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nature reviews rheumatology

SYSTEMIC SCLEROSIS

How does immune cell dysfunction contribute to fibrosis?

The 'precision gap' in rheumatic disease

The factors hindering progress

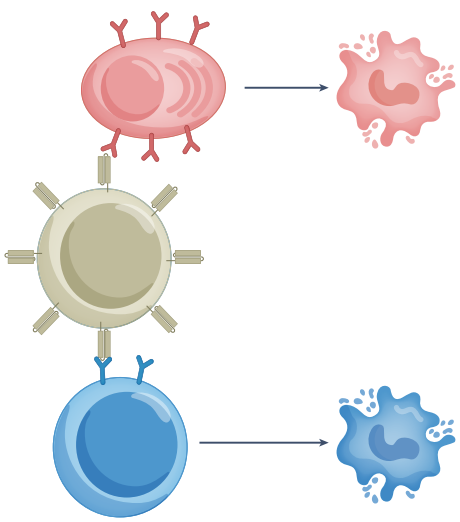
Systemic lupus erythematosus

CAR T cells induce drug-free SLE remission

The results of a new study in a small series of patients with treatment-refractory systemic lupus erythematosus (SLE) provide further evidence that treatment with chimeric antigen receptor (CAR) T cells can achieve sustained drug-free disease remission. Although this novel therapeutic approach still requires long-term validation, for someone who develops severe SLE at a young age it offers hope for relief from a lifetime of medication.

SLE is a prototypic autoimmune disease in which exposure of the immune system to nuclear antigens results in the emergence of antinuclear antibodies, and immune-complex-mediated inflammation in multiple organs. Because of the involvement of B cell-mediated autoantibody production, B cell targeting with monoclonal antibodies is an attractive therapeutic strategy. However, not all patients respond to the currently available therapies.

CAR T cells that eliminate B cells have been developed for cancer therapy, and are now being tested in autoimmune diseases such as SLE. CAR T cells are made by treatment of T cells with recombinant lentivirus, to induce stable expression of a CAR consisting of an extracellular antigen-binding domain and an intracellular T cell-activation domain. The use of CAR T cells targeting CD19, a specific marker of B-lineage cells, has previously shown therapeutic potential in mouse models of lupus and in a single patient with refractory SLE.



In the new study, anti-CD19 CAR T therapy was used in five patients with refractory SLE. The patients were young (18–24 years old), and 80% were female. All had active disease and multiorgan involvement. All the patients had received glucocorticoids, mycophenolate mofetil, hydroxychloroquine and belimumab, among other therapies.

Following leukapheresis for CAR T cell preparation, the patients received lymphodepleting chemotherapy with fludarabine and cyclophosphamide. The autologous CAR T cells were then administered by a single infusion, and they underwent rapid expansion, constituting 11–59% of circulating T cells 9 days after introduction. Over this period, $CD19^+$ B cells were eliminated, and they remained absent from the peripheral blood in the initial assessment period of 30 days, whereas numbers of $CD4^+$ and $CD8^+$ T cells, monocytes and neutrophils initially fell, but then recovered.

CAR T cell therapy improved almost all signs and symptoms of SLE in these patients, resulting in drug-free remission. Autoantibody levels were reduced, and anti-dsDNA antibodies were eliminated. Reconstitution of B cells occurred, but these cells were mostly $CD21^+CD27^-$ naive cells, and they did not result in relapse of SLE over follow-up times of 5–17 months. Measurement of antibody titres from pre-treatment vaccinations identified no substantial decline at 3 months post-therapy, indicating that the depletion of autoantibody-producing B cells did not eliminate all immunoglobulin-producing cells. Mild, treatable cytokine-release syndrome occurred in three patients, but none developed immune effector cell-associated neurotoxicity syndrome, and no infections were observed.

These results are encouraging for the potential use of CAR T cells in SLE. "The fact that a single infusion of CAR T cells led to drug-free sustained remission of SLE is remarkable, as these patients had severe SLE and were receiving several immunosuppressive drugs," notes Georg Schett, corresponding author on the study. "What was observed was a kind of reboot of the immune system of the patients, allowing them to stop all their drugs and face deep immunologic remission of the disease."

Notably, the study of CAR T cells in SLE is still at an early stage. "It is important to understand the long-term safety and efficacy in a larger population, so that we can better define the role of anti-CD19 CAR T therapy in the management pathway for SLE," notes Ian Bruce, an expert in SLE at the University of Manchester, who was not involved in this research. "With other novel therapies in pipeline development for SLE, a key consideration will be the cost-effectiveness of what remains a very expensive therapy."

> **"CAR T cell therapy improved almost all signs and symptoms of SLE in these patients, resulting in drug-free remission"**

In addition to continuing to follow the patients from this study, the researchers plan to initiate a basket trial of CAR T therapy in several autoimmune diseases. "The resulting data could have huge implications for the future treatment of autoimmune disease," adds Schett.

Robert Phillips

Original article: Mackensen, A. et al. Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus. *Nat. Med.* https://doi.org/10.1038/s41591-022-02017-5 (2022)

Experimental arthritis

In-joint photocatalytic hydrogen production prevents RA in mice

In new research, photocatalytic nanorods have been developed and shown to modify the inflammatory synovial microenvironment in a mouse model of rheumatoid arthritis (RA). This development has the potential to enable drug-free treatment of RA.

In the RA synovial microenvironment, synoviocyte proliferation and immune-cell invasion result in pannus formation, with accumulation of metabolites including lactic acid, as well as proinflammatory macrophage polarization and stimulation of invasive phenotypes of fibroblast-like synoviocytes (FLS).

To target synovial acidification, researchers have now developed hydrogen-doped titanium dioxide nanorods that release hydrogen molecules upon near infrared irradiation at 808 nm, to deplete lactic acid and inflammatory reactive oxygen species.

In vitro, nanorod irradiation was able to reverse proinflammatory and invasive phenotypes induced in FLS, macrophages and chondrocytes by treatment with lactic acid. In a collagen-induced arthritis (CIA) mouse model of RA, photocatalytic nanorods were injected into the articular cavities of knee joints, which were irradiated once per week for 30 min at 0.3 W/cm^2. After five irradiation treatments, compared with healthy control mice, concentrations of lactic acid in the joint fluid were elevated in CIA mice, but not in CIA mice with photocatalytic treatment. Nanorod irradiation also resulted in a high concentration of molecular hydrogen in the joint fluid, and attenuated RA symptoms such as paw swelling.

Photocatalytic therapy prevented the development of characteristic features of CIA, including osteoporosis, articular bone erosion and joint-space narrowing. Similarly, intra-articular photocatalysis prevented synovial-tissue hyperplasia and inflammatory infiltration, as well as cartilage erosion.

"We have discovered a new 'dual-brake' mechanism for RA therapy," explain co-corresponding authors Wei Tang and Qianjun He. "Photocatalytically generated hydrogen molecule brakes and even reverses the M2-to-M1 polarization of macrophages in the synovial microenvironment, while the near infrared photocatalytic depletion of lactic acid in the synovial microenvironment brakes the activation of synoviocytes, M1-phenotype macrophages and chondrocytes, blocking their invasion into cartilage, to synergistically correct the synovial microenvironment".

As laser irradiation at near infrared wavelengths might not have sufficient penetration for application in human joints, the researchers are now working to adapt this approach for use with piezoelectric catalysts that can be activated by ultrasonic or mechanical stimulation.

Robert Phillips

Original article: Zhao, B. et al. NIR-photocatalytic regulation of arthritic synovial microenvironment. *Sci. Adv.* **8**, eabq0959 (2022)

Antiphospholipid syndrome

Target neutrophils to treat thrombotic APS?

New research identifies neutrophils as important regulators of both arterial and venous thrombosis, and suggests that therapies directed at neutrophil activation pathways could protect against thrombosis in conditions such as antiphospholipid syndrome (APS).

The researchers demonstrated that activated neutrophils drive arterial and venous thrombosis in a mouse model of APS. They also noted that the expression of the transcription factor KLF2, which acts as a repressor of myeloid cell activation in acute and chronic inflammatory states, was profoundly reduced in neutrophils from patients with APS as well as in neutrophils from mice injected with human antiphospholipid antibodies.

Myeloid-specific deletion of KLF2 worsened arterial and venous thrombotic phenotypes in mice; these phenotypes were rescued by deletion of neutrophils. Further studies showed that loss of KLF2 primed neutrophils for migration, adhesion and the release of prothrombotic factors including neutrophil extracellular traps and tissue factor.

KLF2-deficient neutrophils showed increased uropod formation and clustering of the adhesion molecule PSGL-1. Targeting clustered PSGL-1 using neutralizing antibodies delivered via a nanoparticle-based system attenuated neutrophil-mediated thrombosis in APS and KLF2-knockout models.

Sarah Onuora

Original article: Nayak, L. et al. A targetable pathway in neutrophils mitigates both arterial and venous thrombosis. *Sci. Transl. Med.* **14**, eabj7465 (2022)

Systemic lupus erythematosus

CXCL5 effective in mouse model of SLE

New research shows that serum concentrations of CXCL5 negatively correlate with systemic lupus erythematosus (SLE) activity. Treatment of lupus-prone mice with CXCL5 prevents disease development.

SLE is characterized by loss of immune tolerance to autoantigens, along with neutrophil-mediated immune dysregulation. CXCL5 is a neutrophil chemoattractant, and the CXCL5 concentration gradient determines neutrophil trafficking between the blood and the tissues.

In a new study, serum CXCL5 concentrations in patients with SLE were lower than in healthy individuals, suggesting that low blood CXCL5 causes a pathogenic blood–tissue CXCL5 gradient.

In the SLE model of Fas^{lpr} lupus-prone mice, plasma concentrations of CXCL5 were lower than in healthy control mice, and reduction of CXCL5 correlated with age and disease progression.

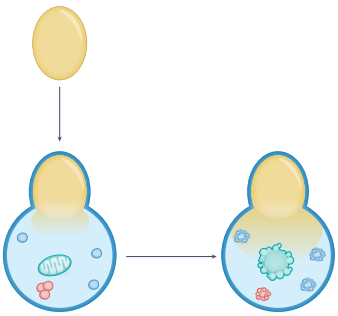
Weekly intravenous administration of CXCL5 to Fas^{lpr} mice with severe lupus improved survival and reduced disease activity and concentrations of anti-dsDNA antibodies, relative to saline-treated mice. CXCL5 treatment also decreased neutrophil activation, proliferation and renal infiltration, and the formation of neutrophil extracellular traps. Furthermore, CXCL5 treatment seemed to prevent delayed toxicity to cyclophosphamide, enabling survival of mice with combination treatment for up to 2 years.

Robert Phillips

Original article: Fan, X. et al. CXCL5 administration dampens inflammation and improves survival in murine lupus via myeloid and neutrophil pathways. *Arthritis Rheumatol.* https://doi.org/10.1002/art.42383 (2022)

Osteoarthritis

Aberrant regulation of autophagy linked to OA



Autophagy, a process for removing damaged or unnecessary cellular components, has a protective function in various diseases. A new study in *Arthritis & Rheumatology* has identified an axis that is involved in the regulation of autophagy in chondrocytes, the disruption of which promotes the development of osteoarthritis (OA) in mice.

Ubiquitination is involved in various processes, including autophagy. In the new study, analysis of cartilage from patients with OA or healthy individuals, as well as of mice with destabilization of the medial meniscus (DMM)-induced or ageing-induced OA, revealed an association between the expression of the E3 ubiquitin ligase HECTD1 and OA progression.

Adenovirus-mediated overexpression of HECTD1 in mice alleviated DMM-induced OA, lowering disease activity and reducing the extent of chondrocyte death in the cartilage. By contrast, conditional knockout of HECTD1 in mouse chondrocytes exacerbated DMM-induced disease and accelerated ageing-induced OA.

In vitro experiments using the chondrogenic cell line ATDC5 showed that HECTD1 regulates autophagy by controlling the expression of Rubicon, a negative regulator of autophagy. HECTD1 interacts with and ubiquitinates Rubicon at lysine residue 534, resulting in proteasome-mediated degradation of Rubicon and autophagy.

Overexpression of HECTD1 or loss of Rubicon expression promotes the formation of autophagosomes in ATDC5 cells, whereas HECTD1 knockdown or Rubicon overexpression has the opposite effect.

Manipulating the expression of Rubicon in the DMM mouse model confirmed that Rubicon-mediated inhibition of autophagy promotes chondrocyte death and OA pathogenesis — a process that was further exacerbated by ectopic expression of a ubiquitination-resistant variant of Rubicon that has an arginine substitution at residue 534.

> **"HECTD1 regulates autophagy by controlling the expression of Rubicon"**

"HECTD1 is a promising candidate for osteoarthritis therapy, and we plan to explore the potential of potent drugs that activate HECTD1 and decrease Rubicon expression for the treatment of OA," explains Hongwei Ouyang, a corresponding author on the study. The researchers also plan to investigate why HECTD1 expression is down-regulated during osteoarthritis, which might reveal further treatment targets.

Jessica McHugh

Original article: Liao, S. et al. HECTD1-mediated ubiquitination and degradation of Rubicon regulates autophagy and osteoarthritis pathogenesis. *Arthritis Rheumatol.* https://doi.org/10.1002/art.42369 (2022)

Osteoarthritis

Single-cell-based platform maps response to candidate DMOADs

To date, no disease-modifying osteoarthritis drug (DMOAD) has been successful in clinical trials despite a number of candidates showing promise in preclinical models, owing at least in part to the complexity of the disease and interpatient heterogeneity. Findings from a new study indicate that a single-cell-based screening platform could provide detailed insights into the effects of preclinical drugs on OA chondrocyte populations, with the aim of identifying which drugs are unlikely to succeed in clinical trials and also which subgroups of patients are likely to benefit from a given drug.

In earlier work, the same researchers had established the first single-cell proteomics atlas of healthy and OA cartilage using cytometry by time of flight (CyTOF), enabling them to identify chondrocyte progenitor populations as well as inflammation-modulating populations that are likely to contribute to OA pathology. In the current study they also identified a senescent cell population in OA cartilage. "Identification of these defined populations made it possible to precisely map how a drug alters the cartilage landscape," explains corresponding author Professor Nidhi Bhutani.

High-resolution mapping by CyTOF was performed to analyse the effects of two pre-clinical drugs, BMS-345541 and kartogenein, on chondrocytes isolated from cartilage tissue taken from patients with end-stage OA during total-knee replacement surgery. The selected drugs, both of which have previously been shown to modulate OA pathogenesis in animal models, have distinct mechanisms of action: BMS-345541 dampens inflammation by selectively inhibiting IkB kinase in the NF-κB pathway, and kartogenin functions as a pro-chondrogenic regenerative drug.

BMS-345541 reduced inflammation and depleted cell numbers in multiple chondrocyte populations, including senescent populations, whereas kartogenin had a modest effect on the OA chondrocyte populations.

In samples from a small subset of patients ($n = 6$), BMS-345541 had a uniform effect on OA chondrocytes, with a clear delineation between responders and non-responders. By contrast, the response to kartogenin was heterogeneous. "This information suggests that kartogenin is likely to fail in a clinical trial where there is no stratification of patients," highlights Bhutani.

The information obtained using this platform could be important for designing future clinical trials for potential DMOADs. "A single cell-based screening platform for patient samples such as we have described will be able to provide more insightful analyses for effective screening approaches for OA therapeutics before clinical trials," says Bhutani. "This can help save both cost and time by making clinical trials more effective."

Sarah Onuora

Original article: Sahu, N. et al. A single-cell mass cytometry platform to map the effects of preclinical drugs on cartilage homeostasis. *JCI Insight* https://doi.org/10.1172/jci.insight.160702 (2022)

TARGETED THERAPIES

Small wonder: nanoparticles feed hydroxychloroquine to activated neutrophils

Somanathapura K. NaveenKumar and Jason S. Knight

Although neutrophils are vital components of the innate immune system, they can also contribute to the inflammation and autoantibody formation that characterize a number of rheumatic diseases. The ability to specifically target neutrophils, and in particular activated neutrophils, could enable the direct delivery of drugs for therapeutic modulation of neutrophil activity.

Refers to Cruz, M. A. et al. Nanomedicine platform for targeting activated neutrophils and neutrophil-platelet complexes using an α_1-antitrypsin-derived peptide motif. *Nat. Nanotechnol.* https://doi.org/10.1038/s41565-022-01161-w (2022).

Neutrophils are an important part of the innate immune system, but they also contribute to the pathogenesis of a number of rheumatic diseases. The results of a newly published study[1] take us one step closer to the therapeutic targeting of neutrophils.

As the most abundant leukocytes in human blood, neutrophils are vital players in the host response to infection. Neutrophils have long been known to neutralize pathogens through a combination of phagocytosis and the production of reactive oxygen species such as hypochlorous acid. In 2004, neutrophils were also found to release microbicidal neutrophil extracellular traps (NETs), sticky spider-web-like structures composed of granule-derived effector proteins adorning a scaffold of massively decondensed chromatin[2]. This decondensation occurs when reactive oxygen species trigger the migration of proteases to the nucleus, where they cleave histones[3]. In parallel, post-translational modifications alter the charge content of histones, most notably through citrullination mediated by peptidylarginine deiminases.

The loss of neutrophil homeostasis and/or unchecked neutrophil activation have been implicated in wide-ranging local and systemic disease states, including defective wound healing, immunothrombosis and COVID-19 (REF.[4]). For numerous rheumatic diseases, neutrophil-mediated inflammation is an important effector of tissue injury. Some obvious examples of this relationship include gout, Behçet disease and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis. Even when neutrophil activation is not obvious, residual activity — including NET release — contributes to the production of cytokines (such as type I interferons and IL-1β) and formation of autoantibodies (including anti-DNA antibodies and anti-citrullinated histone antibodies) that are associated with diseases such as systemic lupus erythematosus and rheumatoid arthritis[5]. There is also strong evidence that neutrophils contribute to accelerated cardiovascular disease in many of these conditions, which can include acute venous and arterial thrombotic events, as are seen in antiphospholipid syndrome (APS)[6].

Despite an emerging consensus that restraining neutrophil hyperactivity would sometimes be beneficial, the best approach to doing so, weighing the benefits and risks, has remained elusive. Some drugs that are already in use in the rheumatology clinic, such as colchicine and Janus kinase (JAK) inhibitors, clearly have direct neutrophil-inhibiting properties. Blockade of cytokines such as IL-23 and IL-17 by monoclonal antibodies would also be expected to reduce neutrophil-mediated inflammation. Of course, development of these drugs has for the most part been optimized with other populations of leukocytes in mind. Therapeutic approaches such as inhibition of chemotaxis or adhesion have imperfect specificity for neutrophils and may carry a high risk of infection[7]. Although the repurposing of drugs such as dipyridamole (and even the use of supplements such as ginger) has also been considered, this concept is again essentially relying on effects that are off-target from the drug's canonical role[8].

In an interesting study published in *Nature Nanotechnology*[1], Cruz and colleagues developed a nanomedicine-based platform that might eventually prove fruitful for the clinical treatment of neutrophil hyperactivity. Nanoparticles can be packaged with drugs, and the surfaces of the particles can be conjugated with ligands that specifically target them to disease-associated cells and tissues. Although this approach has been most extensively characterized in the context of cancer, some recent efforts have focused on developing neutrophil-targeting nanoparticles. However, these approaches have not always been unique to neutrophils (for example, targeting Fc or scavenger receptors that are also found on other cell types), and furthermore they have not had specificity for activated neutrophils.

