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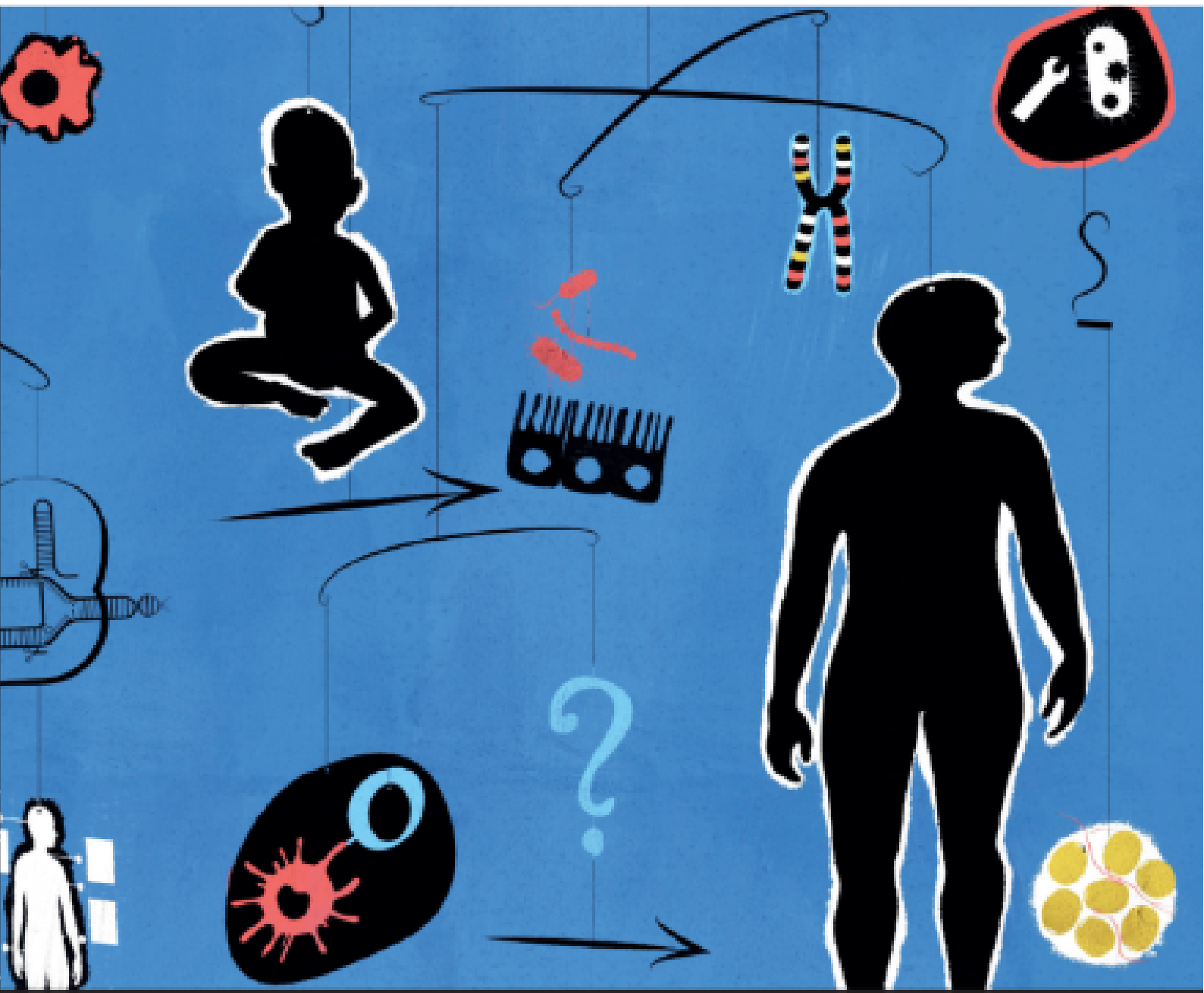

AGE AND ARTHRITIS

How does age matter in the development of arthropathies?

Assessing disease activity in systemic sclerosis

Meeting the challenges

How the Russian invasion affects Ukrainian rheumatological health care

Bohdana Doskaliuk

Individuals with chronic diseases that require constant medical support are particularly vulnerable during wartime and often remain so after the last shot has been fired. What consequences might the Russia–Ukraine war bring for rheumatology?

> War dictates new rules for health-care services, and rheumatology is no exception

On 24 February 2022, Russia's invasion of Ukraine started a new round of full-scale war, escalating a crisis that began in 2014. Since the start of the invasion, the Russian military has repeatedly and methodically shelled and destroyed key infrastructure facilities, residential buildings and even evacuation passages and hospitals in Ukraine[1]. The results of this aggression include numerous civilian injuries and deaths, tremendous ruination of many Ukrainian cities and logistics failures.

War dictates new rules for health-care services, and rheumatology is no exception. Millions of citizens, including people with rheumatological disorders, rheumatologists and related health-care personnel, have been forced to leave Ukraine or have become internally displaced. In the pre-war period, the number of rheumatologists in Ukraine was not sufficient for the size of the population (4 per 500,000 inhabitants)[2], but they were evenly distributed throughout the country. Since the beginning of the Russian invasion, the number of rheumatological practices that are still operating has fallen dramatically, with those practices being located mainly in the western part of Ukraine. The current situation negatively affects access to adequate rheumatological health care in much of the country. Even patients who do have access are unlikely to feel safe while seeking medical help. The World Health Organization (WHO)'s Surveillance System for Attacks on Health Care[3] reported 76 deaths and 59 injuries after 316 attacks on medical facilities and transport, health-care personnel and medical supply missions as of 22 June 2022. Nevertheless, many rheumatologists are trying to build channels of communication to consult with their patients remotely, using tele-rheumatology methods developed as a result of previous experience during the COVID-19 pandemic. Unfortunately, telemedicine is not always an option in times of war, as many patients are restricted in their access to the internet.

A grave issue in the current conflict is the interruption of medical supplies. The Russian invaders have repeatedly demonstrated a complete disregard for the rules of war by shelling humanitarian convoys[1,4]. In the biggest frontline cities of Ukraine, the stores of DMARDs are already insufficient. These shortages raise concerns about the risks of exacerbation of the clinical courses and complications of chronic rheumatic diseases.

The long-term consequences of the war cannot yet be determined. At present, the war has been underway for several months, and it is not known how long it could last. However, the timing of this situation could itself have health-care implications. The time of the acute response to stress in the form of a 'fight or flight' reaction is long past; chronic stress now prevails. Such severe continuous stress can disrupt the proper functioning of the hypothalamic–pituitary–adrenal axis and autonomic nervous system in an individual. This disruption, in turn, might trigger a devastating mechanism of immune-response dysregulation.

The link between stress and the development of autoimmune pathology has long been established as a plausible scientific hypothesis, which is known as the biopsychosocial model[5]. A 2018 study confirmed that people with stress-related disorders, including post-traumatic stress disorder, acute stress reaction and adjustment disorder, are at an increased risk of developing autoimmune rheumatic diseases[6]. Moreover, this risk was more pronounced in patients exposed to stress-related disorders at a younger age[6]. Millions of Ukrainian citizens have been subjected to chronic stress as a result of the hostilities. Thus, a substantial burden of post-traumatic stress disorder and other stress-related disorders among the general population cannot be excluded. A considerable proportion of the Ukrainian population might therefore be at risk of developing autoimmune pathology. The extent of this problem can only be determined accurately by conducting large-scale population studies.

In a tense time of war, when Ukraine needs a helping hand more than ever, it is difficult to overestimate the value of the support provided by our international partners. Since the beginning of the escalation, EULAR has been a pillar of strength for the Ukrainian rheumatological community. EULAR did not limit itself to declaring solidarity with Ukraine and diplomatically condemning

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> The road to recovery will be long, with many challenges ahead

Russia's actions; it also supports Ukrainian refugees with rheumatic and musculoskeletal disorders in their medical needs and supports Ukrainian rheumatologists in their professional growth[7]. The European Federation of Pharmaceutical Industries and Associations (EFPIA) has also adopted measures to improve the provision of essential medicines to Ukrainian[8]. The continuous support of officials, nongovernmental organizations, industry partners and enthusiastic volunteers gives hope and fortifies the Ukrainian resistance to the incessant Russian aggression.

This war will divide the history of Ukrainian rheumatology into 'before' and 'after'. The road to recovery will be long, with many challenges ahead. The direct, urgent mission now is to help and support patients in this challenging time.

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Competing interests
The author declares no competing interests.

RHEUMATOID ARTHRITIS

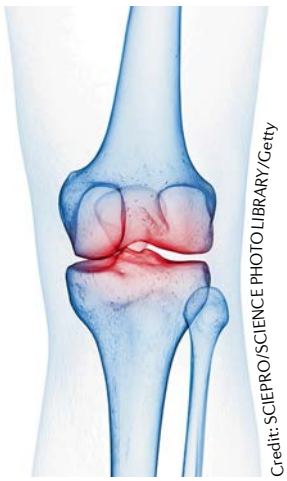
FABP4 exacerbates RA

New research indicates that the adipokine fatty acid-binding protein 4 (FABP4) secreted by synovial macrophages contributes to the progression of rheumatoid arthritis (RA) and could be a target for treatment of the disease. "FABP4 plays a pivotal role in modulating multiple RA pathological changes, including synovitis, pannus formation and cartilage degradation, whereas inhibition of FABP4 can ameliorate these pathological changes in vivo and in vitro," notes Daozhang Cai, corresponding author of the paper reporting the findings.

In the study, the researchers found that FABP4 was upregulated in the synovial tissue, cartilage and serum of patients with RA compared with samples from individuals with osteoarthritis or no history of arthritis. FABP4 expression was also increased in mice with antigen-induced arthritis (AIA), in which levels of FABP4 correlated with cartilage damage. Expression of FABP4 was associated with markers of M1-polarized macrophages in both human RA synovial tissue and mice with AIA. Moreover, stimulation of bone marrow-derived macrophages (BMDMs) with lipopolysaccharide to induce M1 polarization led to increased expression of FABP4 in vitro.

Further experiments demonstrated that FABP4 promoted the proliferation, migration and invasiveness of human umbilical vein endothelial cells and fibroblast-like synoviocytes in vitro and exacerbated AIA in mice. These effects were attenuated by treatment with the FABP4 inhibitor BMS309403.

> FABP4 was upregulated in the synovial tissue, cartilage and serum of patients with RA



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In BMDMs, M1 polarization and FABP4 expression were found to be regulated by activation of the mammalian target of rapamycin complex 1 (mTORC1) pathway. Mice with conditional knockout of Rheb1 (an upstream activator of mTORC1) in myeloid cells had decreased FABP4 expression in M1-polarized macrophages and reduced synovial hyperplasia, cell infiltration and synovitis scores after induction of AIA, compared with control mice. In mice with constitutive activation of mTORC1 owing to myeloid cell-specific deletion of *Tsc1*, treatment with BMS309403 or the dipeptidyl peptidase 4 inhibitor anagliptin could decrease the level of FABP4 in serum and synovial macrophages in mice with AIA and attenuate arthritis severity. "The discovery of the potency of FABP4 inhibition in RA provides a new direction for the development of RA treatment," Cai notes.

Sarah Onuora

ORIGINAL ARTICLE Guo, D. et al. FABP4 secreted by M1-polarized macrophages promotes synovitis and angiogenesis to exacerbate rheumatoid arthritis. *Bone Res.* **10**, 45 (2022)

AUTOIMMUNITY

FOXP3 splice variant is associated with autoimmune disease

FOXP3 encodes a transcription factor that is expressed by regulatory T (T_{reg}) cells. New research indicates that expression of a *FOXP3* exon 2-deficient splice variant results in transcriptional regulation that can induce autoimmunity.

Through alternative splicing, the human *FOXP3* gene produces several different isoforms of the forkhead box P3 (FOXP3) protein, including the full length (FL) isoform and a shorter isoform produced by transcripts lacking exon 2 (FOXP3 ΔE2). These two isoforms have previously been shown to induce differentiation of $CD4^+$ T cells to T_{reg} phenotypes, and their relative expression varies in a number of autoimmune and inflammatory diseases. The specific roles of these isoforms in T_{reg} cells have now been examined.

In the new study, four patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome were identified and found to have mutations resulting in expression of only the FOXP3 ΔE2 isoform, suggesting that this isoform by itself is not able to maintain self-tolerance.

Mice with genomic deletion of *Foxp3* exon 2 were generated for comparison with wild-type mice, which only express FOXP3 FL. The *Foxp3* ΔE2 mice had larger peripheral lymph nodes, expansion of B cells and T cells, and greater numbers of activated T cells and $CD4^+$ T cells expressing IFNγ and IL-4. *Foxp3* ΔE2 mice developed autoimmune disease, with elevation of T follicular helper and germinal centre B cell numbers, seropositivity for antinuclear antibodies and renal deposition of immune complexes, developing splenomegaly and skin lesions consistent with cutaneous lupus erythematosus.

> FOXP3 ΔE2 T_{reg} cells transferred into mice lacking T cell receptor-β … were sufficient to induce systemic autoimmunity

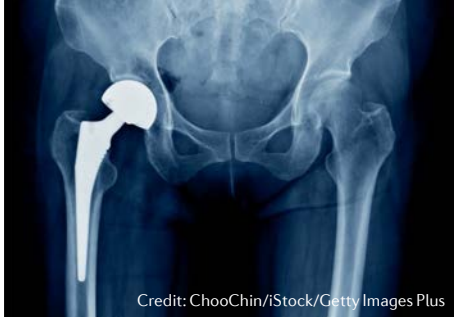
Credit: Alex Whitworth/Springer Nature Limited

FOXP3 ΔE2 T_{reg} cells transferred into mice lacking T cell receptor-β were unstable (demonstrating loss of FOXP3 expression) and were sufficient to induce systemic autoimmunity. In the FOXP3 ΔE2 T_{reg} cells, expression of inflammatory pathway genes including *Ifng*, *Il4* and *Il9* was greater than in wild-type cells.

"Our results demonstrate that the *FOXP3* exon 2-encoded region is indispensable to maintain T_{reg} cell stability and function and immune homeostasis", explains corresponding author Baohua Zhou. "Modulating the expression of FOXP3 FL and ΔE2 isoforms might be a novel approach for treating autoimmune and allergic diseases."

Robert Phillips

ORIGINAL ARTICLE Du, J. et al. FOXP3 exon 2 controls T_{reg} stability and autoimmunity. *Sci. Immunol.* **7**, eabo5407 (2022)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BONE

Annexin A1 could help prevent periprosthetic bone loss

Inflammatory osteolysis in periprosthetic tissue is the leading cause of arthroplasty failure and revision surgery in patients who have undergone total joint arthroplasty. New research suggests that annexin A1, an endogenous regulator of inflammation, could be a therapeutic agent for periprosthetic osteolysis and thus help prolong the lifespan of orthopaedic implants.

"Particulate debris released from materials derived from prosthetic components triggers local chronic inflammatory responses that lead to periprosthetic osteolysis and aseptic loosening," explains Mohamad Alaa Terkawi, corresponding author of the new study. The findings of the study indicate that annexin A1, via the peroxisome proliferator-activated receptor-γ (PPARγ) signalling pathway, attenuates the inflammation and pathological bone resorption induced by this debris.

The researchers found that annexin A1 was expressed by macrophages and neutrophils in periprosthetic tissue and synovial fluid from patients undergoing revision surgery after total hip arthroplasty as well as in calvarial tissue of mice with debris-induced osteolysis. Treatment of isolated human neutrophils with polyethylene debris led to activation of the cells and increased expression of annexin A1.

Studies in human macrophage cultures revealed that annexin A1 inhibits osteoclast differentiation by suppressing NF-κB signalling and by activating the PPARγ signalling pathway. Annexin A1 also inhibited the expression of pro-inflammatory cytokines and bone anabolic factors by TNF-stimulated human synovial fibroblasts and osteoblasts.

In experimental models of debris-induced inflammatory osteolysis, mice treated with the annexin A1 mimimetic peptide Ac2–26 had less extensive osteolytic bone lesions and inflammation in comparison with vehicle-treated mice, although co-administration of a PPARγ antagonist abrogated these effects. Treatment with Ac2–26 was also able to suppress bone loss induced by injection of TNF or RANKL.

> "annexin A1 … could be a therapeutic agent for periprosthetic osteolysis"

Administration of Ac2–26 via a hydrogel that enables locally controlled release of the peptide alleviated TNF-induced bone resorption with effects comparable to those obtained with the mimetic peptide treatment. "Development of safe nanomaterials for the local delivery of Ac2–26 promises to have great potential in clinical applications," notes first author Hend Alhasan.

Sarah Onuora

ORIGINAL ARTICLE Alhasan, H. et al. Inhibitory role of Annexin A1 in pathological bone resorption and therapeutic implications in periprosthetic osteolysis. *Nat. Commun.* **13**, 3919 (2022)

CLINICAL TRIALS

Bimekizumab safe and effective for AS and PsA in long-term trials

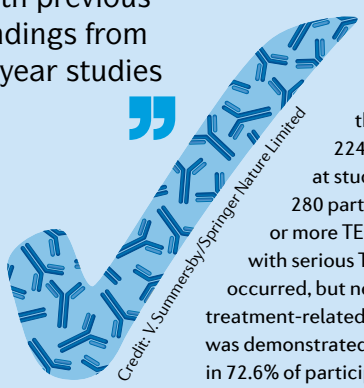
Bimekizumab is a humanized monoclonal antibody that targets IL-17A and IL-17F, potentially giving it greater therapeutic efficacy than inhibitors of only IL-17A. The long-term safety and efficacy of bimekizumab have now been assessed in adults with ankylosing spondylitis (AS) or psoriatic arthritis (PsA) in two international, multicentre phase IIb randomized, controlled trials (RCTs) and their open-label extensions.

BE ACTIVE was a 48-week RCT that enrolled 206 adults with PsA. Its 104-week extension, in which participants received 160 mg bimekizumab every 4 weeks, was completed by 161 patients. Overall, 184 patients had one or more treatment-emergent adverse events (TEAE), including 22 patients with one or more serious TEAE. No deaths were reported.

> "safety profiles for 3 years of treatment … were in line with previous findings from 1-year studies"

A number of patient-reported outcomes were also assessed, demonstrating long-term improvements in pain and fatigue, and high rates of achievement of the patient-acceptable symptom state (75% at week 48, 65% at week 152).

In the BE AGILE study, 303 adults with active AS were enrolled in a 48-week RCT; 255 participated in the extension and 224 were still enrolled at study week 156. Overall, 280 participants had one or more TEAE, including 43 with serious TEAE. Two deaths occurred, but neither was considered treatment-related. Treatment efficacy was demonstrated by ASAS40 response in 72.6% of participants at week 156. Pain, fatigue and quality of life showed sustained improvement.


Credit: V. Summersby/Springer Nature Limited

The safety profiles for 3 years of treatment with bimekizumab were in line with previous findings from 1-year studies in AS and PsA, with no new safety signals or increased risk. The most common TEAEs included nasopharyngitis, upper respiratory tract infection and bronchitis. Fungal infections were of mild or moderate intensity and easily managed. These results indicate that bimekizumab is effective and well tolerated for long-term treatment of AS and PsA.

Robert Phillips

ORIGINAL ARTICLE Mease, P. J. et al. Effect of bimekizumab on symptoms and impact of disease in patients with psoriatic arthritis over 3 years: results from BE ACTIVE. *Rheumatology (Oxford)* https://doi.org/10.1093/rheumatology/keac353 (2022) | Coates, L. C. et al. Safety and efficacy of bimekizumab in patients with active psoriatic arthritis: 3-year results from a phase 2b randomized controlled trial and its open-label extension study. *Arthritis Rheumatol.* https://doi.org/10.1002/art.42280 (2022) | Baraliakos, X. et al. Safety and efficacy of bimekizumab in patients with active ankylosing spondylitis: 3-year results from a phase 2b randomized controlled trial and its open-label extension study. *Arthritis Rheumatol.* https://doi.org/10.1002/art.42282 (2022)

PSORIATIC ARTHRITIS

A protective role for epidermal S100A9 in PsA

A number of S100 proteins are implicated in various rheumatic diseases and could serve as useful biomarkers. New research uncovers a protective role for epidermal S100A9 in psoriasis and psoriatic arthritis (PsA), and highlights this alarmin as a potential biomarker of disease progression.

In the study, the researchers used an inducible conditional-knockout approach to generate mice that lack epidermal expression of c-Jun and JunB (referred to as DKO* mice) or c-Jun, JunB and S100A9 (referred to as TKO* mice). Both the DKO* mice and TKO* mice developed psoriasis and PsA-like disease, but skin disease was more severe and PsA was more frequent in the TKO* mice.

In the DKO* mice, skin disease severity correlated with serum levels of S100A9. The TKO* mice retained expression of S100A9 in dermis-infiltrating immune cells, and circulating levels of S100A9 were similar between DKO* mice and TKO* mice, suggesting that epidermal S100A9 (rather than circulating S100A9) protected against the development of severe skin inflammation in these mice.

> “the serum levels of S100A9-containing dimers correlated with disease activity scores”

Compared with their littermate controls, the skin and inflamed joints of DKO* and TKO* mice contained a greater number of cells expressing Ly6B (a marker of neutrophils, inflammatory monocytes and activated macrophages), including Ly6B^{+}S100A9^{+} cells, implicating neutrophils in potentiating skin and joint disease. Indeed, mass spectrometry-based analysis revealed the presence of a neutrophil activation signature in the skin of TKO* mice.

To investigate the clinical utility of these findings, the researchers measured the expression of S100A9 homodimers and calprotectin (a heterodimer of S100A9 and S100A8) in patients with psoriasis, patients with PsA and healthy individuals. The serum levels of S100A9-containing dimers correlated with disease activity scores and, unlike other pro-inflammatory markers, could be used to discriminate between all three groups.

“The implications for patient care include the use of calprotectin, which is already routinely used for inflammatory bowel disease, in the current panel of biomarkers to identify patients with psoriasis at risk of developing PsA,” says corresponding author Erwin Wagner.

Jessica McHugh

ORIGINAL ARTICLE Mellor, L. F. et al. Keratinocyte-derived S100A9 modulates neutrophil infiltration and affects psoriasis-like skin and joint disease. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2022-222229 (2022)
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SYSTEMIC LUPUS ERYTHEMATOSUS

Risk variant regulates pDC hyperactivation in SLE

Plasmacytoid dendritic cells (pDCs) have an important role in the pathogenesis of systemic lupus erythematosus (SLE). New research attempts to explore the cause of pDC hyperactivation in SLE by focusing on genetic risk variation.

“Previously, we and other groups identified a novel single nucleotide polymorphism (SNP), rs201802880, in neutrophil cytosolic factor 1 (*NCF1*) that was strongly associated with SLE,” explains corresponding author Nan Shen. This missense mutation leads to an arginine-to-histidine substitution at position 90 (p.R90H) of NCF1.

To further explore this genetic variant, the researchers used knock-in mice expressing p.R90H. In vitro, the p.R90H variant promoted pDC activation including the production of type I interferons. Specifically, this variant reduced the affinity of NCF1 to phospholipids and impaired endosomal localization, leading to increased endosomal acidification and reduced reactive oxygen species (ROS) production. Overall, these effects promoted Toll-like receptor cleavage and signalling, and resulted in excessive pDC responses.

In a pDC-dependent mouse model of SLE (imiquimod-induced lupus), the p.R90H variant exacerbated disease: the homozygous knock-in mice had a lower overall survival rate and more serious pathological features (such as larger glomeruli and more infiltrating immune cells) than the heterozygous or wild-type mice. Depletion of pDCs alleviated disease and removed any differences between the genotypes. Interestingly, treatment with hydroxychloroquine also improved the survival of the knock-in mice and eliminated the genotype differences, suggesting that the disease-associated SNP might serve as a useful biomarker for hydroxychloroquine application.

> “the disease-associated SNP might serve as a useful biomarker for hydroxychloroquine application”

Finally, the researchers used single-cell transcriptome profiling of activated human primary pDCs to finely map the pDC subpopulations. They identified four transcriptionally distinct clusters, including an IFN^{hi} pDC cluster that had the lowest expression of NCF1, supporting an inverse relationship between NCF1 expression and pDC activation.

“Given the widespread use of hydroxychloroquine in multiple autoimmune diseases and the presence of inevitable adverse effects, our findings will broadly benefit patients with autoimmune diseases through individualized interventions,” says Shen. “Developing new drugs that directly target ROS and endosomal pH in pDC may also be an effective way to treat SLE and other autoimmune diseases.”

Jessica McHugh

ORIGINAL ARTICLE Meng, Y. et al. The NCF1 variant aggravates autoimmunity by facilitating the activation of plasmacytoid dendritic cells. *J. Clin. Invest.* https://doi.org/10.1172/JCI153619 (2022)

AUTOIMMUNITY

Discontinuing methotrexate to enhance vaccine response

Ana C. Medeiros-Ribeiro and Nadia E. Aikawa

In patients with autoimmune rheumatic disease, methotrexate therapy has been associated with poor immune response to vaccines, including those intended to provide protection against COVID-19. Emerging evidence supports the practice of temporarily discontinuing this treatment in order to improve immunogenicity.

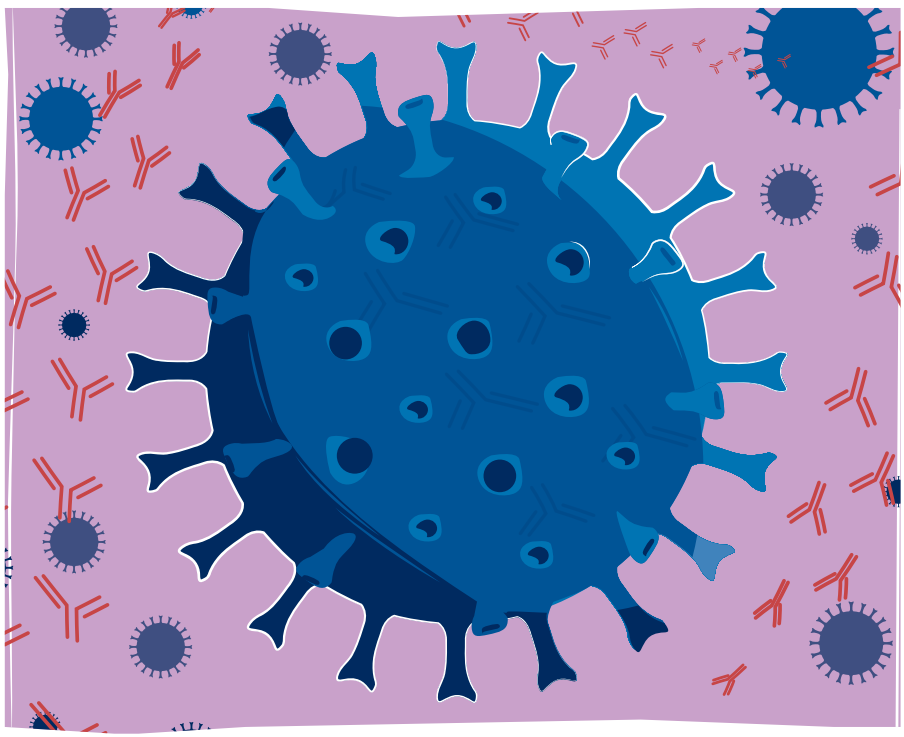
Refers to Arumahandi de Silva, A. N. et al. Pausing methotrexate improves immunogenicity of COVID-19 vaccination in elderly patients with rheumatic diseases. *Ann. Rheum. Dis.* **81**, 881–888 (2022).

Methotrexate is the anchor therapy for inflammatory joint symptoms in many diseases, especially rheumatoid arthritis (RA). It is widely used both as monotherapy and in combination with other agents, owing to its efficacy and adequate safety profile. However, methotrexate has been associated with poor humoral immune responses to many different vaccines, including those against SARS-CoV-2, in patients with autoimmune rheumatic diseases[1,2]. The short half-life of methotrexate and the rapid turnover of naive lymphocyte lineages provide a rationale for temporary discontinuation of this therapy near the time of vaccination as a strategy to enhance immunogenicity. In this context, Arumahandi de Silva et al.[3] retrospectively evaluated the value of this approach for patients receiving mRNA and viral-vector vaccines, and corroborated findings previously reported for influenza vaccines[4–6] and inactivated SARS-CoV-2 virus vaccines[7]. In fact, robust antibody responses are generally induced in a short period (~14 days) after each vaccine dose[3]. The responses can be even faster and stronger with a history of SARS-CoV-2 infection[2].

Evidence from a series of trials of influenza vaccination by Park et al.[4,5] demonstrated that withdrawal of methotrexate for 2 weeks after a single dose of seasonal tetravalent vaccine was associated with enhanced humoral response in patients with RA, without worsening disease activity. However, the challenging scenario for COVID-19 vaccination includes the option of several different vaccines, most of which have a two-dose schedule, with an interval between doses that can vary from 2–4 weeks to 12 weeks. The first version of the ACR guidance for COVID-19 vaccination in patients with rheumatic and musculoskeletal diseases suggested a 1-week discontinuation of methotrexate after each dose of mRNA vaccine and a 2-week discontinuation period after the single-dose viral-vector vaccine for those with well-controlled disease[8]; by contrast, EULAR did not recommend any specific methotrexate-discontinuation strategy. In the German cohort studied by Arumahandi de Silva et al.[3], methotrexate intake schedules around vaccinations were heterogeneous: 33 patients had maintained methotrexate therapy, and 31 had changed their intake in one of several different patterns, including withdrawal before and/or after vaccination.

