge gaps are also substantial and revolve around the safe use and dosing of colchicine, the safety and efficacy of IL-1 inhibitors and the absolute indication for prophylactic therapy in all patients in whom ULT is being initiated.

In order to resolve these issues, it is important that researchers include patients with all stages of CKD in clinical trials of gout management wherever possible, and undertake pre-specified analyses of safety and efficacy according to renal function, using standardized outcome measures.

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Author contributions
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Competing interests
H.K.C. declares that he has received consulting fees from Takeda and Selecta (less than $10,000 each), research support from Horizon on an unrelated project, and is a member of the Data Safety Monitoring Committee for the VA-CSP 594 STOP GOUT clinical trial. R.T. declares that he has received consulting fees from AstraZeneca, Genentech, Horizon, SOBI (less than $10,000 each) and Selecta (more than $10,000). The other authors declare no competing interests. G-CAN is supported at arm's length by unrestricted by grants from pharma companies including AstraZeneca, Horizon and LG within the past 12 months. No specific funding was received from any bodies in the public, commercial or not-for-profit sectors to carry out the work described in this article.

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Publisher Correction: Global epidemiology of systemic lupus erythematosus

Megan R. W. Barber, Cristina Drenkard, Titilola Falasinnu, Alberta Hoi, Anselm Mak, Nien Yee Kow, Elisabet Svenungsson, Jonna Peterson, Ann E. Clarke and Rosalind Ramsey-Goldman

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The originally published article contained errors in Figs. 1 and 2, in which India was not shown. These figures have been corrected in the HTML and PDF versions of the article to reflect that recent data on the estimated incidence and prevalence of systemic lupus erythematosus in India are not available.

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