In the new study[1], liposome-based nanoparticles were labelled with a peptide derived from the reactive-centre loop of alpha-1 antitrypsin, an abundant inhibitor of neutrophil elastase and other serine proteases in solution. Given that neutrophil elastase is only found on the neutrophil surface upon activation and degranulation, the authors posited that this approach would direct the nanoparticles to activated (but not resting) neutrophils. The peptide showed good specificity for elastase as compared with other neutrophil-derived and plasma proteases such as proteinase 3 and plasmin. Peptide-coated nanoparticles associated with the surface of mouse and human neutrophils activated with *N*-formylmethionine-leucyl-phenylalanine in vitro, and a minority of the nanoparticles were internalized and trafficked to lysosomes. When injected into mice, peptide-labelled (but not unlabelled) nanoparticles could be found in close association with lipopolysaccharide-activated neutrophils.

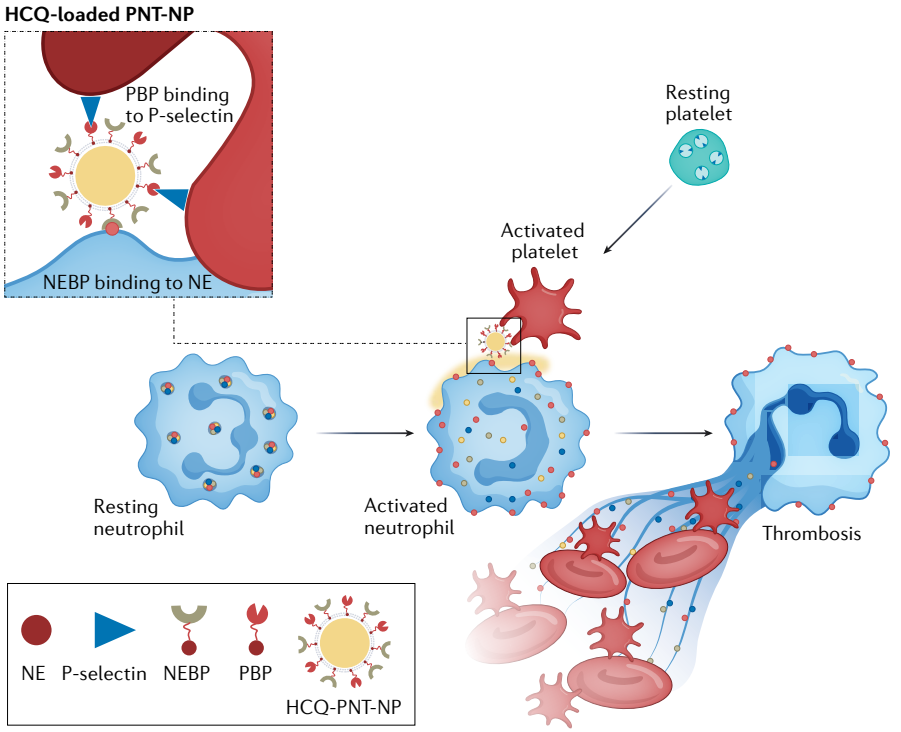


Fig. 1 | **Illustration of therapeutic nanoparticle targeting.** Hydroxychloroquine (HCQ)-containing platelet-neutrophil-targeted nanoparticles (PNT-NP) are conjugated with a neutrophil elastase binding peptide (NEBP) derived from alpha-1 antitrypsin, which engages neutrophil elastase (NE) on activated neutrophils, and with P-selectin-binding peptide (PBP), which interacts with P-selectin on activated platelets. HCQ-PNT-NP have the potential to reduce thrombus formation.

Communication between neutrophils and platelets, including the formation of neutrophil–platelet aggregates, is known to occur in both physiological (for example, in the resolution of infection) and pathological (such as thrombosis) disease states[9]. Through the formation of such aggregates, platelets support various neutrophil effector functions such as chemotaxis and NET release. In the study conducted by Cruz et al.[1], the researchers asked what would happen if nanoparticles were coated with ligands that recognized both neutrophils and platelets (FIG. 1). To add platelet specificity, a peptide was selected on the basis of its known affinity for P-selectin, which comes to the platelet surface upon activation and degranulation. Interestingly, the resultant heteromultivalent nanoparticles, which were capable of targeting both neutrophils and platelets, demonstrated synergistic binding efficacy in activated neutrophil–platelet co-cultures, compared with nanoparticles that targeted either neutrophils or platelets alone.

To assess the potential therapeutic relevance of this approach, nanoparticles with neutrophil, platelet or multivalent specificity were loaded with the antimalarial autophagy inhibitor hydroxychloroquine, which is known to interfere with NET release[10]. The nanoparticles were then tested in a model of inferior vena cava flow-restriction-mediated thrombosis, similar to the model used elsewhere to study APS[8]. Compared with nanoparticles that were either not targeted to cells or not loaded with drug, nanoparticles targeted to any of neutrophils, platelets or neutrophil–platelet aggregates were able to reduce thrombus size in the inferior vena cava model.

Although some progress has been made in defining different neutrophil subsets functionally (such as N1 and N2 neutrophils in the context of cancer), these cells are yet to be fully defined by unique surface markers that would enable them to be specifically targeted[7]. The results of this new study therefore represent an interesting step forward for neutrophil-specific therapeutics, directing nanoparticles only to activated neutrophils with cell-surface expression of neutrophil elastase. One can envision how this approach might be useful in the setting of acute sterile (or overly exuberant infectious) neutrophil activation, including in patients with emergent rheumatic complications such as diffuse alveolar haemorrhage, catastrophic APS, adult-onset Still's disease and likely others, where drugs could be delivered directly to neutrophils in a way that might help mitigate off-target effects. This concept could be most appealing when one considers molecules such as dipyridamole and phosphodiesterase inhibitors, which have an ability to restore neutrophil homeostasis by boosting intracellular cyclic AMP concentrations, but which have numerous other effects when delivered systemically. Going forward, beyond further refining the pharmacokinetics and targeting associated with this approach, an additional consideration is the extent to which nanoparticle internalization and trafficking will need to be optimized in order to maximize the therapeutic benefits.

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Competing interests
The authors declare no competing interests

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PAEDIATRIC RHEUMATOLOGY

Paediatric glucocorticoid toxicity index: new possibilities in assessment

Charlotte King and Daniel B. Hawcutt

Glucocorticoids are common medications that are used in research trials and clinical practice. Measuring the toxicity of glucocorticoids in children is complicated by various factors such as age and growth. A standardized tool could help to record these toxicities across different specialities in a systematic manner.

Refers to Brogan, P. et al. The pediatric glucocorticoid toxicity index. *Semin. Arthritis Rheum.* **56**, 152068 (2022).

Glucocorticoids remain a cornerstone of treatment for a multitude of conditions in paediatric and adult medicine[1]. Within paediatric medicine, these indications include respiratory conditions (such as asthma), gastrointestinal disorders and rheumatological conditions. Children and young people also receive glucocorticoids in research settings — in trials that investigate glucocorticoids as the drug of interest or as a secondary medication. Although the benefit of glucocorticoid treatment in these conditions is often well described, accurately capturing the harms and therefore balancing the risks versus the benefits of glucocorticoids have been challenging. Brogan et al. have now developed a tool to measure glucocorticoid toxicity in children and young adults[2], but what potential does this new tool hold?

Glucocorticoids can have numerous adverse effects[3], and an individual's susceptibility to these effects varies depending on several factors such as the dose, route, potency of glucocorticoid used, route of administration, length of treatment and pharmacogenomics[3,4]. The introduction of glucocorticoid-sparing treatments such as biologic drugs in inflammatory and autoimmune conditions that traditionally used high doses of glucocorticoids has helped some patients to avoid glucocorticoid toxicity[5]. However, expensive therapies such as biologic drugs are limited to well-funded healthcare systems, and even in countries where glucocorticoid-sparing therapies are well established, the use of glucocorticoids in paediatric patients is likely to continue for the foreseeable future owing to the wide range of conditions that glucocorticoids are used for.

The lack of a paediatric glucocorticoid toxicity index (pGTI) has therefore been an important unmet clinical and research need. An adult glucocorticoid toxicity index tool exists that is designed to measure the change in glucocorticoid toxicity between two time points, and has been used both in trials and in clinical practice[5]. However, this tool is not suitable for paediatric use, as various paediatric-only adverse effects, such as effects on growth, are not included. The reporting of paediatric adverse drug reactions using current systems is generally poor[6], and glucocorticoid toxicity in children specifically (both clinically and in research) has been considered in a piecemeal way using a variety of scores. Limited data are available, and the available data are presented in several ways, hindering meaningful interpretation of data between studies or across different centres.

> "the lack of a [pGTI] has therefore been an important unmet clinical and research need"

The pGTI developed by Brogan et al.[2] represents a notable step forward, and has the potential to improve the quality of data collected and standardize the type of data recorded. The researchers use an example of a clinical trial participant when discussing this new tool, but the pGTI might also have clinical applications as well as research applications[2]. The pGTI comprises a set of ten different domains with a weighted scoring system among the sub-domains. A key aspect of this tool is the appreciation that normal physiological parameters in children (such as blood pressure[7], blood test measurements[8] and body mass index (BMI)[9]) change with age[5]. The researchers incorporated these dynamic changes into the pGTI by considering age, growth and the effect of other medications on glucocorticoid toxicity. The pGTI demonstrated good reliability and validity when measured against reported cases of toxicity. The digital platform also provides various beneficial features, including help

with data input, calculations that consider age and developmental changes (such as blood pressure) and the automation of data capture.

> The pGTI demonstrated good reliability and validity when measured against reported cases of toxicity

The development of any tool such as the pGTI requires a considerable effort to obtain appropriate representation. This project has drawn in a considerable range of glucocorticoid uses over various paediatric sub-specialities, ranging from nephrology, rheumatology, oncology, endocrinology, genetics and psychiatry to maternal–fetal medicine. In an ideal world, the development of this tool would also have included other specialities that commonly use glucocorticoids, such as respiratory medicine or dermatology. Nevertheless, the desire for a perfect tool should not prevent the appreciation of what is a notable advance in the field, especially given the lack of any current pGTI or equivalent. It will be interesting to see whether the tool can be used in glucocorticoid toxicity studies in these other specialities, and whether additionally minor tweaks might become necessary. Certainly, within the field of respiratory medicine, patients with asthma seem to struggle with both the local adverse effects (such as hoarse voice and oral candidiasis) and systemic adverse effects (such as adrenal suppression and growth velocity) of glucocorticoids[10]. Although growth is well covered by the pGTI, and oral candidiasis is specifically captured in the infection domain, neither symptomatic adrenal suppression nor hoarse voice are included in the weighting information provided (although symptomatic adrenal suppression is captured in the damage checklist).

A potentially important omission in the development of the pGTI is the voice of the parents, as well as the voice of the children and young people being treated. The weighting given to each symptom seems to have been assigned purely from a medical perspective and will therefore not capture the relative importance of the toxicities to the children and young people affected. Acne or hirsutism, for example, can have a much greater effect on the quality of life and mental health of teenagers than of older adults. It would be interesting to know whether children and young people agreed with the relative weightings created by the adult researchers, and whether the relative weightings change with age for certain domains (for example, whether acne is weighted higher in teenagers than in toddlers). The supplementary data section includes a very helpful video showing a person completing the score and the images and text provided to ensure standardization. The case study used is of an African American teenager, but the images presented involve lighter skinned individuals that might not help to accurately score dermatological outcomes in patients who are not white.

However, despite these minor and addressable points, overall, the pGTI provides a well-constructed system for the systematic recording, and scoring, of glucocorticoid toxicity. We are keen to use this tool both for data capture in research studies and in clinical practice.

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CLINICAL GUIDELINES

The value of comparative efficacy studies in informing rheumatology guidelines

Raveendhara R. Bannuru

New research reinforces the fact that most recommendations in rheumatology are not informed by comparative efficacy randomized controlled trials. Performing these studies is time and resource intensive. Policies and funding to perform these studies in a timely and resource constraint manner are essential.

Refers to Henry, K. et al. Comparative efficacy randomized controlled trials in rheumatology guidelines. *ACR Open Rheumatol.* https://doi.org/10.1002/acr2.11484 (2022).

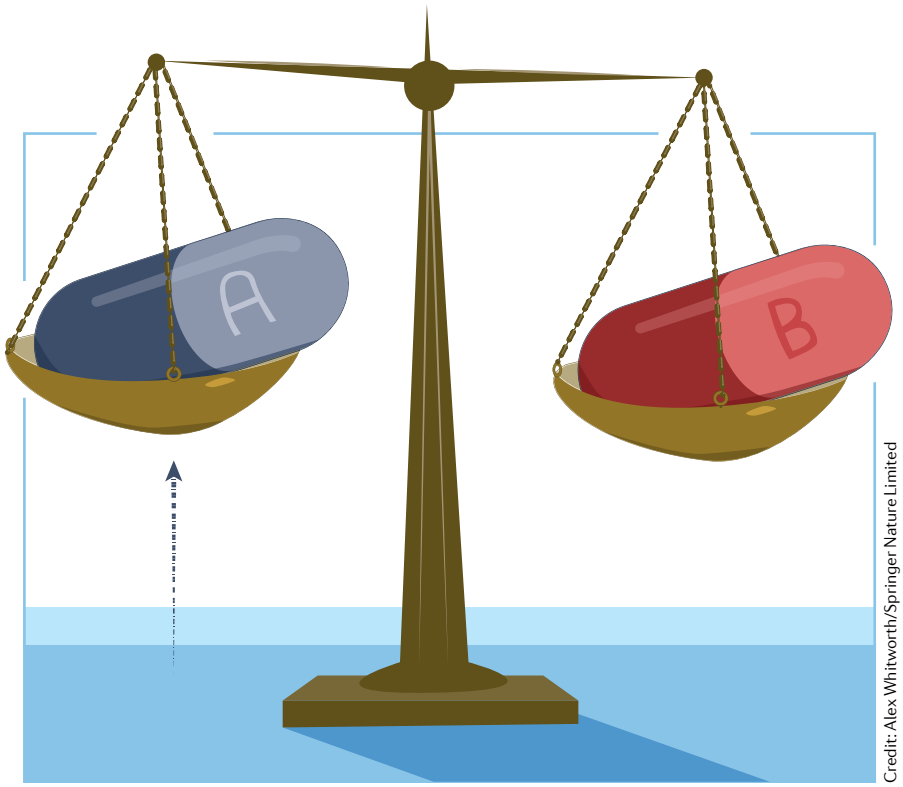
Comparative efficacy randomized controlled trials (RCTs) compare two active interventions in a head-to-head trial design. These studies are ideal to inform most of the treatment decisions made in the clinic and to inform clinical practice guidelines, as they provide high-quality evidence on the relative safety and efficacy of two or more interventions[1]. However, the extent to which recommendations in rheumatology are supported by the evidence from comparative efficacy RCTs is largely unknown. In an article published in *ACR Open Rheumatology*, Henry et al.[2] highlight a lack of such comparative efficacy RCTs within rheumatology guidelines and highlight a need to redirect efforts to large-scale comparative efficacy trials. But is such an approach sustainable in the long term, or are other strategies available to capture such data?

Henry et al.[2] performed a systematic review to characterize the degree to which rheumatology clinical practice guideline recommendations are informed by comparative efficacy RCTs. The researchers examined all the current versions of clinical practice guidelines published by the ACR and EULAR between 1 January 2017 and 12 June 2021 (REF.[2]). They restricted the search to guidelines published using either the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) system for ACR guidelines or Oxford Centre for Evidence-Based Medicine Standards (OCEBM) system for EULAR guidelines to rate the recommendation level of evidence[3,4]. They looked at conventional synthetic DMARDs (such as methotrexate, mycophenolate and hydroxychloroquine), biological and targeted synthetic DMARDs (such as adalimumab, secukinumab and tofacitinib), NSAIDs, steroids, non-pharmacological therapies, urate-lowering therapy and placebos.

The researchers identified 15 guidelines (providing 481 recommendations) pertaining to 10 different rheumatological conditions. Only 15% of these recommendations were supported by at least one head-to-head RCT. Gout (34.0%) and rheumatoid arthritis (32.8%) were the diseases with the largest proportion of recommendations informed by any head-to-head RCT, whereas guidelines for vasculitis and Sjögren syndrome had no recommendations informed by any head-to-head RCTs. The included guidelines referenced 609 RCTs, with only 28% of them being head-to-head RCTs. Most head-to-head trials (63%) were funded by the pharmaceutical industry. This rate was similar to the overall rate of industry-funded trials (66%).

> “guideline committees should come up with future research agendas”

A possible reason why many clinical practice guidelines are not informed by comparative efficacy RCTs is that conducting RCTs is a very time-consuming and resource-intensive process. Hence, once a drug is approved by regulatory agencies such as the US Food and Drug Administration (FDA), the pharmaceutical companies have less incentive to perform a comparative efficacy trial against its competitors. In addition, if the RCT fails to demonstrate the superiority or the non-inferiority of the product under investigation, the company risks losing market share as well as coverage by the payors. Given that major funders such as the National Institutes of Health (NIH) are mostly focused on the novelty and innovation aspect of a product, getting funding for a comparative efficacy RCT is difficult. Without adequate resources, performing any research study is cumbersome and not encouraged by the academic institutions. A potential solution



Credit: Alex Whitworth/Springer Nature Limited

to overcome this barrier could be performing a well-designed and well-executed study using real-world data captured in the electronic health records. Although the evidence generated from such a study might not reach the level of evidence obtained by RCTs, such a pragmatic approach might answer the comparative effectiveness questions using limited resources in a short period of time. With the latest advances in epidemiological methods and new developments in innovative study design, working with large amounts data can generate high-quality evidence[5]. Indeed, the GRADE approach enables the level of evidence obtained from non-randomized studies to be updated if the studies are large and show highly significant benefit from an intervention[6].

> Policies and funding to perform these studies in a timely and resource constraint manner are essential

Another reason that guidelines might not include comparative efficacy RCTs is that the published comparative efficacy RCTs available do not address the specific clinical questions posed by the guideline committees. For example, most of the recently developed cyclooxygenase 2 (COX2) inhibitors for osteoarthritis have been compared against other existing COX2 inhibitors (that is, intra-class comparisons)[7], which might not be of interest to the guideline committees[8]. Usually, the differences in safety and efficacy between the drugs in the same class are subtle and might not reach either statistical or clinical significance to provide a separate recommendation for each of those drugs. Therefore, the guideline committee might decide to provide a recommendation for the entire class rather than each drug in that class. Hence, guideline committees should come up with future research agendas to inform the forthcoming iterations of their guidelines.

Henry et al. suggest that the FDA should take a leadership role and request the industry sponsors to provide comparative efficacy evidence besides placebo-controlled trials as part of the drug approval process[2]. This suggestion is commendable but its implementation would increase the amount of time and resources spent by the industry partners. The use of additional resources would ultimately make its way into the final pricing of the drug and into the overall healthcare costs. Furthermore, this time would be added to the decades-long process of getting these innovative drugs to the market.