> “methotrexate has been associated with poor humoral immune responses to many different vaccines”

This retrospective analysis[3] revealed that methotrexate withdrawal for at least 10 days after the first and/or second dose, but not before, improved the humoral response to vaccination, regardless of the type of vaccine used. This finding is similar to that reported by Park et al. for influenza vaccination[4–6], which demonstrated that the timing of methotrexate interruption is crucial, with discontinuation of methotrexate before vaccination providing no improvement in immunogenicity, even with a pause of 4 weeks[5]; notably, the



Credit: Alex Whitworth/Springer Nature Ltd

timing of the last dose of methotrexate before vaccination did not affect vaccine response[6].

Arumahandi de Silva et al.[3] found that patients who discontinued methotrexate had higher titres of IgG antibodies against receptor binding domain (RBD) proteins, higher vaccine response rates as defined by positive levels of RBD IgG and of SARS-CoV-2-neutralizing antibodies and higher virus-neutralizing capacity, in comparison with those who continued methotrexate therapy[3]. Both seropositivity for anti-RBD IgG and higher titres of SARS-CoV-2-neutralizing antibodies have been associated with increased protection against COVID-19 in patients with autoimmune rheumatic diseases[9]. The ~33% increase in the frequency of vaccine response according to anti-RDB IgG positivity seen in the methotrexate discontinuation group was even greater than the reported 12–34% improvement in response to influenza vaccine[4,5] and to the ~24% increase in IgG response to the inactivated anti-SARS-CoV-2 vaccine[7]. A ~21% higher response rate was also seen with methotrexate withdrawal when neutralization response was measured using a test that directly evaluates inhibition of RBD linkage. This finding contrasts with the modest and non-significant 13% improvement in response observed with the same strategy in patients receiving the inactivated SARS-CoV-2 vaccine[7]. The fact that mRNA vaccines were used in >85% of patients in the analysis by Arumahandi de Silva et al. probably accounts for this difference, since mRNA vaccines induce very specific anti-RDB antibodies at higher titres[3].

> “methotrexate withdrawal for at least 10 days … improved the humoral response to vaccination”

Age is well known to decrease vaccine immunogenicity, and this factor independently influenced vaccine response in all methotrexate-withdrawal trials, including the study by Arumahandi de Silva et al.[3]. Of note, in this study the benefit of pausing methotrexate was limited to elderly patients (≥60 years old); however, the patients aged younger than 60 years, who were underrepresented in the cohort, all responded very well to vaccination, precluding a definitive conclusion about the benefit of the methotrexate-holding strategy in the general adult population receiving COVID-19 vaccines. The results of the largest influenza vaccination study by Park et al., however, support the notion of an overall, age-nonspecific benefit of a methotrexate-holding strategy, as the mean age in the patient groups was approximately 53 years[4].

Although the use of DMARD combination therapy and glucocorticoids has also been associated with decreased response rates in larger COVID vaccine trials[2], their use did not seem to hamper the beneficial effect of discontinuing methotrexate in the analysis by Arumahandi de Silva et al.[3] and by Araujo et al.[7]. Regarding the use of glucocorticoids, methotrexate-holding studies[3–5,7] have solely included patients receiving no or low doses of prednisolone, and in the cohort analysed by Arumahandi de Silva et al. ~80% of patients were not being treated with glucocorticoids[3]. The benefit of methotrexate withdrawal has also been observed regardless of methotrexate being used as monotherapy or in combination with other drugs. However, the biologic DMARDs that interfere profoundly with humoral vaccine response, rituximab and abatacept, were not represented in the study by Arumahandi de Silva et al.[3] and were underrepresented in the other trials[4,5,7].

Regarding methotrexate dose, Park et al.[4] suggested that methotrexate has a dose-dependent detrimental effect on the immune response to influenza vaccine, with the benefit of temporary interruption being apparent in patients receiving doses greater than 10 mg per week. The analysis by Arumahandi de Silva et al.[3] included patients receiving methotrexate with mean doses similar to those in the trials by Park et al. (approximately 13 mg per week), whereas patients in the study of inactivated SARS-CoV-2 vaccine[7] were receiving a slightly greater median dose (20 mg per week) and no patients received a dose lower than 10 mg per week. In both anti-SARS-CoV-2 vaccine papers[3,7], methotrexate doses were comparable between the maintenance and withdrawal groups, and the dose did not impact the effect of methotrexate withdrawal.

Although Arumahandi de Silva et al.[3] did not study the safety of the methotrexate withdrawal strategy or its influence on disease activity, the results of influenza vaccination studies suggested that a 2-week withdrawal period does not affect control of RA disease activity[4,5], whereas in the trial of inactivated SARS-CoV-2 vaccine the frequency of RA flares increased after the second period of methotrexate withdrawal[7], probably owing to the short-term interval between the vaccine doses and therefore the withdrawal periods. This finding is consistent with data on the pharmacokinetics of methotrexate in patients with RA, in whom a 3-week discontinuation of methotrexate would be required for concentrations of its metabolites in red blood cells to fall below the therapeutic threshold, and with the fact that RA disease flares frequently occur approximately 1 month after stopping methotrexate[10].

Overall, the results of the retrospective analysis by Arumahandi de Silva et al. are consistent with those from other trials and support the strategy of discontinuing methotrexate for 10–14 days after each dose of COVID-19 vaccine in patients with well-controlled and stable autoimmune rheumatic disease. This strategy, however, requires close surveillance of disease activity and shared decision-making.

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SYSTEMIC LUPUS ERYTHEMATOSUS

Ikaros, Aiolos and other moving targets to treat SLE

Afroditi Boulougoura and George C. Tsokos

Although the multitude of pathways involved in the pathogenesis of systemic lupus erythematosus (SLE) seem to be interconnected, each predominates in only a fraction of patients. This complexity means that many agents tested in SLE clinical trials — now including iberdomide — produce only a small clinical benefit.

Refers to Merrill, J. T. et al. Phase 2 trial of iberdomide in systemic lupus erythematosus. *N. Engl. J. Med.* **386**, 1034–1045 (2022).

Multiple genetic, environmental and epigenetic factors contribute to the development of systemic autoimmunity, inflammation and multi-organ damage in the disease known as systemic lupus erythematosus (SLE). Practically all domains of the innate and the adaptive immune system are involved, and multiple molecular, biochemical and metabolic aberrations within each domain have been recognized in this disease[1]. Numerous trials have been conducted in people with SLE, each focused on targeting particular molecules and/or pathways under the assumption that they are the dominant disease mechanisms in all, or at least in the majority, of the patients enrolled in the studies. The approval of belimumab and voclosporin for the treatment of lupus nephritis and of anifrolumab for non-renal SLE has attracted interest in conducting even more trials, but without modifying the primary or secondary outcomes or considering who among the enrollees has the best chance of experiencing a clinical benefit. In one of the latest phase II trials, iberdomide, a small molecule drug that promotes the proteosomal degradation of Ikaros and Aiolos, two transcription factors involved in B cell and T cell proliferation and differentiation, reportedly delivered clinical improvement in some patients with SLE[2].

Ikaros (encoded by *IKZF1*) and its family members Aiolos (encoded by *IKZF3*), Helios and Eos/Dedalos are sequence-specific DNA-binding factors that belong to the Kruppel transcription factor family. Ikaros is important in lymphoid development, and its genetic deficiency causes complete lack of lymphoid cells including B cells, T cells, natural killer cells and dendritic cells. At a later stage of lymphocyte differentiation, loss of Ikaros arrests B cell differentiation at the proliferative large pre-B cell stage and prevents transition to the quiescent small pre-B cells, memory B cells and plasmablast differentiation[3] (FIG. 1).

Ubiquitylation is needed to tame inflammation. This important post-translational protein modification involves the covalent conjugation of ubiquitin to lysine residues on specific protein substrates in order to regulate their degradation. The ubiquitylation process is catalysed by three classes of enzymes: E1 enzymes, E2 enzymes and E3 ligases. Iberdomide interacts with cereblon, which is part of the cullin ring ligase 4-cereblon ($CRL4^{CRBN}$) E3 ubiquitin ligase complex, to induce ubiquitylation of Ikaros and Aiolos, resulting in their proteasomal degradation.

The rationale for the reported phase II trial of iberdomide for the treatment of SLE[2] was based on reports that polymorphisms in *IKZF1* and *IKZF3* increase the risk of development of SLE[4]; evidence that Aiolos expression is increased in B cells from patients with SLE and is also associated with increased numbers of circulating double negative B cells and increased levels of BAFF (long considered to be involved in the expression of the disease); and, finally, that iberdomide suppresses antibody production by B cells from patients with SLE in vitro[5].

In the phase II trial, 288 patients with SLE were randomly allocated to receive oral iberdomide (at a dose of 0.15 mg, 0.30 mg or 0.45 mg) or placebo once daily for 24 weeks, in addition to standard care. The percentage of patients who achieved the primary end point of an SLE Responder Index (SRI-4) response at 24 weeks was greater with iberdomide than with placebo only for the group that received the highest dose of the drug (54% versus 35%). Notably, a number of secondary end points were not met, including reduction in the use of glucocorticoids, improvement in joint and skin involvement and no new disease activity as measured using the BILAG index.

A number of observations limit the excitement for the future success of iberdomide. The lack of a dose response curve suggests the two smaller doses were insufficient, and the lack of a dose curve effect in people who had a high Aiolos gene-expression signature at baseline are of concern. The authors acknowledge the disappointing (yet not unprecedented) underrepresentation of Black people in the study. Considering that SLE is a disease with a higher prevalence, morbidity and mortality in patients of African descent, the generalizability of the results remains a serious question. Although it is understandable that patients with severe disease are routinely excluded from phase II studies, no one can predict the utility of iberdomide in patients with severe disease. We should also note that the chosen end point of 24 weeks compromises the utility of the data for a chronic disease such as SLE.

The phase II trial led by Merrill and colleagues[2] used a drug that targets only a certain part of the ubiquitylation processes. In addition to Ikaros and Ailos, several other components of the ubiquitylation process have been linked to SLE and could be considered targets for therapy (FIG. 1). For example, E3 ubiquitin-protein ligase RNF128 (also known as GRAIL) is a negative regulator of T cell receptor (TCR) responsiveness and cytokine production, and is a dominant player in the induction and maintenance of T cell anergy. *Grail*$^{-/-}$ mice display enhanced T cell proliferation and differentiation to T helper 1 (T_H1) and T_H17 lineages, and have a profound multi-organ inflammatory response. GRAIL works in concert with the deubiquitylase Otub1 to balance anergy and activation of T cells[6] (FIG. 1). Rationally, targeting the GRAIL pathway could be anticipated to deliver a broad anti-inflammatory effect.

E3 ubiquitin-protein ligase CBL-B acts as a negative regulator in signalling pathways that involve TCRs and B cell receptors, CD28, CD40 and C-type lectin receptors that are known to regulate innate and adoptive immune responses. CBL-B deficiency or inactivation leads to hyper-activation of canonical and noncanonical NLRP3 inflammasomes[7]. The absence of CBL-B impairs the development of induced T regulatory cells, and causes T cell resistance to anergy by interfering with molecules involved in the TCR signalling pathway (FIG. 1).

T cell surface glycoprotein CD3ζ chain, an important TCR–CD3 signalling protein, is ubiquitylated at increased levels in SLE T cells, and this leads to its degradation. Also increased

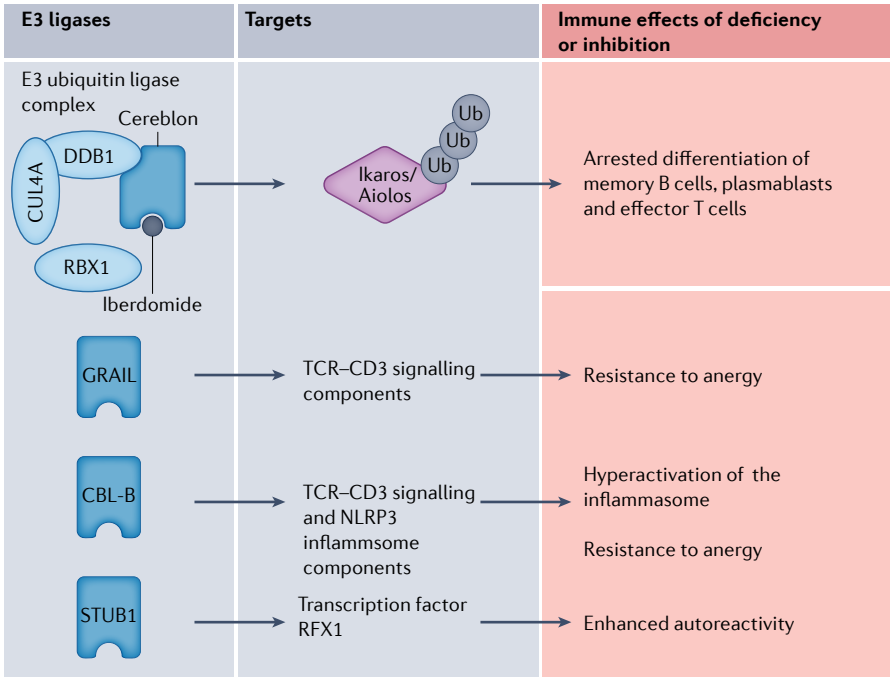

Fig. 1 | **E3 ubiquitin ligases in the pathogenesis of SLE.** The cereblon modulator iberdomide binds the $CRL4^{CRBN}$ E3 ubiquitin ligase complex to induce ubiquitylation of Ikaros and Aiolos, resulting in their proteasomal degradation and ultimately the suppression of B cell and plasmablast differentiation. The E3 ubiquitin ligases GRAIL, CBL-B and STUB1 contribute to T cell and B cell lymphocyte homeostasis by regulating T cell receptor (TCR) signalling, cell proliferation, cytokine production and NLRP3 inflammasome activation. Preliminary data suggest cereblon and STUB1 activity is increased in systemic lupus erythematosus (SLE), whereas GRAIL and CBL-B activity is decreased.

in SLE T cells is the ubiquitylation and degradation of serine/arginine-rich splicing factor 1 (SRSF1), which promotes the upregulation of CD3ζ chain expression in T cells and the production of IL-2 (REF.[8]). The transcription factor MHC class II regulatory factor RFX1 is degraded by the ubiquitin-proteasome system in T cells from patients with SLE via the E3 ubiquitin-protein ligase CHIP (also known as STUB1) (FIG. 1), which is upregulated in $CD4^+$ T cells from patients with SLE[9]. Data in SLE T cells have shown an increase in the expression of TRAF5, a molecule that has been suggested to promote RORγt ubiquitylation, and control T_H17 cell function[8].

The iberdomide trial[2] did not report any notable side effects, but it should not evade a sceptic that this drug is a cereblon modulator, as is thalidomide[10], and although pregnancy avoidance can be achieved in a well-controlled trial environment, it would be of concern if the drug is introduced to the real world where the majority of the patients will be young women. Lastly, as noted above, multiple aspects of ubiquitylation are abnormal in patients with SLE. The decision of which one to target and in what group of patients should be considered in the context of the much needed and delayed precision medicine in SLE. We can keep trying different drugs to treat SLE until we find the panacea or, alternatively, we could spend more time and effort to understand the process involved in this highly heterogeneous disease.

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How does age determine the development of human immune-mediated arthritis?

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Abstract | Does age substantially affect the emergence of human immune-mediated arthritis? Children do not usually develop immune-mediated articular inflammation during their first year of life. In patients with juvenile idiopathic arthritis, this apparent 'immune privilege' disintegrates, and chronic inflammation is associated with variable autoantibody signatures and patterns of disease that resemble adult arthritis phenotypes. Numerous mechanisms might be involved in this shift, including genetic and epigenetic predisposing factors, maturation of the immune system with a progressive modulation of putative tolerogenic controls, parallel development of microbial dysbiosis, accumulation of a pro-inflammatory burden driven by environmental exposures (the exposome) and comorbidity-related drivers. By exploring these mechanisms, we expand the discussion of three (not mutually exclusive) hypotheses on how these factors can contribute to the differences and similarities between the loss of immune tolerance in children and the development of established immune-mediated arthritis in adults. These three hypotheses relate to a critical window in genetics and epigenetics, immune maturation, and the accumulation of burden. The varied manifestation of the underlying mechanisms among individuals is only beginning to be clarified, but the establishment of a framework can facilitate the development of an integrated understanding of the pathogenesis of arthritis across all ages.

The timing of the emergence of chronic inflammatory disorders is one of the unsolved conundrums in medicine. Why do some disease phenotypes present during childhood, and others only later in life? A vivid and timely example of how age matters to the development and phenotype of disease is the current COVID-19 pandemic. As the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus spread around the world, it quickly became clear that severe clinical manifestations of COVID-19 were rare in children[1]. At the same time, a small proportion of SARS-CoV-2-infected children, often with no (or only mild) symptoms in the initial course of the infection, developed a serious and threatening post-infectious hyperinflammatory syndrome known as multisystem inflammatory syndrome in children (also known as paediatric inflammatory multisystem syndrome), which was characterized by fever, skin rash, myocarditis and features reminiscent of Kawasaki disease[2,3]. A similar syndrome is also seen in adults (multisystem inflammatory syndrome in adults), mainly in young adults. For COVID-19, the most important determinant of disease severity and mortality in adults is age. Clearly, both the initial and late immune responses to SARS-CoV-2 infection and the ensuing clinical phenotypes are age dependent.

This example is not unique. Other age associations are well known, for example in reactive arthritis and polymyalgia rheumatica (PMR). Reactive arthritis tends to occur most commonly in men within the age range 20–40 years and PMR typically affects individuals >50 years old and is responsible for rhizomelic muscle and joint pain. PMR results from unknown triggers[4], and its disease pathogeny is thought to be driven by both activated innate immune cells (especially monocytes and macrophages) as potential primary sources of IL-6, and a deregulated adaptive immune compartment (with reduction of numbers of regulatory T (T_{reg}) cells and a shift towards T helper 17 (T_H17) and T_H1 cells[5]. In addition, the T cell compartment exhibits a higher frequency of senescence markers (CD28 expression loss) alongside the expression of activating receptor NKG_2D[6]. Understanding how age-related immune-system modifications contribute to PMR occurrence is an unmet need.

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Key points

- The arthritis-free 'immune privilege' of early childhood is overridden by multiple mechanisms, progressively and age-dependently, generating recognizable patterns of chronic inflammatory arthritis.
- The emergence of arthritis involves interconnected mechanisms related to immune priming, to a situational susceptibility and to the accumulation of an inflammatory burden.
- The accumulation of epigenetic drift may contribute to differences across ages.
- The exposome is expected to contribute to arthritis emergence in adults as well as in children.

A first comprehensive chronological map of human health has been provided recently[7]. On the basis of approximately four million electronic health records of patients in England, from 1 year old to advanced age, cumulative incidence and period prevalence were estimated for 308 common morbidities. In addition to variation related to sex and ethnicity, the data identified age-related differences throughout the decades of life. Over the lifespan, a shift from atopic disorders and acute infections towards cancers and degenerative diseases at advanced ages was seen. From a musculoskeletal point of view, young children (<10 years old) mostly suffered from wrist fractures, enthesopathy and scoliosis, which between them represented about 4% of morbidities in cumulative incidence, compared with >8% for dermatitis, which was the most common condition in this age range. As age increased beyond the 20–29-year-old stratum, the burden of metabolic and degenerative osteoarticular diseases (including osteoarthritis, osteoporosis and gout) progressively increased, reaching an approximate cumulative incidence of 20% in the 70–79-year-old stratum. These data also confirmed that the median age at first diagnosis for two common immune-mediated arthritides in adults, namely rheumatoid arthritis (RA) and psoriatic arthritis (PsA), peaks during the fifth and sixth decades, respectively. By contrast, most juvenile idiopathic arthritis (JIA) subtypes peak in the first decade of life (between 1 year old and 6 years old)[8].

The associations between age and immune-mediated disease are not yet well studied, mainly because of the separation between adult and paediatric patient care and related research programmes. For example, the field of paediatric rheumatology is largely separated from that of 'adult' rheumatology. Age-dependent manifestations of inflammatory arthritis have led to separate disease classifications for paediatric rheumatological diseases that have only recently become the focus of a healthy debate[9]. JIA, which is classified as chronic inflammation involving one or more joints and initiating before the age of 16 years, is only rarely seen before the age of 1 year, and its subtypes differ in their age distributions[10,11]. RA, both seronegative and seropositive, has specific age distributions over the adult lifespan[12], and the course of PsA is also modulated by age. Indeed, skin disease (psoriasis) occurs mostly prior to arthritis in adults yet conversely after arthritis in the juvenile form of PsA[13]. Interestingly, recent data showed that during the transition period from paediatric to adult care, the proportion of patients fulfilling adult immune-arthritis classification criteria (the ACR–EULAR 2010 criteria for RA, the Yamaguchi criteria for adult-onset Still's disease, the Assessment of SpondyloArthritis international Society criteria for spondyloarthritis and the Classification Criteria for Psoriatic Arthritis) differ substantially among different subtypes of childhood arthritis, at least at the classification level[14]. Increased understanding of underlying mechanisms and pathways of immune-mediated arthritis has re-opened the discussion of the relevance of the different classification systems and, along with the development of novel bioinformatics strategies, has created an opportunity to move towards classification with a more biological and molecular basis, as reviewed previously[15].

Clearly, age matters in the development of human arthropathies. In this Review, we explore the underlying mechanisms of age-specific arthropathy manifestations and, most importantly, what we can learn from differences and similarities across the age spectrum. We focus on JIA (excluding the predominantly autoinflammatory subtype systemic JIA) and on the most prevalent adult counterparts, namely RA and PsA. We develop three hypotheses (which are summarized in FIG. 1) on how age-related mechanisms trigger chronic arthritis across ages. These hypotheses are probably not mutually exclusive, and likely represent processes that act synergistically and interact with shared molecular mechanisms. By understanding the key immune mechanisms that drive arthropathies in children and adults, we will be able to explore new avenues of diagnostics, treatment and, ultimately, maybe even prevention.

A 'critical window in (epi)genetics'

Although the exact trigger (or triggers) and pathogenic mechanisms that underlie the development of chronic arthritis in humans are still largely unresolved, it is generally thought that chronic immune arthritis, in keeping with other immune-mediated inflammatory diseases (IMIDs), is the result of exposure of a genetically susceptible host to various environmental stimuli. JIA and RA are both complex, polygenic diseases, in which the timing of specific environmental exposures in individuals with specific genetic backgrounds seems to be critical for disease development. We can therefore hypothesize that differences in age at onset might be explained by inherited predisposition for so-called 'critical windows' for environmental influence.

Shared genetic components in arthritis. A major determinant of the emergence and severity of IMIDs is an individual's genetic background[16]. The most common inflammatory arthropathy in adults is RA, and results from seminal studies have identified an association with

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HLA class II alleles in this disease[17], leading to the definition of the shared epitope (SE)[18], and also identifying a role for the *PTPN22* locus[19]. For JIA, associations with specific HLA regions (which include *PTPN22* (REF.[20]), and which differ depending on disease phenotype) were identified >40 years ago[21–23]. *PTPN22* is an ethnicity-specific genetic factor, and the *PTPN22* R620W-encoding allele is mostly observed in white European and white North American populations[24,25]. Altogether, currently available results point to a striking overlap in risk loci between JIA and other forms of arthritis[20,22], informing ongoing discussions on the transformation of the current classification criteria for JIA[15], as reviewed elsewhere[15,26]. The overlap of genetic susceptibility loci suggests shared mechanisms among immune arthropathies. Notably, this overlap involves immune arthritis specific to childhood as well as IMIDs specific to adulthood[26], and the identification of genetic susceptibility for human arthropathies[19,27–30] has translated into therapeutic targeting of IL-6, IL-17, CTLA-4–CD28 and Janus kinases[31]. However, many genetic associations are still poorly understood, and shared genetic backgrounds are sometimes associated with opposite outcomes, such as the protective nature of *HLA-DRB1:04* in polyarticular JIA, in contrast to its status as a risk allele in seropositive adult RA[20,22,23,31–33]. Similarly, *HLA-DRB1:08* is a risk allele for polyarticular JIA, whereas it is protective against adult seropositive RA. By contrast, the *PTPN22* rs6679677 single-nucleotide polymorphism (SNP) confers risk in both conditions. Novel bioinformatics strategies may help to develop our understanding of these findings and to identify relevant non-coding variants, in particular those that function by modulating the binding of DNA regulatory proteins such as transcription factors, including epigenetic marks and predicted and/or experimentally determined transcription factor binding motifs.

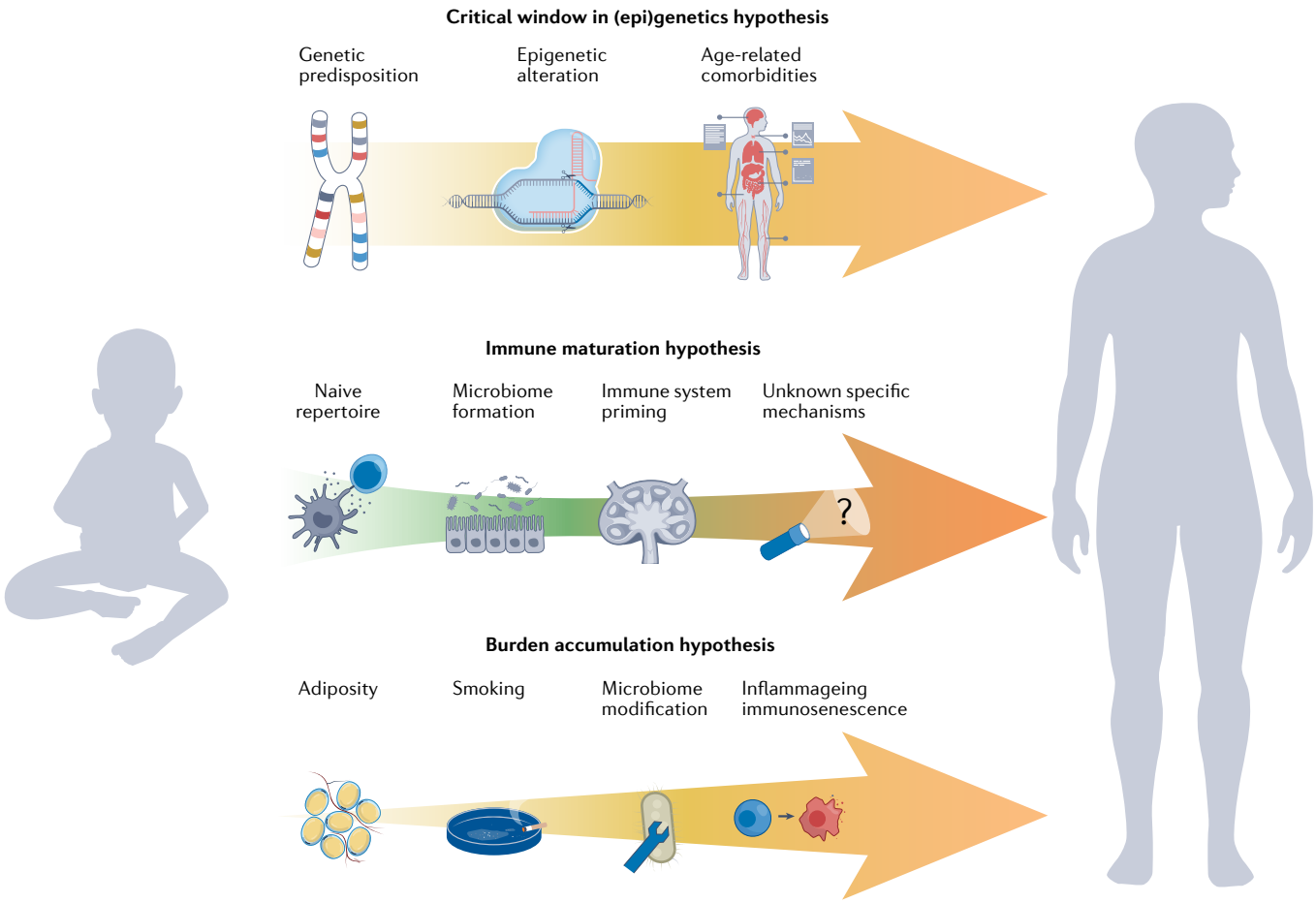

Fig. 1 | **Model of age-related arthritis emergence.** Emergence of arthritis is a multifactorial phenomenon. Given the striking differences observed between paediatric-onset and adult-onset arthritis, age-specific mechanisms are likely to be present. How these mechanisms articulate in individuals is still only beginning to be clarified. We propose an age-related model of arthritis emergence, based on three hypotheses that are not mutually exclusive, and that are potentially even interconnected. The 'critical window' hypothesis relates to pro-arthritogenic factors that might define a set point for arthritis emergence. As such, genetic predisposition, epigenetic status and associated comorbidities represent relevant components for this critical window. The immune-maturation hypothesis relates to the fact that the first year of life is usually free of arthritis, suggesting an immune 'privilege'. Currently available data suggest a low propensity at this age to autoimmune inflammatory responses that is related to regulatory controls such as the presence of a naive immune repertoire, with tolerogenic T lymphocytes, naive dendritic cells and a diverse regulatory T (T_{reg}) cell repertoire. Early microbiota composition, built from fetal stages onwards, may also contribute to this 'tolerogenic' environment. However, aberrant priming of the immune system, especially resulting from infection with pro-arthritogenic pathogens (and other unknown mechanisms) could also affect the emergence of arthritis. Finally, the accumulation of a pro-inflammatory burden resulting from acquired influences such as adiposity, smoking, microbiome dysbiosis, immunosenescence and inflammageing, and more widely by the exposome, represent additive hits upon regulatory control, leading to breach of tolerance, and inflammation.