In the second part of the study, the researchers identified a positive association between the presence of comparative efficacy RCTs and the strength and level of evidence, but such an association is not surprising or particularly informative. A lack of comparative efficacy RCTs that directly address the concerned clinical question will inevitably lead to a downgrading of evidence because of indirectness[9]. Furthermore, the availability of a high-quality comparative efficacy non-inferiority RCT might not result in a high level of evidence as the evidence can be downgraded for imprecision (meaning that there is no clear evidence of benefit from one intervention over the other)[10].

Comparative efficacy studies are certainly needed to inform clinical practice better. The results of the study by Henry et al.[2] highlight the lack of comparative efficacy RCTs that are currently informing recommendations in rheumatology. Policies and funding to perform these studies in a timely and resource constraint manner are essential. Putting the entire burden of generating the comparative efficacy evidence on the pharmaceutical industry might not be a sustainable option in the long term. Effective strategies to leverage the wealth of data captured in the electronic health records to perform well-designed comparative effectiveness studies are needed. Guideline committees are advised to come up with the future research agendas to inform the future iterations of their guidelines.

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AUTOINFLAMMATORY DISEASES

Keeping immunostimulatory self-RNA under the rADAR

Christine Wolf and Min Ae Lee-Kirsch

Structural modification of RNA by adenosine-to-inosine editing renders self-RNA invisible to RNA sensors of the innate immune system. Lack of RNA editing unleashes inflammatory responses that can lead to autoinflammation. A deeper understanding of the associated signalling pathways might reveal potential targets for drug discovery for autoinflammatory diseases.

Refers to Hubbard, N. W. et al. ADAR1 mutation causes ZBP1-dependent immunopathology. *Nature* **607**, 769–775 (2022) | de Reuver, R. et al. ADAR1 prevents autoinflammation by suppressing spontaneous ZBP1 activation. *Nature* **607**, 784–789 (2022) | Jiao, H. et al. ADAR1 averts fatal type I interferon induction by ZBP1. *Nature* **607**, 776–783 (2022)

Pathogen-derived RNA represents a key molecular pattern recognized by pathogen recognition receptors of the innate immune system to fight infections. However, RNA is also an integral component of the host, necessitating efficient strategies that can reliably distinguish self-RNA from nonself-RNA to avoid aberrant immune activation and subsequent autoinflammation. One such strategy involves biochemical or structural modifications of RNA by enzymes such as adenosine deaminase acting on RNA-1 (ADAR1), which render self-RNA invisible to RNA-sensing receptors. Mutations in *ADAR* (which encodes ADAR1) cause the type I interferonopathy Aicardi-Goutières syndrome (AGS), an infancy-onset autoinflammatory disease characterized by brain and skin inflammation owing to constitutive activation of antiviral type I interferon signalling[1]. ADAR1 functions by editing adenosine to inosine (A-to-I) in double-stranded RNA (dsRNA), thereby introducing mismatches and bulges within the double helix structure that prevent erroneous recognition of endogenous dsRNA by the RNA sensor melanoma differentiation-associated protein 5 (MDA5)[2] (FIG. 1). However, embryonic lethality of *Adar*$^{-/-}$ mice is not fully rescued by deletion of MDA5 or its downstream signalling adaptor MAVS[3,4], which suggests that ADAR1 limits the activation of other immune sensors. Three new studies in *Nature* by Hubbert et al.[5], de Reuver et al.[6] and Jiao et al.[7] implicate Z-DNA binding protein 1 (ZBP1) as an additional pattern recognition receptor in this process, and provide insights into the pathways governing RNA sensing that might lead to the discovery new therapeutic targets for autoinflammatory diseases.

ZBP1 is an innate sensor of viral infections and a central regulator of pro-inflammatory immune responses and programmed cell death by inducing RIPK3–MLKL signalling-dependent necroptosis and RIPK1–caspase-8 signalling-dependent apoptosis[8] (FIG. 1). ZBP1 and the long p150 isoform of ADAR1 are the only mammalian proteins known to contain a Zα domain, which binds RNA that has a left-handed double-helix structure (Z-RNA). Notably, patients with AGS commonly carry a proline-to-alanine substitution (P193A) within the Zα domain of ADAR1, together with a loss-of-function mutation on the second allele[1]. Hubbard et al.[5] demonstrated that ADAR1 interacts with ZBP1 through a common nucleic acid ligand via their Zα domains, which suggests that ADAR1 and ZBP1 might be functionally related.

> these findings establish ZBP1 as a bona fide pattern recognition receptor for dsRNA

To investigate the role of ZBP1 as a possible mediator of autoinflammation in ADAR1 deficiency, all three groups turned to hemizygous mice with an *Adar* allele containing a mutation of the RNA-binding Zα domain and an *Adar* null allele. These mice succumb to inflammatory organ pathology shortly after birth, a phenotype that could be largely rescued by concomitant deletion of ZBP1 or by knock-in of a Zα-mutant ZBP1 allele, confirming that the Zα domain is required for ADAR1-mediated restriction of ZBP1. Notably, the expression of interferon-stimulated genes in ADAR1-deficient mice was still present after loss of ZBP1, but fully abrogated with additional loss of MDA5, which suggests that ZBP1 and MDA5 bind to the same dsRNA ligand. Consistent with a cooperative nature of ZBP1 and MDA5 in activating innate immune responses, additional deletion of MAVS in hemizygous *Adar*-mutant mice lacking a functional form of ZBP1 prevented both immunopathology and the induction of interferon-stimulated genes[5,7].

Given that ADAR1 deficiency causes activation of type I interferons, and that ZBP1 expression is induced by type I interferon, Jiao et al.[7] and Hubbard et al.[5] assessed whether ZBP1 is also involved in the immunopathology of *Trex1*$^{-/-}$ mice — another mouse model of AGS[9]. However, neither deletion of ZBP1 nor deletion of its downstream signalling molecule RIPK3 ameliorated the immunopathology of TREX1-deficient mice, which suggests that type I interferon-mediated ZBP1 upregulation is not sufficient to drive autoinflammation.

> future work will need to unravel the complex regulatory signalling network downstream of ZBP1

To further dissect the contribution of distinct signalling pathways downstream of ZBP1 to ADAR1-driven inflammatory disease, the three groups deleted individual inflammatory or cell death signalling molecules, including RIPK1, RIPK3, MLKL, FADD and caspase-8, either alone or in different combinations, in hemizygous *Adar*-mutant mice. However, unlike ZBP1 ablation, which fully rescued the immunopathology of hemizygous *Adar*-mutant mice, individual deletion of MLKL or RIPK3 only partially phenocopied the loss of ZBP1, probably reflecting the pleiotropic effects of ZBP1 signalling that might involve other yet unknown signalling pathways (FIG. 1). Moreover, de Reuver et al.[6] and Hubbard et al.[5] showed that further ablation of caspase-8 even promoted lethal inflammation, possibly owing to a loss of caspase-8-dependent suppression of ZBP1–RIPK1 inflammatory signalling.

Finally, Jiao et al.[7] and de Reuver et al.[6] set out to characterize the nature of the dsRNA ligand of ZBP1 by comparing the A-to-I-editing profiles of mouse and human RNA from ADAR1-deficient cells. Notably, most ADAR1 targets were derived from mouse short interspersed nuclear elements (SINEs) or human Alu repeats — repetitive DNA sequences within the mouse and human genomes that represent remnants of ancient retroviral infections. Furthermore, de Reuver et al.[6] showed that unedited Alu repeat dsRNA, previously shown to function as a ligand for MDA5 (REF.[10]), could also activate ZPB1, further

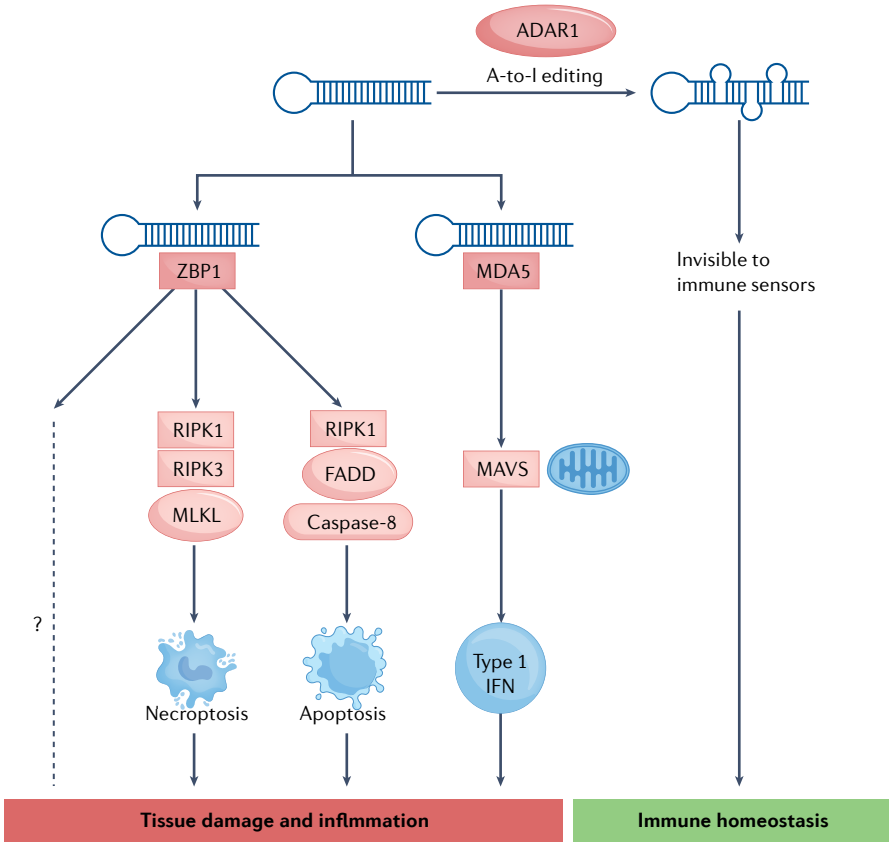

Fig. 1 | **ADAR1 restricts activation of the innate immune sensor ZBP1 by endogenous dsRNA.** Endogenous double-stranded RNA (dsRNA) containing inverted Alu repeats is modified by adenosine deaminase acting on RNA 1 (ADAR1) via adenosine-to-inosine (A-to-I) editing, resulting in mismatches and bulges within the RNA double helix structure. This modification renders self-dsRNA invisible to immune sensors of the innate immune system. In the absence of functional ADAR1, unedited self-dsRNA is recognized by Z-DNA binding protein 1 (ZBP1), the engagement of which induces diverse inflammatory responses and cell death, including RIPK1–RIPK3–MLKL-dependent necroptosis and RIPK1–FADD–caspase-8-dependent apoptosis as well as other yet undefined inflammatory signalling pathways. In addition, unedited self-RNA is also recognized by melanoma differentiation-associated protein 5 (MDA5), which leads to MAVS-dependent type I interferon signalling and potentiates ZBP1-induced inflammation.

indicating that ZBP1 and MDA5 bind to the same dsRNA ligand.

Together, these findings establish ZBP1 as a bona fide pattern recognition receptor for dsRNA, expanding the arsenal of the innate immune system in antimicrobial defence, and provide mechanistic insights into how ZBP1 and ADAR1 cooperate to achieve discrimination between self-RNA and nonself-RNA. Given that loss-of-function mutations of *ADAR* are implicated in rare type I interferon-driven autoinflammatory phenotypes in humans, it will be of interest to learn whether perturbations of ADAR1-associated and/or ZBP1-associated signalling pathways are also seen in common inflammatory conditions such as rheumatoid arthritis or systemic lupus erythematosus. However, future work will need to unravel the complex regulatory signalling network downstream of ZBP1, which will probably vary in a cell-type-specific and context-specific manner. Moreover, understanding the molecular underpinnings of ZBP1 signalling, including positive and negative regulatory cues provided by intersecting pathways, might reveal potential targets for drug discovery for autoinflammatory diseases.

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Review article

Immune cell dysregulation as a mediator of fibrosis in systemic sclerosis

Dan Fang[1,4], Beidi Chen[1,4], Alain Lescoat[2], Dinesh Khanna[3] & Rong Mu[1]

Abstract

Systemic sclerosis (SSc) is a destructive connective tissue disease characterized by dysregulation of the immune system and fibrosis in the skin and internal organs. The pathogenesis of SSc is complex and remains to be determined. So far, limited specific disease-modifying treatments are available for the effective control of fibrosis in patients with SSc. Studies from the past few years hint at the importance of immune dysfunctions, including the dysregulation of innate and adaptive immune cells, as well as the aberrant secretion of inflammatory and fibrotic cytokines, in the pathogenesis of SSc fibrosis. In this Review, we summarize the most pertinent findings concerning the involvement of dysregulated immune responses in fibrosis of the skin and lungs in SSc and highlight the current and potential immune-based targets for SSc therapeutics.

Sections

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Key points

- Immune abnormalities, including aberrantly activated immune cells and overproduction of pro-inflammatory and pro-fibrotic molecules, are the main hallmarks of systemic sclerosis (SSc) pathogenesis.
- Advances in our knowledge of the immune cells and mediators in SSc fibrosis have revealed an increasing number of possible treatment targets.
- Clinical trial data suggest that targeting immune cell types and cytokines involved in fibrosis has beneficial effects.
- Tocilizumab, an antibody that targets the IL-6 receptor, is now approved for the treatment of SSc-associated interstitial lung disease owing to its effectiveness in preserving pulmonary function.
- The high heterogeneity of the immune phenotypes that occur in SSc might contribute to the limited effect of some immune-targeted therapies, which might be overcome by combined or individualized therapy.

Introduction

Systemic sclerosis (SSc, also known as scleroderma) is an autoimmune-mediated connective tissue disease. Immune abnormalities, including aberrantly activated immune cells and overproduction of pro-inflammatory molecules, are one of the main hallmarks of SSc pathogenesis[1]. Both innate and adaptive immune responses are implicated in SSc development[2–4]. Another outstanding feature of SSc is progressive fibrosis.

The interplay between abnormal immunity and fibrosis in SSc is complicated and has received mounting attention by rheumatologists in the past few years. Studies of these interactions indicate that aberrantly activated immune cells instigate the transition of fibroblasts into myofibroblasts[5], the key effector cells involved in the fibrotic progression of SSc. Numerous pro-fibrotic cytokines secreted by various immune cells, including IL-1, IL-6, IL-17, IL-33, transforming growth factor-β (TGFβ) and TNF, are enriched in the serum and local tissues of patients with SSc and correlate positively with the production of extracellular matrix (ECM)[6].

A number of drugs that target the immune system have beneficial therapeutic effects in SSc. For example, both cyclophosphamide and mycophenolate mofetil, two representative immunosuppressive drugs, can improve the lung function of patients with SSc-associated interstitial lung disease (ILD)[7], and CD20-targeted therapy (rituximab) can reduce skin fibrosis in patients with SSc[8]. Furthermore, in 2021, the FDA approved an IL-6 receptor inhibitor (tocilizumab) for the treatment of SSc-ILD[9,10] owing to its effectiveness in preserving pulmonary function in these patients[10]. Thus, these effects further implicate immune dysregulation in the initiation and perpetuation of fibrotic processes in SSc.

In this Review, we summarize findings from the past decade concerning the role of immunological abnormalities in fibrosis and discuss how immune cells can contribute to SSc development (Fig. 1). We also discuss current and potential therapies that target the immune cell compartment in patients with SSc. A better understanding of the immunopathogenic mechanisms underlying fibrosis might facilitate the discovery of novel and effective therapies for patients with SSc.

Immunological abnormalities in SSc

A growing number of studies have revealed the involvement of immunological abnormalities, including dysregulated immune cells and cytokines, in the pathogenesis of SSc fibrosis. Both the innate and adaptive immune compartments have critical roles in the fibrotic process and contribute to disease progression in patients with SSc.

Innate immune cell compartment

Macrophages. Macrophages are one of the main types of effector cells in SSc pathogenesis. The expression levels of various macrophage biomarkers (for example, CD14 and IL13RA) are high in the skin of patients with diffuse cutaneous SSc (dcSSc) and are associated with progressive skin fibrosis[11]. Macrophages have a high plasticity and polarizing potential, which results in a broad spectrum of macrophage phenotypes. Two extreme ends of the polarized states of macrophages can be induced in vitro: classically activated (M1) macrophages and alternatively activated (M2) macrophages[12]. In a gene expression study of 103 patients with SSc and 33 healthy individuals, the researchers identified gene signatures associated with both M1 and M2 macrophages in the skin of patients with early-stage dcSSc[13]. Various other studies implicate M2 macrophages in particular in SSc pathogenesis. For example, macrophages from patients with SSc express higher levels of M2 polarization markers (including CD163 and CD206), cytokines (including TGFβ and IL-6) and fibrotic factors (including various components relating to the ECM, matrisome and collagen formation) than macrophages from healthy individuals and patients with non-inflammatory diseases[14,15]. Knockout of IRF8 in myeloid cells specifically leads to an M2 phenotype and can aggravate dermal fibrosis in bleomycin-treated mice, suggesting that M2 macrophages have a pro-fibrotic role in SSc[16]. Furthermore, depletion of macrophages with a CSF receptor 1 inhibitor can alleviate skin fibrosis in a mouse model of SSc[17], highlighting the therapeutic potential of targeting macrophages in SSc. Notably, in a phase II clinical trial of patients with SSc, the efficacy of tocilizumab in improving skin and lung fibrosis was predominantly mediated by the inhibition of M2 macrophages, reflected by the sustained serum reduction of CCL18 (ref.[18]).

The introduction of droplet-based single-cell RNA sequencing (scRNA-seq) has challenged the dichotomies of macrophages, helped to reveal novel macrophage subtypes and provided more information regarding SSc heterogeneity[19]. Using scRNA-seq, researchers have identified a specific *FCGR3A*$^+$ macrophage subpopulation in the skin of patients with dcSSc that is absent in normal skin[20]. The *FCGR3A*$^+$ macrophages express high levels of M2 markers (such as CD163 and MS4A4A) and other markers (such as MSR1, F13A1, CCL2 and LILRB4) whose expression levels in the skin correlate with disease severity[20]. Pseudo-time analysis of the *FCGR3A*$^+$ macrophages suggested that these macrophages were derived from *CCR1*$^+$ and *MARCO*$^+$ macrophages present in healthy skin[11,20]. In a separate study of patients with SSc and late-stage ILD[21], scRNA-seq revealed a population of *SPP1*^{hi} macrophages that were thought to have pro-fibrotic effects in the lung and had already been implicated in idiopathic pulmonary fibrosis[22]; this population accounted for the largest macrophage cluster in the lungs of the study patients. Single-cell epigenomic analysis uncovered key transcription factors, including ATF5 and TFEB, that were capable of promoting the differentiation of normal macrophages into *SPP1*^{hi} macrophages[21]. Novel macrophage subsets revealed by these technologies can provide insight into immune heterogeneity in SSc and related molecular mechanisms.

Macrophages contribute to SSc fibrosis via various pro-fibrotic and pro-inflammatory mechanisms. Macrophages are important sources of TGFβ1, the most pro-fibrotic isoform of TGFβ[23]. TGFβ1 can promote fibrosis by inducing the recruitment and proliferation of fibroblasts, promoting their differentiation into myofibroblasts and fostering ECM deposition[24]. In addition, both *FCGR3A*$^{+}$ macrophages in the skin and *SPP1*$^{+}$ macrophages in the lungs of patients with SSc express pro-fibrotic mediators, including IL-6 and CCL18 (refs.[20,21]). Type I interferon signalling is also upregulated in profibrotic *SPP1*^{hi} macrophages in patients with SSc-ILD[25]. Osteopontin, encoded by *SPP1*, is both an ECM phosphoglycoprotein and a cytokine that stimulates type I collagen production by fibroblasts. Secreted by the macrophages differentiated from blood monocytes, osteopontin can recruit fibroblasts and promote lung fibrosis, and serum osteopontin can function as a prognostic biomarker for SSc[26]. The Toll-like receptor (TLR) signalling pathway is an important mediator of the persistent fibrotic response in SSc. Specifically, TLR4 and its exogenous ligand tenascin C are upregulated in the skin of patients with SSc compared with healthy individuals, with TLR4 being highly expressed by fibroblasts and vascular cells within lesional tissue[27,28]. Macrophages also express TLR4 on their surface. TLR4-mediated macrophage activation via tenascin C stimulation can substantially contribute to collagen synthesis and maintain the integrity of the ECM[29].

During the evolution of SSc, various factors influence the fate of macrophage subtypes and their development towards acquiring either a pro-inflammatory phenotype or a pro-fibrotic phenotype. The identification of specific macrophage populations and related pro-fibrotic mechanisms in SSc tissues should lead to more specific macrophage-based anti-fibrotic therapy in future. Immune abnormalities can serve as targets for new precision therapies aimed at reprogramming and switching the function of the pro-fibrotic macrophages.

Dendritic cells. Dendritic cells are also important effector cells in SSc pathogenesis. Dendritic cells can be divided into conventional dendritic cells and plasmacytoid dendritic cells (pDCs), the latter of which is the main producer of type I interferons in autoimmune diseases such as SSc[30]. Aberrant pDC infiltration occurs not only in the skin lesions but also in the lungs of patients with SSc[20,31,32]. Enrichment of pDCs in the blood and skin of patients with dcSSc is associated with a higher modified Rodnan Skin Score (mRSS)[33]. Furthermore, depletion of pDCs considerably reduces the skin thickness of bleomycin-treated mice[4], whereas transplantation of human pDCs into immunodeficient mice enhances the inflammatory and fibrotic response to bleomycin treatment[34], indicating an essential role for pDCs in SSc fibrosis. In 2022, a scRNA-seq analysis identified another subset of dendritic cells found exclusively in the skin of patients with SSc; these *FCN1*$^{+}$ monocyte-derived dendritic cells mainly existed in the perivascular regions and correlated positively with the mRSS, highlighting the potential pro-fibrotic nature of these cells[20].

SSc-associated single-nucleotide polymorphisms are highly enriched in accessible DNA regions (that is, DNA regions characterized by accessible chromatin) of skin-resident dendritic cells[31]. For example, in an Assay of Transposase Accessible Chromatin using sequencing (ATAC-Seq) analysis of primary cells from healthy individuals and patients with SSc, among the various immune cells assessed, the dendritic cells had the greatest number of SSc-associated changes in chromatin accessibility, suggesting a role of epigenetic modifications in dendritic cell-associated SSc pathogenesis[31]. Notably, the expression of *RUNX3* is notably decreased in pDCs from patients with SSc compared with pDCs from healthy individuals owing to hypermethylation of the gene, and deletion of *RUNX3* in dendritic cells aggravates skin fibrosis in a bleomycin-induced mouse model of SSc[35,36]. Thus, epigenetic mechanisms can regulate the pro-fibrotic functions of dendritic cells.

The CXCL4–IFNα signalling pathway in pDCs also has a crucial role in SSc fibrosis. Accumulating evidence indicates that pDCs infiltrate the skin in SSc and primarily secrete CXCL4 and IFNα (a type I interferon)[4,20,37]. In a multi-centre study, plasma levels of CXCL4 were increased in patients with SSc compared with healthy individuals and were positively associated with pulmonary arterial hypertension and lung fibrosis[38]. The overproduction of CXCL4 in pDCs from patients with SSc causes overproduction of IFNα[4]; indeed, serum levels of IFNα are elevated in SSc and are associated with lung fibrosis[39]. Furthermore, inhibition of type I interferon secretion by pDCs using monoclonal BDCA2 antibodies can alleviate bleomycin-induced skin fibrosis in mice, implicating the importance of type I interferons in SSc pathogenesis[34]. Intriguingly, the CXCL4–DNA and CXCL4–RNA complexes are present in the serum and tissues of patients with SSc and can stimulate B cells to produce anti-CXCL4 autoantibodies, another potent stimulator of IFNα production, forming a vicious cycle that maintains the type I interferon signature in SSc[40].

An important next step in the investigation of immune cells in SSc is the identification of the presence and cell fate of the pro-fibrotic macrophages and dendritic cells in different SSc subtypes. Blockade of these pathogenic cells within the mononuclear phagocyte system might also present an attractive strategy for controlling SSc.

Mast cells. Mast cells have an essential pro-fibrotic role in organs such as the lung and heart[41,42]. Infiltration of mast cells into the skin occurs during both the onset[43] and the later stages of SSc, implicating a role for these cells in SSc development[44]. Mast cells possess granules that contain a large number of pro-fibrotic cytokines, including IL-4, IL-13 and TGFβ. When secreted, these cytokines can directly influence the generation and function of myofibroblasts[45]. The suppression of mast cell degranulation by a cannabidiol derivative (VC3-004.3) inhibits the differentiation of fibroblasts to myofibroblasts and attenuates bleomycin-induced skin fibrosis in mice[46]. Mast cells might also promote fibrosis by producing serotonin[47], a substance that accelerates fibroblast activation and ECM synthesis via its receptor 5-HT_{2B} and downstream TGFβ–SMAD signalling[48]. Serotonin is also capable of decreasing the apoptosis of skin fibroblasts and promoting their migration towards lesions[49]. Additionally, an antagonist of serotonin (SB 204741) reduces myofibroblast accumulation and collagen deposition in the skin of bleomycin-treated mice[48]. Another product of mast cells is histamine, which has long been known to stimulate skin collagen synthesis[50], suggesting its potential to promote skin fibrosis. Collectively, mast cells are important contributors to SSc fibrosis through these mediators.

Neutrophils. As important members of the innate immune defence, neutrophils are promptly recruited to sites of damage in response to inflammation. In SSc, neutrophil-mediated release of reactive oxygen species can activate fibroblasts and induce fibrosis through TGFβ signalling and ECM overproduction[51]. SSc neutrophils also produce various pro-fibrotic cytokines, such as TGFβ, IL-6 and vascular endothelial growth factor (VEGF)[45]. Neutrophil elastase has also been linked to fibroblast-to-myofibroblast differentiation[52], and inhibition of neutrophil elastase protects mice against pulmonary fibrosis induced by bleomycin[53]. Neutrophils from patients with SSc have an increased tendency to release large amounts of neutrophil extracellular traps

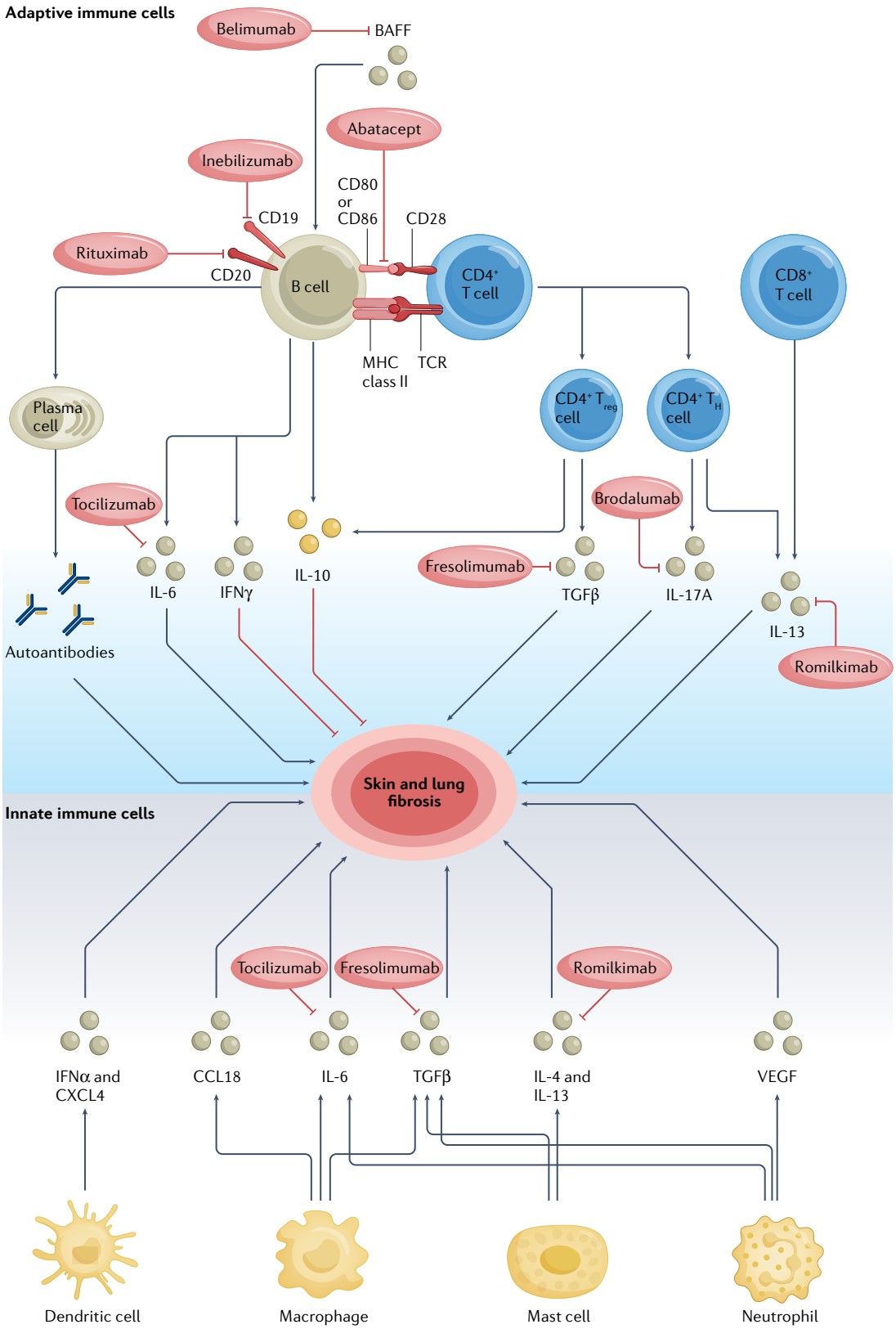
Adaptive immune cells
Belimumab
BAFF
Abatacept
Inebilizumab
CD80
or
CD86
CD28
CD19
Rituximab
CD20
B cell
CD4+
T cell
CD8+
T cell
MHC
class II
TCR
Plasma
cell
CD4+ Treg
cell
CD4+ TH
cell
Tocilizumab
Brodalumab
IL-6
IFNγ
IL-10
Fresolimumab
TGFβ
IL-17A
IL-13
Romilkimab
Autoantibodies
Skin and lung
fibrosis
Innate immune cells
Tocilizumab
Fresolimumab
Romilkimab
IFNα and
CXCL4
CCL18
IL-6
TGFβ
IL-4 and
IL-13
VEGF
Dendritic cell
Macrophage
Mast cell
Neutrophil

Fig. 1 | Roles of immune cells in the pathogenesis of SSc fibrosis. Diverse immune cell types are activated in systemic sclerosis (SSc) and secrete numerous cytokines that either promote or restrain fibrosis in the skin and/or lung. This figure summarizes the main cells, cytokines and mechanisms involved in SSc fibrosis, and their interactions, along with the corresponding drugs that target these components and that have undergone (or are undergoing) clinical trial assessment. BAFF, B cell-activating factor; CCL18, chemokine (C-C motif) ligand 18; IFN, interferon; CTLA4, cytotoxic T lymphocyte Antigen 4; CXCL4, C-X-C-motif ligand 4; TGFβ, transforming growth factor-β; T_H, T helper; T_{reg}, regulatory T; VEGF, vascular endothelial growth factor.

(NETs) compared with neutrophils from healthy individuals[54]; notably, NETs can induce fibroblast differentiation into myofibroblasts[55]. The past few years have seen mounting interest in the pathogenic role of neutrophils in autoimmune disease and strategies for targeting these cells, such as interference of neutrophil recruitment, cell degranulation and NET formation, which has until recently been a previously understudied therapeutic avenue[56]. Future exploration of the pro-fibrotic role of neutrophils should provide further insight into immunopathogenesis of SSc.

Other innate immune cells. Other innate immune cells have also been implicated in the immunopathogenesis of SSc, such as monocytes, innate lymphoid cells (ILCs)[2], γδ T cells[57], natural killer (NK) cells and invariant natural killer T cells[58]. The number of group 2 ILCs (ILC2s) is increased in both the peripheral blood and damaged skin of patients with SSc compared with that of healthy individuals, and circulating numbers of these cells correlate positively with the severity of both skin and lung fibrosis[59]. An expanded number of γδ T cells of the $Vγ9^+$ subtype is present in the circulation of patients with SSc compared with that of healthy individuals, especially in patients with pulmonary fibrosis[57]. $Vγ9^+$ γδ T cells derived from patients with SSc produce upregulated amounts of anti-fibrotic cytokines, including TNF and IFNγ, compared with $Vγ9^+$ γδ T cells from healthy controls[60]. Finally, both NK cells that express high levels of CD56 and invariant natural killer T cells are reduced in number and functionally impaired in patients with SSc[61,62]. However, whether these cells participate in the fibrosis of SSc remains unclear. Further mechanistic insights and understanding of these innate immune cells in SSc fibrosis will provide future directions for developing novel diagnostic and therapeutic targets for patients with SSc.

Adaptive immune cell compartment

T cells. $CD4^+$ T cells are considered the main contributing T cell type in SSc pathogenesis. Myriad $CD4^+$ T cell subsets are implicated in SSc development. Specifically, $CD4^+$ T cells in the skin of patients with SSc have a T helper 2 (T_H2) cell phenotype[63], and T_H2-associated immunity promotes ECM production and is associated with fibrosis[64,65]. Compared with healthy individuals, patients with SSc have a decreased T_H1 cell to T_H2 cell ratio, as well as an increased T_H17 cell to regulatory T (T_{reg}) cell ratio, in their circulation[66,67]. Both circulating T_H17 cells and $IFNγ^+$ $IL\text{-}17^+$ T_H17 cells are enriched in patients with SSc and correlate positively with disease activity[68,69], but the exact role of T_H17 cells in SSc fibrosis remains understudied[70]. Evidence also suggests that circulating T_{reg} cells have dysregulated functions in patients with SSc as the secretion of IL-10 and TGFβ is impaired in these cells[71]. However, the majority of studies have found that patients with SSc have an increased number of T_{reg} cells[68,72], implicating a possible compensatory but ineffective T_{reg} cell expansion in SSc. The immune disequilibrium among those T cell subsets might be an important determinant of SSc progression.

T follicular helper (T_{FH}) cells, another subset of $CD4^+$ T cells, are enriched in the circulation of patients with SSc and have an upregulated B cell-stimulating capacity[21]. Intriguingly, the skin of patients with SSc also contains a T_{FH}-cell like population. These $ICOS^+$ $PD1^+$ $CXCR5^+$ T cells are associated with skin fibrosis, and have pro-fibrotic activities, as demonstrated in a graft-versus-host disease–SSc mouse model[73]. ScRNA-seq has identified another cluster of T_{FH} cell-like $CXCL13^+$ T cells with a B cell-promoting phenotype that were found exclusively in the skin lesions of patients with SSc[74]. These skin-derived $CXCL13^+$ T cells were mostly $CD4^+$ cells, existed in nearly 50% of patients with SSc and diminished in number after treatment with immunosuppressants. They were positively associated with the presence of SSc-ILD rather than the skin score, implicating a potential systemic role for these cells[74].

Although $CD4^+$ T cells are the predominant infiltrating T cell subset in the dermis of patients with SSc, $CD8^+$ T cells also participate in SSc pathogenesis and have pro-inflammatory, pro-fibrotic and cytotoxic effects[75]. In a gene expression analysis of skin samples from 48 patients with early dcSSc, more than half of the patients had upregulated gene signatures of both $CD8^+$ T cells and $CD4^+$ T cells, and the signatures were more prominent in those patients with a shorter disease duration[13]. Additionally, $CD8^+$ T cells are enriched in the skin and lungs of patients with SSc[76], whereas the number of these cells in the circulation is decreased[77,78]. Enrichment of $CD8^+$ T cells in lesional tissue indicates the presence of local factors that recruit pro-inflammatory cells, including $CD8^+$ T cells, to the affected tissues in SSc.

T cells mainly induce and maintain SSc fibrosis by secreting cytokines. IL-17A, a pro-inflammatory cytokine predominantly produced by T_H17 cells, has a profibrotic role in different mouse models of SSc[79,80]. The influence of IL-17A on fibrosis remains controversial in human studies[69,81]. Nevertheless, in a phase III clinical trial, brodalumab, a completely human anti-IL-17 receptor A monoclonal antibody, reduced skin sclerosis and had beneficial therapeutic effects on lung function, digital ulcers and gastroesophageal reflux disease symptoms in patients with SSc[82,83]. These results suggest that IL-17 signalling is a promising therapeutic target for SSc.

IL-13 is another profibrotic mediator derived from T cells. Serum levels of IL-13 are increased in patients with SSc compared with healthy individuals[84] and levels of this cytokine correlate positively with the severity of skin fibrosis in these patients[85]. IL-13 can induce the transition of fibroblasts to myofibroblasts in vitro, leading to ECM production and tissue stiffening[86]. T_H2 cells are the main source of IL-13, the expression of which is upregulated by the transcription factor GATA3 (refs.[6,87]). $CD8^+$ T cells and $CCR7^-$ $CD4^+$ memory T cells are also important IL-13 producers in SSc[64,88]. IL-13-producing $CD8^+$ T cells infiltrate the skin in SSc and can promote ECM production by dermal fibroblasts in vitro[89].

Hence, accumulating evidence suggests that T cells have an important role in the initiation and maintenance of the fibrotic network, and thus provide potential targets for the treatment of SSc.

B cells. B cells contribute to SSc pathogenesis predominantly through antigen presentation, antibody production and cytokine secretion. Effector B cells secrete pro-inflammatory cytokines, such as IL-6, granulocyte–macrophage colony-stimulating factor (GM-CSF) and IFNγ[90]. Among these cells, IL-6-producing effector B cells infiltrate the skin

lesions of mice with bleomycin-induced fibrosis[91]. By contrast, regulatory B (B_{reg}) cells suppress immune responses via the production of anti-inflammatory cytokines such as IL-10. Evidence suggests that B_{reg} cells are numerically reduced and functionally impaired in patients with SSc[90]. Thus, an imbalance between activated effector B cells and impaired B_{reg} cells contributes to SSc immunopathology and might promote SSc fibrosis[92].