Although the common dogma is to classify paediatric and adult rheumatological IMIDs separately, genetic data from the past decade have challenged this age-dependent partitioning of arthritis, raising the question of why these conditions should be separated rather than being grouped together. Evidence of similarities in genetic backgrounds in child and adult immune arthritis has led to the proposal of a new classification system[26]. This proposal combines genetic patterns, demographic and clinical data to generate four clusters spanning both paediatric and adult variants of inflammatory arthritis: seropositive, seronegative, spondyloarthritis and systemic. This classification needs further development to decipher intra-cluster heterogeneity and to determine the involvement of particular immune pathways, which could translate to different therapeutic targets. However, this new paradigm is valuable, and an open mind and pragmatic approach might help us to develop the current step-up treatment approach into more tailored strategies[15].

Epigenetics and phenotypes. Control of gene expression can be postulated to account for immune arthritis onset and outcome. The discovery of epigenetic regulation — defined as heritable (and acquired) modifications of (regulatory) DNA sequences, affecting the expression of genes without changing the coding sequence itself — has revolutionized our understanding of immune arthritis[34–36]. Epigenetic mechanisms are thought to act at the interface between disease risk factors (including environmental factors, nutrition, infection and socioeconomic factors) and the implementation of the genetic information encoded in DNA[37–39].

DNA methylation and histone modification are well described epigenetic marks. Current technologies can provide accurate and reproducible genome-wide analysis of DNA methylation patterns[40]. Notably, methylation seems to induce stable, long-term gene repression[41]. The deregulation of methylation in RA might account for aggressive fibroblast phenotypes[42,43]. In murine arthritis, repeated inflammatory insults with zymosan or monosodium urate crystals induce metabolic, transcriptomic and epigenetic changes in synovial fibroblasts. These changes are mediated by complement components C3 and C3a receptor, and result in pathogenic inflammatory fibroblast functionality[44]. An elegant experimental approach has enabled differentiation of methylation signatures that cause RA from those that result from the disease. In this approach, peripheral blood leukocytes from 354 anti-citrullinated protein antibody (ACPA)-positive patients with RA and 337 healthy individuals from the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study were assessed[45]. Results were adjusted according to blood-cell proportions, age, sex and smoking status. Ultimately, the results of a series of conditional correlation analyses identified causal inference on RA occurrence for a set of 10 differentially methylated positions, 9 of which were located in the MHC cluster, emphasizing the relevance of MHC genes, and hence core immunological function (as opposed to primary non-immunological stromal-cell dysregulation) in RA susceptibility. Results from a landmark study of disease-discordant monozygotic twins support the importance of DNA methylation signatures in the emergence of autoimmune diseases such as RA[46].

In JIA, evidence suggests that epigenetic profiles and alterations have important roles in disease development[47–49]. Immunoprecipitation targeting a specific histone modification (acetylation of lysine at residue 27 of histone H3) has demonstrated that synovial fluid-derived $CD4^+$ effector memory T cells display a disease-specific signature of both enhancers and super-enhancers, which are non-coding regulatory elements in *cis*-acting DNA sequences ranging in size from a few hundred base pairs to some 50 kb, to which transcription factors and co-factors can bind to control transcription[48]. In systemic lupus erythematosus (SLE), a specific mechanism that contributes to T cell and B cell hyperactivity involves reduction of expression of the transcription factor RFX1, which affects DNA methylation and histone acetylation in $CD4^+$ T cells[50]. Clinical trials with compounds that affect epigenetic alterations, including enhancer and super-enhancer activity, are ongoing in the field of oncology[51], demonstrating that it is possible to target disease-specific signatures of regulatory elements that affect immune-related genes. Important reader proteins at enhancer and super-enhancer regions are the bromodomain and extraterminal (BET) proteins, which can bind to acetylated histone and non-histone proteins, thereby regulating gene transcription. BET inhibitors impair differentiation of naive $CD4^+$ T cells into effector T cell subsets[52,53].

Further evidence of the potential for therapeutic modulation of epigenetic markers in T cell-mediated autoimmune diseases is provided by the observation that the DNA methyltransferase inhibitor 5-azacytidine can modify the surface glycoproteins (T4 molecules) expressed upon maturation of thymocytes[54]. Methotrexate, the cornerstone of arthritis treatment, is also expected to modulate DNA methylation. Methotrexate inhibits folate metabolism and, as such, should reduce regeneration of methionine, which is an essential methyl-group donor for DNA methylation[55]. Results indicate that the DNA hypomethylation profile that is found in RA and JIA is modified by methotrexate treatment[56,57]. Furthermore, in a study of patients from the Scottish Early RA cohort and additional samples from an independent cohort, a chromosome conformation signature was found to predict response to methotrexate prior to treatment initiation[58]. This approach identified at baseline non-responders to a 6-month course of methotrexate, with a true-negative response rate of 86% and 90% sensitivity.

Age-related epigenetic drift. The epigenome is particularly susceptible to deregulation at pivotal and extreme ages, especially during early embryonic, neonatal, pubertal and elderly periods[59]. In this respect, lessons from Alzheimer disease may be valuable for arthritis. One theory is that Alzheimer disease originates early in life[60]. Although it is associated with mutations (in genes such as *APP*, *PSEN1* and *PSEN2*), Alzheimer disease develops with a sporadic non-Mendelian pattern, strongly suggesting a role for epigenetic regulation. The accumulation of epigenetic modification (including histone

modulation and DNA methylation) in the susceptible early embryonic and neonatal periods, or later in life, is thought to contribute to Alzheimer disease[61]. Whether and how non-Mendelian IMIDs, such as the human chronic arthropathies, similarly arise from an individual's heritable epigenetic drift is not yet understood[62]. In the differentiation of human T_{reg} cells into effector T_{reg} cells in arthritis, the cells display a transcriptional profile similar to that of naturally occurring (suppressive) T_{reg} cells, along with characteristics of an effector program (including transcription of *TNFRSF18*, *PRDM1* and *BATF*). This profile suggests that the specific transcriptional and epigenetic signature of T_{reg} cells from the site of inflammation, the synovial fluid and the joint, is developed or shaped through epigenetic changes and environment-specific adaptations[47].

Age-related comorbidities. Both in paediatric arthritis and in adult arthritis, specific, strikingly different, comorbidities exist or develop. These comorbidities can provide information about specific disease mechanisms across the age spectrum (BOX 1).

Chronic anterior uveitis (CAU) is the most common extra-articular disease manifestation of JIA, and although its prevalence differs between subtypes, it can be seen in as many as 25–30% of anti-nuclear antibody (ANA)-positive patients with oligoarticular JIA[63,64]. CAU presents completely asymptomatically, so it must be actively screened for, and it is common in all JIA subtypes except for systemic JIA and rheumatoid factor-positive polyarticular JIA. In adults, acute uveitis is usually symptomatic (with pain and red eye) and largely limited to the spondyloarthropathies. Acute uveitis can also develop in adolescents with spondyloarthropathy. Known risk factors for the development of CAU in patients with JIA are JIA subtype (patients with oligoarticular JIA are at highest risk), younger age at disease onset and the presence of ANAs[63]. Evidence has linked JIA-associated uveitis with specific HLA haplotypes (*HLA-DR5* and *HLA-DRB*1104*)[65,66], whereas other HLA haplotypes (*HLA-DR1* and *HLA-DQA*0101*) seem to be protective. Although B cells are generally considered not to have a central role in the pathogenesis of JIA, results from transcriptomic and proteomic analysis of iris tissue and aqueous humour suggest the presence of disease mechanisms involving B cells[67]. Appreciation of specific disease manifestations or even disease subtypes has resulted in stratified clinical trials in JIA[68]. Although CAU associated with JIA is different from acute anterior uveitis that is seen in adult spondyloarthropathies, it seems for both comorbidities that adalimumab and other anti-TNF monoclonal antibodies are preferable to etanercept (a TNF-binding receptor fusion protein), which indicates the potential for the development of targeted treatment modalities[69].

Apart from uveitis, other symptomatic comorbidities in adults accumulate over time and substantially influence arthritis. Psoriasis is a hallmark of PsA. With a prevalence of 6–42% among patients with psoriasis, PsA is the most frequent comorbid condition that is associated with psoriasis[70]. In most cases, the skin disease precedes the articular disease. Importantly, pathogenic pathways governing psoriasis and PsA do not fully overlap, and some differences (such as the discrepancies between the clinical responses to DMARDs) may occur as a result of tissue-specific responses[71,72]. However, leakage of immune deregulation from the skin to the joint is suspected[73], potentially involving lymphocyte subsets such as $CD8^+$ T effector memory CD45RA cells[74] and $CD8^+$ $CCR10^+$ T cells[75]. DNA methylation patterns of $CD8^+$ T cells can differentiate patients with psoriasis from those with PsA, suggesting the potential for early patient stratification for therapy[76]. Another comorbidity in adult chronic arthritis is an increased cardiovascular risk. Psoriasis is also associated with ischaemic heart disease, cerebrovascular and peripheral vascular disease, and with subsequent mortality[77–79].

Results from multiple studies suggest that psychological disorders influence the immune system and the course of autoimmune diseases. The occurrence of depression is a common comorbidity in adult patients with psoriasis[80], and the risk of developing psoriasis is higher in patients suffering from depression than in those without depression, suggesting a bidirectional interaction. The influence of depression on the development of arthritis is exemplified in RA, in which it is the most frequent comorbidity, and is responsible for a dramatic effect on quality of life[81,82]. In addition, results from population-based studies have shown that depression is associated with elevation of the risk of developing RA, with any protective role of antidepressant medication being unclear[83,84]. This association is not epidemiologically and pathophysiologically limited to adulthood[85,86]. Exposure to adverse childhood experiences is associated with the risk of reporting JIA, with an adjusted odds ratio of 9.4 (95% CI 4.0–22.1) for arthritis versus health in individuals with four or more adverse childhood experiences[87]. Current hypotheses to explain this association note the potential role of stress in the induction of pro-inflammatory cytokines and in insufficient glucocorticoid-related negative feedback on inflammation in adulthood[88,89].

Box 1 | **Main comorbidities associated with arthritis and their putative influence on arthritis course**

Smoking
- Increases the generation of lung citrullinated peptides that are targeted by anti-citrullinated-protein antibodies in patients with rheumatoid arthritis who carry HLA shared epitope alleles
- Modulates CpG methylation, with a potential functional impact

Adiposity
- Favours inflammatory polarization of immune cells

Uveitis
- Shared antigens and a role for B cells

Psoriasis
- Migration of arthritogenic $CD8^+$ T cells from the skin to the joint

Depression
- Increases pro-inflammatory cytokines and insufficient glucocorticoid-related negative feedback on inflammation

Gut microbiota deregulation
- Provides a pro-inflammatory trigger

Ageing
- Immune senescence reduces homeostatic functions
- Inflammageing increases production of pro-inflammatory cytokines

The immune-maturation hypothesis

Throughout early life, individuals face immunological challenges that shape the immune system, building on the base of the innate immune system[90] and

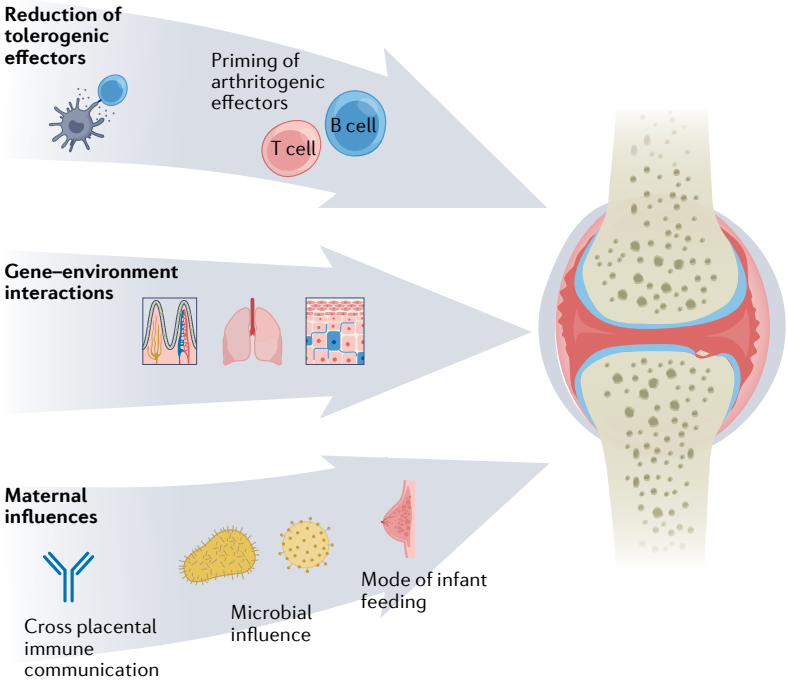

Fig. 2 | **Factors influencing immune-system maturation and arthritis development.** The adaptive immune system of the fetus and the child gradually acquires the ability to respond to specific antigenic challenges. By nature, the naive immune system harbours tolerogenic cells, including naive dendritic cells and regulatory T (T_{reg}) cells. Priming of the immune system reshapes the immune response and the predisposition to later inflammatory arthropathies. This influence starts in utero with exposure to maternal microbiota, and continues with breastfeeding and with environmental exposures. Altogether, these factors are responsible for large modifications of the microbiota during growth, especially in the gut, and for the subsequent activation of the immune system, potentially triggering the emergence of arthritis in children.

gradually acquiring and refining an adaptive immune repertoire[91,92]. This acquisition begins in utero and potentially shapes later autoimmune outcomes. The development of chronic arthritis in humans probably involves multiple potential triggers both in the joint and in lymphoid organs.

Age-related features of synovial tissue. Little is known about changes affecting synovium and immune-cell surveillance of the joint during growth and ageing. Normal fetal synovium sampled postpartum (at term) is histologically similar to adult synovium[93]. In morphological analysis, normal juvenile synovium seems to be similar to adult tissue[94]. Microarthroscopic and microscopic synovium assessment demonstrate that, compared with adult synovium, that of teenagers (15–19-years old) displays less 'roughness' of the whole tissue (fewer villi), with a regular vascular network, a preponderance of elongated and evenly distributed cells, and fewer macrophages. In addition, adipocyte content decreases with age[95]. These observations may, at least in part, explain features such as the differences in antibody-dependent and immune-complex-mediated involvement in synovitis and arthritis across the age spectrum[96,97]. From a molecular point of view, the autoantibodies in seropositive RA can result in immune-complex deposits and formations on cartilage, which can subsequently potentiate joint inflammation. This phenomenon might be facilitated in cartilage of older people, which is theoretically more susceptible to complement-binding through loss of sialic acids in the surface section[97–99]. To date, no comprehensive comparison of the composition and specific phenotype of paediatric and adult synovial cells exists.

Maternal influences. The maternal imprint may affect the immune system of the child and bias its propensity to trigger immune arthritis (FIG. 2). This phenomenon is apparent at various levels, such as in the vertical transmission of immunity to specific pathogens (by the transplacental passage of maternal antibodies and immune cells)[100] and in the maternal influence on the child's microbiota. In the second trimester of pregnancy, human foetuses are exposed in utero to live bacteria, including staphylococci and lactobacilli, which can induce in vitro activation of memory T cells in lymph nodes[91], suggesting that in utero microbial exposure contributes to acquisition of T cell immune memory and priming before birth. Supporting the hypothesis that infections in pregnancy can have subsequent effects on immunity and predisposition to inflammatory disorders in offspring, evidence from a murine model has shown that maternally restricted and transient infection with attenuated *Yersinia pseudotuberculosis* is associated with elevation of numbers of T_H17 cells in the lamina propria of adult offspring, which have enhanced reactivity to microbiota and susceptibility to mucosal inflammation[101]. This tissue imprinting is dependent on IL-6, and in the presence of IL-6 it is independent of maternal microbiota.

Breastfeeding affects a child's microbiota[102,103]. Milk contains immunomodulatory components, such as secretory IgA, which shape the intestinal microbiota, favouring beneficial bacteria. Milk also contains cytokines, such as IL-6, IL-10, transforming growth factor β1 (TGFβ1) and TGFβ2, which affect the infant's microbiota[103]. Another potential mechanism for specific maternal influence on the offspring's risk of developing autoimmune responses involves maternal–fetal microchimerism, resulting from the bidirectional passage of fetal and maternal cells across the placenta. Studies of maternal microchimerism in relation to juvenile autoimmune diseases[104–106] have not yet demonstrated any contribution to a loss of tolerance, although evidence specific to JIA or RA has not yet been published.

Regulation of autoimmune responses. Naive T cells from healthy babies retain a propensity to become T_{reg} cells, while they develop the capacity to differentiate into T_H17 cells, until the age of at least 12 months. In this way, as immunity against pathogens develops[91], the immune system maintains a regulatory profile[107]. Moreover, besides naturally occurring thymus-derived T_{reg} cells[108], T_{reg} cells can be induced in the periphery from naive T cells in the presence of TGFβ[109].

Patients with JIA have T cell-compartment dysregulation. T cell diversity (especially in relation to T_{reg} cells) in patients with refractory JIA seems to be restricted when compared with healthy individuals[110], and conserved and pathogenic T cell clones can be found in both blood and synovial fluid[47,110,111]. Results from seminal studies that

followed the reconstitution of the T cell compartment after a 'hard reset' of the immune system in patients severely affected by JIA and undergoing autologous stem cell transplantation suggested functional renewal of T_{reg} cells and T cell receptor diversification as potential mechanisms underlying the beneficial effect of this treatment in a substantial number of the patients[112,113]. These observations suggest a role for a diverse (naturally occurring and induced) T_{reg} cell repertoire (early in life) in prevention of the development of chronic autoimmune responses (FIG. 2).

Microbiota in health and disease. The gut is the main interface of the immune system with the environment, and the gut microbiota affect health and disease. Its composition is subject to important inter-individual and intra-individual variability[114]. Notably, in conditions of systemic immune dysregulation, gut, respiratory and oral microbiota are largely interconnected with the immune system[115–117] (FIG. 2). From experimental models in chronic inflammatory bowel disease, it seems clear that the microbiota influence immune activation, but also that chronic inflammation in turn shapes the gut microbiota and contributes to dysbiosis[118]. In RA, evidence suggests that the transcriptome of cell infiltrate from highly inflamed synovial tissue reflects synovial macrophage activation by microbial (bacterial and fungal) sources, potentially originating from the gut[119]. Other results from studies in humans have emphasized the pronounced effect of age on microbiota, and the existence of a threshold between child and adult microbiota in the first years of life[120–123]. Such modifications may account for differences in the behaviour of the immune system in immune arthritis associated with ageing.

(Epi)genetic–environmental interactions. As discussed in relation to the 'critical window in (epi)genetics' hypothesis, human immune arthritis has a significant (epi)genetic component, which is suspected to be differentially modulated in adults and in children, but this component is not sufficient to explain all major age-related differences. In addition to genetic and epigenetic factors, multiple parameters may contribute to the differences between adults and children, such as (for example) parental circumstances, social class, lifestyle, nutrition and diet, development stage, pharmacology and cultural behaviours. These interactions are multidirectional and complex, and the molecular and clinical integration of these factors is not yet fully understood. However, age-related differences in disease expression suggest that the gene–environment interaction is unlikely to drive arthritis equally or similarly across the lifespan of an individual. Conceptually, priming of the immune system early in life must be one of the most important factors that affect autoimmune responses in immune-mediated arthritis.

Burden-accumulation hypothesis

This hypothesis centralizes the accumulated burden of both environmental and host-related factors in ageing and their consequences for shaping and refining immune responses, potentially resulting in chronic immune-mediated arthritis at different ages. Growing and then ageing are also associated with progressive joint 'priming', in which the experience of articular challenges (re) models the local tissue and potentially the articular immune response. This phenomenon results from mechanical stress or damage, microbial challenges or any other extra-articular influence, and it suggests a heterogeneity in the development of arthritis that depends on the baseline status of the joint ('naive' versus 'primed') (FIG. 3).

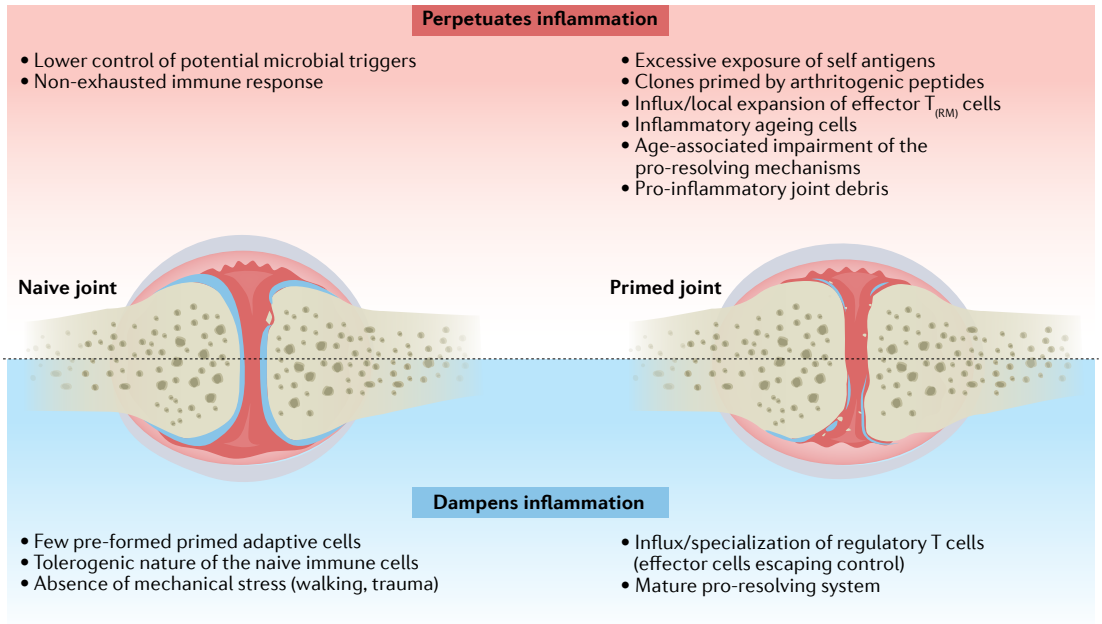

Fig. 3 | **Influence of prior joint priming on arthritis fate.** Proposed (age-dependent) factors determining arthritis 'heterogeneity', depending on the baseline status of the joint ('naive' versus 'primed'). In naive and in primed joints, some factors are presumed to have a dampening influence on joint inflammation, and similarly, in naive and in primed joints, other factors are thought to have a perpetuating influence on inflammation. $T_{(RM)}$ cells, tissue-resident memory T cells.

Oestrogens. Host-related factors participate in the generation of an inflammatory burden in IMIDs. Women usually have stronger adaptive immune responses than men and greater susceptibility to autoimmunity, as observed after puberty in relation to RA and SLE[124]. This sex bias is not observed in enthesitis-related arthritis (ERA)-JIA or its adult counterpart spondyloarthritis. Underlying mechanisms for the sex bias involve the sex steroid hormone 17β-oestradiol (E2), acting through oestrogen receptor-α (ERα) in plasmacytoid dendritic cells to enhance the type I interferon response to activation of TLR7 and TLR9, so that this response is stronger in women than in men[125]. Oestrogens also enhance the $CD4^+$ effector T cell response[126] and increase B cell activation and autoantibody production[127]. Notably, the *TLR7* gene is located on the X chromosome. In women, one of the two X chromosomes in each cell is physiologically inactivated. However, as recently demonstrated, *TLR7* can escape from X-inactivation in B cells and myeloid cells in women and individuals with Klinefelter syndrome (47, XXY)[128]. This biallelic *TLR7* expression is functionally responsible for enhancement of TLR7-dependent B cell proliferation and immunoglobulin class-switching. These observations might explain female susceptibility to SLE and potentially to other IMIDs.

Obesity and autoimmune inflammation. Obesity is a relevant comorbidity with the potential to contribute to chronic inflammation, as a result of the accumulation in adipose tissue of pro-inflammatory immune cells such as M1 macrophages, mast cells, neutrophils and $CD4^+$ and $CD8^+$ T cells[129]. The involvement of some of these cell types in IMIDs raises the question of whether common or intersecting pathways are associated with obesity and IMIDs.

The association of psoriasis and PsA with obesity suggests the existence of a causal relationship. Notably, a genetic risk score incorporating 97 SNPs can account for ~2.7% of variation in adult BMI[130]. The relationship between BMI and psoriasis was analysed in 753,421 patients from the UK Biobank and the Nord-Trøndelag Health Study[131], using a Mendelian randomization approach based on the 97-SNP genetic risk score as an optimized surrogate for BMI. This analysis showed that a 1 kg/m^2 increase in BMI is associated with a 9% increase in the odds of psoriasis. This causality link was unidirectional, with no strong evidence of an effect of psoriasis genetic risk on BMI. However, a limitation of this approach was that BMI SNPs are a stronger instrument for adult BMI than for child BMI[132]. Genetic background and biological pathways involved in adiposity and obesity only partly overlap between adults and children. Thus, direct transposition of adult analysis tools to childhood is still not straightforward.

The differential effects of smoking. The relationship between smoking and *HLA-DRB1* SE alleles is a well-described gene–environment interaction with regard to the development of ACPA-positive RA[133,134]. The current dogma is that individuals carrying *HLA-DRB1* SE alleles are dose-dependently predisposed to developing a rheumatoid self-reactivity to citrullinated peptides[12], which are more prominent in lungs of smokers than in those of non-smokers. Another major genetic risk factor that is associated with ACPA-positive RA is the *PTPN22* polymorphism encoding the R620W substitution. Data from the EIRA, North American RA Consortium and Leiden Early Arthritis Clinic studies have highlighted complex gene–gene (*HLA-DRB1* SE–*PTPN22*) and gene–environment (*HLA-DRB1* SE–smoking and *PTPN22*–smoking) interactions. Notably, these interactions are associated with seropositive, but not seronegative, RA[135].

An illustration of how environmental factors can act upstream of gene regulation and disease phenotype is epigenetic modulation induced by smoking. Results of a meta-analysis of genome-wide DNA methylation highlight the association between smoking and DNA methylation[136]. Smoking can either increase or reduce CpG methylation. CpGs in DNA derived from whole blood, $CD4^+$ T cells or monocytes are differentially methylated in current versus never smokers. This pattern of alteration persists in former smokers (defined as having stopped ≥12 months prior to the blood test) within 5 years of stopping smoking for most CpGs. A minority of altered CpGs persist 30 years after smoking cessation, and some of these CpGs are associated with genes that are potentially involved in RA pathogeny (such as *AHRR*)[137–139]. Interestingly, enrichment in the smoking methylation phenotype is also observed in genome-wide association studies in RA, osteoporosis and inflammatory bowel disease[140]. These findings suggest a functional relevance of smoking-related CpG methylation patterns in arthritis, especially in RA.

Microbiota. Microbiota affect many aspects of human physiology, and their composition varies throughout the lifespan of an individual. The first 3 years of life are critical for mature microbiota constitution, and might represent a window of opportunity for microbial modulation[120–122]. In adulthood and old age, diet, lifestyle and medication affect the microbiota[123].

Microbiota are heritable from the mother, and this vertical transmission is influenced by the birth mode[141]. Other factors that affect microbiota transmission include maternal health status and lifestyle during pregnancy, antibiotic use around birth, geographical location, family environment, type of feeding, duration of lactation and use of antibiotics in infancy[142]. Microbiota composition varies according to infant body sites and age[143]. Importantly, inheritance of particular species can affect later pathological outcomes such as the development of obesity (with specific changes in *Christensenella minuta, Akkermansia muciniphila, Methanobrevibacter smithii* and *Blautia* spp. operational taxonomic units)[144]. Disturbances in the microbiota early in life might be a risk factor for the development of autoimmune responses and disease later on. Results from two large case–control studies (in the UK and Finland) have identified increased risk of the development of JIA in children with cow's milk allergies[145] and in children with antibiotic use early in life[146,147]. The association with antibiotic use was dose-dependent, had the strongest association with antibiotic use in the first 12 months of

life and did not substantially change when adjusted for the number and type of infections. In a Dutch–Italian inception-cohort study of patients with new-onset JIA, signs of dysbiosis were found in the gut microbiota of patients with JIA relative to healthy individuals (siblings or age-matched, sex-matched schoolmates)[148].

The human virome (as part of the microbiome) clearly represents key modulators of human health[149,150], especially for the emergence of autoimmunity, as exemplified by the association between Epstein–Barr virus (EBV) and the onset of multiple sclerosis[151]. The proposed mechanisms for EBV-driven development of multiple sclerosis are molecular mimicry, B cell transformation and induction of B cell trafficking to the central nervous system[152]. The association with EBV might also be applicable to autoimmune arthritis, such as RA. The antibody response to EBV (IgG against the viral early antigen) is specifically elevated in the serum of patients with preclinical RA, and its increase correlates with rheumatoid factor seroconversion[153], suggesting an EBV reactivation in preclinical RA and a role for RA development.

From development to senescence

Modifications in the behaviour of the immune system are likely to account, at least in part, for differences between adult and childhood IMIDs. In the first year of life, naive T cells will encounter a large variety of non-self antigens as they are exposed to an increasing variety of both food antigens and pathogens (as they go through their first infectious episodes). The developing immune system in healthy individuals has developed mechanisms to avoid autoimmune responses and aberrant response to non-pathogenic non-self antigens.

Immunosenescence. During ageing, the immune system undergoes profound changes in a process called immunosenescence, which is responsible for susceptibility to infection, impairment of antivaccine responses, a weakened defence against malignancies and impaired wound repair[154]. At the cellular level, immunosenescence is associated with reduced maintenance of immune and haematopoietic cells by haematopoietic stem cells, the output of which is reduced in diversity and functionally biased[155]. In a longitudinal study of blood samples from 135 healthy adults over a period of 9 years by mass cytometry, dynamic changes in circulating immune-cell subpopulations were assessed, enabling identification of a gene set, the expression of which correlated with scores for the trajectory of immune ageing (IMM-AGE)[156]. The IMM-AGE score, which was a summary of the rates of change of numbers of immune cells in an individual, correlated functionally with all-cause mortality, suggesting that it is a better measure than chronological age for assessment of immune ageing.

RA is associated with several extra-articular features that shape (or reshape) the immune response. These features include premature immunosenescence, which might result from genomic instability linked to DNA damage, telomere shortening, impaired autophagy and protein homeostasis, mitochondrial dysfunction (with mitochondrial DNA mutations and production of reactive oxygen species) and/or stem cell exhaustion[157]. In the context of intense rheumatoid expansion of the immune cells (also termed advanced replicative stress), all these mechanisms merge to lead to a premature immunosenescence with altered function of leukocytes (especially T cells)[158]. Interestingly, in cohorts with suspected early RA, individuals with younger and older age of onset differ in terms of genetic background, clinical presentation and prognosis. Older age at RA onset is usually associated with lower rates of positivity for rheumatoid factor and ACPA, lower rate of remission, higher radiographic progression and lower functional score during follow-up[159,160]. These differences may be partly explained by immunosenescence.

Viral stimuli have long been suspected to trigger arthritis, especially RA. In a gerontological context, human cytomegalovirus (HCMV) infection accelerates some features of immunosenescence, notably by promoting the expansion of senescent T cells ($CD28^-$)[161,162]. Interestingly, in addition to immunosenescence, RA is associated with age-related diseases such as cardiovascular disease. HCMV and HCMV-specific T cells are also involved in cardiovascular disease, a typical age-related condition[163,164]. More studies are necessary to understand the role of chronic viral infection during clinical progression in RA[165–167]. Seroprevalence for HCMV infection ranges from 40% to almost 100%, with higher prevalence associated with female gender, low socioeconomic status and specific geographic locations (such as South America, Africa, Asia and the Middle East)[168–170]. Moreover, HCMV seroprevalence increases with age, with estimated positivity of 21.5% in children at ages 1–2 years and 32.0% at ages 14–17 years[171]. Given its ability to establish a lifelong latent infection, and to reshape the immune response for immune escape, a reasonable hypothesis is that HCMV represents one of the triggering factors for chronic inflammatory arthritis. Differential effects on T cell effector subsets are observed in patients with JIA and in healthy children following HCMV infection[172].

Inflammageing. Ageing immune cells contribute to age-related pathological conditions such as osteoarthritis, atherosclerosis and neurodegenerative diseases. In addition, the pro-inflammatory burden, carried by ageing cells and tissues, and resulting in age-related chronic comorbidities (also termed inflammageing), occurs earlier in IMIDs such as RA[173,174]. This phenomenon, extensively reviewed previously[175], is associated with high levels of pro-inflammatory cytokines, especially IL-6, and might contribute to the differentiation between adult disease and the onset of disease in children, in whom the accumulated inflammatory burden is likely to be a lot less.

Conclusions

Improved understanding of age-related mechanisms shaping the immune system could help to bring together adult and paediatric perspectives on the development of immune-mediated arthritis. In this Review, we have discussed several age-dependent physiological mechanisms that modulate disease presentation, by presenting

three (not mutually exclusive) hypotheses. These mechanisms are potentially synergistic and are likely to explain some of the differences and commonalities between age groups. By defining these hypotheses, we aim to encourage a more unified view of the diagnosis and treatment of both childhood and adult arthritis. Notably, other Reviews have discussed the genetic classification of primary arthritis[9,26], supporting the premise that distinct biological signatures will enable the partitioning of patients into groups that are amenable to mechanism-based intervention[15]. New classification systems for juvenile and adult inflammatory arthritis can help to unite clinical perspectives. However, classifications do not (and are not meant to) explain what lies beneath clinical phenotypes. As discussed here, knowledge of the underlying genetic influences certainly helps to clarify important risk factors in IMIDs, but it does not tell the full story. Further understanding is necessary of how, against a specific genetic background, the immune system interacts with the environment, leading to epigenetic changes and the ensuing autoimmune response. In this paradigm, age is an important, but mostly overlooked, factor. When and how an environmental trigger activates the developing immune system determines the ultimate immune response. The first step in unravelling this complex, multidimensional process would be to build a comprehensive framework to describe the development of adaptive and innate immunity over time, as was done long ago for normal growth and development[176]. Although such an endeavour would be complex and challenging, the rewards would be considerable, in terms of better understanding of the emergence of IMIDs, and possibly also the identification of the potential for truly innovative interventions. Following on from studies of genetics, epigenetics and environmental interactions, inflammageing could be the next frontier in inflammatory arthritis. Whatever else, the hope is that the molecular medicine revolution can provide effective solutions for the treatment of IMIDs in young and old alike.

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Sex- and gender-related differences in psoriatic arthritis

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Abstract | Psoriatic arthritis (PsA) is an inflammatory musculoskeletal disease with a chronic, progressive course. Various aspects of PsA, including its clinical features, disease course and response to treatment, are influenced by sociodemographic characteristics of the patient. This includes patient sex, the biological attributes associated with being male or female, and gender, a sociocultural construct that comprises attitudes, traits and behaviours associated with being a man or a woman. An understanding of sex- and gender-related differences in PsA, as well as their underlying mechanisms, is therefore important for individualized care. In this narrative review, the influence of sex and gender on PsA manifestation and course, patient function and quality of life, and their association with comorbidities are described. Sex- and gender-related disparities in response to advanced therapies and their potential underlying mechanisms are delineated. Differences in pathophysiological mechanisms between male and female patients including genetics, immune and hormonal mechanisms are discussed. Finally, fertility and pregnancy outcomes in PsA are outlined. By adopting sex and gender lenses, this review is aimed at highlighting key differences between male and female patients with PsA and uncovering mechanisms underlying these differences, ultimately promoting individualized care of men and women with PsA and informing future research in this area.

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Psoriatic arthritis (PsA) is an immune-mediated inflammatory musculoskeletal disease with a chronic, progressive course, resulting in joint destruction and disability in severe cases. PsA develops in up to a third of the patients with psoriasis[1], a common inflammatory skin disease that affects 1–3% of the general population[2]. Ever since Moll and Wright described PsA as a unique category of inflammatory arthritis in 1973 (REF.[3]), the clinical presentation, disease course, risk factors and pathophysiological mechanisms of PsA have been explored in great detail[1]. With a better understanding of the disease came the development of novel treatment options and outcome measures to assess treatment efficacy[1]. Furthermore, investigations into the distinct domains of PsA, such as pain and fatigue, as well as the involvement of patient research partners in clinical research, has led to a more wholesome understanding of how the disease impacts daily function and quality of life. Notably, the accumulating scientific knowledge of PsA has revealed several differences between men and women, which might have implications for understanding how the disease is managed by health care providers.

PsA affects male and female patients in different ways, giving rise to sex- and gender-related differences in clinical presentation, disease course and response to treatment. Sex and gender are often used interchangeably in the medical literature, but their meanings are distinct and have important implications for understanding the mechanisms of disease differences between men and women (BOX 1). Sex refers to biological characteristics associated with being male or female, a straightforward measure that is often considered in medical research. On the other hand, gender is a sociocultural construct and refers to social norms and experienced dimensions of 'femaleness' or 'maleness' that determine roles, relationships and positional power in society[4,5]. Assessment of gender-related attributes, such as risk-taking behaviour and social support, is complex and thus often not considered in patient registries or clinical trials. Instead, the absence of gender dimension data results in a simplified classification of 'males' and 'females' in studies that have assessed sex/gender disparities in PsA, missing opportunities to disentangle the effect of biological sex from that of sociocultural gender.

The impact of sex and gender on various aspects of PsA is modulated by sociodemographic characteristics of the patient, including age, race or ethnicity and socioeconomic status (SES), all of which are important determinants of health (FIG. 1). For example, the higher prevalence of osteoarthritis in older female patients[6]

Key points

- The majority of studies show that peripheral arthritis is more common in female patients with PsA, whereas axial disease and severe psoriasis are more common in male patients, emphasizing the need for specific sex-gender considerations in diagnosis and treatment selection.
- Female patients with PsA are less likely to develop radiographic damage in axial and peripheral joints than male patients, and therefore require more sensitive imaging modalities (such as MRI and ultrasound) for diagnosis.
- Higher levels of pain, fatigue, poor functional status and worse quality of life in female patients with PsA have been reported compared with male patients. Women with PsA might require psycho-social support, occupational modification and targeted pain management.
- Female patients with PsA are at a higher risk of discontinuing or switching advanced therapy than male patients, thereby necessitating closer monitoring after drug initiation.
- Parameters of PsA disease activity improve during pregnancy and flare up during the postpartum period, emphasizing the need for close monitoring after pregnancy.

shifts the diagnostic suspicion away from PsA, thereby delaying diagnosis and resulting in poor disease outcomes. Moreover, SES is influenced by sex of the patient, with women often belonging to a lower SES than men[7]. A Canadian study reported that patients of high SES were more likely to visit rheumatologists, whereas those of low SES were more likely to visit family physicians for care of their arthritis[8], despite rheumatologists being more experienced in diagnosing arthritis. Thus, by virtue of common differences in SES, male patients might be diagnosed in a timelier fashion than women.

Incorporating both sex and gender dimensions in PsA research and analysing outcomes through a sex–gender lens is needed to better understand the underlying mechanisms driving differences between men and women with PsA. Such an approach might help to diagnose patients earlier, inform treatment guidelines and address the gaps in care of men and women. Moreover, delineating sex- and gender-related disparities is a further step to achieving the goal of personalized medicine with implications for clinical, scientific, social and economic spheres. This Review presents a comprehensive overview of the influence of sex and gender on the clinical presentation, disease course and treatment response in PsA, as well as to discuss sex-related differences in its underlying pathophysiological mechanisms. First, we review the literature concerning sex and gender differences in epidemiology, clinical and imaging features, comorbidities and response to treatment in PsA. Then, we discuss potential sex- and gender-related mechanisms underlying these differences, and summarize the relationship between PsA and the reproductive system. Finally, we provide suggestions for future PsA research to focus on sex and gender.

Box 1 | **Sex and gender**

- Sex refers to a set of biological attributes that encompass physical and physiological features, including chromosomes, gene expression, hormones and reproductive anatomy. It is typically categorized as 'male' or 'female'.
- Gender refers to socially constructed norms that determine roles, relationships and positional power in any society. It is a complex construct comprising gender identity, gender roles, gender relationships and institutionalized gender[5].
- Sex influences disease inheritance, immune system dysregulation, pain processing and perception, and pharmacokinetics of drugs.
- Gender impacts disease outcomes by influencing the perception of illness, type of coping mechanisms, health care-seeking behaviour, access to care and the nature of treatment and interaction with care providers[195].

Epidemiology of PsA

PsA and psoriasis have marked variation in incidence and prevalence rates according to age, studied population and geographical area. The sex ratio in PsA is largely considered to be equal[1], although discrepancies exist in the literature. Although early studies tended to show male predominance of PsA, with male-to-female ratios ranging from 1.4 to 1.9 (REFS.[9–13]), a change in sex distribution with higher female predominance has been reported over the last decade, including in cohorts of PsA[14–23], both in the general population[14–20,23] and in disease registries of psoriasis[21,22], with female-to-male ratios ranging from 1.2 (REF.[16]) to 2 (REF.[21]) (Supplementary Table 1). Such a trend was shown in a recent epidemiological study of temporal trends in the incidence of PsA across five decades in a US population[11]. In that study, although an overall increase in incidence rates of PsA was found in both sexes, in more recent decades (2000–2017) overall incidence rates remained relatively stable in men but increased by approximately 3% per year in women. This contrasts with an equal rate of increase (4% per year) of PsA incidence in both sexes in earlier decades (1970–1999)[11]. A similar disproportionate increase in incidence rates of PsA among women during recent decades was reported in a Danish population from 1997 to 2011 (REF.[19]). Although the reasons behind such an increase are uncertain, it might reflect either a change in disease epidemiology or, perhaps more likely, an increased diagnosis among women. The latter possibility may be explained by the evolution of the classification criteria for PsA, which are now more sensitive than previous criteria, as well as the use of more sensitive imaging modalities that might better suit women. Possible differences in prescription patterns between men and women with psoriasis, which can affect subsequent risk of developing PsA, may also be a contributing factor.

Although less studied, there may also be differences in the age at onset of PsA between men and women, with PsA onset occurring later in women. A study of US adults reported peak incidence of PsA to occur in the fifth decade (ages 40–49 years) in male patients, whereas in female patients peak incidence occurred roughly a decade later[11]. A similar trend with potential earlier age at onset of PsA in males was reported using UK administrative data. Indeed, prevalence of PsA was found to be higher in males in the 30–49 age range, with male-to-female ratios ranging from 1.18 to 1.23, but this ratio was reduced to 0.84 to 0.94 in the 50–69 age groups[24]. Finally, in a Swedish study of early PsA, approximately 41% of women were diagnosed before

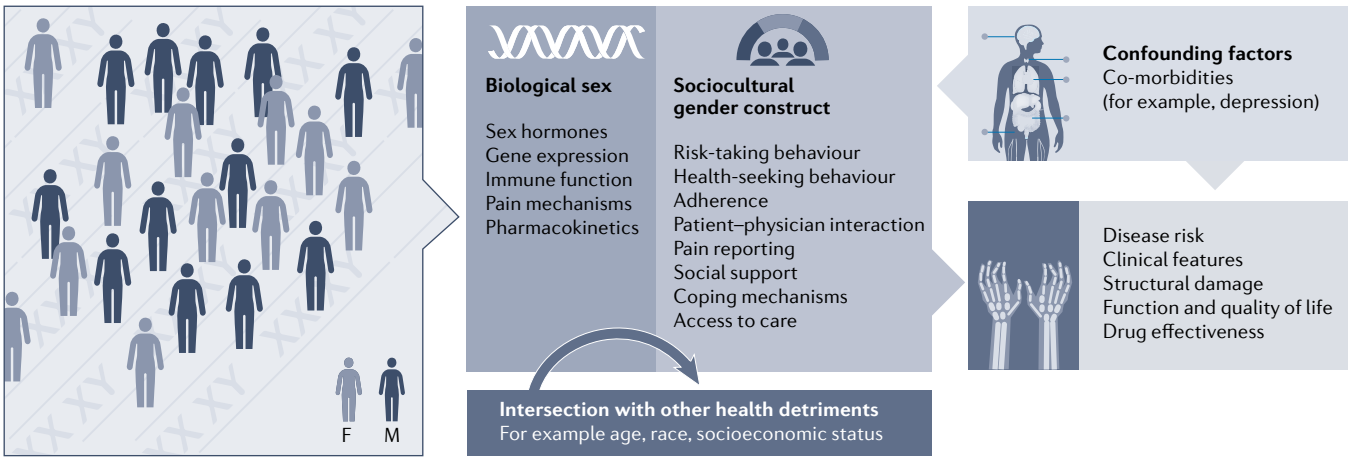

Fig. 1 | **Sex- and gender-related mechanisms and disease outcomes in PsA.** Biological sex can influence PsA progression by affecting sex hormones, gene expression, immune function, pain mechanisms and pharmacokinetics of medications. Gender, a sociocultural construct, also affects PsA, such as by influencing risk-taking behaviour, health care-seeking behaviour, adherence to medications, patient–physician interactions, pain reporting, social support, coping mechanisms and access to care. Other health determinants, such as age, race and socioeconomic status, interact with sex and gender to modulate disease course and characteristics. Moreover, both sex and gender interact to determine disease risk, clinical features, structural damage, function and quality of life, and the effectiveness of medications in PsA. Comorbidities, such as depression, have confounding effects on the relationship between sex, gender and disease domains.

the age of 45, whereas 55% of men were diagnosed by that age[25]. Thus, the above data show that reported cases of female PsA is increasing, perhaps due to improved diagnostics, and that men have an earlier age at onset of the disease.

Sex differences in clinical features

Musculoskeletal involvement

The clinical presentation of PsA is strongly influenced by the sex of the patient[25–29] (Supplementary Table 2). Our group studied sex-specific differences in clinical and radiographic features among 345 men and 245 women with PsA[26]. Female patients predominantly presented with oligoarthritis and polyarthritis, whereas male patients more commonly presented with oligoarthritis. Moreover, the prevalence of axial involvement, defined by radiographic sacroiliitis, was higher in male patients (43% in men versus 31% in women; odds ratio (OR) 1.8). Such sex-specific differences might be related to the difference in age of peak prevalence between men and women; indeed, given that joint involvement increases over time in PsA[23,30], the later age of peak prevalence in women might allow for more joint involvement and presentation with polyarthritis. In a Turkish study of over a thousand patients with PsA, 69% of female patients presented with peripheral arthritis compared with 59% of male patients; only 9% of female patients had axial disease versus 11% of male patients[27]. On the other hand, no sex differences in axial involvement were reported in other studies in patients with severe psoriasis[31] and PsA[32]. Instead, tender and swollen joint counts were reported to either be higher in women[28,29] or similar in both the sexes[26,27]. Evidence of sex-related differences in enthesitis and dactylitis is conflicting, with some studies reporting no differences between male and female patients[26–29] and others reporting a female predominance of enthesitis[33–35] and dactylitis[34]. Overall, male and female patients with PsA show some differences in clinical features of the disease, which may be due to sex differences in immune mechanisms and its interaction with external environmental factors.

Extra-articular manifestations

A remarkable feature of PsA is the extra-articular manifestations (EAMs), predominantly consisting of cutaneous, ocular and gastrointestinal features, all of which are thought to share common immunopathogenic mechanisms with PsA[36]. Of the various EAMs, sex-related differences in the most common EAMs — psoriasis, anterior uveitis and inflammatory bowel disease (IBD) — are summarized below.

Psoriasis and psoriatic nail disease. Psoriasis is considered to be one of the core domains of PsA[37] and its activity should be evaluated in every patient. Most studies report greater severity of psoriasis in men than in women[2,28,29,34,38,39]. For example, a Swedish cohort study from 2017 found that women had significantly lower Psoriasis Area Severity Index scores, a measure of psoriasis severity[39]. A similar finding was observed in studies of large patient registries of female patients with psoriasis and PsA who begin biologic medications[40–42]. Like psoriasis, psoriatic nail disease, which affects up to 80% of patients with PsA[43], has an 11–12% higher prevalence in men than in women[26,38]. Interestingly, despite the generally greater psoriasis severity in men, female patients with PsA seem to report a worse impact of psoriasis on their health-related quality of life[2,22,44], highlighting the different effect that gender has on disease impact, which may be influenced by issues such as body image.

Uveitis. Uveitis is an inflammatory disease of the eye that is sometimes associated with PsA, with studies reporting rates of up to 25%[45]. Compared with spondyloarthritis (SpA), uveitis in PsA tends to be more insidious in onset and can be chronic, often affecting different regions of

the eye. A retrospective study of 71 patients with PsA detected a slight predominance of uveitis in women compared with men[46]. Moreover, the prevalence of uveitis seems to be linked to disease phenotype. In a small study of 16 patients with uveitis and PsA, it was found that patients with axial disease and uveitis were more likely to be male, whereas patients with peripheral disease and uveitis were more likely to be female[47]. Of note, patients with psoriatic axial disease are at an 8- to 17-fold increased risk of developing uveitis, possibly because of the association of HLA-B27 antigen with both axial disease and uveitis[46,48]. The predominance of psoriatic axial disease in men may result in a parallel predominance of uveitis. Interestingly, one study reported a higher prevalence of ocular manifestations in male patients (OR 1.89 versus female patients; 95% CI 1.09–3.30), although conjunctivitis was the most common form of ocular involvement, with uveitis accounting for only a third of the ocular cases[49]. However, a population-based Swedish study of 22,667 patients with PsA reported no excess of uveitis in men or women[50]. Overall, no clear sex-related susceptibility was found for uveitis among patients with PsA.

Inflammatory bowel disease. Patients with PsA have a high risk of IBD, although studies reporting sex-specific relative risk have shown conflicting results. One study reported an increased incidence of Crohn's disease in female patients with PsA (incidence rate ratio [IRR] 4.16), whereas the incidence rate in men with PsA was significantly lower and similar to the general population[51]. By contrast, the incidence rate for ulcerative colitis was similar in women (IRR 2.64) and men (IRR 2.08) with PsA, and both these rates were significantly higher than the general population[51]. Moreover, conflicting with these results, a Swedish population-based study from 2021 found no sex difference in prevalence or incidence of IBD in PsA[50]. Discrepancies between findings might be due to differences in case definitions or environmental and genetic differences across populations.

Functional status and quality of life

Patient-reported outcomes in PsA have consistently shown a sex difference (Supplementary Table 3), with women often experiencing greater pain, fatigue, functional disability, and worse quality of life with and without axial disease[26–29,34,35,52–55]. For example, pain in the metatarsophalangeal joints is associated with female sex (OR 2.07; 95% CI 1.20–3.58)[56]. However, conflicting results have been reported. Studies from Singapore reported no sex difference in fatigue[57] and no association of sex with physical function measures[58]. Although unclear, these findings may be specific to the Asian population or might be explained by the particular measures of fatigue under investigation. Work disability associated with PsA has also been found to be greater in women than in men[59–61] (Supplementary Table 3). In a Norwegian study of 271 patients with PsA, 33% of female patients were work disabled compared with 17% of male patients[60]. Involvement of peripheral joints of the hands and feet, higher levels of pain, fatigue and physical limitations could all contribute to the higher work disability in women with PsA. Moreover, several factors, both biological and sociocultural, possibly contribute to worse pain scoring in women, as discussed below. Finally, it is important to consider whether subjective measures of disease activity are related to manifestations of fibromyalgia, which is more commonly associated with female sex[62], rather than PsA; this area remains to be explored.

Patient–physician interaction

Discrepancies between physician and patient assessments of PsA disease activity are not uncommon. Often, patients will give a higher estimation of disease activity compared with physicians[63], which can have an impact on therapeutic management, such as by delaying treatment initiation and escalation, as well as by having a negative effect on treatment retention rates and remission[64]. The extent to which physician–patient assessment discrepancies are mediated by sex or gender factors remains to be determined. Conflicting effects of sex and gender on patient–physician discrepancies have been reported in the literature, including both positive associations[63] and null associations[65,66]. Sex and gender of the physician also influence access to health care services and, thus, may potentially affect disease outcome. Although not specifically studied in PsA, a study investigating access to rheumatologists in patients with rheumatoid arthritis (RA) found that patients of male family physicians were less likely to have a timely rheumatology consult[67]. Yet, the sex of the physician was not associated with physician–patient discrepancies in assessment in another study[63]. One potential explanation for this sex discrepancy is that male physicians might have more confidence in managing RA in primary care, and patients may have more confidence in male physicians, making them less likely to seek specialty care. Such influences of physician's sex and gender remain to be explored in PsA.

Biomarkers of PsA

Even though there is no specific biomarker for reliably screening or diagnosing PsA[68], numerous studies have explored possible biomarkers; however, by and large, these studies failed to report sex disaggregated results. In day-to-day rheumatology practice, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are commonly assessed, as these acute phase reactants are markers of active inflammation and poor prognosis[69]. Higher ESR values in female patients with PsA compared with male patients have been reported in several studies[28,29,34,53], but this finding could be offset by the fact that normal physiological ESR levels are higher in women than in men[70]. On the other hand, only a single PsA study reported that elevated CRP levels occurred more frequently in men than in women (45% vs 34%)[35], although this finding was not corroborated in other studies[28,29,53]. Future work is needed to clarify whether sex influences levels of commonly used inflammatory biomarkers and to identify new biomarkers that accurately reflect disease activity in male and female patients with PsA.

Autoantibodies have been evaluated in biologic-naïve patients with PsA. Although anti-nuclear antibodies and antibodies to extractable nuclear antigens

occasionally occur in PsA, no differences between sexes have been observed[71].

Imaging

Musculoskeletal imaging is frequently used to diagnose PsA, to monitor treatment response and to assess disease progression. In the following section, sex-related differences in radiographic, MRI and sonographic features of PsA are discussed (Supplementary Table 2).

Radiography

Compared with female patients, male patients with PsA often have more severe radiographic structural damage in both axial and peripheral joints, and greater progression of damage, both in early and established disease[26,52,72]. Female patients are more likely to have non-erosive disease and lower degrees of structural damage[26,52,72]. One study reported that men were more likely to develop grade 3 or 4 sacroiliitis (28.8% in men versus 17.6% in women), syndesmophytes in the cervical, thoracic and lumbar spine (10.3% to 19.1% in men versus 2.9% to 6.2% in women) and higher radiographic damage scores in peripheral joints (OR 1.6) than women[26]. A study from the Swedish Early Psoriatic Arthritis registry investigated radiographic progression in a cohort of patients with early PsA over the course of 5 years and found that male patients had more severe radiographic damage in peripheral joints than female patients, both in early disease (Wassenberg score 3.2 in men versus 1.6 in women) and in established disease (Wassenberg score 8.0 in men versus 3.4 in women)[52]. The odds of accruing joint damage were four times higher in men than in women (OR 4.1; 95% CI 1.2–13.8) and joint damage in men was strongly associated with dactylitis[52]. Last, a study that examined an Ibero-American SpA cohort found significantly higher spinal damage scores in male than in female patients with PsA (BASRI score 7.2 in men versus 4.8 in women)[72]. One possible explanation for the greater structural damage in male patients is their involvement in high-impact occupational and recreational physical activities, which may result in greater biomechanical stress and the 'Deep Koebner' phenomenon[73], whereby physical trauma triggers inflammation in musculoskeletal structures.

MRI

Limited information exists regarding MRI spinal abnormalities in PsA, although other types of arthritis have been studied in this context. Multiple MRI studies of axial SpA, including psoriatic spondylitis, show male sex to be associated with sacroiliitis[74,75] and spondylitis[75]. Studies of the SPondyloArthritis Caught Early (SPACE) cohort reported that 30% of male patients with axial SpA had sacroiliitis on MRI, compared with 17% of female patients[74]. The DESIR cohort of patients with inflammatory back pain suggestive of SpA reported 59% of men with sacroiliitis on MRI compared with 40% of women and 35% of men with spondylitis on MRI versus 18% of women[75]. An analysis of MRI axial features in 93 patients with PsA identified male sex to be the only factor associated with MRI spondylitis (OR 6.9; 95% CI 1.4–33.6)[76]. However, diagnostic pitfalls must be taken into consideration when analysing MRI data for axial involvement. For example, MRI of the sacroiliac joints in women may show bone marrow oedema consistent with the Assessment of Ankylosing Spondylitis (ASAS) definition for sacroiliitis in the postpartum period[77]. Structural lesions, such as fatty infiltration and erosions, are uncommon in these situations and can help to differentiate post-partum changes from true sacroiliitis[78]. Overall, the sex of the patient influences the prevalence and pattern of MRI abnormalities in SpA including PsA, which can have significant implications for patient care.

Ultrasound

Ultrasound is used predominantly to assess PsA disease activity and damage in peripheral joints, tendons and entheses[79]. It is particularly useful in the case of enthesitis, as its clinical assessment is subjective and relies on eliciting tenderness at entheseal regions. Importantly, such an examination is affected by the patient pain threshold, a limitation that might promote apparent sex differences, given that fibromyalgia, which is more commonly found in women[80], can lead to higher measures of tender entheses in women with SpA[78,81] and PsA[34]. In contrast, ultrasound studies of enthesitis, which involves the detection of inflammatory and structural lesions, have reported higher enthesitis scores in male patients. In a study comparing ultrasonographic scores of enthesitis between 79 patients with ankylosing spondylitis and 85 patients with PsA, inflammation scores in men were significantly higher than in female patients (12.3 in men and 8.9 in women)[82]. In support of these findings, a recent study examining the correlation between clinical and sonographic indices of enthesitis in PsA reported male sex to be independently associated with sonographic damage and female sex to be associated with clinical enthesitis scores[83].