CD19 is a common biomarker for B cells and might also have relevant functions in SSc. $CD19^+$ B cells are enriched in both the peripheral blood of patients with SSc and the spleen of mice with hypochlorous acid-induced fibrosis[76]. CD19 is an important B cell activator, and its expression level in bleomycin-induced mice is positively associated with fibrosis[93]. Furthermore, in a tight-skin mouse model of SSc, CD19 inhibition leads to decreased skin thickness, collagen deposition and autoantibody production[94]. Notably, in systemic lupus erythematosus (SLE), treatment with CD19-targeted chimeric antigen receptor (CAR)-modified T cells can deplete the patient's B cells and induce rapid remission of refractory SLE[95,96]. Therefore, CD19 is a promising target for B cell depletion in other autoimmune conditions, including SSc. CD22 and CD72 are two specific membrane molecules of B cells. In mouse models of bleomycin-induced or hypochlorous acid-induced fibrosis, the levels of various pro-fibrotic cytokines IL-6, TGFβ, CTGF, IL-1β, IL-13 and TNF are reduced, and skin and lung fibrosis are attenuated, in $CD22^{-/-}$, $CD72^{-/-}$ and $CD22^{-/-}/CD72^{-/-}$ mice compared with wild-type mice[97], indicating that CD22 and CD72 contribute to fibrosis development by controlling the secretion of various cytokines.

Table 1 | Targeted therapies in clinical trials for treating SSc

Drug	Target	Mechanism	Phase	ClinicalTrials.gov identifier
Abatacept	T cells	Binds to CD80 or CD86, blocks their interaction with CD28 and inhibits T cell activation	II	NCT02161406
Rituximab	B cells (CD20)	Binds to CD20 on B cells and depletes these cells	II	NCT01086540
			II–III	NCT04274257
Inebilizumab	B cells (CD19)	Binds to CD19 on B cells and depletes these cells	I	NCT00946699
Belimumab	BAFF	Binds to soluble BAFF and inhibits its B cell-activating potential	II	NCT01670565
Tocilizumab	IL-6	Binds to the IL-6 receptor and inhibits its activities	II	NCT01532869
			III	NCT02453256
Romilkimab	IL-4 and IL-13	Binds to and neutralizes IL-4 and IL-13	II	NCT02921971
Fresolimumab	TGFβ	Blocks TGFβ signalling	I	NCT01284322

APC, antigen-presenting cells; BAFF, B cell-activating factor; SSc, systemic sclerosis; TGFβ, transforming growth factor-β.

The pro-fibrotic role of B cells partly relies on B cell-activating factor (BAFF), an important B cell regulator mainly secreted by T cells and dendritic cells[98]. In co-cultures of B cells and dermal fibroblasts from patients with SSc, BAFF can boost the B cell-induced pro-fibrotic activities of fibroblasts, including mRNA expression of *COL1A1*, *COL1A2*, *COL3A1*, *α-SMA* and *TIMP1*, and the release of IL-6, TGFβ1 and collagen[99]. Levels of BAFF are elevated in the serum of patients with SSc[91], compared with that of healthy individuals, and a BAFF antagonist can alleviate skin and lung fibrosis in a bleomycin-induced SSc model by regulating the balance between effector B cells and B_{reg} cells[91]. BAFF can boost IL-6-producing effector B cells and suppress IL-10-producing B_{reg} cells[91], which is important, as IL-6 promotes fibrosis whereas IL-10 inhibits collagen synthesis by skin fibroblasts[100]. Accordingly, skin and lung fibrosis are attenuated in mice with IL-6-deficient B cells but aggravated in mice with IL-10-deficient B cells[91]. Furthermore, adoptive transfer of IL-10-producing B_{reg} cells during the early stages of disease results in a reduced skin score and disease severity in mice with sclerodermatous chronic graft-versus-host disease[101]. Indeed, B cells from patients with SSc produce more IL-6 and less IL-10 than B cells from healthy individuals[102]. In addition, a preliminary randomized controlled multi-centre trial conducted by Grassegger et al.[103] showed that the IFNγ secreted by effector B cells had a mild anti-fibrotic effect in patients with SSc.

Some data also suggest that B cells can directly participate in the polarization of macrophages towards the M2 signature, as B cell depletion in the bleomycin-induced mouse model of SSc is associated with a decreased number of pro-fibrotic M2 macrophages, suggesting an interplay between adaptive and innate immunity in the pathogenesis of SSc[104].

Therapeutic targets for SSc

Clinical options for ameliorating the manifold clinical symptoms of SSc and postponing the progression of this disease are limited. Although wide-acting immunosuppressants, such as methotrexate, cyclophosphamide and mycophenolate mofetil, have some beneficial effects in patients with SSc, none of these therapies can notably improve the survival of these patients[105,106]. Accumulating studies provide evidence that specific immune cells and their immune mediators are promising and safe targets for limiting SSc disease progression. Encouragingly, targeting the IL-6 receptor with tocilizumab is approved by the FDA for the treatment of SSc-associated ILD, indicating the great potential of immune-related targeted therapies for patients with SSc. In this Section, we summarize the available data on the effect of various targeted therapies on SSc development and highlight potential immune-modulating strategies (Table 1).

Targeting T cells

Abatacept, the most frequently studied T cell-targeting therapy for patients with SSc, inhibits T cell co-stimulation by the well-known surface molecule CD28. The T cell surface molecule cytotoxic T lymphocyte antigen 4 (CTLA4) binds to CD80 or CD86 on B cells and other antigen-presenting cells with higher avidity than CD28 and functions as a negative regulator of T cell activity[107]. Abatacept is a fusion protein made up of the ligand-binding domain of CTLA4 and an IgG1-derived Fc domain[107]. Therefore, abatacept can competitively block CD28-mediated T cell activation. Importantly, this drug is well tolerated in patients with SSc[108,109]. Abatacept can reduce the infiltration of activated T cells in skin lesions and suppress dermal fibrosis in bleomycin-treated mice[110]. Consistently, the mRSS of 27 patients with SSc was reduced following treatment with abatacept in a retrospective observational

study[108]. Abatacept has also been evaluated for the treatment of dcSSc in a randomized, double-blind, placebo-controlled, 12-month phase II clinical trial[111]. In this trial, the mean mRSS change from baseline to 1 year after treatment initiation, the primary outcome, showed no statistical difference between the abatacept and the placebo control group (−6.24 versus −4.49, $P = 0.28$). Nevertheless, the secondary outcomes, including the Health Assessment Questionnaire-Disability Index and the ACR Combined Response Index in Systemic Sclerosis, showed notable improvement in favour of abatacept. Given these encouraging results, a phase III clinical trial is warranted.

Targeting B cells

Rituximab. Rituximab is an anti-CD20 monoclonal B cell-depleting antibody, and treatment with this antibody led to improvement in cutaneous fibrosis and pulmonary function in six clinical trials. In a prospective study, the 254 patients with SSc who had received treatment with rituximab were more likely to show improvements in skin fibrosis than the 9,575 untreated patients[112]. Similarly, in a double-blind, randomized, placebo-controlled, phase II–III trial in Japan, the patients in the rituximab group had a greater reduction in mRSS after 24 weeks than the patients in the placebo group (−6.30 versus 2.14, $P < 0.0001$)[113]. In addition, the utility of rituximab in patients with SSc-associated pulmonary arterial hypertension (SSc-PAH) has been tested in a multicentre, double-blind, and randomized phase II trial[114]. The primary outcome measure (mean change of 6-min walking distance) favoured the rituximab group over the placebo group, although the difference between the two groups was not statistically significant (23.6 ± 11.1 m versus 0.5 ± 9.7 m, $P = 0.12$). Rituximab is now approved in Japan for the treatment of SSc and provides a new treatment option for refractory SSc.

Inebilizumab. Inebilizumab (also known as MEDI-551) is a synthetic monoclonal anti-CD19 antibody with a B cell-depleting ability[115]. In a phase I trial, inebilizumab was well tolerated and safe in patients with SSc[116]. The mean mRSS change (from baseline to day 85) was −5.4 in the inebilizumab group compared with 2.3 in the control group, indicating that inebilizumab has beneficial effects on skin fibrosis. Compared with anti-CD20 therapy, this anti-CD19 antibody can target a wider range of B cells, including plasma cells and plasmablasts, the main producers of pathogenic autoantibodies[95,96]. Exploration of the safety and efficacy of inebilizumab in patients with SSc by a more stringent phase II–III clinical trial with a larger number of patients is warranted, in addition to investigations of how targeting CD19 compares with targeting CD20 in SSc.

Targeting cytokines

Belimumab. Belimumab is a recombinant human monoclonal antibody that targets BAFF and is approved by the FDA for the treatment of systemic lupus erythematosus[117,118]. Belimumab can promote B cell apoptosis and reduce autoantibody secretion via neutralizing BAFF[119]. Different from the non-selective depletion of B cells with anti-CD20 or anti-CD19 antibodies, BAFF neutralization limits the abundance and function of over-activated, and potentially pathogenic, B cells. In a phase II trial, treatment with mycophenolate mofetil and belimumab led to a greater improvement in skin fibrosis (as assessed by mRSS) in patients with early dcSSc than treatment with mycophenolate mofetil and placebo (−10.0 versus −3.0, $P = 0.411$)[120]. The expression of B cell signalling molecules, as well as genes involved in pro-fibrotic pathways (such as *COL4A1* and *IL6)* and TGFβ signalling, were also reduced in the skin of patients in the belimumab group. The occurrence of adverse events did not differ between the two groups, and no deaths occurred during the trial period. Future phase II or III clinical trials of belimumab treatment in patients with SSc are warranted.

Tocilizumab. Tocilizumab, an IL-6 receptor inhibitor, is effective in the treatment of numerous autoimmune diseases, especially rheumatoid arthritis[121]. Tocilizumab can limit fibrosis in the skin and lungs of mice with bleomycin-induced fibrosis[122]. Furthermore, in a phase II trial of patients with SSc, tocilizumab could stabilize the lung function of patients by partially restoring their forced vital capacity[18]. A subsequent phase III trial demonstrated the safety and efficacy of tocilizumab in SSc-ILD[10]. In this trial, tocilizumab treatment showed a tendency to alleviate skin thickness (as evaluated by mRSS), although the primary skin fibrosis end point was not met. Furthermore, the secondary end points (such as measures of restrictive ventilation dysfunction by percentage predicted forced vital capacity and pulmonary fibrosis by high-resolution computed tomography) indicated that lung function was significantly preserved after 48 weeks in patients being treated with tocilizumab[10], consistent with the results of the previous phase II trial[18]. Based on the positive effects of this therapy on pulmonary function in this trial, tocilizumab was approved by the FDA for the treatment of adults with SSc-ILD in 2021 (refs.[9,10]).

Romilkimab. IL-4 and IL-13 are pro-fibrotic cytokines that are greatly increased in the serum of patients with SSc[64]. Notably, anti-IL-4 and anti-IL-13 antibodies can prevent the progression of skin fibrosis in mice[64,123]. Romilkimab (SAR156597), an engineered IgG4 antibody, is able to combine and neutralize both IL-4 and IL-13. The efficacy and safety of romilkimab in patients with early dcSSc has been evaluated in a randomized, double-blinded, placebo-controlled, 24-week phase II trial[124]. In this trial, the mean change of mRSS from baseline to week 24 was greater in the romilkimab group than in the placebo group (−4.76 versus −2.45; one-sided $P = 0.0291$). Additionally, the safety profile of romilkimab was similar to that of placebo when treating patients with dcSSc. Therefore, romilkimab is a promising option for the treatment of skin fibrosis in patients with SSc.

Fresolimumab. TGFβ is an important mediator of fibrosis[125,126]. For example, TGFβ can trigger fibroblast migration, fibroblast-to-myofibroblast differentiation and ECM synthesis[23]. Blockade of TGFβ signalling has robust efficacy in the prevention of fibrosis in preclinical models of lung, liver, heart and renal fibrosis[23,127], and thus this cytokine is a promising target for SSc fibrosis. Fresolimumab is a neutralizing antibody that can target all three active forms of TGFβ[128]. In an open-label trial that included 15 patients with early dcSSc, fresolimumab treatment decreased the expression of various TGFβ-regulated genes (*THBS1*, *COMP*, *SERPINE1* and *CTGF*) in the skin, reduced local myofibroblast infiltration and decreased the amount of skin fibrosis (as evaluated by mRSS)[129]. The results were encouraging, but this small trial was only exploratory and had a limited number of participants. Collectively, these observations support a key role for TGFβ in SSc pathogenesis. Further research is necessary to determine the safety of the long-term use of this therapy, as well as the efficacy of fresolimumab in treating skin, lung and gastrointestinal tract fibrosis in a large SSc cohort.

Stem cell therapies

Mesenchymal stem cell transplantation. Mesenchymal stem cells (MSCs) exert immunomodulatory functions primarily through interacting with immune cells, such as macrophages and lymphocytes,

via cell-to-cell contact and paracrine activity[130,131]. MSCs can increase the expression of IL-10 and other anti-inflammatory mediators in macrophages and inhibit $CD4^+$ and $CD8^+$ T cell proliferation[132,133].

The efficacy of combined plasmapheresis and allogeneic MSCs in patients with SSc has been evaluated in a phase I–II trial[134]. The 14 recruited patients received plasmapheresis and cyclophosphamide on days 1, 3 and 5 and MSC transplantation on day 8. After a year of follow-up, the mRSS of the patients had decreased, and both the pulmonary function and appearance of the lungs by computed tomography had improved in those patients with SSc-associated ILD. The anti-Scl70 antibody titre and TGFβ level in the serum had also decreased. Additionally, in a randomized clinical trial of adipose-derived regenerative cell transplantation in patients with SSc, the hand function of patients had improved from baseline after 24 and 48 weeks compared with the placebo control group, although the difference was not statistically significant[135]. Therefore, MSC-based therapies are a promising option in the treatment of SSc.

Haematopoietic stem cell transplantation. Since 1996, haematopoietic stem cell transplantation (HSCT) has been used for the treatment of patients with dcSSc owing to its long-term clinical benefits[136,137]. The rationale of using HSCT for the treatment of SSc is that this approach eliminates autoreactive immune cells, resulting in reconstitution of the immune system with novel and tolerant cells[137]. Autologous HSCT can restore the number and suppressive ability of IL-10-producing B_{reg} cells and reduce the number of IL-6 and TGFβ1-producing B cells in the peripheral blood of patients with SSc[138,139]. Additionally, autologous HSCT in patients with SSc is followed by a regain in $CD8^+$ T cell frequency and an increase in T_H1 cell to T_H2 cell ratio in the blood mononuclear cell compartment[140]. In a comparative study, autologous HSCT decreased the interferon and neutrophil signatures and increased the cytotoxic–NK signature of patients with SSc, which correlated with an improvement in lung function and a reduction in mRSS[141].

Importantly, pilot studies for autologous HSCT have demonstrated its effectiveness in the treatment of skin fibrosis and lung function in patients with SSc[142–145]. Three phase II or III randomized clinical trials provide evidence of the long-term benefits of HSCT in improving skin fibrosis and pulmonary function when compared with standard immunosuppressive therapies[144,146,147]. HSCT also improves the event-free and overall survival of patients with dcSSc[144,146,148]. However, because of the risk of transplantation-related mortality, HSCT should only be provided to patients with rapidly progressive SSc or those patients with a high risk of SSc-related organ failure[149]. Importantly, a standardized procedure that includes precise patient screening, pre-transplant examination and post-transplant management of HSCT remains to be established.

Future perspectives

SSc is a devastating autoimmune disease mainly characterized by vasculopathy, immune abnormalities and progressive fibrosis. Among these features, immune dysregulation has a central role by regulating both vascular damage and the fibrotic progress. In the past 5 years, a large number of experimental studies have demonstrated the infiltration of aberrantly activated immune cells and the over-production of related pro-inflammatory and pro-fibrotic cytokines in the serum or affected organs of patients with SSc. These studies provide sufficient evidence that various immune cells, especially some of the disease-specific cell types that are only present in a subset of patients, have a critical and non-negligible role in SSc fibrosis.

Intriguingly, in a population-scale scRNA-seq analysis of the blood and lesioned skin of patients with SSc, the various changes in immune subsets were inconsistent across different patients, whereas the patients did share a specific and global perturbation of the stromal compartment (specifically, within a population of cells referred to as SSc-associated fibroblasts)[33]. This lack of an apparent culprit or culprits within the immune cell compartment could be due to the high heterogeneity of the immune cell phenotypes that can occur in SSc, which might mask alterations in immune subsets that only occur in particular patient subgroups defined by the disease subtype, duration, activity and/or other temporarily unidentified factors. This variability might also explain the limited beneficial effects of some immune-targeted therapies in SSc, which might be overcome by combined and individualized therapy. For instance, the skin inflammatory signatures of patients with SSc are most evident in the early stages of disease (less than 3 years from the first symptom other than Raynaud syndrome)[150], which might be an ideal 'window of opportunity' for preventing disease progression through immune-targeted therapy.

In addition to the heterogeneous immune mechanisms in different patients with SSc, a common causative immune cell type might exist in different autoimmune diseases. For example, a similar cluster of T_{FH}-like cells with B cell-promoting ability is present in both the skin of patients with SSc and the synovium of patients with rheumatoid arthritis[151]. In this scenario, those autoimmune diseases with common autoimmunogenic mechanisms might be treated by targeting the same cell subset or signalling pathway. Notably, beyond treatment with monoclonal antibodies, targeted immune therapy can also be achieved using cell therapy, which is already showing promise for other indications. In systemic lupus erythematosus, B cell depletion using therapeutic antibodies has only limited therapeutic efficacy[152], whereas B cell depletion using CD19-targeted CAR T cells has achieved huge success in some patients[95,96], encouraging the investigation of CAR T cell usage in patients with SSc or other autoimmune conditions.

Conclusion

Immune dysregulation promotes the fibrotic process and has a central role in the initiation and progression of SSc. Numerous immune cells and molecules are involved in the pathogenesis of SSc, with different patients possessing distinct types of immune dysregulation. Continuing insights into the immunological and fibrotic mechanisms of SSc and their interactions should help to uncover the intricacies of this disease and enable the development of more effective targeted treatments. For heterogeneous diseases such as SSc, it is wise to 'treat the same disease with different drugs' (the concept of personalized medicine) and 'treat the different diseases with the same method', a principle recorded by the Synopsis of Golden Chamber, an ancient Chinese medical book written approximately 1800 years ago[153,154]. Further mechanistic and clinical studies are warranted to deepen our understanding of the role of immune cells in regulating fibrosis and to exploit this knowledge to develop effective immunomodulatory treatments in SSc.

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Review article

Age-related mechanisms in the context of rheumatic disease

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Abstract

Ageing is characterized by a progressive loss of cellular function that leads to a decline in tissue homeostasis, increased vulnerability and adverse health outcomes. Important advances in ageing research have now identified a set of nine candidate hallmarks that are generally considered to contribute to the ageing process and that together determine the ageing phenotype, which is the clinical manifestation of age-related dysfunction in chronic diseases. Although most rheumatic diseases are not yet considered to be age related, available evidence increasingly emphasizes the prevalence of ageing hallmarks in these chronic diseases. On the basis of the current evidence relating to the molecular and cellular ageing pathways involved in rheumatic diseases, we propose that these diseases share a number of features that are observed in ageing, and that they can therefore be considered to be diseases of premature or accelerated ageing. Although more data are needed to clarify whether accelerated ageing drives the development of rheumatic diseases or whether it results from the chronic inflammatory environment, central components of age-related pathways are currently being targeted in clinical trials and may provide a new avenue of therapeutic intervention for patients with rheumatic diseases.