Diagnosis of PsA

As there is no single diagnostic test for PsA, its diagnosis is based on a combination of clinical findings suggestive of musculoskeletal inflammation in the context of a personal or family history of psoriasis. Laboratory tests and imaging may, on occasion, follow up this assessment to establish the diagnosis[1]. Numerous sex- and gender-related factors contribute to difficulties in PsA diagnosis. In particular, several factors are more common in women with PsA, such as presentation with vague complaints of pain and fatigue[26,84], clinical features overlapping with those of osteoarthritis[85], RA[86] and fibromyalgia[62,87], all of which are female-predominant conditions[88–90]. Additional features that are more common in female patients include the absence of PsA-related radiological changes in early disease, and the presence of radiological features related to pregnancy, such as bone marrow oedema in the sacroiliac joint in the postpartum period[77]. On the other hand, male patients have more objective evidence of radiographic damage[26] and more severe psoriasis[2], necessitating dermatology referrals, thereby providing an alternate route for rheumatology referral and diagnosis.

When considering gender-related characteristics, evidence suggests that women have a tendency to seek healthcare[91], whereas men tend to be more reluctant to

seek care[92]. Men are also more likely to ignore symptoms, have a high threshold for reporting pain, self-medicate, present to health care providers only when disease is severe or symptoms are intolerable, and lack a source of usual care, such as a family physician[92]. Women may navigate the health care system more successfully and with comparative ease, perhaps because of a more frequent interaction with the health care system for obstetric and gynaecological reasons. Furthermore, physicians also tend to misinterpret subjective complaints of pain and fatigue to be psychosomatic in female patients or to be related to mental health disorders, such as depression, rather than reflecting disease manifestations[92,93]. Thus, the higher age at diagnosis of PsA in women may not only indicate a biological difference between women and men, but may also suggest delays in diagnosis possibly because of the misinterpretation of early symptoms of PsA and delayed access to rheumatology care in female patients. To the best of our knowledge, this topic has not been specifically studied in PsA.

Comorbidities

PsA is associated with various comorbidities, in particular cardiovascular disease and related cardio-metabolic abnormalities, infections, mental disorders and osteoporosis[36,94]. Sex-related differences in comorbidities in PsA might reflect the general sex-specific disease susceptibilities rather than being specific to PsA.

Cardiovascular disease

PsA is associated with a higher risk of atherosclerotic cardiovascular disease (CVD), which has been associated with both higher prevalence of traditional cardiovascular risk factors and persistent systemic inflammation[95]. Similar to that seen in the general population[96], the increase in cardiovascular events in women with PsA is generally seen a decade later than men with PsA (7th decade in women and the 6th in men)[97]. Cross-sectional studies have reported that male sex is associated with major adverse cardiovascular events in PsA[98]. Indeed, CVD is the leading cause of mortality in PsA, similar to the general population[36]. A British study exploring mortality in patients with severe PsA reported that mortality from circulatory disease, especially coronary heart disease, is significantly higher in male patients than in the general population (circulatory disease: standardized mortality ratio (SMR) 2.24; 95% CI 1.03–4.27; coronary heart disease: SMR 2.80, 95% CI 1.13–5.78) but not in female patients[99]. No direct comparison of cause-specific mortality was made between male and female patients. Among non-traditional risk factors of CVD[36], ESR was found to be a significant predictor in women only[97]. Among the traditional risk factors for CVD, the prevalence of metabolic syndrome[100], diabetes mellitus[101] and hypertension[102] has been reported to be higher in women with PsA.

Although the effect of systemic therapies for PsA, including conventional and targeted DMARDs, on cardiovascular morbidity has been summarized in several meta-analyses, it is unclear whether the effect reported is modified by sex as these studies do not report sex-disaggregated results[103,104]. Future work in this area will be important.

Malignancy

Most studies that have investigated sex-related difference in rates of malignancies in patients with PsA have found no difference between men and woman[105–107]. However, one exception comes from a study investigating malignancy in patients with severe PsA requiring TNF inhibitors, which reported a higher risk of non-melanoma skin cancers in female patients (standardized incidence ratio (SIR) 2.41, 95% CI 1.10–4.58) than in the general population, but no such excess in male patients[99]. Thus, conflicting evidence exists regarding differences in cancer risk between male and female patients with PsA, and more research is needed to clarify this matter.

Infections

Although no sex-related differences in the type of infections have been reported, the risk of infection in PsA is significantly higher in women than in men[108,109]. In a population-based matched cohort study in the UK, female patients with PsA had more than double the incidence rate of opportunistic infections than male patients (incidence rate per 1,000 person-years: 37.1 in women; 95% CI 34.8–39.5; 13.6 in men; 95% CI 12.3–14.9)[108]. A Canadian study exploring the incidence and predictors of infections in patients with PsA similarly found that female sex is a predictor of infections rather than male sex (OR 0.47; 95% CI 0.36–0.61)[109], with the most common infections being of the lung, sinus, skin and the genitourinary system. Precisely why women are more likely to experience infections in PsA is unknown, but it might be influenced by both biological sex-related factors (such as sex differences in immune responses) and sociocultural gender-related factors (such as exposure to infectious agents through work, leisure activities or children)[110]. Future work is needed to gain insight into the underlying mechanisms.

Mental health conditions

Depression and anxiety affects more than 30% of patients with PsA[111]. Similar to the general population[112,113], the risk of anxiety and depression is higher in women with PsA than in men (adjusted OR for depression: 1.64–3.47)[98,111]. Importantly, depression and anxiety are associated with higher pain scores, worse quality of life and poorer responses to advanced therapies[114]. The higher prevalence of these comorbidities in female patients with PsA may therefore explain some of the sex differences observed in PsA clinical presentation and treatment outcomes.

Mortality

Studies examining sex differences in the risk of premature mortality in PsA have shown conflicting findings. A population-based study from Taiwan reported a significantly higher risk of premature mortality in both men and women with PsA than in the general population, with men tending to be at the greatest risk (SMR 1.53 in men; 1.32 in women)[115]. Similarly, two studies reported that men with PsA had a significantly higher mortality risk (SMR 1.41–1.75) than the general population, but not women with PsA[99,116]. On the other hand, SMR of female patients with PsA was significantly higher than in

the general population (SMR 1.96; 95% CI 1.14–2.77) in Hong Kong, but risk in male patients was not significantly different from that of the general population (SMR 1.40; 95% CI 0.89–1.90)[117]. Overall, conflicting evidence exists regarding the effect of sex on risk of premature mortality in PsA. Differences across studies may be related to differences in study design, case definition, as well as geographical and genetic differences across populations.

Treatment outcome

Some evidence exists of the sex-related disparities in prescription patterns and treatment response in PsA. Although in the general population women have a higher likelihood of receiving prescriptions for NSAIDs and opioids than men[118,119], to the best of our knowledge this pattern of prescription has not been specifically investigated in PsA. Even though there is no particular reason to expect otherwise in PsA, large population-based studies could best address this issue. Regarding the efficacy of conventional synthetic DMARDs in PsA, little work has examined whether sex-related differences exist in the efficacy of these agents in PsA[120]. Similarly, clinical trials on biologic DMARDs (bDMARDs) and targeted synthetic DMARDs do not typically report results stratified by sex[121,122].

Observational studies investigating the effectiveness of bDMARDs, predictors of treatment response and drug persistence rates of TNF inhibitors[123–140], secukinumab[141], ustekinumab[142,143] and apremilast[144], have consistently reported that female sex is associated with poorer treatment outcomes and lower drug persistence rates. This association is fairly consistent across the different phenotypes of PsA (peripheral versus axial disease, polyarticular versus oligoarticular disease) and across various measures of treatment response (such as American College of Rheumatology (ACR) 20/50/70 criteria; European Alliance of Associations for Rheumatology (EULAR) criteria; minimal disease activity (MDA) scores; and Disease Activity Index for Psoriatic Arthritis), both in the short term and in the long term (3 months to 10 years), irrespective of prior biologic exposure. An exception is a study from the Corrona Psoriatic Arthritis/Spondyloarthritis Registry, which reported no sex difference in achievement of MDA following biologic therapy[145]. The reason for this disparity is unknown. In multiple studies, the likelihood of response to TNF inhibitors in male patients with PsA ranged from 1.7 up to 4.48 (REFS.[135,146]) for MDA, 1.5 to 2.2 for EULAR good response[127,130] and 1.8 for ACR 50 response[130]. Similarly, odds of response to secukinumab were also higher in men (MDA 1.6) at 6 months[141]. The lower rates of remission in women are not merely attributable to worse patient-reported outcomes, such as higher pain and physical dysfunction scores, although these factors do play an important role. In fact, persistent inflammation in female patients, as evidenced by a lesser reduction in joint counts and CRP levels after 6 months of treatment with TNF inhibitors, resulted in lower treatment response rates (by EULAR and ACR response criteria) than in male patients[140].

Perhaps relating to worse treatment responses in women, evidence suggests that female patients are more likely to switch therapies or discontinue treatment altogether compared with male patients[147] (Supplementary Tables 4 and 5). In one study, the probability of early treatment discontinuation was higher in women, with hazard ratios (HR) ranging from 1.29 to 2.65 for TNF inhibitors, interleukin (IL)-17 inhibitors and IL-12/23 inhibitors[124–128,131–134,136,137,139–143,146,148–151]. When examining the cause of treatment discontinuation in female patients, lack of effectiveness (HR 2.2) and adverse events (HR 1.8–3) seem to be the major contributors[124,127,128,139].

Although most studies have evaluated sex in addition to other predictors of drug effectiveness, one study specifically examined sex-related disparities in response to TNF inhibitors in patients with PsA. The authors conducted an observational cohort study of 1,750 patients with PsA treated with infliximab, adalimumab and etanercept, with 15 years of prospectively collected data from a national patient registry in Denmark[140]. Response rates to TNF inhibitors were significantly lower in women than in men, both at 3 and at 6 months, across the different PsA domains (clinical measures, patient-reported outcomes) as well as in a subgroup of patients with axial disease. EULAR good or moderate response at 6 months was significantly higher in men than in women (81% versus 69%, respectively; adjusted OR 3.15). Persistence of TNF inhibitor therapy was also found to be significantly lower in women with a median of 1.44 years versus 3.83 years in men. No significant differences were found among the reasons for treatment discontinuation across the sexes in this particular study.

Mechanisms of treatment responses

Despite remarkable differences in treatment effectiveness between men and women, little attention has been given to understanding the underlying sex- and gender-related mechanisms. It is likely that the underlying causes are, at least partly, disease neutral, as sex-specific differences have been reported in other types of inflammatory arthritis[152,153]. As discussed below, sex differences in immune function and pain mechanisms may explain the differences in treatment outcomes between men and women with PsA.

Sex-based differences in immune responses have been reported in patients. Women naturally have a more pronounced innate and acquired immune response than men, which generally renders female patients more susceptible to certain immune-mediated diseases but more resistant to infections[154]. Interestingly, immunotherapies that stimulate the immune system (such as vaccines) tend to be more effective in women, whereas those that suppress immune function (such as cytokine inhibitors in rheumatology and checkpoint inhibitors in cancer) tend to be more effective in men[155]. A possible reason for differential effectiveness of immunotherapies between sexes is differences in immunogenicity — the propensity of a biologic therapy to generate an immune response and form anti-drug antibodies[156]. Critically, generation of antibodies may reduce the therapeutic efficacy of specific biologic therapies. The greater likelihood of antibody production by the female immune system could therefore be hypothesized to result in higher levels of anti-drug antibodies treated with bDMARDs and thus poorer

treatment responses in women. However, studies exploring generation of anti-drug antibodies to bDMARDs did not report sex to be a predictor of immunogenicity in rheumatic conditions, including PsA[156,157].

Medication adherence is an important factor determining treatment response. A systematic literature review assessing medication adherence and persistence in RA, psoriasis and PsA found female sex to be associated with lower levels of adherence[158]. Only one study on PsA was included in this review thereby limiting the generalization of this finding to PsA.

Disparities in health care-seeking behaviour owing to gender norms, such as women actively seeking health care more often than men, might lead to under reporting of adverse events in men[155]. Another issue is the perception and reporting of adverse drug reaction in men and women. Body- or self-image-related adverse drug reactions are considered more burdensome in women owing to societal expectations associated with gender[159]. For example, hair loss is accepted as a physiological phenomenon in aging men but is considered a problematic adverse drug reaction in women.

Much of the sex- and gender-related differences in treatment response of PsA are rooted in the type of composite outcome measures assessed, such as the ACR response, which incorporates both subjective measures including patient-reported outcomes of pain and function, and objective measures of disease activity, such as joint counts and inflammatory markers. Both randomized controlled trials and observational cohort studies use composite outcome measures to report treatment response and achievement of low disease activity states. Since pain perception and reporting are higher in women[160,161], composite outcome measures would therefore be inherently biased to report higher scores in women. Women also tend to report poorer physical function scores on the Health Assessment Questionnaire[152], which further shifts the composite outcome measures towards higher scores. Thus, women consistently have higher scores on composite outcomes measures of PsA disease activity, which translate into lower levels of remission or low disease activity[162]. It is conceivable that this difference in disease activity between men and women with PsA emerges, at least in part, from the measures of disease activity rather than from the disease itself.

PsA development and disease course

Sex- and gender-related differences in the development of symptoms and disease in PsA are discussed below and summarized in TABLE 1.

Risk factors for PsA

Risk of PsA development has been associated with a combination of genetic and environmental factors. Sequencing analyses has revealed that gene mutations within the MHC-class I region of the genome confers the greatest risk of PsA[163]. Additional non-HLA genes that are implicated in PsA include those that encode for proteins involved in the TNF and IL-23/IL-17 pathways[163]. Unfortunately, limited information exists regarding any sex differences in genetic risk, as most relevant studies have not stratified their results by sex. Nonetheless, a potential role for sex in PsA inheritance was previously described in one study, which suggested a paternal mode of transmission[164]. The authors found that probands with PsA had significantly more affected fathers than mothers, with a similar trend observed among their offspring[164]. Interestingly, probands without affected first-degree relatives had affected second-degree relatives descended from the paternal side of the family rather than the maternal side. The notion of a parent-of-origin effect was later reinforced by a subsequent study that reported higher rates of transmission to sons rather than to daughters when the proband was male, but no such effect in the case of female probands[165]. This form of transmission is termed genomic imprinting, an epigenetic phenomenon that causes genes to be expressed in a parent-of-origin-specific manner. In support of this hypothesis, an Icelandic study of 178 patients with PsA that used an imprinting based scoring system in their analysis identified a susceptibility gene for PsA on chromosome 16 (REF.[166]). This study lends further support to the role of genomic imprinting in PsA susceptibility, although it remains unclear which genes are affected by this epigenetic phenomenon.

Several environmental risk factors, co-morbidities and psoriasis characteristics have been associated with PsA risk, including physical trauma, obesity, uveitis, severe psoriasis, flexural and scalp psoriasis and psoriatic nail lesions[167]. Whether the risk of PsA conferred by these factors differs between men and women remains to be determined. In addition, few studies have evaluated female-specific risk factors for PsA. Of the data that are available, no association has been found between risk of developing PsA and post-menopausal state, use of oral contraceptives and hormone replacement therapy, fertility treatments, breastfeeding and postpartum period[73,168].

The effect of sex hormones in PsA

Historically, oestrogen has been associated with increased T helper 1 cell response and B cell activation, whereas testosterone has been linked with reduced immune response[169]. More recent studies from the last decade have shown that sex hormones have complex and multilayered effects on the immune system[154,170]. Although a detailed discussion of the effect of sex hormones on the immune system is beyond the scope of this review, we briefly summarize sex differences in key cytokines involved in the pathogenesis of PsA. The roles of IL-17/IL-23 and TNF pathways in PsA have been highlighted in recent studies[1]. Production of pro-inflammatory cytokines, such as TNF, IL-1β and IL-6, is enhanced in low oestrogen concentrations, as occurs in women before puberty or after menopause, but is suppressed by high oestrogen levels during the reproductive period of women, as well as by progesterone and testosterone[154,171]. On the other hand, anti-inflammatory cytokines, such as IL 4, IL 5, IL 10 and transforming growth factor-β (TGFβ) production is increased by progesterone and testosterone[154,171]. Both testosterone and high-dose oestrogen inhibit IL-17 secretion, a key mediator in the pathogenesis of PsA[1], whereas low-dose

Table 1 | **Sex and gender differences in development and symptoms of psoriatic arthritis (PsA)**

Factors related to PsA development	Sex and/or gender related	Evidence in the literature
Risk factors		
Genetic susceptibility	Sex related	Parent-of-origin effect (genomic imprinting); probands are more likely to have an affected father than mother[164,165].
Environmental	Sex and gender related	No sex difference in known risk factors, such as physical trauma, obesity, uveitis, severe psoriasis, flexural and scalp psoriasis and psoriatic nail lesions[167].
Sex hormones		
Testosterone	Sex related	Inversely correlated with disease activity in male patients with PsA[174].
		Low levels associated with chronic pain[180].
Oestrogen	Sex related	Not associated with disease activity in PsA[174].
		Inhibits development of arthritis in animal models[178].
		Has both pro-nociceptive and anti-nociceptive properties[181].
		Periods of fluctuation of female sex hormones in a woman's life, such as postpartum, breastfeeding and post-menopause, are not associated with PsA development[73,168].
		Sex hormone-containing medications, such as oral contraceptives, hormone replacement therapy and fertility treatments are not associated with PsA development[73,168].
Progesterone	Sex related	Has both pro-nociceptive and anti-nociceptive properties[181].
Prolactin	Sex related	Possible mediator of arthritis pain[160].
Selective oestrogen receptor modulator	Sex related	Inhibits joint inflammation in animal models[179].
Pain		
Pain processing	Sex related	Higher pain stress response in ovulating female mice[160].
		Spinal microglial cells mediate persistent pain hypersensitivity in male mice only[160].
		Significant upregulation of μ-opioid receptor in male mice only[160].
		Anti-depressant (fluoxetine) exerts analgesic effect in chronic pain in female mice only[160].
Pain perception	Gender related	Women experience pain in more sites, with greater severity, higher frequency and longer duration than men[160].
Pain reporting	Gender related	Women have less tolerance of pain and report pain more often than men[161].
		Men tend to ignore, suppress, or withstand pain.
		Pain sensitivity is influenced by childhood sexual abuse and family history of pain only in women[181].

oestrogen has the opposite effect[154,171]. On the contrary, a study from 2019 investigating the influence of the X chromosome on inflammatory cytokine production found higher levels of inflammatory cytokines, such as IL-1β, IL-6, IL-8, IL-10, TNF and IFNα, in men compared with women after stimulation of receptors on immune cells[172]. Another study on B10.RIII mice reported development of arthritis, psoriasis-like skin disease, colitis, weight loss and osteopenia after IL-23 injection, but arthritis was more severe in female mice than in male mice[173]. Of note, IL-23 is central to the pathogenesis of PsA[1].

Information on the effect of sex hormones on PsA risk and disease course is scarce. In a small study, oestradiol levels did not correlate with PsA disease activity whereas testosterone levels did correlate inversely with disease activity in males[174]. Menopause results in a decline in female sex hormone levels, leading to significant modulation in immune cell function, which may have clinical implications. RA has been reported to worsen after menopause and lead to progressive disability[175]. Risk of development of psoriasis risk and disease severity have also been associated with surgical menopause[176] and with sex hormone levels[177]. Using SKG mice that underwent ovariectomy, one study demonstrated that oestrogen inhibits the development of SpA-like manifestations, possibly through decreased expression of TNF and interferon gamma and by modulating IL-17-producing cells[178]. The same group also investigated the efficacy of the selective oestrogen receptor modulator, lasofoxifene, on SpA activity in mouse models and found that the drug inhibited joint inflammation in a similar manner to oestrogen[179]. Overall, the effect of menopause on PsA remains unknown.

Sex and gender differences in pain

Sex- and gender-related differences in pain have been established by decades of clinical and experimental research. For the same disease condition, women tend to experience pain in more sites, with greater severity, higher frequency and longer duration than men[160]. Pain perception and reporting are known to be influenced

by both the sex and gender of the individual. Multiple preclinical and clinical studies have concurred that low testosterone levels make subjects susceptible to chronic pain, which is reversed upon administration of testosterone[180]. Naturally occurring high levels of testosterone in males could thus have an anti-nociceptive effect in inflammatory arthritis such as PsA. Oestradiol and progesterone, on the other hand, have complex effects on pain sensitivity, with studies reporting both pro-nociceptive and anti-nociceptive properties[181]. Prolactin has also been suggested to be a mediator of arthritis pain[160].

Pain processing in the central nervous system is modulated by sex. Animal studies have demonstrated a higher pain stress response in ovulating female mice than in males[160]. Only in male mice, spinal microglial cells have been found to mediate persistent pain hypersensitivity, probably through Toll-like receptor 4 (TLR4), which is dependent on male hormone testosterone[160]. Significant upregulation of μ-opioid receptor, which mediates analgesia, has also been reported in male mice[160]. On the other hand, analgesic effects of fluoxetine in formalin-induced chronic pain have been demonstrated in female mice only[160].

Gender-related sociocultural factors have also been implicated in differences in pain reporting. Greater degrees of femininity or female social roles are associated with a lower threshold for pain, less tolerance to pain and a greater likelihood of reporting pain, irrespective of ethnicity, sexual orientation or type of pain stimulus. Men, on the other hand, tend to suppress or withstand pain, congruous with stereotypical masculinity[182]. Environmental stressors, such as a past history of childhood sexual abuse and a family history of pain, influence pain sensitivity in women but not in men[181].

Box 2 | **Summary of sex- and gender-related differences in PsA**

- Prevalence: historically, the prevalence of PsA has been reported to be equal in male and female patients[1], but studies in the last few decades have reported higher prevalence in female patients[14–21,23], perhaps because of better diagnosis in women.
- Clinical features: male patients have more axial involvement[25–29], more severe psoriasis[2,28,29,34,38,39] and psoriatic nail lesions[26,38], whereas female patients frequently have peripheral joint involvement[25–29].
- Quality of life and function: male patients typically have a better quality of life and less of a work disability[59–61], whereas female patients suffer from more pain, fatigue and poor functional status[26–29,34,35,52–55]. These worse patient outcomes in women might be related to PsA or, alternatively, to co-existing conditions that are more common in the female sex, such as fibromyalgia. We recommend that simple imaging techniques (such as ultrasound) be used to differentiate between PsA and other conditions.
- Imaging: male patients develop more severe structural changes both in axial and in peripheral joints, whereas female patients exhibit less structural damage[26,52,72].
- Treatment response: male patients respond better to treatment with advanced therapy (biologic DMARDs and targeted synthetic DMARDs) and are more likely to achieve and sustain remission or low disease activity states. On the other hand, female patients have a poorer response to these medications, with frequent premature discontinuation of therapy owing to a lack of effectiveness or to adverse events[123–144].

PsA and the reproductive system

Fertility in PsA

Studies investigating fertility abnormalities among patients with PsA have found conflicting results. In a retrospective survey of women with PsA, infertility was reported in about a third of women, mainly because of polycystic ovarian syndrome[183]. These findings were not corroborated in another observational study, which reported no change in fertility or rates of miscarriage in patients with PsA[184]. The reasons for these differences are unknown. On the other hand, male patients with PsA were found to have lower levels of testosterone, reduced sperm count and motility, as well as evidence of sexual accessory gland inflammation, which might produce fertility abnormalities[185], including low sperm count and abnormal motility.

Pregnancy in PsA

Given that PsA affects women of childbearing age, it is important to understand how the disease and its treatments relate to pregnancy, including changes to disease severity and risk of adverse pregnancy outcomes. For unknown reasons, PsA disease activity levels tend to decline during pregnancy and flare in the postpartum period, as reported in a large prospective Norwegian study of 103 pregnant women with PsA followed from preconception to 1 year postpartum[186]. In that study, disease activity levels decreased during pregnancy and flared in the postpartum period, reaching a peak at 6 months despite 40% of women receiving TNF inhibitors at that time point. Improvement of arthritis[183,187–189] and psoriasis[183,187] during pregnancy followed by a flare in the postpartum period have also been described in other studies on PsA. Finally, women who continue TNF inhibitor therapy during pregnancy have low levels of disease activity and a lower risk of flares during pregnancy and in the postpartum period, in comparison with women who discontinue the medication[188,190].

Although pregnancy with PsA is associated with a few adverse maternal outcomes, neonatal outcomes seem to be generally favourable with no increase in congenital anomalies, neonatal mortality or stillbirth[191]. Few studies evaluated pregnancy outcomes in PsA, showing a higher risk of preterm birth (adjusted OR 1.63; adjusted risk ratio (aRR) 1.81), Caesarean section deliveries (adjusted OR 1.43; aRR 1.63)[189,192] and oligohydramnios (aRR 3.79)[189] but no excess pre-eclampsia.

Conclusions and future directions

In summary, the immunopathogenesis, clinical manifestations, imaging features and treatment outcomes of PsA are influenced by the sex and gender of patients to a considerable extent (BOX 2). Clinicians need to consider these differences when managing patients with suspected PsA or those with established disease. Adopting sex- and gender-based analyses in clinical research using

validated gender tools and considering both sex and gender dimensions in analyses are key to moving beyond the simple assessment of differences between women and men towards an understanding of why these differences exist and how to address them. Such an approach has resulted in the development of gender-specific recommendations for risk stratification and drug treatment in osteoporosis and cardiology[193,194].

Future research into segregating the effects of biological sex from sociocultural gender on PsA risk, disease course and response to treatment would help to identify underlying mechanisms for these disparities. Investigating sex-specific risk factors for development of PsA, as well as designing sex-specific tools for the screening of PsA, would facilitate early intervention to prevent irreversible structural damage and disease progression. Research into the identification of sex-specific biomarkers as well as the use of more sensitive imaging modalities, such as MRI or ultrasound, in female patients may be needed to detect objective signs of PsA and expedite diagnosis. Additional basic science and translational research into pathophysiological mechanisms of immune response and pain perception between men and women with PsA could help to identify novel therapeutic targets that may differ between sexes. Moreover, appropriate treatment targets should be investigated according to sex and gender of the individual with more emphasis on pain, fatigue and other factors that tend to be more severe in women. Finally, identifying and exploring links between sex and comorbidities, and finding optimal ways of addressing these conditions, would be pivotal in improving quality of life of patients with PsA.

All studies, both observational studies and clinical trials, should report results stratified by sex. Despite the limited power of an individual trial to detect sex disparities in treatment responses, such sex-stratified reporting would allow data from multiple studies to be pooled to explore whether different classes of advanced therapies affect men and women with PsA differently. Better understanding of the sex- and gender-related mechanisms that affect therapeutic effectiveness could inform the development of sex gender-specific approaches to optimizing the management of patients with PsA.

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Assessment of disease outcome measures in systemic sclerosis

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Abstract | The assessment of disease activity in systemic sclerosis (SSc) is challenging owing to its heterogeneous manifestations across multiple organ systems, the variable rate of disease progression and regression, and the relative paucity of patients in early-phase therapeutic trials. Despite some recent successes, most clinical trials have failed to show efficacy, underscoring the need for improved outcome measures linked directly to disease pathogenesis, particularly applicable for biomarker studies focused on skin disease. Current outcome measures in SSc-associated interstitial lung disease and SSc skin disease are largely adequate, although advancing imaging technology and the incorporation of skin mRNA biomarkers might provide opportunities for earlier detection of the therapeutic effect. Biomarkers can further inform pathogenesis, enabling early phase trials to act as reverse translational studies through the incorporation of routine high-throughput sequencing.

Systemic sclerosis (SSc) is a rheumatic disease with a high case mortality at present[1]. This high mortality rate is driving continued intense interest in developing a deeper understanding of SSc pathogenesis and pharmaceutical company interest in finding therapeutics targeting SSc and its pleiotropic manifestations. The main clinical features of SSc — vascular injury and fibrosis — are common to many other diseases, whereas two manifestations of SSc, pulmonary fibrosis and pulmonary arterial hypertension (PAH), are found in isolated single-organ forms as the related diseases idiopathic pulmonary fibrosis (IPF) and idiopathic pulmonary arterial hypertension, respectively. More broadly, inflammation leading to vascular injury or fibrosis is a common feature of many diseases, which means that an improved understanding of SSc pathogenesis might provide insights into vascular disease such as atherosclerosis and into fibrotic diseases including liver cirrhosis, bone marrow fibrosis and end-stage diabetic renal fibrosis.

Despite some recent successes, most clinical trials of therapeutics for SSc have failed to show efficacy[2,3]. These failures highlight the need for improved outcome measures that are directly linked to disease pathogenesis. Recent observations, emerging technologies and novel approaches to clinical outcomes are improving clinical trial design[4,5]. Here, we review clinical and biochemical outcome measures of distinct skin and lung manifestations of SSc. We consider how biomarkers can inform pathogenesis, enabling clinical trials to serve as reverse translational studies. Finally, we consider how biomarkers could be applied as surrogate outcome measures in SSc.

Considerations for SSc outcome measures

As a starting point, the FDA defines clinical end points as "a characteristic or variable that reflects how a patient feels, functions, or survives"[6]. Clinical end points are typically acceptable for drug approval. However, this definition could be broadened to include physical and laboratory findings. For instance, the National Cancer Institute defines a clinical end point as an event or outcome that can be measured objectively to determine whether the intervention being studied is beneficial[7]. The wide array of clinical manifestations in SSc provide an equally wide array of potential clinical and molecular end points. The majority of clinical trials in SSc thus far have assessed three disease complications: skin disease (SSc skin disease), SSc-associated interstitial lung disease (SSc-ILD) or SSc-associated PAH (SSc-PAH), but some trials have examined digital ischaemia, assessing the severity of Raynaud's disease or numbers of digital ulcers. Measures of disease outcomes and approved therapeutics are less common for SSc manifestations beyond the skin and lung (TABLE 1).

The challenges in assessing patients with SSc in clinical trials stem from the disease heterogeneity, the variable rate of disease progression and/or regression, and the relative paucity of patients. None of these problems is unique to SSc, but the combination of these issues leads to distinct challenges. Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are also known to be heterogeneous diseases, and the underlying pathogenesis is thought to vary between patients. Heterogeneity in RA might account for patients' differing responses to various medications[8]. The biological basis for differences

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Key points

- The majority of clinical trials for systemic sclerosis (SSc) focus on skin disease, interstitial lung disease (ILD), or pulmonary arterial hypertension, with modified Rodnan skin score (MRSS), forced vital capacity and 6-min walk distance being the primary clinical end points assessed, respectively.
- Increased variability in the natural history of SSc skin disease impedes the identification of treatment-related effects, and strategies for shorter duration trials might improve the separation of treatment effect from variation in disease natural history.
- Forced vital capacity is an FDA-accepted surrogate outcome measure for SSc-ILD, whereas novel imaging technologies and serum biomarkers present opportunities for future additional SSc-ILD outcome measures to show significant changes at earlier timepoints.
- Skin mRNA biomarkers, including *THBS1* and *COMP*, could be considered as surrogate outcome measures in early-phase clinical trials, to more rapidly assess target engagement by therapeutic efficacy.

in pathogenesis and identifying markers for patients likely to respond to a medication, defined as predictive biomarkers, underlie the concept of precision medicine. In RA, predictive biomarkers are emerging for approved medications; for example, one study showed that among patients whose synovial biopsy samples were classified as being B cell poor by RNA sequencing, response rate was higher in those who received tocilizumab than in those who received rituximab[9].

The variability in disease progression is a particular challenge in SSc. Formerly thought to be relentlessly progressive, we now know that skin disease in most patients with diffuse cutaneous SSc (dcSSc) will regress after some period of time, and the majority of patients show improved skin disease within a year after entry into trials for dcSSc[10]. SSc-ILD also stabilizes spontaneously in many patients[10]. By contrast, SSc-PAH is relentlessly progressive and of note is that therapeutics for SSc-PAH emerged sooner than did those for SSc-ILD or SSc skin disease, and that no FDA-approved therapy is yet available for SSc skin disease. This lack of approved treatments likely partly results from the increased variability in the natural history of SSc skin disease, which makes it hard to see treatment-related effects. Notably, PAH trials of vasodilators are also much shorter, typically 12–16 weeks. Trials of shorter duration can provide improved opportunities to detect treatment-related effects rather than variation from the natural history of disease. However, some treatments might require longer to show clinical effects and it is also important to demonstrate sustained effects beyond a few months.

Disease heterogeneity is evident at the level of clinical disease, with different autoantibodies associated with different rates of skin disease progression and organ complications. For example, patients with antibodies to centromere protein B (CENPB) typically show limited cutaneous disease, whereas patients with antibodies to RNA polymerase 3 (RNAPol3) typically show severe and rapidly progressive skin disease[11]. The relationship between these and other SSc-associated autoantibodies and the associated clinical manifestations has been reviewed elsewhere[11].

Disease heterogeneity is also evident at the level of skin pathology (FIG. 1), and at the level of gene expression[12]. Some patients with SSc have pronounced perivascular inflammation, whereas others show relatively little inflammation. Deep learning computer analyses of skin are emerging[13], but we do not yet know whether this heterogeneity reflects different underlying pathogeneses or different stages of disease. However, we do know that markers of fibroblast and macrophage activation are associated with progressive skin disease, which suggests that skin inflammation, particularly by macrophages, drives fibrosis, and that skin inflammation and fibrosis can resolve together in later stages of disease. Thus, temporal changes in skin pathology might explain much or all of the observed heterogeneity when patient biopsy samples are examined at a single point in time.

An important question is whether SSc patient heterogeneity can or should be used prospectively to stratify patients in clinical trials. Autoantibodies, skin pathology and gene expression classifiers — although not outcome measures — are important considerations for clinical trial design and will be considered below. The basic stratification into diffuse and limited cutaneous subsets serves well as a starting point, with diffuse cutaneous disease defining a patient subset with more skin disease, higher disease-related mortality, and more likely to develop ILD, scleroderma renal crisis, and lower gastrointestinal manifestations[14]. Limited cutaneous disease, the most prevalent form of SSc, defines a subset with a limited distribution of skin fibrosis but pronounced vascular manifestations including digital ischaemia and PAH[15].

Disease assessment in SSc skin disease

Skin disease in patients with SSc leads to considerable morbidity, comparable with patients with psoriasis or atopic dermatitis[16]. Yet patients do not generally verbalize their concerns about the thickness of their skin. Patients' experience of skin disease is quite variable, reflecting considerable limitations imposed by skin tightness (for example, functional limitations imposed on hands and decreased mouth aperture), physical effects (for example, pain, dryness and tightness), as well as emotional and social effects[17].

Clinical assessment. The modified Rodnan skin score (MRSS) has been the standard method for assessing SSc skin disease for years. The MRSS is calculated by assessing skin thickening at 17 sites on an ordinal 0–3+ scale. The MRSS correlates with skin thickness and with other measures of disease severity such as forced vital capacity (FVC) in ILD[18]. The MRSS meets Outcome Measures in Rheumatology (OMERACT) criteria[19], and has been the primary outcome measure in multiple SSc clinical trials, yet has failed to lead to regulatory approval of any therapeutics. Owing to its reliance on subjective observer assessments, much discussion has centred on the reproducibility of MRSS by physician assessors, and considerable effort has been made to standardize the assessment of MRSS in clinical trial settings[20]. The MRSS performs reasonably well in terms of accuracy, referring to the ability of the instrument to measure skin disease severity, and responsiveness, the movement with disease improvement or decline. However, variability of the natural history of skin disease in patients with SSc places

a constraint that cannot be solved by just improving accuracy and responsiveness of the outcome measure[10]. Despite its limitations and concerns about its utility, the MRSS does change with effective therapy, as shown by the improved MRSS following immunoablation and stem cell transplant[21].

The skin alterations occurring in SSc skin disease include changes to skin thickness, skin tightness and skin tethering. Of these, the MRSS is only designed to detect skin thickness (TABLE 2), which is most closely associated with collagen and other extracellular matrix deposition[22]. Skin tightness reflects the effects of myofibroblast tension, whereas skin tethering occurs owing to the replacement of subcutaneous fat by fibrotic tissue and adhesion of skin to the underlying fascia. High-frequency and ultra-high-frequency ultrasound can detect skin thickness but require specialized instruments and have not been studied sufficiently for agreement on their implementation as outcome measures in SSc[23–26]. Durometry, measuring skin hardness, correlates highly with the MRSS and demonstrates higher intraobserver and interobserver intraclass correlation than the MRSS[27], but requires further testing in clinical trial settings[28].

Skin disease also leads to contractures in both small and large joints. Contractures most commonly affect fingers and hands, but can also affect large joints including the elbows, shoulders and — rarely — the knees and hips. These contractures are in part caused by skin traction across joints, but also tendon involvement. Several measures of hand function have been developed for other diseases, notably RA[29], but hand disability in SSc differs from that in RA, in which synovitis and joint subluxation are prominent. Although patients with SSc also develop synovitis, tendon inflammation is more prominent, including friction rubs and limited range of motion, particularly finger extension. Tenosynovitis can be detected on MRI and ultrasound[30].

SSc hand disease manifests in many ways: ischaemia and digital loss, ulcers, contractures, calcinosis and arthritis[31]. Several clinical outcomes for hand function have been tested in patients with SSc: grip strength, finger-to-palm and hand mobility in scleroderma (HAMIS). Finger-to-palm, measuring the flexion of the third finger to the wrist, has more recently been modified as delta finger-to-palm, examining the distance between extension to flexion[32], and validated[33]. HAMIS has been modified to a more simplified outcome[34,35].

Patient-reported outcomes. Quality of life assessments are increasingly emphasized by regulatory authorities and patients as important outcome parameters in clinical trials. Patient-reported outcome (PRO) measures

Table 1 | **Measures of systemic sclerosis disease complications**

Complication of systemic sclerosis	Medications	Outcome measures of disease activity
SSc-PAH[a]	Prostacyclin analogues[b], endothelin receptor antagonists[b] and phosphodiesterase type 5 (PDE5) inhibitors[b]	6MWD has been the most commonly used measure leading to FDA approval in trials of vasodilators for PAH. Time to event and mortality have also been examined, with time to event particularly important. In PAH, events were defined as worsening of pulmonary arterial hypertension, initiation of treatment with intravenous or subcutaneous prostanoids, lung transplantation, atrial septostomy or death from any cause[155]. Brain natriuretic peptide is a useful biomarker
Gastroesophageal reflux disease	Proton pump inhibitors, H2 blockers, sucralfate	A gastrointestinal patient reported outcome, the Scleroderma Clinical Trial Consortium GIT 2.0 (REF.[156]) has begun to be tested in interventional clinical trials[157], and appears promising for assessing the burden of gastrointestinal symptoms[158]
Gastrointestinal dysmotility	Erythromycin, azithromycin, metoclopramide, domperidone, cisapride	Scleroderma Clinical Trial Consortium GIT 2.0 (REF.[156])
Scleroderma renal crisis	Angiotensin-converting enzyme inhibitors[159]	Outcomes of blood pressure and renal function have only been used in open-label trials
Fibrotic heart disease	No proven therapy	Although SSc myocardial disease has not been studied in controlled clinical trials, observational trials are tracing a path for such studies[160]
Conduction abnormalities and arrhythmias, including fatal ventricular arrhythmias	Standard therapies	No outcome measure has been developed for cardiac arrhythmias
Calcinosis[c]	No proven therapy. Potential therapies include: intralesional sodium thiosulfate, diltiazem, rituximab and minocycline[161,162]	Radiographic outcome measures are described and under development[163]

SSc-PAH, systemic sclerosis-associated pulmonary arterial hypertension. [a]Early trials of vasodilators for PAH have had the advantage of measurement of pulmonary vascular resistance, essentially an effective biomarker for clinical response. The pulmonary vascular resistance changes rapidly (minutes to hours) for these vasodilators[164–166], providing immediate feedback about likely efficacy. [b]FDA approved. [c]Calcinosis remains one of the most poorly understood and challenging complications of scleroderma[167,168].

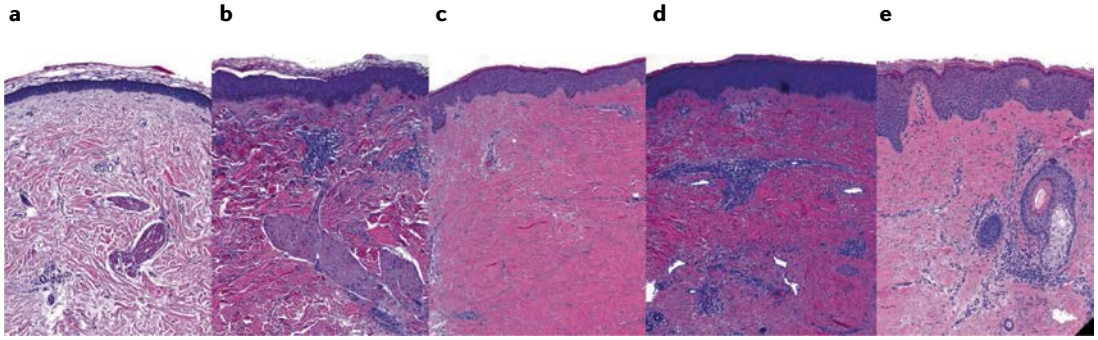

Fig. 1 | **Heterogeneity of SSc skin pathology.** Examples of skin biopsy samples from five patients with systemic sclerosis showing relatively preserved collagen structure and lack of inflammation (**a**), moderate inflammation and increased collagen (**b**), densely packed hyalinized collagen without inflammation (**c**), dense collagen with moderate inflammation (**d**) and dense collagen with dense perivascular inflammation and epidermal hypertrophy (**e**). Histology images are owned by Dr. Lafyatis and have not been previously published elsewhere. Magnification × 4.

are collected directly from the patient and encompass patients' experience of their own health and quality of life, capturing different perceptions from those measured by physiology studies[36,37]. The patient perspective of their skin disease has been evaluated and captured in the Scleroderma Skin Patient-Reported Outcome (SSPRO). This outcome is divided into measures of physical limitations imposed by skin tightness, physical effects, emotional effects and social effects[17]. The SSPRO has been further validated in a placebo-controlled trial of lenabasum in patients with dcSSc[38].

Several hand PRO measures have also been validated for use in patients with SSc. The most frequently studied PRO measure in this setting is the Cochin Hand Function Scale (CHFS)[39], a questionnaire with 18 items concerning daily activities; a shortened six-question version, the CHFS-6, has now been validated[40]. The Michigan Hand Outcomes Questionnaire, a more extensive assessment of hand function, was validated in a controlled, randomized interventional study in 2016 (REF.[41]). In 2020, the Hand Disability in Systemic Sclerosis-Digital Ulcers (HDISS-DU) was developed and validated for evaluating digital ulcers[42]. Of these hand PRO measures, CHFS is considered fully validated for use in clinical trials[43]. Although hand contractures can lead to irreversible damage, measures of hand function should be included as secondary or exploratory outcome measures in all SSc skin disease trials to better understand their utility as outcome measures.

A more comprehensive PRO measure assessing multiple disease facets including hand, skin and Raynaud's phenomenon — the EULAR ScleroID questionnaire — has been developed and validated in both cross-sectional and longitudinal cohorts[44]. More discrete PRO measures have also been developed and tested for assessing lung disease associated with SSc and are discussed below. Given that regulatory agencies need to know the effects of medications on a patient's perception of disease, PROs represent a key outcome measure for all SSc clinical trials.

Disease assessment in lung disease

ILD is a common and highly variable manifestation of SSc and is associated with considerable morbidity and mortality. Although some patients exhibit stable disease or progress slowly, others have rapid loss of lung function resulting in respiratory failure[45]. ILD remains the leading cause of disease-related mortality in SSc and is responsible for SSc having the highest case-specific mortality among the rheumatic diseases[46,47]. The availability of new therapeutics for SSc-ILD increases the desire to accurately identify those patients who will develop ILD at the time of an SSc diagnosis, and increases the wish to rapidly detect disease progression in order to consider escalation or changes in therapy. Reliable outcome measures are necessary to accurately assess the benefits of pharmacological and other treatments, yet few have been validated in SSc-ILD. Given the absence of validated prognostic biomarkers for SSc-ILD, clinical assessment based on both pulmonary function testing and thoracic imaging is currently recommended[48], although no consensus exists on the methods and time course utilized for diagnosis and monitoring of progression.

Clinical assessment. Given the similarities in the presentation and outcomes of SSc-ILD and IPF, many of the clinical trial and observational study outcomes in SSc-ILD mirror those in IPF. Akin to studies in IPF, pulmonary physiology is the favoured primary outcome, including the pulmonary function test (PFT) measures of FVC, total lung capacity (TLC) and diffusing capacity of the lung for carbon monoxide (DLCO) (TABLE 3). FVC is the most broadly utilized variable for measuring restrictive impairment[49], with TLC also measuring lung restriction. For example, the annual rate of decline in FVC and/or FVC as an absolute value showed significant differences between intervention arms in the SLS-I (cyclophosphamide versus placebo)[50], SLS-II (cyclophosphamide versus mycophenolate mofetil)[51], and SENSCIS trials (nintedanib versus placebo)[52] and led to the FDA approval of nintedanib for SSc. In a 2018 systematic review of the use of PFTs as outcome measures in SSc-ILD, FVC (% predicted) was the primary end point in 70.4% of 169 outcome studies and 50 validation studies, with DLCO (% predicted) being the primary outcome in 11.3% of the studies[53]. Only five studies sought to validate PFT measurements by cross-sectional assessment against ILD severity as measured

by high-resolution computed tomography (HRCT)[54–56], with two studies concluding that DLCO was the best predictor of the presence of ILD, and the other studies not identifying a particular PFT measurement as being best. The predominance of the use of FVC as the primary end point of choice despite a lack of evidence of its superiority over other physiological measures might reflect its greater precision and ease of measurement than TLC and DLCO.

DLCO is influenced by changes not only in lung parenchyma, but also by pulmonary vascular disease and anaemia. Given the confounding effect of pulmonary hypertension, FVC is regarded as more specific (than DLCO) for detecting ILD[57,58], whereas DLCO may be more sensitive than FVC for detecting ILD[55,56]. The variability of FVC is still a concern, especially as the minimal clinically important difference (MCID) in SSc-ILD (3% to 5.3% for FVC% improvement, –3% to –3.3% for FVC% decline — calculated at the group level from a pooled cohort of SLS-I and SLS-II participants) is within the range of measurement error for an individual[59]. A systematic review of 27 studies found that DLCO is more consistently associated with mortality in SSc-ILD than is FVC[60], with a subsequent cohort of 264 individuals with SSc-ILD similarly finding that 1-year decline in DLCO of ≥15% is the best predictor of adverse outcomes, including death[61]. Indeed, DLCO's inherent decreased specificity might make it a superior predictor of adverse outcomes, as it encompasses other clinical factors.

Composite end points incorporating multiple physiological parameters might better predict outcomes than any isolated single measure. A 2017 UK cohort examining 15-year mortality in 162 patients with SSc-ILD concluded that 1-year trends in an FVC and DLCO composite end point and 2-year trends in gas transfer were the most predictive measures of mortality[62]. The 2015 OMERACT connective tissue disease-associated ILD (CTD-ILD) working group proposed a decline in FVC of ≥10% or a <10% to ≥5% decline in FVC combined with a ≥15% decline in DLCO as a clinically meaningful outcome in CTD-ILD, including SSc-ILD[63]. These ranges of sustained pulmonary function that declines over 1 year have been proposed as criteria for the progression of SSc-ILD[48,62], although not formally validated. However, both the 2017 EULAR Scleroderma Trials and Research (EUSTAR) treatment recommendations and the 2020 Delphi-process European consensus statements for the management of SSc-ILD rely on FVC as being the primary outcome measure to define progressive SSc-ILD[64,65].

Despite their limitations, and given their associations with mortality and progression, FVC and DLCO have clinically meaningful prognostic value, and should inform therapeutic decisions. It is important to consider that patients with early SSc-ILD might have preserved lung volumes and be asymptomatic, despite clear structural disease on HRCT. More than 60% of patients with SSc-ILD diagnosed by HRCT have normal spirometry at the time of chest imaging[54,66]. In both SSc-ILD and other fibrotic ILDs, DLCO is frequently the first PFT parameter to decline (as shown during a negative clinical trial for *N*-acetylcysteine in patients with SSc)[67] and is a harbinger of other changes to come if not swift intervention is not successful.

Functional assessment with the 6-min walk distance (6MWD) is an accepted primary end point in pulmonary vascular disease clinical trials, and often included as a secondary end point in ILD trials. However, in SSc, the 6MWD does not directly correlate with physiological parameters of ILD (FVC and DLCO), as it influenced by several disease manifestations including pulmonary hypertension, myocardial fibrosis, musculoskeletal disease, conditioning, peripheral vascular disease and pain[68–70]. This lack of correlation does not negate the utility of the 6MWD as a measure of limited-exercise

Table 2 | **Outcome measures for skin disease and hand function in systemic sclerosis (SSc)**

Skin and musculoskeletal feature	Outcome measure	Validity and limitations
Skin thickness	Modified Rodnan Skin Score	Validated in clinical trial settings, but continuing concern about reproducibility across centres
	Ultrasound	Incompletely assessed in clinical trial settings. Feasibility of this outcome measure for multicentre trials is uncertain
	Durometry	Incompletely assessed in clinical trial settings
Tenosynovitis	MRI	Not studied in clinical trial settings
	Ultrasound	Not studied in clinical trial settings
Global hand function	Delta finger-to-palm	Partially validated, not tested in clinical trial settings
	Hand mobility in scleroderma	Validated for use in SSc clinical trials
	Cochin Hand Function Scale	Validated for use in SSc clinical trials
	Michigan Hand Outcomes Questionnaire	Partially validated in SSc clinical trial settings
	Hand disability in systemic sclerosis-digital ulcers	Validated for use in SSc clinical trials
Patient-reported outcomes	Scleroderma skin patient-reported outcome	Partially validated in SSc clinical trial settings

Table 3 | **Outcome measures for SSc-ILD**

Assessment category	Outcome measure	Validity and limitations
Pulmonary physiology	FVC	Endorsed for use in RCTs[169]
	TLC	Requires further validation
	DLCO	Lacks sufficient evidence of clinical trial discrimination, confounded by extrapulmonary and pulmonary vascular disease
Exercise physiology	6MWD	Confounded by extrapulmonary and pulmonary vascular disease
	CPET	Confounded by extrapulmonary and pulmonary vascular disease
Radiology	Quantitative chest HRCT	Feasibility limited by lack of standardized imaging measure across clinical practice and trials
Patient-reported outcomes	HAQ-DI, SHAQ, FACIT-dyspnoea, Mahler's dyspnoea score	Robustly validated in SSc-ILD
	SGRQ, Leicester cough score, SF-36, FACIT-fatigue, KB-ILD	Reproducibility needs further validation in SSc-ILD

6MWD, 6-minute walk distance; CPET, cardiopulmonary exercise testing; DLCO, diffusing capacity of the lung for carbon monoxide; FACIT, functional assessment of chronic illness therapy; FVC, forced vital capacity; HAQ-DI, Health Assessment Questionnaire Disability Index; HRCT, high-resolution computed tomography; KB-ILD, Kings Brief Interstitial Lung Disease Questionnaire; SF-36, 36-item short-form survey; SGRQ, St. George's Respiratory Questionnaire; SHAQ, Scleroderma Health Assessment Questionnaire; SSc-ILD, systemic sclerosis-associated interstitial lung disease; TLC, total lung capacity.

capacity in SSc, but emphasizes that 6MWD is influenced by more than pulmonary disease alone in SSc. Rather than serve as an isolated surrogate end point for PAH or ILD, the 6MWD might be better utilized as part of a composite end point or secondary outcome measure in SSc-ILD. Cardiopulmonary exercise testing (CPET) has also been assessed in SSc: decreased peak oxygen consumption (VO_2) and increased slope of ventilatory efficiency for carbon dioxide (V_E/VCO_2) were associated with decreased survival in a retrospective cohort ($n = 210$)[71] and correlated with an annual decrease in FVC in a smaller prospective cohort ($n = 29$)[72]. Similar to 6MWD, CPET is a composite outcome influenced by several manifestations of SSc.

Multiple clinical trials have included measures of ILD radiographic burden as a secondary outcome[51,73], but a single standardized imaging outcome measure has yet to be employed consistently across studies or in clinical practice. A systematic review identified the extent of ILD on HRCT as the only variable to independently predict both SSc-ILD progression and mortality, although the studies in this systematic review utilized different measures to quantify disease extent[60]. For instance, the phase III FocuSSed trial, which compared weekly subcutaneous tocilizumab with placebo in patients with early SSc and elevated acute-phase reactants, demonstrated decreased lung fibrosis in tocilizumab-treated patients in a quantitative analysis of fibrosis by HRCT and resulted in the FDA approval of tocilizumab for SSc-ILD[73]. Autologous haematopoietic stem cell transplantation also significantly reduced ILD extent in the phase II ASSIST trial of patients with rapidly progressing SSc[74]. Quantitative assessment of the extent and/or severity of ILD by lung texture analysis and other radiographic artificial intelligence techniques will continue to be incorporated in future studies as these methods become more widely available and advance to providing additional information on pulmonary vasculopathy and the quantification of distinct ILD patterns (for example, reticulations, ground glass and honeycombing).

Alternative imaging modalities to traditional chest X-ray and HRCT have gained increasing interest in clinical medicine in order to limit cost and radiation exposure and to better predict progressive disease. Point-of-care lung ultrasound is now routinely used in critical care and has been proposed in the assessment of CTD-ILDs. Despite the publication of several methods for the ultrasound assessment of SSc-ILD[75–77], a 2020 systematic review by the OMERACT Ultrasound Group concluded that insufficient evidence currently exists for it to be used as an outcome measure in SSc-ILD and that further validation of criterion validity, reliability and sensitivity to change are first needed[78]. A retrospective study of ^{18}F-fluorodeoxyglucose (FDG) PET scanning in 36 patients with SSc identified higher FDG uptake in patients with SSc who also had ILD than in those without ILD, and that pulmonary FDG uptakes correlated positively with ILD severity[79], expanding the findings of an earlier case series[80]. With further prospective studies, 18F-FDG PET might have a role in detecting and quantifying early changes in SSc-ILD, such as identifying therapeutic response in a pharmaceutical trial prior to a detectable change in FVC. Preclinical studies of single photon emission computed tomography/computed tomography imaging of pathogenic disease processes, including $\alpha v\beta 3$ integrin, somatostatin receptor 2 and apoptosis, have demonstrated utility in detecting early ILD in animal disease models[81,82], with plans to expand these to human trials.

Patient-reported outcomes. The St. George's Respiratory Questionnaire (SGRQ) is the most broadly used lung-specific QoL assessment. Although initially developed for obstructive lung disease, the SGRQ has since been validated for use in restrictive lung disease, having

acceptable reliability, construct validity and responsiveness to change for use in patients with dcSSc[83]. Other PROs commonly used in SSc-ILD include the Health Assessment Questionnaire Disability Index (HAQ-DI), functional assessment of chronic illness therapy (FACIT)-dyspnoea, FACIT-fatigue, Mahler's Dyspnoea Score, the Leicester Cough Score, the Short Form (SF)-36, the Scleroderma Health Assessment Questionnaire (SHAQ), and the Kings Brief Interstitial Lung Disease Questionnaire (KBILD); these PROs have been reviewed elsewhere[84,85].

No clinical trials in SSc-ILD have shown statistically significant differences in these PRO questionnaires when used as secondary outcome measures for treatment effect. The lack of significant effects on PROs in clinical trials meeting their primary end points might reflect limitations of both the currently available tools and the medications.

Biomarkers in SSc skin disease

Use of biomarkers to study SSc skin disease provides clear advantages compared with use of biomarkers in other organs where biopsy and/or repeat biopsy are not practical. In particular, in vitro studies establishing the biological activity and molecular targets affected by a therapeutic provide pharmacodynamic biomarkers of in vivo drug activity. Pharmacodynamic biomarkers associated with disease severity also provide information regarding drug efficacy. Currently, both pharmacodynamic and prognostic biomarkers of SSc skin disease remain investigational and have not been incorporated into clinical practice.

Pharmacodynamic biomarkers of SSc skin disease. mRNA expression of a four-gene biomarker (*THBS1*, *COMP*, *SIGLEC1* and *IFI44*) from a mid-forearm skin biopsy sample correlates highly with the MRSS[86]. This finding was a seminal and surprising observation in that the MRSS measures skin disease throughout the body, whereas the four-gene biomarker assesses mRNA expression at only the forearm[86]. Matrix genes, postulated to be mainly associated with skin fibroblasts (*THBS1* and *COMP*), correlate most highly with the local skin score (that is, the 0–3+ score at the site of the biopsy), consistent with the notion that *THBS1* and *COMP* are molecular markers closely associated with skin fibrosis. Subsequent studies examining the correlation of specific genes with the MRSS longitudinally identified a weighted combination of *THBS1* and *MS4A4A* as the best longitudinal biomarkers of SSc skin disease. The expression of these markers changed in a clinical trial of an anti-TGFβ antibody (fresolimumab) and an anti-IL-6 antibody (tocilizumab), but not in studies of other therapies[2,87,88].

Single-cell RNA (scRNA) sequencing studies have provided deeper insights into the cell sources of biomarker genes in SSc skin disease, by enabling the comprehensive examination of gene expression on a cell-by-cell basis[89,90] (FIG. 2). For example, *THBS1* and *COMP*, which correlate strongly with the MRSS, are expressed mainly in a discrete fibroblast subpopulation and are highly upregulated in SSc fibroblasts[89]. By contrast, the macrophage marker *MS4A4A* is expressed exclusively in the myeloid cell cluster and is upregulated in SSc myeloid cells[91]. Other markers of SSc skin disease — including *VWF* and *SELP* — are selectively upregulated in endothelial cells[89]. Biomarkers previously shown to correlate with progressive skin disease, for example, *IL13RA*[92], show little difference in gene expression in SSc myeloid cells compared with control myeloid cells[91], suggesting that *IL13RA* is marking the number of infiltrating myeloid cells rather than there being an increase in the expression of *IL13RA* in the cell population. Previous studies of bulk skin RNA expression were unable to distinguish these differences in the number of cells expressing a gene versus higher expression by a stable number of cells[93].

scRNA sequencing provides unparalleled insights into altered gene expression in the skin and, in particular, can define discrete cell populations expressing previously defined biomarker genes. Particularly important is the observation that the biomarkers correlating the most highly with the MRSS are expressed by a subpopulation of fibroblasts, characterized by their expression of *SFRP2* and *DPP4* (REF.[89]). A further subpopulation of these cells — myofibroblasts — are characterized by their expression of *SFRP4*, *ADAM12*, *CTGF* and other genes[89]. Future interventional studies incorporating skin scRNA sequencing into the clinical protocols will provide insight into mechanisms of actions and the cellular and molecular targets of therapeutics.