Sections

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Key points

- Rheumatic disease prevalence is increased as a consequence of population ageing.
- Rheumatic diseases share common ageing characteristics and molecular pathways, which enables their classification as premature-ageing or accelerated-ageing diseases.
- Ageing and inflammation form a self-perpetuating, vicious cycle to advance rheumatic disease in patients and accelerate ageing phenotypes.
- Anti-ageing drugs may have therapeutic potential for the management and treatment of patients with rheumatic disease.

Introduction

Ageing is a complex biological process that is characterized by cellular and functional decline over a lifetime, leading to loss of homeostasis under stress conditions, and increased risk of many age-related morbidities that comprise the diseasome of ageing (neurodegenerative disorders, cancer and cardiovascular disease), with negative effects on quality of life and the likelihood of mortality[1–3]. Although lifespan has, globally, increased in the past decades, considerable variation exists according to geographic, socio-economic, lifestyle and environmental factors[3]. The cumulative exposure to these factors across a life, which is often referred to as the 'exposome', substantially affects healthspan and life expectancy[4]. Furthermore, caring for older adults affected by the diseasome of ageing imposes a considerable financial burden on the health care system[5–7]. Therefore, studies in the ageing field are needed to identify new strategies to treat or prevent these diseases and promote healthy ageing.

Understanding the central mechanism underlying ageing is challenging because of the complex interplay between factors and the considerable heterogeneity among individuals. However, over the past 30 years, research into the biology of ageing has made considerable progress, leading to identification of the cellular and molecular hallmarks of ageing[2]. Nine proposed hallmarks are generally considered to characterize the ageing process: genomic instability, epigenetic alterations, telomere attrition, mitochondrial dysfunction, loss of proteostasis, deregulated nutrient sensing, cellular senescence, stem cell exhaustion and altered intercellular communication (Fig. 1). Ageing is classically defined as the inevitable accumulation of cellular damage, but initial age-related changes in cellular and tissue functions may be compensated for by resilience factors to sustain organismal function and survival[8]. These resilience mechanisms counteract stress derived from the exposome and help to maintain health and organismal functionality. Because the balance between stress and resilience can be regarded as a dynamic equilibrium, one may hypothesize that age-induced reduction in resilience can be the result of impaired health and fragility[9]. Accordingly, sustained stress and activation of age-related pathways ultimately leads to classic age-related decline. Through a better understanding of key age-related molecular and cellular pathways, we can exploit them to access new treatment opportunities and make lifestyle changes to support healthy ageing.

Rheumatic diseases are a heterogeneous group of diseases affecting joints, bones and muscles, and they can also have systemic manifestations. More than 200 different conditions are currently labelled in this category, including osteoarthritis (OA), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), psoriatic arthritis, primary Sjögren syndrome (pSS), systemic juvenile idiopathic arthritis, ankylosing spondylitis, gout, fibromyalgia, dermatomyositis and polymyositis, mixed connective tissue disease and other overlap syndromes. Some of these conditions are among the most prevalent diseases and leading causes of disability worldwide[10,11]. Rheumatic diseases are generally characterized by inflammation, pain, swelling and stiffness, which subsequently lead to tissue destruction and reduction of patients' mobility. Disease onset can often be observed at a young age or even during childhood, but prevalence increases with age[12,13]. Although most rheumatic diseases are not yet classified as being age-related, age is a well-known risk factor for the development and management of several rheumatic diseases[14,15]. The current paradigm is that both genetic and exposome factors underlie the pathogenesis of rheumatic diseases. However, several changes in age-related pathways have been characterized in rheumatic diseases, including epigenetic alterations, stem cell exhaustion, loss of metabolic homeostasis and alteration of intercellular communication[13]. Nevertheless, our understanding of the ageing processes that are involved in disease pathogenesis is still at an early stage. Although ageing and rheumatic diseases share common underlying features, causality has not been proven. Whether ageing drives the development of rheumatic diseases, or the induction of age-related pathways is simply a secondary effect to disease chronicity, remains to be conclusively addressed.

In this narrative Review, we dissect each hallmark of ageing with respect to its occurrence in a variety of rheumatic diseases, and further propose a classification of rheumatic-disease-associated ageing phenotypes on the basis of their main ageing features. Finally, we shed light on the effects of current anti-rheumatic therapies on age-related pathways, and summarize current clinical approaches to target these pathways for treatment of rheumatic diseases.

Rheumatic disease hallmarks of ageing

Identification of molecular and cellular processes causative for ageing has been ongoing for several decades. Cumulatively, these research efforts have led to the recognition of key hallmarks of ageing[2,16], which are summarized in Fig. 1. These hallmarks can be classified into three categories: molecular alterations that might be the primary causes for ageing (genomic instability, epigenetic alterations, telomere attrition and loss of proteostasis); ensuing cellular dysfunction (mitochondrial dysfunction, deregulated nutrient sensing and cellular senescence); and dysregulation of tissue homeostasis (stem cell exhaustion and intercellular communication). In the following section, we discuss each hallmark of ageing with its implications for a variety of rheumatic diseases.

Molecular alterations of ageing

Genomic instability. The diversity of longevity and disease resistance within a species is governed by a complex interplay of genetic and epigenetic mechanisms, in addition to environmental factors[2,17–20]. The lifespans of mammalian species correlate inversely with their mutation rates, providing evidence of the influence of genomic instability on longevity[21]. Underlining the role of genetics in ageing, results from twin studies and studies with families including centenarians have revealed that ageing phenotypes may be driven by genetic predisposition[22]. Although polymorphisms in genes such as those encoding proteins of the insulin and insulin-like growth factor 1 (IGF-1) pathway are involved

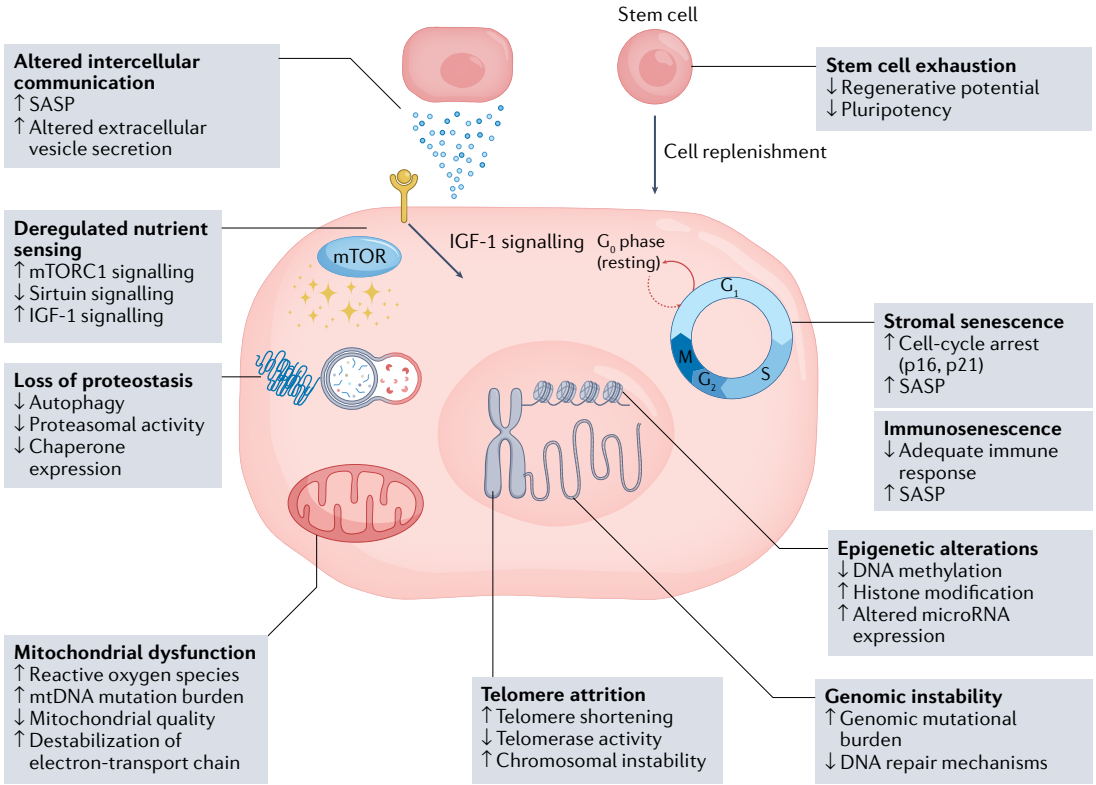


Fig. 1 | Classic hallmarks of ageing. Representation of the nine classic cellular hallmarks of ageing and their associated key features and effects. Genomic instability, epigenetic alterations, telomere attrition and loss of proteostasis are considered to be primary hallmarks, and represent the causes of cellular damage. This damage can consequently promote antagonistic hallmarks such as deregulated nutrient sensing, mitochondrial dysfunction and cellular senescence. Dysregulation of all these hallmarks contributes to alteration of intercellular communication and furthers stem cell exhaustion. IGF-1, insulin-like growth factor 1; mtDNA; mitochondrial DNA; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; SASP, senescence-associated secretory phenotype.

in extreme longevity in several model organisms[23], most premature ageing diseases (such as progeria and Werner syndrome) are the consequence of increased DNA damage accumulation[17,24]. The accumulation of genetic damage throughout life results from impairment of DNA repair mechanisms, which are consequently unable to maintain the integrity of telomeres and mitochondrial DNA (mtDNA)[25,26]. Oxidative stress promotes DNA damage and, together with disruption of DNA repair pathways, is a driver of genomic instability in immune cells from patients with RA[27–30], SLE[31–34] or SSc[35,36]. DNA damage promotes an aberrant immune response in these patients, which in SLE is characterized by autoantibody generation owing to excessive apoptosis and type I interferon response[28]. In RA, DNA damage renders T cells sensitive to apoptosis and leads to premature immunosenescence and T cell-driven autoimmunity[30]. Dysfunctional DNA repair was recently proposed to be central to the development of senescent $CD4^+$ T cells and their pro-arthritogenic effector functions[37]. In patients with SSc, DNA damage is associated with fibrosis and type I interferon production[35]. In OA chondrocytes, accumulation of DNA damage causes upregulation of cell cycle inhibitors, such as the cyclin-dependent kinase inhibitors $p16^{INK4a}$ and $p21^{Cip1}$, which maintain cell arrest associated with senescence[14,38]. In addition, oxidative DNA damage occurs in patients with fibromyalgia and correlates with physical and mental health status[39]. Taken together, genome instabilities caused by defects in DNA repair and oxidative stress are commonly found in rheumatic diseases and impair cellular homeostasis, leading to cellular senescence and aberrant immune responses, implicating them in rheumatic disease development and pathology.

Epigenetic alterations. Epigenetic mechanisms regulate gene expression at the transcriptional level through histone modifications, DNA methylation and transposable elements. Further post-transcriptional regulation via non-coding RNAs such as microRNAs (miRNAs) fine-tunes gene expression. Epigenetic processes are sensitive to environmental factors such as stress and diet. Unsurprisingly, ageing leads to distinct epigenetic modifications[40,41], which are also readily observed in rheumatic diseases[42].

RA synoviocytes exhibit several epigenetic changes that are associated with their aggressive phenotype[43–46], including global hypomethylation[44] and enhancement of histone deacetylase (HDAC) activity and hyperacetylation[47,48]. In addition, disruption of the balance between histone acetylases (HATs) and HDACs in patients with RA prompts chronic histone hyperacetylation, leading to *IL6* expression[48]. Likewise, HDAC2 expression and global H4 acetylation in B cells from patients with SSc correlate with skin thickness and disease activity, respectively[49]. In addition, global hypomethylation occurs in salivary gland epithelial cells and T cells from patients with pSS[50,51], and in B and T cells in SLE[52–54].

Reduction of DNA methyltransferase (DNMT) activity is thought to be responsible for the loss of DNA methylation during ageing. Similarly, DNMT levels are lower in T cells from individuals with SLE than from healthy individuals[55,56].

Several miRNAs (a subtype of non-coding RNAs), such as miR-29, miR-30 and miR-146a/b, are potent regulators of post-transcriptional gene expression in ageing[57]. These miRNAs are also implicated in rheumatic diseases. For instance, the miRNA-29 family has a pivotal role in skin fibrosis in patients with SSc[58], and in IFNγ-activated SSc fibroblasts miRNA-30a-3p controls the synthesis of B cell-activating factor (BAFF)[59]. In addition, dysregulation of miRNAs such as miRNA-146a in the salivary glands and peripheral blood mononuclear cells (PBMCs) from patients with pSS can enhance type 17 T helper (T_H17) cell differentiation and drive inflammation[60–62]. By contrast, overexpression of miRNA-29b and miRNA-21 in SLE $CD4^+$ T cells indirectly induces hypomethylation by targeting DNMT1, thereby promoting inflammatory gene expression[63,64]. In OA, delivery of miR-29b-5p promotes recruitment of synovial stem cells and their differentiation into chondrocytes, improving cartilage repair[65].

Cellular senescence promotes the expression of transposable elements such as long interspersed nuclear element (LINE-1). Because of the resemblance of transposable elements to viral RNA, they trigger innate pathways and promote expression of type 1 interferons, which contribute to inflammageing[66]. In several rheumatic diseases, including SLE and pSS, LINE-1 is hypomethylated and its expression is higher than in patients without autoimmune disease[67]. Furthermore, LINE-1 activity contributes to genomic instability through its multiplication and integration at other genomic loci[68]. The expression patterns and activity of transposable elements represent important age-related mechanisms that have been extensively reviewed elsewhere[69].

Epigenetic alterations that overlap with those observed during ageing are frequently observed in rheumatic diseases. The causal impact of these modifications in disease development remains to be addressed, but available data underline the potential implications of epigenetic modifications in the clinical manifestations of rheumatic diseases. Moreover, these data indicate that epigenetic modifications may be disease specific, thereby explaining some of the differences in immunopathology that are observed in rheumatic diseases.

Telomere attrition. Telomeres are regions of repetitive DNA sequence at chromosome termini that preserve the integrity and stability of the genome. As such, their attrition ultimately leads to replicative cellular senescence and chromosomal instability, which are two of the main hallmarks of ageing. Telomere shortening is part of the ageing process, both in humans and in mice[70]. In fact, in animal models, telomere shortening is associated with lifespan reduction[71–73]. Telomere shortening can be accelerated by a deficiency in telomerase, the enzyme responsible for the maintenance of telomere length.

Telomere shortening and telomerase deficiency occur in several rheumatic diseases. In RA, available data are conflicting, with most results indicating telomere shortening[74–76], whereas some have identified longer telomeres in RA leukocytes than in healthy controls[77]. In addition, peripheral blood lymphocytes and synovial infiltrating lymphocytes from patients with RA exhibit high levels of telomerase activity, whereas this enzyme activity is absent in RA synoviocytes[78]. By contrast, telomerase activity in peripheral blood lymphocytes, synovial lymphocytes and synoviocytes of patients with OA is lower than in unaffected individuals[78]. In patients with SLE, telomere length in whole-blood cells is less than in healthy controls, and is associated with anti-Ro antibodies (anti-nuclear autoantibodies that are associated with SLE)[79,80]. Similar to RA, contrasting results exist for telomere length in peripheral blood lymphocytes from patients with SSc[81–83]. Cross-comparison of patients with rheumatic diseases indicates that telomerase activity is higher in SLE and RA than in SSc, suggesting that SSc is associated with telomere attrition[84]. Notably, telomere length in patients with fibromyalgia tends to be shorter than in healthy individuals, and is associated with pain[85].

Accurate measurement of telomere length is difficult to achieve[86]. Opposing reports of telomere lengths in rheumatic disease may result from variations in the healthy tissues used for comparison, in detection methods and in statistical analysis[87]. Unsurprisingly, telomere shortening marks several rheumatic diseases as a consequence of sustained lymphocyte activation and proliferation. Further research is required, to unequivocally identify telomere alterations in rheumatic diseases, and to dissect their functional effects on inflammation, pain and fragility.

Loss of proteostasis. Protein homeostasis (proteostasis) is maintained through a fine balance of protein synthesis, conformational stability and degradation[88]. Ageing disrupts cellular proteostasis, and it is also altered in several rheumatic diseases. Alterations in the autophagic–lysosomal and ubiquitin–proteasomal degradation pathways are the best-studied elements of proteostasis during ageing. Age-related decline of autophagy in OA chondrocytes promotes cartilage loss through secretion of extracellular matrix-degrading enzymes such as matrix metalloproteinase 13 (MMP13), as well as through upregulation of apoptosis[89,90]. Similarly, SSc-derived fibroblasts demonstrate reduction of autophagic flux, which is associated with elevation of cellular senescence[91]. Correspondingly, expression of mammalian target of rapamycin (mTOR) complex 1 (mTORC1), an inhibitor of autophagy, increases with age in human OA chondrocytes[92]. Mice with deletion of the gene encoding mTOR are protected from cartilage loss[92]. However, in diseases such as SLE and RA, in which the pathological conditions involve autoantigen production, autophagic flux is elevated in B cells and fibroblast-like synoviocytes (FLS), respectively, thereby supporting cell survival and immunogenicity[93,94]. Together, these studies suggest a causal link between rheumatic disease and dysregulation of proteostasis.

Age-related decline in the proteasomal activity in OA chondrocytes reduces expression of extracellular matrix molecules (such as aggrecan) and promotes expression of MMP13 and inflammatory cytokines[95,96]. Proteasomal function is indispensable for healthy ageing, because upregulation of autophagy cannot compensate for ageing-related loss of proteasomal function, which disrupts chondrogenic protein expression and enhances OA-mediated cartilage degradation[95]. In addition to protein degradation, age also influences protein folding, which is mediated through chaperone proteins. Reduction of expression of several chaperones occurs in chondrocytes with age, resulting in endoplasmic reticulum stress and apoptosis[97]. However, further study is necessary to facilitate understanding of the role of the unfolded protein response during ageing, and its influence on rheumatic disease. Although protein synthesis decreases with age, leading to reduction of collagen deposition in cartilage[98], it remains unclear whether these changes contribute meaningfully to the age-related manifestations of rheumatic disease.

Cellular dysfunction during ageing

Mitochondrial dysfunction. Because of the pivotal role of mitochondria in cell homeostasis, their dysfunction has a profound effect on the ageing process, and is known to accelerate ageing in a number of

species[2,99,100]. During ageing, mitochondrial dysfunction manifests in several molecular and cellular alterations; namely, the destabilization of the respiratory chain, elevation of the mtDNA mutation burden, reduction of mitochondrial biogenesis, alteration of mitochondrial dynamics, dysregulation of mitophagy (mitochondrial turnover) and elevation of the production of reactive oxygen species (ROS)[100–102].

Many of the characteristics of age-related mitochondrial dysfunction also occur in rheumatic diseases. For instance, disruption of mitochondrial homeostasis stimulates cellular oxidative stress, accumulation of damaged proteins and DNA, deregulation of cell death and the overactivation of immune cell functions that characterize several rheumatic disorders[103–108]. Interestingly, a mutation affecting mitochondrially encoded NADH dehydrogenase 1 (MT-ND1), a subunit of complex I of the electron-transport chain, results in generation of non-self MHC peptide epitopes, which are recognized by the immune system and which exacerbate synovial inflammation in RA[109].