Prognostic biomarkers of SSc skin disease. It is challenging to predict progression versus regression of skin involvement in SSc. As mentioned, the presence of autoantibodies to RNAPol3 is associated with severe and rapidly progressive skin disease[11], but does not provide notable prognostic information about the likelihood of future progression. Gene expression data from placebo-treated patients in the faSScinate phase II study showed that expression of *IL13RA1*, *OSMR*, *SERPINE1*, *CTGF* and *CD14* correlates positively with progressive skin disease and that high expression of these genes are markers of a trajectory of progressive disease[92]. However, we do not believe that gene expression data should be used to stratify or select patients for inclusion in early-phase trials, but rather encourage inclusion and post hoc analysis of predictive biomarkers. The overlap of biomarkers of progressive disease with biomarkers of disease severity is consistent with the notion that patients with relatively high skin scores are most likely to show a differential response to therapy[94].

Biomarkers in SSc lung disease

Unlike the situation in SSc skin disease, tissue is not easily accessible for directly assessing biomarkers in SSc-ILD lungs in clinical trial settings. Serum and broncho-alveolar lavage fluid biomarkers have been sought for the diagnosis and prognostication of SSc-ILD, but (with the exception of Krebs von den Lungen (KL-6) in Japan[95]) autoantibodies are the only markers used in current clinical practice. The biomarkers discussed in this section correlate with the presence or progression of disease but have been used only rarely as exploratory

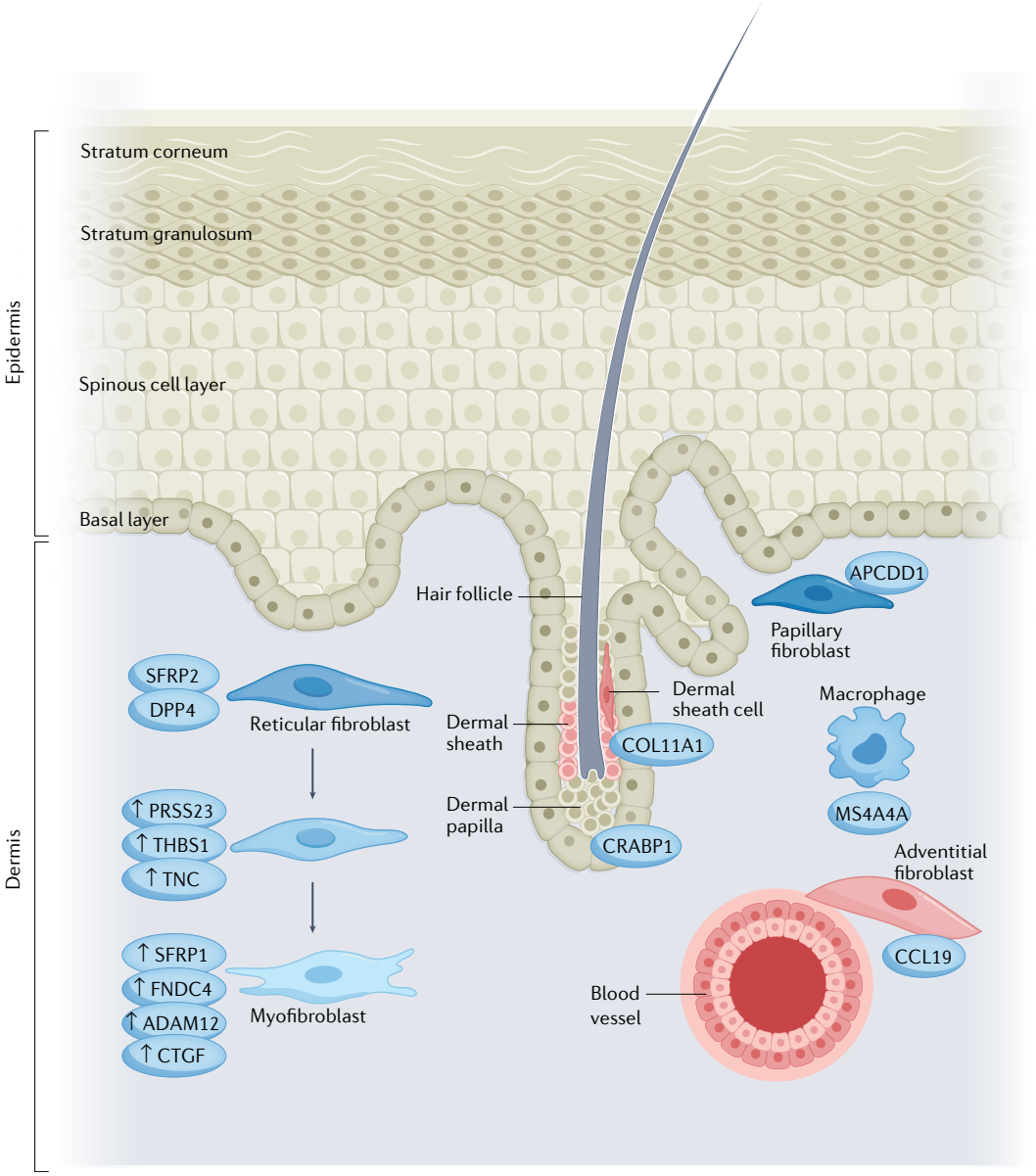

Fig. 2 | **Skin cell populations expressing biomarkers in SSc skin disease.** Single-cell RNA sequencing of skin biopsy samples from patients with diffuse cutaneous systemic sclerosis (SSc) shows that fibroblast and myeloid cell (MS4A4A-expressing macrophages) populations express biomarkers of SSc skin disease. The biomarkers correlating most highly with the modified Rodnan skin score (MRSS)[86,94] are expressed by a population of SFRP2/DPP4-expressing dermal fibroblasts that differentiate in two steps into myofibroblasts. Both steps in the fibroblast-to-myofibroblast differentiation are associated with biomarker genes that correlate with the MRSS. In the first step, SFRP2/DPP4-expressing fibroblasts upregulate expression of PRSS23, THBS1 and TNC. In the second step, SFRP2/DPP4-expressing fibroblasts additionally upregulate expression of SFRP4, FNDC4, ADAM12 and CTGF[89]. Other fibroblast populations and their associated gene markers are also shown[90,170].

outcome measures in SSc-ILD therapeutic trials[96,97], and remain investigational at this time. scRNA sequencing on explanted SSc-ILD lungs has established the cell-specific origins of these biomarkers[98] (FIG. 3).

Biomarkers of lung epithelial cells. KL-6 and surfactant protein D (SP-D), the products of the *MUC1* and *SFTPD* genes, respectively, are selectively expressed by epithelial cell populations in the lungs, with *MUC1* expressed by several epithelial cell populations, whereas *SFTPD* expression is primarily restricted to alveolar type II cells[95]. KL-6 is associated with FVC decline and with the development of end-stage lung disease (defined as FVC percent predicted <50%, need for supplemental oxygen, or death from respiratory failure)[97,99]. In a multicentre observational study of 427 individuals with SSc, baseline KL-6 levels correlated with FVC, DLCO and the extent of lung fibrosis on imaging, and could thus be used to evaluate the severity of SSc-ILD[97]. Unlike previous studies, in this multicentre cohort, KL-6 did not have predictive significance for a decline in pulmonary function. In the SLS-II study, baseline and 12-month KL-6 levels

and C-C motif chemokine ligand 18 (CCL18) correlated with the extent of pulmonary fibrosis on imaging and declined significantly with immunosuppressive treatment (with either cyclophosphamide or mycophenolate mofetil) at 12 months[100]. Higher baseline levels of CCL18 or KL-6 predicted decline in FVC and DLCO over 1 year, even with immunosuppressive treatment.

SP-D is a surfactant lipoprotein elevated in the setting of alveolar–capillary barrier damage[101]. SP-D serum levels are elevated in patients with SSc-ILD, and were previously identified to be more sensitive than KL-6 but less specific for detecting ILD[102]. Significant decline in SP-D levels in the setting of cyclophosphamide and prednisolone treatment was found to be predictive of a positive treatment response in patients with SSc-ILD[103]. In a European multicentre cohort that included 427 patients with SSc-ILD, SP-D level correlated with the presence of SSc-ILD but was not predictive of decline; it was posited as a possible diagnostic biomarker for SSc-ILD[97].

Serum biomarkers for profibrotic macrophages: CCL18 and osteopontin. CCL18 and osteopontin are primarily produced in the lung by macrophages[98] and are thought to be minimally influenced by extra-pulmonary organ involvement[104]. CCL18 is a macrophage-derived chemokine that acts as a chemotactic factor for other immune cells. CCL18 levels are elevated in the serum and broncho-alveolar lavage fluid in patients with SSc-ILD, and elevated serum CCL18 level at baseline predicts a decline in FVC[97,105,106] and is a risk factor for progression of SSc-ILD (despite treatment) and death[97,100,107,108]. CCL18 levels decreased significantly in patients treated

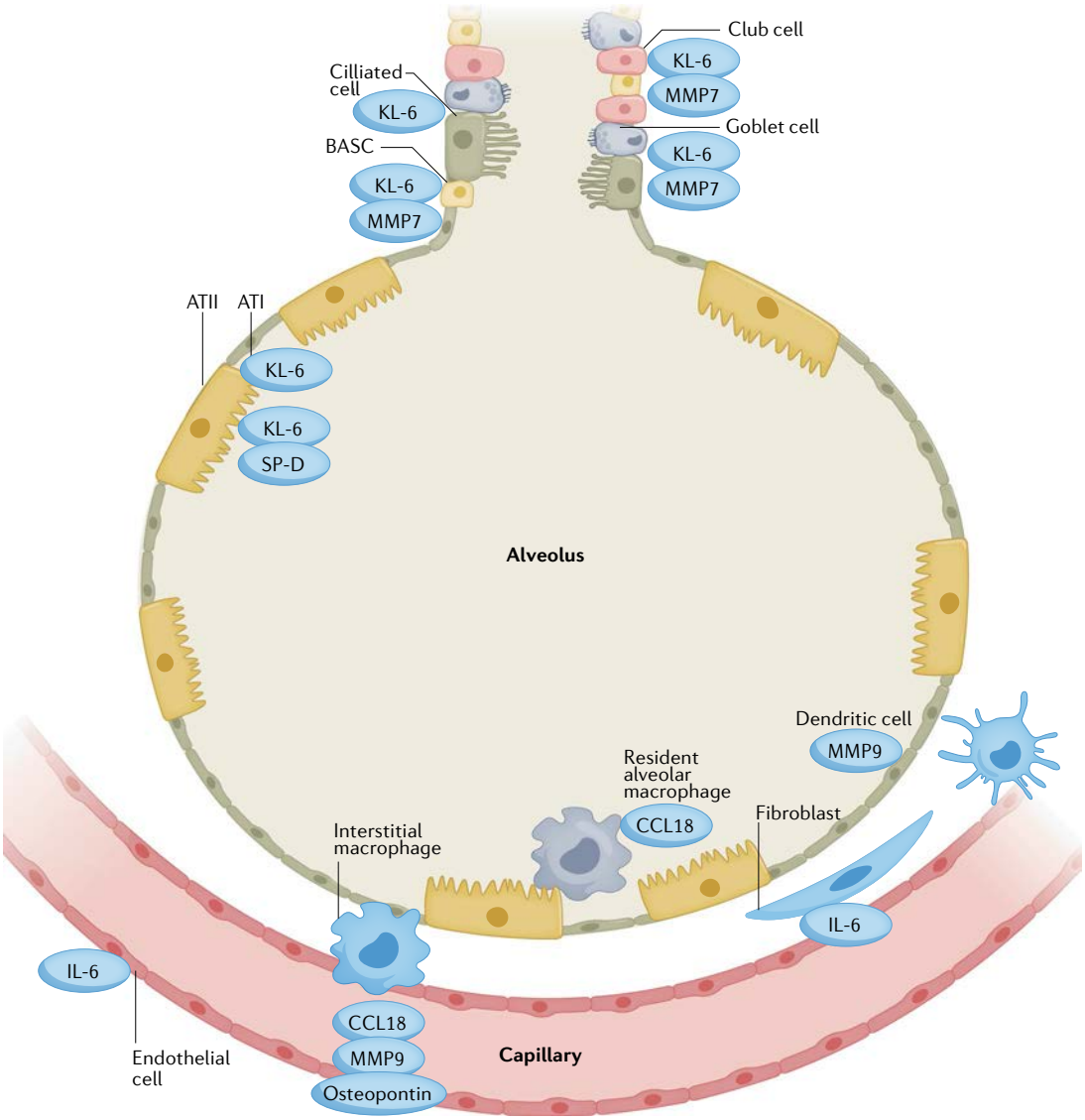

Fig. 3 | **Lung cell populations expressing biomarkers in SSc-ILD.** Single-cell RNA sequencing of explanted lungs from patients with systemic sclerosis-associated interstitial lung disease (SSc-ILD) show myeloid, epithelial and mesenchymal cell populations expressing biomarkers of SSc-ILD[98]. Krebs von den Lungen (KL-6) and matrix metalloproteinase 7 (MMP7) are expressed by multiple bronchial epithelial cell populations, whereas the alveolar epithelial cells express KL-6 and surfactant protein D (SP-D) (alveolar type II cells only). The interstitial *SPP1*^{hi}-expressing macrophages prominently express C-C motif chemokine ligand 18 (CCL18), MMP9 and osteopontin (*SPP1*), with alveolar macrophages only expressing CCL18. Both fibroblasts and endothelial cells express IL-6.

with the IL-6 receptor antagonist tocilizumab, which is associated with the stabilization of lung function[88]. This finding is consistent with findings from an in vitro study showing that IL-6 markedly reduced expression of CCL18 in vitro in alternatively activated macrophages[109]. Whether CCL18 is a specific pharmacodynamic biomarker for the effect of IL-6 or more generally a marker for SSc-ILD remains unclear. Osteopontin, the gene product of *SPP1*, has been less frequently studied but is associated with ILD in patients with SSc[110]. High levels of *SPP1*-expressing macrophages are present in the lungs of patients with SSc-ILD, and in those with IPF, and *SPP1*-expressing macrophages are strongly implicated as being profibrotic through their expression of *SPP1* and *CCL18* (REFS.[98,111,112]).

C-reactive protein, a prognostic biomarker. C-reactive protein (CRP) is an acute phase reactant produced mainly by liver hepatocytes in response to IL-6 secretion by myeloid and lymphoid populations[113]. Increased CRP levels are associated with decreased survival and with progression of SSc-ILD as measured by a decline in PFTs[114,115], as well as with early progressive SSc-ILD[116]. In the Genetics versus Environment in Scleroderma Outcome Study (GENISOS), which included 266 patients with SSc and 97 controls, baseline CRP was higher in patients with SSc than in controls, with higher CRP levels associated with a decline in FVC and decreased survival[114]. Similarly, in the Australian Scleroderma Cohort Study (involving 1,545 individuals) increased CRP was associated with a significant decline in FVC[115]. Elevated CRP levels were used as an enrichment criterion to prospectively select a study population with a greater likelihood of detecting a drug effect in the phase II and III trials of tocilizumab for the treatment of early SSc[73,117].

Plasmacytoid dendritic cells, interferon and CXCL4. Plasmacytoid dendritic cells (pDCs) have been clinically implicated in SLE through their known role in secreting high levels of type I interferon-α[118,119]. Proteome-wide analyses of SSc pDCs identified C-X-C motif chemokine ligand 4 (CXCL4, also known as platelet factor 4) as a prominent protein secreted by pDCs in the skin and peripheral blood[120]. In patients with SSc, pDCs are depleted in the peripheral blood and accumulate in target organs, with the frequency of pDCs correlating with fibrosis severity on HRCT[121]. Elevated levels of CXCL4 correlate with the presence of ILD in patients with SSc, with higher levels predicting a more rapid decline in DLCO[120]. In a post hoc analysis of the SLS-II participants, CXCL4 was higher in patients with SSc-ILD than in healthy controls, but no significant correlation was found between CXCL4 levels and baseline disease severity[122]. CXCL4 levels decreased significantly in all patients receiving immunosuppressive therapy (with no between-treatment differences observed between cyclophosphamide and mycophenolate mofetil), and patients with the greatest decline in CXCL4 at 12 months had the most improvement in pulmonary function during the subsequent 12 months[122]. CXCL4–DNA complexes are present in vivo and have been found to activate pDCs in a toll-like receptor 9 (TLR9)-dependent manner[123], independent of CXCR3 signalling. Although a marked increase in pDCs has been shown in skin from patients with SSc, single-cell analyses did not detect *CXCL4* expression by pDCs from SSc skin[91].

Other proposed biomarkers for SSc-ILD include insulin-like growth factor binding protein 2 (IGFBP2)[124], CCL2 (REF.[125]), IL-6 (REF.[126]), matrix metalloproteinase 7 (MMP7) (REF.[127]), and MMP9 (REF.[128]). Composite biomarkers seeking to incorporate multiple organ manifestations such as the enhanced liver fibrosis score, which includes three extracellular matrix components[129,130], and multiple interferon-inducible chemokine scores, have also been investigated for prognostication and risk stratification in patients with SSc[131,132].

Future directions

Considerations for early-phase trials. Although historically fibrosis was considered an irreversible process, this is clearly not the case, as reversibility of skin fibrosis is well described[10]. This sense of irreversibility led to the notion that the only approach to effective therapy would be to block disease progression. From this standpoint, data from the EUSTAR database suggested that the best patients to enter into clinical trials would be patients with relatively mild skin disease[133,134]. By contrast, trajectory analyses of patients entered into the US faSScinate trial indicated that in this cohort patients with more severe disease at study onset tend to show progressive disease, and so might be more suitable for entry into clinical trials[92]. This discrepancy might reflect the entry criteria for faSScinate, which required evidence of progressive skin disease and elevated inflammatory markers, or might reflect differences in biology between Europe and the US.

Only about 30% of patients with dcSSc show progressive disease, yet a large percentage of these patients have moderately severe to severe skin involvement[10]. The severity of skin disease — as assessed by the MRSS — correlates strongly with biomarkers of disease and not with disease duration[94]. In view of these considerations, we suggest that the goal of skin treatment in SSc trials should not be limited to blocking progression, but rather to improving skin disease, and that patients most likely to show improvement are those with more severe skin disease.

The biggest limitation to using the MRSS as an outcome measure for SSc skin disease seems to be that we have not yet found the correct drugs. Most recent trials have used medications that work in other diseases that have a different pathogenesis and no prominent fibrosis. Targeting pathways that are more clearly implicated in SSc pathogenesis would more likely lead to the discovery of active therapeutics. Biomarkers of skin disease have two important roles in this process, helping to identify likely targetable pathways and providing pharmacodynamic biomarkers for assessing patient responses. The major advantages of biomarkers are that they are objectively and precisely quantifiable, and that they are theoretically responsive to short treatment periods. These qualities mean that skin biomarkers are particularly well suited to being the primary outcome for early-phase clinical trials, a current critical bottleneck in SSc drug development.

Skin mRNA biomarkers have the potential to aid in clinical trials in several ways. First, they are objective measures and not affected by the scorer like the MRSS is. Second, they in theory might change more rapidly, enabling evaluation after shorter periods of time. Our experience in a limited number of clinical trials suggests that although pharmacodynamic biomarkers can aid in evaluating drug efficacy, as they are selected based upon their representation of the skin score, they do not circumvent the variability of skin disease progression or regression. However, most concerning has been the difficulty in interpretating underpowered clinical trials. Trials of rituximab for SSc-PAH[135] and abatacept for SSc-skin disease[136] have illustrated this concern. Although the primary outcome measure was not met in either case, in both studies biomarker changes in the treated groups suggest treatment efficacy that might have been detected with studies enrolling larger numbers of patients[136,137]. Indeed, studies showing efficacy of drugs for PAH generally enrol ≥200 patients[138,139], compared with 57 patients entered into the study of rituximab in SSc-PAH[137]. Thus, early-phase trials must have adequate sample sizes for evaluating safety and trends in biomarkers as well as clinical indicators of efficacy. As these trials are rarely powered to detect efficacy with a P value <0.05, the results should be interpreted in this context.

It has generally been accepted that phase II/III trials in patients with SSc skin disease should last 6–12 months. However, the difficulty in detecting drug effects owing to spontaneous improvements in skin disease could in theory be circumvented in early-phase trials by examining biomarker changes after a shorter period of time. Such considerations need to be tailored to the therapeutic target. We might expect that therapies that directly target myofibroblasts (such as inhibitors of transforming growth factor beta (TGFβ), integrin activators of TGFβ and rho-kinases), which show effects in vitro in hours to days, might affect skin gene expression in vivo after 2–4 weeks, as was reported for fresolimumab[87]. For drugs targeting inflammatory mediators thought to act with other cell types before affecting myofibroblasts, the timing for assessing skin gene expression should be delayed. For example, the effects of tocilizumab on macrophages were shown to be statistically significant after 24 weeks[2,88].

Combined outcome measures. A key question is whether a composite or a single measure is superior in detecting the effects of an intervention. Composite measures broadly include combined measures, such as the Composite Response Index in dcSSc (CRISS) and the European Scleroderma Study Group Activity Index (EScSG-AI), and/or a composite outcome most commonly examining time to event. Challenges with composite indices include weighting and comparing individual outcomes on a continuous scale. Lupus studies provide some context for the use of combined outcome measures. The approval of the novel calcineurin inhibitor voclosporin for lupus nephritis was based on a dichotomous complete renal response outcome[140], a composite outcome assessing urine protein, renal function, rescue medication or need for continued high-dose prednisone. Extending the study of belimumab (a recombinant human IgG-1λ monoclonal antibody that inhibits B cell activating factor) to lupus nephritis (from SLE) used a similar renal composite outcome to define preserved renal function[141]. Although using composite outcomes, these measures were all designed to assess the same organ, the kidney. Thus, these composite outcome measures are different from the CRISS and EScSG-AI, which seem more similar in construct to the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), a composite measure including indices of disease activity across nine organ systems[142].

The CRISS is a composite outcome with five scaled measures as well as organ damage components, thus designed to assess multiple organs[143]. The CRISS also includes physician and patient global indices (the latter a PRO) potentially capturing disease manifestations, ranging from digital ulcers to gastrointestinal disease. The methodology and careful development of the CRISS suggest that it is at a minimum a strong starting point for SSc trials. Its lack of success so far might be related more to inadequate trial power and lack of application to the right therapeutic. As a composite measure, it might be most appropriate for studies involving medications thought to be disease modifying, particularly drugs likely to affect both of the most highly weighted components: skin disease (MRSS) and SSc-ILD (FVC).

Another composite measure, the EScSG-AI[144], was originally developed to determine how aggressively patients should be treated, rather than as an outcome measure for clinical trials[145]. Disease severity assessed by chart review correlated with select disease parameters. Four of the 11 composite criteria are based on patient-assessed change in skin, vascular, articular/muscular and cardiopulmonary disease in the preceding month (essentially PROs), two are laboratory measures (ESR and hypocomplementaemia), the remainder are total skin score >20, arthritis, scleredema and depressed DLCO. The EScSG-AI correlates with physician assessments of patient disease activity and acts as a prognostic marker of organ damage[146–148]. Revision of the EScSG-AI in 2017 resulted in the EUSTAR-AI[149], showing higher predictive correlations with future risk of organ damage[150]. The correlation with future organ damage fulfils a key regulatory desire for drug approval. The EUSTAR-AI includes a series of dichotomous measures: a PRO (Δ-skin; that is, patient-perceived change in skin disease from the preceding month); physical findings (for example, digital ulcers and tendon friction rubs); a laboratory measure (CRP >1 mg/dl); a lung function measure (DLCO <70%); and an MRSS score, consisting of either a single score weight for MRSS >18 or a weighted value for MRSS <18. Although not explicitly developed as an outcome measure, the EUSTAR-AI changes in patients over time[150]. Its performance in a clinical trial setting has yet to be shown. The use of DLCO rather than the FVC measure in the EUSTAR-AI is notable as DLCO captures pulmonary vascular disease while reducing specificity for SSc-ILD compared with FVC. However, like the CRISS, the EUSTAR-AI is strongly weighted towards detecting skin and lung disease.

In time-to-event trials, composite outcomes lead to more events and the potential to increase power based

on higher event rates, thus enabling smaller sample sizes or shorter study duration[151]. Could time-to-event be utilized effectively as a composite outcome in SSc? What would be appropriate events? Some events could be defined easily: death, scleroderma renal crisis, and decline or improvement in FVC or MRSS beyond a certain percentage of baseline. This approach could convert the challenges in precisely assessing the MRSS to the time to achieve some threshold of improvement or deterioration. Gastrointestinal events might also be defined. Whether or not this approach would provide a more robust outcome measure would require extensive development and testing. A major limitation of combined outcomes in general is that outcomes in the composite outcome that show an effect might be diluted by outcomes that show no effect[151].

Surrogate outcome measures. The FDA has described a formal process for the regulatory approval of surrogate outcome measures, referred to as qualification[39]. Once approved, these surrogate outcome measures become acceptable for regulatory approval. The FDA has described three stages of biomarker progression to surrogate end point: first, a candidate surrogate end point; second, a reasonably likely surrogate end point; and third, a validated surrogate end point. The FDA states "Reasonably likely surrogate end points are supported by strong mechanistic and/or epidemiologic rationale, but the amount of clinical data available is not sufficient to show that they are a validated surrogate end point. Reasonably likely surrogate end points can be used to support FDA's Accelerated Approval program, which is intended to provide patients with serious diseases more rapid access to promising therapies"[152].

FVC is the only measure for SSc-ILD listed on the FDA Table of Surrogate Outcome measures[153]. Lupus nephritis is the only other rheumatic disease complication with a listed surrogate outcome: a complete renal response, defined as a decrease in urine proteinuria and improvement or stabilization of renal function. The commonly used measures for SSc disease, MRSS, FVC and 6MWD, are consistent with surrogate outcome measures. Although they don't directly measure how a patient feels, functions or survives, the MRSS does correlate with survival, the FVC correlates with how a patient feels and the 6MWD correlates with how a patient functions.

For a biomarker to be considered as a surrogate outcome, it must correlate with a clinical outcome and be directly involved in the pathogenic process. Otherwise, the biomarker might change with treatment without indicating an underlying benefit to the patient. Thus, the FDA qualification of skin mRNA biomarkers requires that we understand the correlation with clinical measures, as well as the role in pathogenesis. Several of the biomarkers of fibroblast activation, such as *COMP* and *THBS1*, correlate with MRSS, change over time, and are directly implicated in the pathogenic process. Thus, there are candidates that might be considered as surrogate outcome measures. As a minimum, they seem to meet the FDA designation of a reasonably likely surrogate end point and could be considered for accelerated approval.

Research agenda. A core set of outcomes needs to be established for future SSc clinical trials in order to assess both clinical and biomarker outcomes. The current deficiency in this area is highlighted in a 2020 study assessing the extent and consistency of measures and outcomes reported in SSc clinical trials, which found reported measures to be very inconsistent, with all five measures included in the CRISS reported in only 11% of trials[154]. The core set of outcomes should as a minimum include the component measures included in the EUSTAR-AI and CRISS. Such a core set of outcomes would enable evaluation of both CRISS and EURSTAR-AI, even if these were not the primary outcomes, and would enable investigators to assess the sensitivity to change of individual components of these outcome measures. Similarly, a core set of biomarkers should be established for inclusion in all SSc clinical trials. Understanding the relationship between biomarkers and clinical outcomes is critical to assessing biomarker utility. Open access to skin biomarker results should become a clinical trial standard. Many patients with SSc have had gene expression studies on skin biopsy samples as part of clinical trials only to have the results remain unreported and/or inaccessible. This loss of data represents tragic lost opportunities for moving research and patient care forward. In the future, we anticipate that biomarkers developed from scRNA sequencing will become a new standard for assessing both efficacy and the pathways affected by interventions in SSc.

Conclusions

Current outcome measures in SSc-ILD are largely adequate, but outcome measures of SSc skin disease have yet to lead to drug approval. Further study of skin outcome measures is warranted, but the MRSS might yet prove a robust outcome measure for efficacious medications in adequately powered clinical trials, such as those seen in immunoablation and stem cell transplant[21]. Further development of promising biomarkers and composite end points as outcome measures is needed. Use of FVC as an end point in trials in SSc-ILD has led to approval of two medications (nintedanib and tocilizumab)[52,73], and use of 6MWD as an end point in trials has led to multiple medications for SSc-PAH. Further study of skin mRNA biomarkers might enable them to qualify as surrogate outcome measures, although their utility is likely to be primarily in the context of early-phase clinical trials, where they might be used to assess target engagement by study medication as well as efficacy. However, their effective utilization requires careful consideration of expected cell and gene targets as well as the anticipated speed of their effect on fibroblasts, the final mediators of skin fibrosis. ScRNA sequencing dramatically expands the breadth of information about drug activity and should probably be used routinely in early-phase SSc skin studies to understand drug effects. Further studies will show whether emerging imaging technologies can inform clinical trials of lung disease where direct access to tissue is not feasible.