The accumulation of mtDNA damage and reduction of mtDNA repair capacities are well documented in rheumatic diseases such as fibromyalgia[110], SSc[111] and OA[112], and correlate with disease severity. Characteristically, prevalence of the 4977-bp common deletion of mtDNA (which often occurs in aged individuals) in knee cartilage is associated with idiopathic OA[113]. Functionally, mitochondrial dysfunction prompts exacerbation of the inflammatory and oxidative response through the production of inflammatory mediators and ROS in both in vitro and in vivo models of rheumatic disease[107]. Age-related decline in autophagy has wide-ranging effects on the clearance of defective mitochondria. Malfunctioning mitochondria promote mitophagy[114], and reduction of levels of the key mitophagy initiating protein phosphatase and tensin homologue-induced putative kinase 1 or mitochondrial deacetylase sirtuin 3 is associated with organ fibrosis in SSc[115,116]. PBMCs from patients with fibromyalgia have greater numbers of dysfunctional mitochondria than those of unaffected individuals, and consequently induce mitophagy to maintain cellular health[110,117]. In addition, the combination of mitochondrial damage with impairment of mitophagy may contribute to the development of pulmonary and organ fibrosis[115,116].

Available evidence suggests that dysfunctional mitochondria are prevalent in most rheumatic diseases, thereby associating this key hallmark of ageing with pathogenesis. The precise pathogenic role of mitophagy requires further investigation, to determine whether a reduction in mitophagy can promote rheumatic disease pathology.

Deregulated nutrient sensing. Integration of systemic and cellular nutrient availability dictates cell activities such as metabolism, cellular functionality and proliferation. Nutrient-sensing pathways have critical roles in ageing, as demonstrated by the beneficial effects of caloric restriction in extending lifespan[118]. Critical nutrient pathways affecting ageing are the insulin and IGF-1 signalling, mTOR and sirtuin 1 (SIRT1) pathways, which sense and control cellular glucose, amino acid and NAD^+ status, respectively.

Loss of mTORC1 activity supports longevity[119,120]. However, in rheumatic diseases, mTORC1 signalling is often greater than in individuals without rheumatic disease[121–128]. For example, mTOR enhances the invasive properties of synovial fibroblasts in RA, and rapamycin treatment inhibits synovial fibroblast invasion and therefore reduces FLS-mediated articular damage[121]. In patients with spondyloarthritis (SpA), mTOR mediates production of IL-17A and TNF by PBMCs[124]. In addition, rapamycin inhibits the development and severity of spondylitis in a transgenic rat model of SpA by reducing articular bone erosions and suppressing IL-17A expression[124]. In OA chondrocytes and RA-derived osteoclasts, mTOR signalling is an important contributor to cartilage and bone erosion[92,129–131].

In addition to mTOR signalling, sensing of nutrient scarcity (via cellular NAD^+ accumulation) by SIRT1 promotes longevity and reduces inflammation. Curiously, SIRT1 levels decline in human aged and OA chondrocytes, contributing to disease progression[132–134]. In RA, PBMCs have lower activity and expression of SIRT1 than in healthy individuals[135], which may promote myeloid-driven pro-inflammatory phenotypes in RA[136,137]. By contrast, elevation and reduction of expression of SIRT1 in RA FLS have both been reported[138,139]. Systemic loss of *Sirt1* expression in mice leads to autoimmune and lupus-like disease, whereas administration of the SIRT1 activator resveratrol improves inflammation in a pristane-induced model of SLE[140,141], suggesting a causal link between the SIRT1 pathway and SLE development.

Deregulation of insulin and IGF-1 signalling pathways is commonly found during ageing, leading to a high prevalence of type 2 diabetes mellitus, which is associated with many rheumatic diseases, including OA[142], RA[143,144], SLE[145] and SpA[146]. Although the nature of this interaction remains unclear, it is likely to be bi-directional, with chronic inflammation contributing to the development of type 2 diabetes mellitus, and diabetes worsening rheumatic disease outcome, as exemplified by the enhanced inflammation associated with experimental OA in a diabetic-mouse model compared with non-diabetic controls[147].

Changes in systemic and cellular nutrient sensing are widely implicated in rheumatic disease, preventing longevity and extension of healthspan. It remains unclear whether nutrient provision locally changes with age and contributes to deregulation of nutrient-sensing pathways[148], raising questions as to whether select nutritional changes could suppress the outcome of rheumatic diseases.

Cellular senescence. Malfunctioning and damaged cells or cells with shortened telomeres enter the state of cellular senescence, which is defined as irreversible cell-cycle arrest accompanied by elevation of metabolic activity and a secretory profile known as the senescence-associated secretory phenotype (SASP). Senescent cells are identified on the basis of a variety of cellular markers including, but not limited to, upregulation of expression of the cyclin-dependent kinase inhibitors $p16^{INK4a}$ and $p21^{Cip1}$, β-galactosidase activity and SASP (such as production of IL6, TNF and MMPs). Cellular senescence has beneficial and detrimental effects, and is central to multiple physiological and pathological processes[149]. Although cellular senescence prevents the proliferation of damaged cells, thus protecting from cancer, clearance of senescent cells in an efficient and timely manner is essential for the resolution of inflammation and for tissue homeostasis. Defective clearance of senescent cells results in their accumulation, thereby sustaining SASP production and promoting chronic low-grade inflammation, in a phenotype known as 'inflammageing'. During ageing, accumulation of senescent cells is observed in several tissues and occurs in both immune and stromal cells. In contrast to stromal-cell senescence, senescence in immune cells (often referred to as 'immunosenescence') is predominantly characterized by the failure of leukocytes to respond adequately to infection, malignancy and vaccination. Immunosenescence further promotes hyperinflammation, thereby contributing to age-related tissue malfunction.

Immunosenescence occurs in several rheumatic diseases. In RA, SLE, pSS and SpA, T cells undergo premature ageing marked by telomere attrition, p16 expression and downregulation of surface expression of the co-stimulatory molecule CD28 (refs.[75,150–153]).

In fact, senescent $CD4^+CD28^-$ T cells promote bone destruction by bolstering osteoclastogenesis through elevation of RANKL expression[154]. Furthermore, their interaction with fractalkine-expressing synovial fibroblasts promotes degranulation and IFNγ production, contributing to synovial inflammation[155]. Accumulation of senescent $CD28^-FOXP3^+$ regulatory T (T_{reg}) cells impairs the control of T cell proliferation in patients with RA, thereby further exacerbating inflammation[156]. Senescence in myeloid cells also contributes to the inflammatory environment in rheumatic diseases. Neutrophils, monocytes, macrophages and dendritic cells become increasingly dysfunctional with age, with typical hallmarks such as reduction of phagocytic capacity and aberrant cytokine production. In addition, aged macrophages exhibit reduction of autophagic flux, and autophagy deficiency mimics several age-related features of aged macrophages, including reduction of phagocytosis and enhancement of the inflammatory cytokine response[157]. With increasing age, non-classic monocytes increase in number and exhibit telomere shortening, reduction of proliferative potential and elevation of SASP production[158]. Although myeloid cells contribute to rheumatic diseases (such as RA and SLE) through chronic activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), excessive cytokine production and MMP expression[159], direct evidence of the role of senescent innate cells in rheumatic disease pathogenesis is still lacking. It is possible that innate-cell dysfunction is secondary to SASP expression in other surrounding cells (such as chondrocytes), rather than being a prerequisite for their pathogenesis. Nonetheless, it is almost certain that age-associated low-grade inflammation contributes to rheumatic disease progression and severity.

Beyond the immune system, senescent mesenchymal cells have been implicated in the development of OA. Transfer of senescent fibroblasts exacerbates murine models of OA, and induction of post-traumatic OA is reversed by depletion of senescent cells[160,161]. Accumulation of senescent, p16-expressing fibroblasts and macrophages with inflammatory phenotypes occurs in young patients with RA[162]. However, this effect may represent a beneficial adaptation, because in adenoviral transfer studies, overexpression of p16 or p21 ameliorates disease pathology in rodent models of RA[163,164]. Notably, p16 expression in senescent macrophages suppresses IL-6 production[165]. In patients with SLE, bone-marrow-derived mesenchymal stem cells (MSCs) are senescent and inhibit T_{reg} cell differentiation ex vivo in a p16-dependent manner, which could explain the low numbers of circulating T_{reg} cells that are observed in SLE[166,167]. Results indicate that fibroblasts from patients with SSc exhibit oxidative-stress-induced senescent phenotypes, but their involvement in disease progression is not yet known[91,105].

The functional role of senescence phenotypes in rheumatic disease pathology should become a more central focus of this research area. Separating beneficial and maladaptive changes could be decisive in the use of therapeutics to target cellular senescence.

Tissue homeostasis during ageing

Stem cell exhaustion. Maintenance of tissue homeostasis requires constant replenishment of dysfunctional cells through progenitor and stem cell populations. Decline of the regenerative potential is a common feature of ageing tissues[2]. MSCs are multipotent progenitors that reconstitute cells of the connective-tissue compartment, and are therefore crucial to bone and joint maintenance and recovery. MSCs isolated from patients with OA have lower proliferative capacity and impaired differentiation potential compared with MSCs from unaffected individuals[168,169]. In mice with deletion of the gene encoding fibroblast growth factor 2 (a potent activator of stem cell proliferation), spontaneous and surgically induced OA development is accelerated[170]. MSC dysfunction in RA and SLE has been reported and correlates with reduction of immunoregulation and elevation of senescence[171,172].

In contrast to MSCs, exhaustion of haematopoietic stem cells (HSCs) during ageing is well documented, and may be involved in the development and progression of rheumatic diseases. Ageing is associated with elevation of the proportion of HSCs among $CD34^+$ cells, with HSCs from older individuals showing several markers of cellular dysfunction, including a higher burden of somatic mutations, epigenetic alterations and proliferative stress, relative to HSCs from younger donors[173,174]. A key influence on HSC expansion is the effect of somatic mutations in genes such as *DNMT3A* and *TET2*, which encode epigenetic regulators. These mutations control genes involved in HSC self-renewal at the expense of differentiation[175]. Consequently, *Dnmt3a* mutations confer HSC fitness, promoting their clonal expansion. Takeover of the bone marrow by myeloid-biased HSCs is also associated with clonal HSC expansion and the aged bone-marrow niche[176]. Clonally expanded HSC populations can have a wide-ranging influence on inflammatory processes in rheumatic diseases (as reviewed elsewhere[177]). For example, loss-of-function mutations in *Dnmt3a* and *Tet2* facilitate epigenetic accessibility and expression from inflammatory cytokine loci such as *Il1b*[178,179]. Mutations in *DNMT3A* and *TET2* are readily detectable in peripheral blood cells of patients with RA, although their involvement in disease pathology is not yet known[180]. Total $CD34^+$ haematopoietic stem and progenitor cell (HSPC) numbers are lower in both the circulation and the bone marrow of patients with RA than in those of healthy individuals, which is attributed to their reduced proliferative potential in RA[181–183]. Additionally, HSPCs in the RA bone marrow are more apoptotic, likely because of excessive production of TNF by the bone-marrow stroma, which can be improved by anti-TNF therapy[181]. Similarly, compared with healthy individuals, patients with SLE exhibit greater $CD34^+$ HSPC TNF expression and apoptosis, but a link between these factors has not yet been demonstrated[184,185]. Nevertheless, results with the collagen-induced arthritis model in mice demonstrate that the pro-inflammatory environment can contribute to the myeloproliferative and anaemic phenotype observed in RA, which can be partially rescued by anti-TNF therapy[186]. Indeed, high TNF expression is associated with the development of anaemia in patients with RA or psoriatic arthritis[187,188]. Although anaemia occurs in several rheumatic diseases, the mechanism by which TNF exerts its effects (whether directly or indirectly) requires further investigation. Anti-TNF therapy can reduce levels of hepcidin, the key transcription factor of iron homeostasis, possibly as the result of an indirect effect of reduction of overall inflammation, including expression of IL-6, a well-known inducer of hepcidin. Strikingly, high concentrations of hepcidin are also observed in patients with RA, and they can induce cellular iron sequestration and further promote anaemia in chronic inflammation[189].

The links between stem cell exhaustion and rheumatic disease have only recently been explored, and currently available evidence is mostly correlative. However, initial results suggest that stem cell exhaustion does have a role in rheumatic disease, although identifying the precise contribution (which may be secondary to chronic inflammation) will require further investigation. Notably, the identification of mutation in the *UBA1* gene in HSCs as the cause for the vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic (VEXAS) syndrome, a late-onset autoimmune disorder, demonstrates the power of clonal haematopoiesis in the pathogenesis of rheumatic diseases[190].

Altered intercellular communication. Well-balanced intercellular communication is imperative for the maintenance of tissue homeostasis. Thus, aberrant release of soluble mediators such as cytokines, chemokines, extracellular vesicles and metabolites can promote tissue damage.

Senescent cells have high SASP expression, which has important implications for surrounding cell types and which feeds into a self-perpetuating inflammatory cycle[191]. In health, SASP recruits immune cells to aid tissue repair and the removal of senescent cells. However, during ageing, senescent cells are ineffectively cleared, thereby exacerbating the inflammation. This sustained SASP eventually subverts the physiological resolution processes and aggravates the disease[192]. Although SASP has been described in key cell types in OA (chondrocytes[193] and synovial fibroblasts[162]), RA (fibroblasts[162]), SLE (bone-marrow MSCs[194]) and SSc[105], its effect on intercellular crosstalk requires further exploration.

Extracellular vesicles have emerged as important mediators of communication between different cell types and tissues. Age shifts the abundance and cargo of extracellular vesicles[195,196]. A proteomic analysis comparing SASP and extracellular vesicle contents showed little overlap, demonstrating the presence of two distinct communication pathways used by senescent cells to influence the local tissue environment[197]. Most extracellular vesicles from senescent cells were enriched in membrane proteins, which might still be functional once taken up by recipient cells, thereby altering their cellular identity[197]. For example, senescent-cell-derived extracellular vesicles act in a paracrine manner through transmission of interferon-induced transmembrane protein 3 to neighbouring cells, thereby inducing senescence and SASP[198]. In addition to proteins, extracellular vesicles are often enriched in small non-coding RNAs or lipids. The extracellular-vesicle-mediated transfer of miR-217 and miR-21-5p from senescent cells to recipient cells can regulate *SIRT1* and *DNMT1* expression and thereby reduce cell proliferation and increase cellular senescence and SASP[199]. Few studies have investigated the lipid contents of extracellular vesicles and their effects, but results from one study indicated that extracellular vesicles enriched in the C24:1 ceramide increase in the circulation with age, which can trigger senescence in bone-marrow MSCs[200]. Age-related disruption of MSC-derived extracellular vesicles results in a loss of their regenerative potential and promotes senescent phenotypes in recipient cells[201]. Similarly, incubation with circulating extracellular vesicles from patients with SLE promotes a senescent phenotype in bone-marrow-derived MSCs through sustained NF-κB activation[202]. Results have also implicated extracellular vesicles in other rheumatic diseases, including RA, SLE and SSc[203–208]. Moreover, transfer of healthy-MSC-derived extracellular vesicles (as well as engineered extracellular vesicles) can improve pathology and suppress inflammation in murine models of OA, RA and SSc, supporting its therapeutic potential[209–212]. Although extracellular vesicles clearly seem to be involved in rheumatic-disease pathogenesis, whether their cargos and functions are the same as in ageing or may simply resemble changes observed in ageing will require further investigation. Nonetheless, inflammatory-cell signalling, in conjunction with aberrant extracellular-vesicle communication, is considered to be an important contributor to rheumatic disease.

Altered microbiota: an emerging hallmark

Ageing leads to alteration of microbial composition, with reduction of its diversity. Recolonization studies in model organisms point to an important role of the microbiota in control of the ageing process through regulation of gut permeability and the production of microbial metabolites[213,214]. In mechanistic studies, the transplantation of faecal content from young mice into aged mice leads to an increase in production of bile acids and polyamines, which are contributors to healthy ageing[215]. The faecal matter of aged mice is enriched with a distinct subset of microbes that includes *Prevotella* species, which metabolize choline to produce trimethylamine[215]. In the liver, trimethylamine is further oxidized to trimethylamine-*N*-oxide, which has pro-inflammatory and atherosclerotic effects, and as such may contribute to inflammageing and the development of comorbidities[216], via NF-κB-dependent elevation of expression of IL-6 and TNF and production of NLR family pyrin domain containing 3-inflammasome-dependent IL-1β[217,218]. Interestingly, centenarians have a distinct metabolic profile with a lower abundance of *Prevotella* species than in younger individuals, suggesting a role for the microbiome in the ageing process[219]. Notably, *Prevotella* species are enriched in individuals with preclinical RA. Indeed, administration of *Prevotella copri* to germ-free mice exacerbates arthritis[219]. However, despite the experimental evidence from studies of faecal-matter transplantation, the causal relationship between microbiota and ageing remains incompletely understood. Furthermore, clear evidence that trimethylamine and trimethylamine-*N*-oxide can directly contribute to rheumatic disease is still lacking. Because inflammation can equally provoke microbial changes, the nature of this interaction is likely reciprocal[220].

Organismal effects of ageing

Expression of the hallmarks of ageing is the product of the antagonistic relationship between the exposome and an individual's resilience. This concept holds up on a biological and socio-behavioural level[221]. Cognitive–behavioural changes (such as pain avoidance) that patients with rheumatic disease can undergo, as well as social-resilience factors (such as support in daily life), are good predictors of long-term physical and psychological well-being[221]. Understanding biological and social resilience mechanisms should reveal new methods of improving healthspan in rheumatic diseases and preventing disability.

When resilience mechanisms can no longer keep up with sustained cellular damage, its accumulation will result in the loss of physiological integrity, impairment of tissue function and occurrence of comorbidity and mortality. Frailty, which manifests as weakness, weight loss, reduction of muscle mass and strength (sarcopenia), exhaustion, low physical activity and reduction of walking speed, contributes greatly to development of disability[222,223]. In addition, comorbidities such as cardiovascular disease are risk factors for frailty[224,225]. Together, frailty and comorbidities contribute to mortality[222,223,226,227]. The term frailty was initially used synonymously with ageing itself, but ageing and frailty may actually represent two distinct concepts, as advanced age does not necessarily equate with vulnerability, and frailty can manifest in populations with specific diseases or chronic conditions, but is not associated with age per se[228]. Nonetheless, frailty is highly prevalent in old age, and it may be considered as an ageing hallmark at the organismal level[223,229]. Equally, the prevalence of frailty ranges from 24% to 60% in patients with OA, and is estimated as 16% in patients with RA[230–234], indicating higher prevalence than the 8% observed in healthy, age-matched individuals, and the occurrence of frailty can correlate with disease activity[233,235,236]. Similarly, the diseasome of ageing (consisting of cardiovascular disease, type 2 diabetes mellitus, neoplastic conditions, cancer and hypertension) is prevalent in individuals with rheumatic disease, as reviewed elsewhere[237,238].