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Author contributions
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When underlying biology threatens the randomization principle — initial gout flares of urate-lowering therapy

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Abstract | Flare is the dominant feature of gout and occurs because of inflammatory response to monosodium urate crystals; prevention of gout flares should be the major goal of gout care. However, a paradoxical increase in the risk of flare following initiation of urate-lowering therapy presents considerable challenges for proving the expected long-term benefits of flare prevention in clinical trials. Nevertheless, excluding from enumeration flares that occur in the initial post-randomization period (which can last several months to 1 year) can threaten the core benefits of randomization: the characteristics of the remaining participants can differ from those who were randomized, introducing potential bias from confounding (both measured and unmeasured); participants who drop out or die are excluded from the analysis, introducing potential selection bias; and, finally, ignoring initial flares underestimates participants' experience during the trial. This Perspective discusses these issues and recommends measures that will allow for high-level evidence that preserves the randomization principle, to satisfy methodological scrutiny and generate robust evidence-based guidelines for gout care.

The gout flare is the dominant presentation of gout and occurs because of an inflammatory response to monosodium urate (MSU) crystals. The intense pain and impact of the gout flare mean that it is central to the patient experience of gout, and prevention of gout flares should be the major goal of effective gout management. However, the risk of flare paradoxically increases in the period after initiation of urate-lowering therapy (ULT), presenting considerable challenges related to proving the expected flare-prevention benefits of ULT over the long term in randomized trials. Nevertheless, excluding from enumeration flares that occur in the initial post-randomization period of randomized controlled trials (RCTs), which has been done in all RCTs to date, can threaten the core benefits of the randomization principle. For instance, the characteristics of participants remaining in the RCT after this initial period (lasting several months to 1 year in RCTs) might be different from the characteristics of those who were randomized, introducing potential bias from measured and unmeasured confounding; moreover, participants who drop out or die during the initial period cannot be included in the analysis, introducing potential selection bias; finally, ignoring initial flares underestimates the burden of gout flares experienced by participants over the entire trial.

In this Perspective, we discuss several measures to accommodate this characteristic biology of paradoxical gout flares while preserving the benefits of randomization, including careful planning for entire-period analyses (as opposed to analyses of a specified post-randomization period), effective flare prophylaxis, sufficient trial duration, maximum efforts and mechanisms for participant retention, use of adherence-adjusted per-protocol analysis (in addition to intention-to-treat (ITT) analysis) and collection of high-quality longitudinal data to predict non-adherence. Implementation of these measures in gout RCTs will lead to high-level evidence of ULT effects for flare prevention to generate robust evidence-based guidelines for gout care.

Flares are central to gout

The gout flare is the most common and dominant presentation of gout, and occurs because of the activation of the innate immune system in response to MSU crystals[1,2]. The gout flare is experienced as the rapid onset of acute joint inflammation, with severe pain and associated tenderness, swelling, warmth and erythema. The patient experience of the gout flare is multidimensional, as it affects activities of daily living (including difficulty with walking, self-care, driving and sleeping); social and family life (by restricting social participation, employment, independence and intimacy); and psychological health (contributing to irritability, anxiety, fear, depression, isolation and financial worry)[3]. The intensity of the pain and the impact of the gout flare mean that it is central to a patient's experience of gout, and prevention of gout flares should be the major goal for effective gout management. Nevertheless, high-quality data from trials with gout flares as a primary end point remain scarce, which has contributed to conflicting guidelines on gout care for primary care (American College of Physicians)[4] and for rheumatology, as reviewed elsewhere[5–8]. Rheumatology guidelines emphasize a treat-to-target serum urate approach (for example, serum urate concentration <6 mg/dl, a urate sub-saturation point)[9,10]; however, citing the absence of evidence, serum urate is not even measured during ULT in the vast majority of patients with gout in primary care practice, where >90% of gout care occurs[11].

Initiating ULT triggers flares

Although long-term ULT leads to the prevention of gout flares (through dissolution of deposited MSU crystals), the frequency of gout flares increases at the start of ULT. This common, paradoxical pattern of initial worsening, which can last for months (FIG. 1), followed by improvement of the same disease end point, is unique in modern rheumatology therapeutics, although it is often underappreciated and poorly explained to patients, contributing to premature discontinuation of ULT[12].

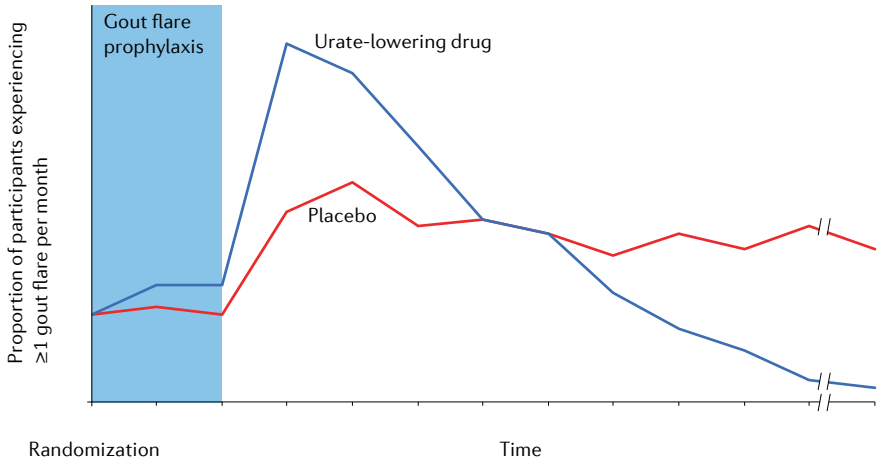

Fig. 1 | **Gout flare trends after initiation of a potent urate-lowering agent in a hypothetical placebo-controlled randomized controlled trial.** The risk of flares in the urate-lowering drug group increases after the initial anti-inflammatory prophylaxis phase of the trial (for example, 3 months) dissipates. This paradoxical worsening is followed by a substantially lower risk of flares over time. By contrast, the placebo group is expected to have a similar (or higher) level of flares over time, once the initial anti-inflammatory prophylaxis effect discontinues.

This phenomenon was recognized from the initial reports of ULT, with Yue and Gutman reporting in their early descriptions of allopurinol from the 1960s "The most troublesome problem we encountered with allopurinol therapy was the precipitation of acute gouty arthritis. The incidence of acute attacks provoked by allopurinol must be considered excessive"[13]. Gout flares occur in up to three-quarters of patients in the first 6 months of allopurinol treatment without anti-inflammatory prophylaxis[14].

Some investigators have termed gout flares occurring soon after initiation of ULT 'mobilization flares', reflecting that these flares are thought to occur as MSU crystals are shed or mobilized from intra-articular deposits when the serum urate level falls, leading to interactions between crystals and resident synoviocytes and initiation of the acute inflammatory response[15,16].

Increased frequency of gout flare has been reported with all currently approved urate-lowering drugs, and occurs more often in the setting of more rapid and intensive reductions in serum urate[17,18]. For this reason, gradual dose escalation of ULT and anti-inflammatory prophylaxis is recommended for the first 3–6 months of ULT[19]. The most common strategy for anti-inflammatory prophylaxis is low-dose daily colchicine, which reduces the frequency and severity of gout flares, and the likelihood of recurrent flares in those starting allopurinol[14]. In an RCT of patients with gout starting allopurinol, colchicine prophylaxis reduced the number of flares over 6 months (0.5 in the colchicine

Table 1 | **Design and gout flare reporting in pivotal clinical trials of urate-lowering therapy for gout**

Trials	Trial summary	Trial duration (dropout rate)	Flares as outcome measure	Anti-inflammatory prophylaxis	Gout flare outcome reporting and time periods	Ref.
FACT	RCT comparing fixed-dose febuxostat and fixed-dose allopurinol	52 weeks (35%)	Secondary end point	Low-dose colchicine or naproxen for 8 weeks	The proportion of participants requiring treatment for acute gout flares from weeks 9 to 52 (specified) The proportion of participants requiring treatment for acute gout flares from weeks 49 to 52 also reported	24
C0405 and C0406	Placebo-controlled RCT of pegloticase	6 months (30%)	Secondary end point	Low-dose colchicine or NSAIDs for 6 months (entire trial period)	The proportion of participants with gout flare (gout flare incidence) during months 1–3 and 4–6 of the trial The number of flares per participant during months 1–3 and 4–6 of the trial	26
CLEAR-1[a]	Placebo-controlled RCT of lesinurad (in combination with allopurinol)	12 months (25%)	Secondary end point	Low-dose colchicine or NSAID for 5 months	Mean rate of gout flares requiring treatment from the end of month 6 to the end of month 12	33
Doherty et al. 2018	RCT comparing nurse-led gout care with usual care	2 years (9% vs 21%)[b]	Secondary end point	Timing not standardized, low-dose colchicine used in 3/255 (1%) of participants in the nurse-led group	Frequency of gout flares during years 1 and 2	25
CSP594	Non-inferiority RCT comparing febuxostat and allopurinol using a treat to serum urate target approach	72 weeks (20%)	Primary end point	Low dose colchicine, NSAIDs, or glucocorticoids for 48 weeks	Primary end point: proportion of participants experiencing one or more flares during weeks 49 to 72 Secondary end point: rate of gout flares (events/person-years) during the entire period as well as each phase of the trial: titration (weeks 0 to 24), maintenance (weeks 25 to 48) and observation (weeks 49 to 72)	27

Trials in this table represent the major phase III trials for urate-lowering therapy approved by the FDA since 2009 or large (>500 participants) investigator-initiated strategy trials. RCT, randomized controlled trial. [a]US-based trial; same design used for CLEAR-2 multinational trial[41]. [b]9% in nurse-led gout care and 21% in usual care.

Table 2 | **Flare outcomes during pivotal clinical trials of urate-lowering therapy for gout**

Trials	Flare outcomes in the early trial period	Flare outcomes in the late trial period	Flare outcomes for the entire trial period	Ref.
FACT (Becker et al. 2005)	Day 1 to week 8: more participants with gout flare in the febuxostat 120 mg group than in the allopurinol group (36% vs 21%; $P < 0.001$)	Weeks 9–52: no difference in the proportion of participants with gout flare in the febuxostat 120 mg group compared with the allopurinol group (70% vs 64%) Weeks 49–52: no difference in the proportion of participants with gout flare in the febuxostat 120 mg group compared with the allopurinol group (6% vs 11%)	Not reported	24
C0405 and C0406 (Sundy et al. 2011)	Months 1–3: higher gout flare incidence in the bi-weekly pegloticase group than in the placebo group (75% vs 53%; $P = 0.02$) Months 1–3: more gout flares in the bi-weekly pegloticase group than in the placebo group (mean 2.3 vs 1.2; $P = 0.001$)	Months 4–6: lower gout flare incidence in the bi-weekly pegloticase group than in the placebo group (41% vs 67%; $P = 0.007$) Months 4–6: fewer gout flares in the bi-weekly pegloticase group than in the placebo (mean 0.8 vs 1.3; $P = 0.06$)	Not reported	26
CLEAR-1 (Saag et al. 2017)	Not reported	Months 7–12: no difference in the lesinurad 400 mg group and the placebo group (mean 0.51 vs 0.58; $P = 0.61$)	Not reported	33
Doherty et al. 2018	Year 1: more participants in the nurse-led group than in the usual care group experienced ≥2 gout flares (54% vs 40%, risk ratio 1.36 (95% CI 1.05–1.77))	Year 2: fewer participants in the nurse-led group than in the usual care group experienced ≥2 gout flares (8% vs 24%, risk ratio 0.33 (95% CI 0.19–0.57))	Not reported	25
CSP594 (O'Dell et al. 2022)	Weeks 0–48: proportion of participants experiencing one or more gout flares not reported Secondary end point: weeks 0–24: no difference in gout flare rate in the allopurinol group and the febuxostat group (2.09 vs 2.25 flares per person years; rate ratio 0.93 (95% CI 0.81–1.06)) Secondary end point: weeks 25–48: no difference in gout flare rate in the allopurinol group and the febuxostat group (1.60 vs 1.59 flares per person years; rate ratio 1.00 (95% CI 0.85–1.18))	Primary end point: weeks 49–72: fewer participants with gout flares in the allopurinol group than in the febuxostat group (36.5% vs 43.5%; risk ratio −7 (95% CI −∞ to −1.2); $P < 0.001$ for non-inferiority of allopurinol) Secondary end point: weeks 49–72: fewer gout flares in the allopurinol group than in the febuxostat group (1.48 vs 2.02 flares per person years; rate ratio 0.73 (95% CI 0.63–0.86))	Proportion of participants experiencing one or more gout flares over the entire study period not reported Gout flare rate over the entire study period reported in the manuscript: fewer gout flares in the allopurinol group than in the febuxostat group (1.73 vs 1.97 flares per person-years, rate ratio 0.88 (95% CI 0.81–0.96))	27

Trials in this table represent the major phase III trials for urate-lowering therapy approved by the FDA since 2009 or large investigator-initiated strategy trials (>500 participants). Where more than one dose was tested, data for the highest dose are presented.

group and 2.9 in the placebo group; $P = 0.008$)[14]. Low-dose NSAIDs can also be used as anti-inflammatory prophylaxis[20]. Anti-IL-1 therapies, such as canakinumab and rilonacept, have also been shown in clinical trials to reduce gout flares at the time of initiating ULT[21,22], but are not approved for this indication.

How are flares assessed in trials?

The Outcome Measures in Rheumatology (OMERACT) group has recognized the central importance of the gout flare in its core set of outcome domains for long-term studies of gout[23]. However, pivotal phase III trials of ULT in the modern era of drug approval and major strategy trials have segmented out flare analyses to focus on flare reporting later in the course of treatment, when the risk of flares has subsided (summarized in TABLE 1). Some of these trials have reported an increased risk of flares with the investigational product in the early stages of the trial[24–26], but the approach of reporting the flare experience over the entire period of the trial has not been adopted (TABLE 2). The comparative efficacy trial of febuxostat and allopurinol published in early 2022 is the first ULT trial to report gout flares as the primary end point[27]. However, this primary end point (the proportion of participants experiencing one or more flares) only covered the third phase of the study (weeks 49 to 72), after urate-lowering had been established, but not for the entirety of the trial or for the first phase (weeks 0 to 24) or second phase (weeks 25 to 48) individually. Nevertheless, flare rate (as opposed to risk proportion), one of the trial's pre-specified secondary end points, was reported over the entire period as well as for each of the three phases[27].

These analytic approaches[27] are in contrast to those used in trials of other interventions with time-dependent (non-proportional) effects (TABLE 3). For example, similar time-dependent trade-offs arise with the use of initially intrusive interventions, such as transplantation, surgeries or other invasive procedures, which can have immediate adverse effects — even mortality, initially — but subsequently lead to benefits among those who survive. For example, the 2014 ASTIS RCT of autologous haematopoietic stem cell transplantation for systemic sclerosis produced crossing survival curves for both death and organ failure, as expected, but the primary end point (event-free survival) was reported over the entire period (median follow-up 5.8 years) as well as at year 1, year 2 and year 4, starting from randomization[28] (TABLE 3). Other trials have similarly reported all adverse effects and benefits that occur during the entire period since randomization[29,30].

To accommodate the expected lag in biological effect of the COVID-19 vaccination, two trials[31,32] ascertained the primary end point (COVID-19 infection) from a pre-specified time after randomization (7 or 14 days after the second dose of vaccine) (TABLE 3) in a per-protocol analysis, which excluded ~3%

Table 3 | **Clinical trials with post-randomization landmark time or time-dependent relative risks**

Trial	Trial summary	Trial duration	Primary outcome measure	Outcome reporting	Ref.
ASTIS (van Laar et al. 2014)	RCT of ASCT vs cyclophosphamide for diffuse systemic sclerosis	5.8 years	Event-free survival (death or persistent major organ failure)	Events during the entire follow-up period and also at year 1, year 2 and year 4 Outcomes initially worse with ASCT and then better; survival curves crossed at year 2	28
SCOT (Sullivan et al. 2018)	RCT of myeloablative ASCT vs cyclophosphamide for severe scleroderma	54 months (primary) and up to 72 months	Global rank composite score (including death and major events)	ITT and 'per-protocol'[a]: event-free survival of the entire follow-up period. ASCT had longer-term benefits in events and mortality, but treatment-related mortality was 6% in the ASCT group vs 0% in the cyclophosphamide group	29
MEDIC (Skou et al. 2015)	RCT of TKR vs non-surgical treatment	12 months	Change in OA outcome scale scores (0–100) over 12 months	OA outcome scale score over 12 months improved with TKR Adverse effects over 12 months were worse in the TKR group	30
Polack et al. 2020	RCT of BNT162b2 mRNA vaccine vs placebo	Varied owing to ethical concern, although designed to be up to 2 years	COVID-19 onset ≥7 days after second dose of vaccine	Risk of COVID-19 after dose 1, between doses 1 and 2, 7 days after dose 2, and ≥7 days after dose 2 (primary)	31
Baden et al. 2021	RCT of mRNA-1273 vaccine vs placebo	Varied; median follow-up duration of ≥2 months, per FDA	COVID-19 onset ≥14 days after the second dose of vaccine	Risk of COVID-19 any time after randomization, between randomization and 14 days after dose 1, 14 days after dose 1 to dose 2, dose 2 to 14 days after dose 2, and ≥14 days after dose 2 (primary)	32

ASCT, autologous stem cell transplantation; ITT, intention-to-treat; OA, osteoarthritis; RCT, randomized controlled trial; TKR, total knee replacement. [a]Defined as participants who received a transplant or completed nine or more doses of cyclophosphamide.

to 7% of the ITT population as randomized. Both trials also conducted ITT analyses for the same end points over the entire period of follow-up, starting from the point of randomization (TABLE 3), the results of which were consistent with the primary analyses. Thus, these vaccine trials adopted similar landmark analysis strategies to the gout trials discussed above (TABLES 1,2), although the duration of the unaccounted period was shorter in the vaccine trials (4–6 weeks), resulting in lower dropout rates; additionally, there was no initial paradoxical worsening in the intervention group, and the same primary end points were reported over the entire period after randomization (using ITT analysis).

Landmark analysis can bias results

The randomization in clinical trials (RCTs), when done properly and with a sufficiently large sample size, guarantees that the potential confounders, known or unknown, are evenly distributed between the comparison groups, providing a powerful advantage over observational studies. This advantage is valid at the time of randomization (the index date) and can be sustained when all events are counted after randomization during the entire trial period, without biased follow-up (for example, from participants dropping out or switching treatment). However, all gout trials to date, including the pivotal trials in TABLE 1, started counting flare events (as a primary end point in one trial[27] and a secondary end point in the other trials[24–26,33]) at a specific time that was substantially after the time of randomization (that is, a post-randomization landmark time), and they also had notable dropout rates (TABLE 1). Such post-randomization analysis (also called landmark analysis)[34] resets the trial 'clock', by moving the index date from the time of randomization to the landmark time. As a result, the characteristics of participants who survive and are retained to the landmark date could be different from the characteristics of those who were randomly assigned to a treatment group on the index date[34,35], introducing potential confounding bias (including both measured and unmeasured confounders). Furthermore, participants who are not retained to the landmark date (for example, owing to dropout or death) cannot be included in the analysis, introducing potential selection bias. Finally, this approach ignores the outcome (that is, gout flare) that occurs between the index date and the landmark date, resulting in underestimation of the risk of flares that participants experience over the entire study period[34,35], as stated above.

ITT can underestimate effect

ITT analysis of events during the entire study period guarantees a valid estimate of the effect of the treatment on the outcome, provided that there is no treatment misclassification and no selection bias. However, in the context of notable non-adherence to treatment assignment, including loss-to-follow-up, particularly in long-term RCTs (which is often the case with gout trials) (TABLE 1), ITT analysis would, in general, underestimate the effect of treatment. As such, if the outcome of interest concerns undesirable events, such as gout flare or safety (for example, toxicity), the results could be incorrectly interpreted as lack of evidence of harm[36]. A better approach to account for non-adherence in this context (including loss to follow-up) is an adherence-adjusted per-protocol analysis. By predicting non-adherence using appropriate statistical methods[37], this approach enables the investigators to assess the effect that would have been observed if all participants (as randomized) had received their assigned treatment during the study period[37] (BOX 1). This adherence-adjusted per-protocol analysis should not be confused with conventional on-treatment or as-treated analysis, which jeopardizes the central purpose of randomization, unlike ITT or adherence-adjusted per-protocol analysis (BOX 1). Nevertheless, adherence-adjusted per-protocol analysis relies on available prognostic factors to predict the risk of non-adherence, necessitating pre-planned collection of high-quality longitudinal data including health care utilization, comorbidities and medication use[38] (TABLE 4).

Solutions and associated issues

To accommodate the characteristic biology of flares in gout while retaining the advantages of randomization, future RCTs of ULT with gout flares as end points would be well served by several considerations (TABLE 4). To take full advantage of the RCT design and avoid potential biases that could interfere with identifying causal relationships, it would be desirable to include flares over the entire trial period (starting from randomization) as the primary outcome. As a minimum, investigators should include in their report entire-period data for the occurrence of flares. Analysis of a pre-specified specific period after initial flare early in the course of treatment (that is, landmark analysis)[34] should consider including measures to appropriately address expected non-adherence and dropout by the landmark time and to adjust for potential confounders, as the intervention and comparison groups are no longer the same as the groups that were randomized. These issues can threaten the validity of an RCT, particularly when there are notable dropouts or switching of treatments. In other words, although the data are generated from an RCT, the study ends up having the vulnerabilities of observational studies and the severe loss of the advantages of an RCT, which can provide misleading data under the guise and perceived weight of the RCT label. To overcome these issues, we recommend analyses of pre-specified periods that start counting flares from the time of randomization, although the follow-up time can be stopped at different time points of interest to demonstrate the lagged effect of ULT after the expected initial worsening (TABLE 4).

One difficulty to consider is that the effect of ULT on flares analysed over the entire study period could be diluted by flares that occur after ULT initiation. Also complicating outcome analysis is that non-adherence could arise as a consequence of the adverse effect of a flare following ULT initiation. These difficulties can be mitigated by the use of highly effective anti-inflammatory flare prophylaxis and gradual dose escalation of ULT[17]. The duration of prophylaxis has varied substantially in gout trials to date, ranging from nearly zero prophylaxis[25] to prophylaxis for up to 11 months[27] (TABLE 1). We recommend prophylaxis for a duration of 3–6 months as a minimum, as recommended in the ACR guideline for the management of gout[19].

Trial duration is another important consideration, as the clinical benefits of ULT for flare outcomes are usually observed after more than 1 year of therapy[39]. The trial must therefore be long enough to overcome the initial worsening of flares, particularly with potent ULT, although longer trials tend to suffer from higher rates of non-adherence and dropout than shorter ones. Nevertheless, as with all RCTs and even more so in gout trials (which tend to have notable dropouts), investigators should maximize efforts to avoid dropouts by the use of intense retention strategies, including repeated engagement of patients by research staff such as nurses, wherever feasible and appropriate. For example, in a UK trial the dropout rate in a group receiving nurse-led intervention was less than 10% over 2 years[25].

In terms of analytical approach, we recommend a priori specification of the statistical analysis plan for adherence-adjusted per-protocol analysis over the entire trial period starting at randomization, in addition to ITT analysis. The adherence-adjusted per-protocol analysis will be directly relevant in accounting for expected dropouts and non-adherence during the trial while accommodating the initial flare phase and the pre-specified partial period analyses. For example, in a 2022 trial that used gout flare as the primary end point[27], the dropout rate by the time the investigators enumerated the primary analysis end point (end of the third phase) was 20%. Furthermore, for the adherence-adjusted per-protocol analysis to effectively account for dropouts, high-quality longitudinal data should be collected by planning ahead (or be available through linked electronic medical records) (TABLE 4). Finally, we recommend using rates (number of events per person-time) as the primary end point, as opposed to the proportion of participants experiencing one or more events (or risk estimate), as the latter would be difficult to implement, particularly given that flares will be frequent during the initial months of ULT, overwhelming the first-event analysis. To that end, Poisson distribution would reflect gout flare events well by accommodating the event counts in rates (TABLE 4).

Other end points in ULT trials

Although the central importance of the gout flare for long-term gout trials is recognized[23], the current practice of using serum urate concentration as the primary end point in pivotal trials for the approval of new ULT drugs for gout care[40] is likely to continue, as long as the effect of the treat-to-serum-urate-target approach is firmly established with the determination of clinically meaningful, quantitative improvement in serum urate levels over the entire duration of treatment. To that end, it would be desirable to quantify the value of such an approach for clinical end points in comparison with alternative strategies in a high-quality RCT. Once this effect and its magnitude are clearly established, future studies could rely on serum urate response as a powerful surrogate for gout flare risk, similar to the way in which serum cholesterol levels came to be used as a surrogate end point in the development and approval of cardiovascular drugs (after several large trials confirmed its strength as a surrogate for 'hard' end points) . This approach might ultimately reduce the cost of future development programmes for urate-lowering drugs. Furthermore, serum urate levels start improving, usually within days of starting ULT, without initial worsening (in contrast to flare end points)[40], avoiding many of the issues we discuss above. In terms of other core clinical end points of gout in the evaluation of ULT, tophus burden reduction

Box 1 | **Adherence-adjusted per-protocol analysis and conventional on-treatment or as-treated analysis**

- Adherence-adjusted per-protocol analysis uses inverse probability weights to account for non-adherence to treatment. The denominator of the inverse probability weight is the probability that a participant adhered to their assigned treatment, obtained from logistic regression. The predictors in the logistic regression model consist of the baseline covariates and post-randomization time-varying covariates[34]. By accounting for non-adherence[34], this method allows for the assessment of the effect that would have been observed if all participants (as randomized) had received their assigned treatment during the study period[34].
- By contrast, traditional naive 'per-protocol analysis' (or complete set) only includes participants/person-time that adhere to assigned treatment by excluding those who deviate from the protocol. If non-adherence is not random, a treatment effect is susceptible to confounding or selection bias (as discussed in the main text). Similarly, the conventional as-treated or on-treatment approach defines a participant's treatment status according to the treatment they received regardless of their randomized allocation. This approach is vulnerable to confounding bias because it ignores the random assignment of treatment.

Table 4 | **Recommendations for future ULT trials with gout flare end points**

Recommendations	Purpose
Plan on reporting entire-period flare results (primary period of interest) as well as pre-specified partial periods (starting from the time of randomization) (secondary period of interest)	To accommodate gout-specific biology while retaining the advantage of the RCT design
Implement effective flare prophylaxis during the initial period of ULT, when risk of flare is paradoxically increased	To minimize dilution of the effect of the intervention in analysis of the entire trial period
Conduct long-term trials to overcome the initial worsening of flares in the intervention group	To avoid false-negative results while quantifying the clinical benefits and risks of ULT, given that ULT is a long-term care medication
Design and carry out the trial to minimize dropouts	To maximize the validity of the RCT in both entire-period and partial-period analyses
Specify a priori the statistical analysis plan for adherence-adjusted per-protocol analysis[a] for the entire period, as well as pre-specified partial-period analyses, in addition to ITT analysis	To account for dropouts and treatment adherence
Collect high-quality longitudinal data, including health care utilization, comorbidities and medication use	To effectively predict and account for treatment adherence
Consider using flare rate as the primary end point, as opposed to flare risk (proportional), and employ Poisson regression models	To best accommodate for recurrent events of gout, the time-dependent (non-proportional) risk and the paradoxical increase in flare risk after ULT initiation
Collect data on the use of prophylaxis and medication for acute gout care	To serve as a secondary end point and as a key variable to account for non-adherence and censoring events for primary flare end point

ITT, intention-to-treat; RCT, randomized controlled trial; ULT, urate-lowering therapy. [a]See BOX 1 for further explanation of adherence-adjusted per-protocol analysis.

does not involve initial worsening in the same way as flare end points, although patient-reported-outcome and quality-of-life measures would be partially affected by the initial worsening of flares[40]. As such, these clinical end points would also be better served by long-term trials, such as those of 2 years' duration.

Conclusions

The period of increased flare risk occurring after ULT initiation, which lasts for months, presents considerable challenges in proving the expected flare-prevention benefits of ULT in the long term. Excluding flare outcomes that occur in the initial post-randomization period can threaten the randomization property that allows for causal conclusions to be drawn from RCTs. To accommodate this rare biological phenomenon with the randomization property intact, we recommend careful planning for entire-period analyses, adequate trial duration, effective flare prophylaxis, maximum retainment efforts/mechanisms, adherence-adjusted per-protocol analysis (in addition to ITT), and high-quality longitudinal data collection for adherence prediction. These approaches will allow for high-level evidence of the ULT effect that preserves the randomization principle, which will satisfy methodological scrutiny and generate solid evidence-based guidelines for optimal gout care.

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