A rheumatic disease ageing phenotype

The evidence presented in this Review indicates that ageing and rheumatic diseases share common molecular and cellular pathways (Table 1). These commonalities manifest in a considerable number of ageing-related clinical features (the 'ageing phenotype'), which could lead to the consideration of rheumatic diseases as a class of premature or accelerated ageing diseases. On the basis of their ageing phenotypes, we can classify rheumatic diseases into two categories. The first category encompasses acute and chronic inflammation, marked by a systemic (or stromal–immune) ageing phenotype. This category is characterized by hyperinflammation and accelerated proliferation of immune cells as well as the microenvironment of these immune cells in the affected organs, and includes rheumatic diseases such as RA, SLE, pSS and SSc. The second category comprises a local (or stromal) ageing phenotype. Pathological conditions in this category are less associated with systemic inflammation, and they instead exhibit low-level chronic inflammation and senescence in the resident stromal cells of the affected organs. This category includes OA and fibromyalgia[239]. The discrimination of rheumatic diseases on the basis of their ageing phenotypes can enable the identification of targeted intervention strategies focussing on the central pathways of these phenotypes. In addition, it can enable further elaboration of the mechanical relationships between ageing and rheumatic diseases, to facilitate the development of new therapeutics.

Overall, it seems likely that there is a reciprocal relationship between the inflammatory processes observed in patients with rheumatic diseases and their ageing phenotypes. Accordingly, we propose that disease-specific inflammation enhances premature-ageing phenotypes, and these imprinted alterations in turn exacerbate inflammation and promote disease progression. Currently available data suggest that there is a central, self-perpetuating vicious cycle of ageing and inflammation (Fig. 2). The inflammatory milieu in rheumatic diseases is marked by ROS production, which alters the expression of proteins involved in DNA repair and results directly and indirectly in DNA damage, including telomere erosion, replication errors and mutations in cellular DNA and mtDNA. This damage can further promote mitochondrial impairment, resulting in oxidative stress and, subsequently, damaged protein and DNA, telomere attrition and deregulation of programmed cell death. Eventually, these damaged cells enter a senescent state with concomitant SASP accelerating inflammation. The sustained local and systemic inflammation depletes stem cell pools and results in the loss of their regenerative capacity, preventing adequate repair and leading to degenerative disease. In parallel, damaged cells can survive and replicate, promoting the overactivation of immune cells that characterizes many rheumatic disorders. These processes will be enhanced by epigenetic modifications, such as histone modification, DNA methylation and miRNA dysregulation, leading to a pathogenic transcriptional profile that is linked to the aggressive phenotypes of these diseases. Release of disease-promoting factors (such as MMPs), SASP-associated cytokines (such as TNF and IL-6) and alteration of intercellular mediators (such as extracellular vesicles) widens the cellular involvement in the inflammatory process and may eventually even lead to disease manifestations in other tissues, and the development of comorbidities. Although rheumatic diseases all have complex interactions between different hallmarks of ageing, the disease-specific relationships require further analysis to enable identification of new therapeutic avenues to break the vicious cycle of inflammation and ageing in rheumatic diseases.

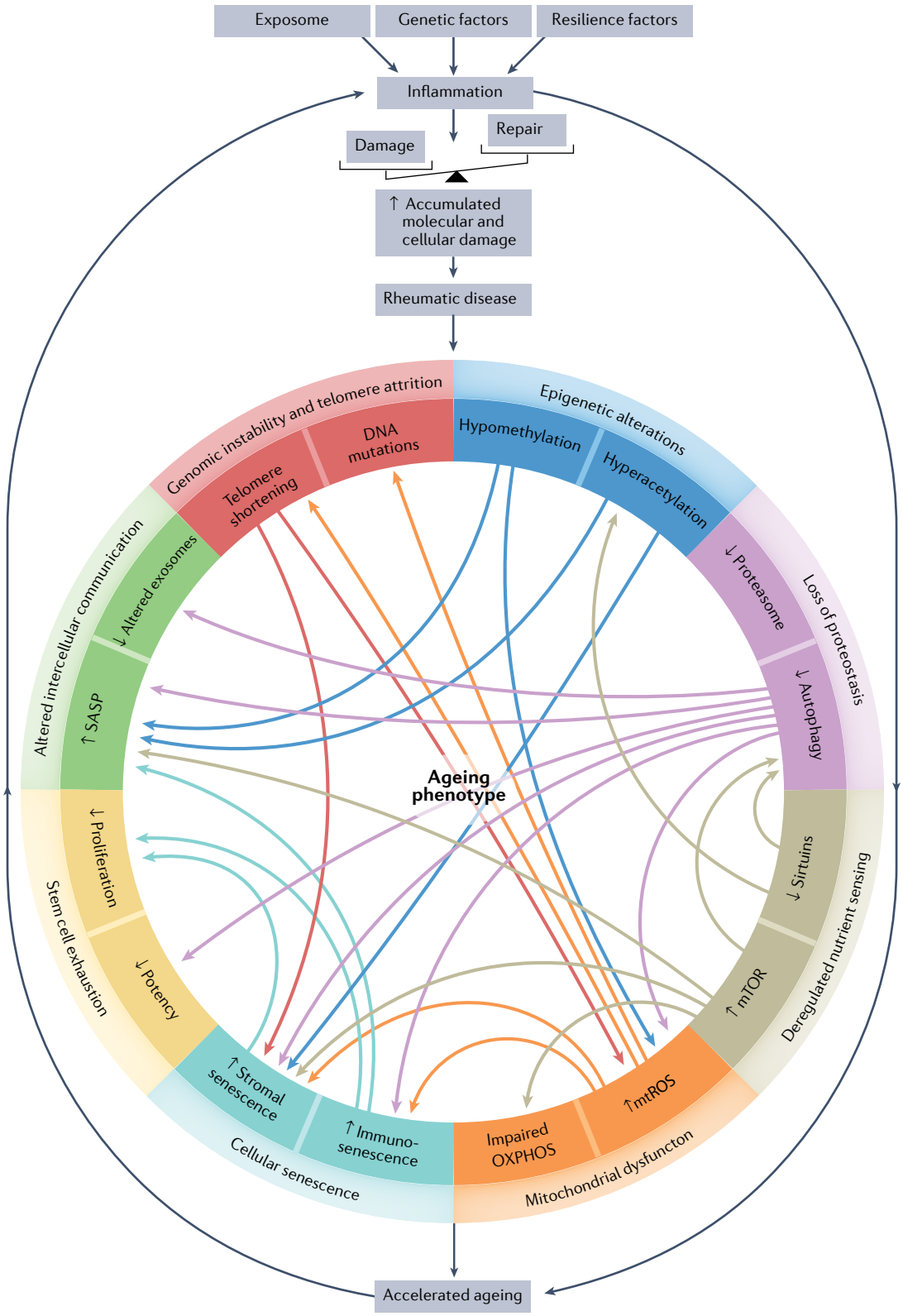
DMARD effects on ageing phenotypes

In this Review, we have proposed that rheumatic diseases can be considered to be diseases of premature or accelerated ageing. Currently, DMARDs represent the first-line therapies for several rheumatic diseases. However, the safety profiles of DMARDs include several adverse effects, such as fatigue and frailty, which may reinforce ageing phenotypes[240,241].

Table 1 | Overview of age-related hallmarks in rheumatic disease

Conditions	Cell types	Genomic instability and/or telomere attrition	Epigenetic alterations	Mitochondrial dysfunction	Loss of proteostasis	Deregulated nutrient sensing	Cellular senescence	Stem cell exhaustion	Altered intercellular communication
Rheumatoid arthritis	Immune cells	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
	Synovial fibroblasts	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
	MSCs and HSCs	No	No	No	No	No	No	Yes	No
Systemic lupus erythematosus	Immune cells	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
	MSCs and HSCs	No	No	No	No	No	No	Yes	No
Systemic sclerosis	Immune cells	Yes	Yes	Yes	No	No	No	No	Yes
	Fibroblasts	No	Yes	No	Yes	Yes	Yes	No	No
Primary Sjögren syndrome	Immune cells	Yes	Yes	No	No	No	Yes	No	Yes
	Salivary gland epithelial cells	No	Yes	No	No	No	No	No	Yes
Osteoarthritis	Immune cells	No	No	No	No	No	Yes	No	No
	Chondrocytes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
	Synovial fibroblasts	No	No	No	Yes	No	No	No	Yes
	MSCs	No	No	No	No	No	No	Yes	No

HSCs, haematopoietic stem cells; MSCs, mesenchymal stem cells.



The question of whether DMARD treatment has an anti-ageing or pro-ageing effect in rheumatic diseases remains largely unanswered. Most currently available data are derived from patients with RA, and demonstrate reversibility of premature ageing of HSCs and immune cells upon DMARD treatment[181,187,242–244]. Furthermore, DMARD therapy reduces oxidative stress, DNA damage and mitochondrial dysfunction

Fig. 2 | Vicious cycle of premature ageing and inflammation in rheumatic diseases. The exposome (including mechanical stress, smoking and other lifestyle factors), resilience factors and genetic predisposition promote and sustain inflammatory processes. Although short-term inflammation can be resolved, chronicity of inflammation associated with rheumatic disease will result in the accumulation of molecular and cellular damage, triggering ageing phenotypes, which interact, and which can promote each other. Clinical establishment of the ageing phenotype results in the overall acceleration of systemic ageing processes and, in turn, promotes and sustains inflammation. mTOR, mammalian target of rapamycin; mtROS, mitochondrial reactive oxygen species; OXPHOS, oxidative phosphorylation; SASP, senescence-associated secretory phenotype.

in patients with RA[245–248]. Although data are still limited with respect to DMARD effects on the ageing phenotype, initial results in patients with RA demonstrate that reduction of inflammation can indeed modulate the ageing phenotype (Table 2).

The effects of DMARDs on ageing phenotypes are predominantly studied ex vivo in immune cells or cell lines, in attempts to mimic in vivo processes. Conclusive studies would require biopsy-derived samples from patients before and after DMARD treatment, which are understandably hard to obtain because of the invasiveness of these procedures. However, recent technological advances, such as microsurgery and single-cell 'omics' analyses, can provide the surgical and analytical tools needed to make use of limited amounts of tissue. Enabling the investigation of DMARD effects in prospective patient cohorts using paired analyses and access to tissue from affected sites will represent a major step forward to answering the question of whether DMARDs affect the ageing phenotype.

Anti-ageing-drug effects on rheumatic disease

Most current therapies for chronic diseases treat symptoms but fail to address underlying causes, partly because much of the molecular and cellular damage has already occurred by the time of diagnosis. Ageing is one of the main risk factors for many chronic illnesses. Interventions that extend lifespan can prevent or delay the onset of chronic disease, which has given rise to the field of geroscience and its aims to identify age-related pathways leading to chronic disease and to develop new therapeutics to target these pathways. In contrast to an individualist, disease-based approach, early targeting of central pathways that cause the diseasome of ageing and its comorbidities has the potential to prevent age-related frailty, to extend healthspan and to decrease the economic health burden resulting from an ageing population. As such, the presence of age-associated features in stromal and immune cells in rheumatic diseases suggests the potential to target them using classic anti-ageing drugs.

As outlined above, several ageing-associated molecular pathways have been identified as potential drug targets, including aberrant mTOR signalling, autophagic–lysosomal dysfunction, sirtuin-dependent epigenomic regulation, impaired clearance of senescent cells and unbalanced metabolism. Many drugs targeting these pathways have undergone or are currently undergoing clinical trials (Table 3).

Although ageing research tends to focus on the identification of compounds that increase lifespan and healthspan, the use of such compounds to treat rheumatic diseases in a human setting has only just begun. However, these substances are predicted to have great beneficial potential for the treatment of rheumatic diseases because of the shared age-related pathways. Future research will certainly provide further insights into the discovery of new anti-ageing interventions and their applicability to rheumatic diseases.

Nutrient-sensing and metabolic targets

Rapamycin. The mTOR inhibitor rapamycin (sirolimus) and its derivatives (such as everolimus) have been tested against a plethora of rheumatic diseases, with varying clinical efficacy. In RA, the addition of everolimus to conventional methotrexate therapy improved response rates[249]. Similarly, sirolimus treatment reduced SLE disease activity index scores relative to pre-treatment scores, and resulted in an expansion of circulating T_{reg} cells[250]. The expansion of T_{reg} cells is

Table 2 | DMARDs and their effects on the hallmarks of ageing in rheumatic disease

Hallmarks of ageing	Diseases	Treatment	Effects	Refs.
Genomic instability	RA	Etanercept; infliximab; tocilizumab	Reduction of production of oxidative stress markers of DNA damage, lipid peroxidation and protein glycosylation	245–247
Epigenetic alterations	RA	Methotrexate	Reversal of DNA hypomethylation in peripheral blood mononuclear cells; restoration of regulatory T cell function through demethylation of *FOXP3* enhancer	270,271
	SLE	Tocilizumab	Downregulation of IL-6 in B cells by induction of DNA hypermethylation	272
Mitochondrial dysfunction	RA	Anti-TNF	Reduction of mitochondrial DNA mutation frequency	248
Loss of proteostasis	RA	Etanercept; adalimumab; tocilizumab	Reduction of autophagosome levels with disease remission	273
Cellular senescence	RA	Infliximab; abatacept; etanercept	Restoration of CD28 expression in $CD4^+$ cells; reduction of circulating $CD28^-$ T cells; reduction of circulating $CD14^{bright}/CD56^+$ monocytes	242–244,274
Stem cell exhaustion	RA	Infliximab	Reduction of apoptosis in $CD34^+$ cell fraction; elevation of cell growth potential and frequency of $CD34^+$ cells	181,187
Altered intercellular communication	RA	Tocilizumab; infliximab; tofacitinib; baricitinib	Inhibition of IL-6 signalling (tocilizumab), TNF signalling (infliximab); multiple cytokines (tofacitinib, baricitinib)	275–278
	SLE	Tocilizumab	Inhibition of IL-6 signalling	279,280
	SSc	Tocilizumab	Inhibition of IL-6 signalling	281

No effects of DMARDs on telomere attrition have yet been reported. RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

Table 3 | Effects of anti-ageing drugs on rheumatic diseases

Drug	Mode of action	Disease	Effects	Refs.
Rapamycin (sirolimus) and its derivates (including everolimus)	mTOR inhibitor	RA	Combination therapy with methotrexate showed a good safety profile and improved ACR20 response rate	249
		SLE	Reduction of SLEDAI on 12-month treatment with sirolimus, with concurrent elevation of regulatory T cell and $CD8^+$ memory T cell numbers and reduction of IL-4 and IL-17 production	250
		SSc	Rapamycin had a good safety profile, with improvement from baseline in patients with diffuse SSc in a pilot study	282
		Others	Sirolimus had limited effect in severe psoriasis, but may enable concomitant therapy with cyclosporin (reducing cyclosporin toxicity)	283
Metformin	Activator of AMP-activated protein kinase	RA	Improvement of quality of life, reduction of C-reactive protein concentrations, good tolerability	259
		OA	Reduction of progression of knee OA in obese patients	258
		SLE	Acceptable safety profile with no significant difference in SLE flares vs control; post hoc analysis of two trials indicated reduction of flares	284,285
Resveratrol	Interacts with many stress-related targets, including mammalian NAD^+-dependent deacetylase sirtuin 1	RA	Improvement of disease activity scores and reduction of C-reactive protein, TNF, matrix metalloproteinase 3 and IL-6 concentrations in patients with RA	286
		OA	Improvement in OA-associated pain with resveratrol as an adjuvant to the NSAID meloxicam in treatment of patients with mild-to-moderate knee OA	287
Dasatinib+quercetin	Dasatinib is a tyrosine-kinase inhibitor; quercetin is a PI3K and serpin inhibitor (senolytics)	SSc	Small increase in physical function in patients with idiopathic pulmonary fibrosis	288
UBX0101	Inhibits interaction between cellular tumour antigen p53 and E3 ubiquitin-protein ligase Mdm2 (senolytic)	OA	Reduction in OA-associated pain in phase I, but not phase II (NCT04129944)	289
Tocilizumab	Anti-IL-6 antibody (senomorphic)	RA	Improvement in RA symptoms and signs	290
		OA	No effect on hand OA	291
		SLE	Improvement in disease activity and reduction of numbers of circulating plasma cells, suggesting specific effect of IL-6 inhibition on autoantibody-producing B cells	279,280
		SSc	Softening of skin sclerosis observed in two patients with diffuse cutaneous SSc; preservation of lung function	281,292,293
		Other	Improvement of disease activity and reversal of growth retardation in systemic juvenile idiopathic arthritis	294
Clazakizumab	Anti-IL-6 antibody (senomorphic)	PsA	Improvement of arthritis, enthesitis and dactylitis, but not skin disease	295
PF-04236921	Anti-IL-6 antibody (senomorphic)	SLE	Some evidence of response, acceptable safety (10 mg every 8 weeks)	296

ACR20, ACR 20% improvement criteria; OA, osteoarthritis; PsA, psoriatic arthritis; pSS, primary Sjögren syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index; SSc, systemic sclerosis.

a well-documented feature of rapamycin-mediated immune effects, which shifts the T_H17–T_{reg} cell balance and enables the control of inflammatory processes[251–253]. This aspect of rapamycin activity may also ameliorate other T_H17-driven pathological conditions, such as pSS and SSc[254]. However, rapamycin is also immunosuppressive, making it unsuitable for lifespan extension in humans. Long-term rapamycin administration causes a number of adverse health effects in patients, including impaired wound healing, anaemia, proteinuria, pneumonitis and hypercholesterolaemia. Future studies into intermittent or low-dose rapamycin regimes in the elderly may enable exploitation of the beneficial effects of mTOR inhibition while avoiding unwanted immunosuppression and adverse effects.

Metformin. The AMP-activated protein kinase (AMPK) activator metformin promotes reduction of pro-inflammatory cytokine expression and T_H17 cell differentiation, putatively through AMPK-dependent repression of mTOR[255]. Furthermore, metformin exerts AMPK-independent anti-inflammatory and anti-oxidative effects via regulation of key transcription factors such as NF-κB and nuclear factor erythroid 2-related factor 2 (NRF2)[256]. Metformin-induced inhibition of insulin and IGF-1 signalling and the electron-transport chain further contribute to deceleration of the ageing process[256]. In two large-scale clinical studies, Targeting Ageing with Metformin[257] and the Metformin in Longevity Study (NCT02432287), the effects of metformin on healthy ageing and its comorbidities are being studied.

Metformin has shown chondroprotective effects in several preclinical models of OA, and its efficacy in patients with OA is currently under investigation (NCT05034029). Results from a prospective cohort study suggest that metformin provides protection against cartilage loss and knee-replacement surgery in obese patients with OA[258]. Safety trials of metformin in patients with RA have demonstrated a good safety profile associated with improvement of self-reported quality of life and reduction of systemic inflammation[259]. Results from an ongoing phase II clinical trial testing the effect of metformin in combination with the first-line DMARD methotrexate (NCT04196868) will provide valuable insights into the potential of this drug as an anti-rheumatic therapy.

Resveratrol. The antioxidant resveratrol mimics some metabolic effects of calorie restriction, and is able to protect against age-associated diseases in preclinical models[260]. Currently, a phase III randomized, controlled trial of its effects on knee OA is ongoing (NCT02905799).

Spermidine. A promising anti-ageing drug is spermidine, a naturally occurring polyamine with the potential to extend lifespan and healthspan via activation of autophagy[261–263]. Notably, spermidine can reverse T cell and B cell senescence in an autophagy-dependent manner, thereby conferring improvements in immune function and vaccine efficacy[264,265]. In the destabilized medial meniscus mouse model of OA, administration of spermidine ameliorates cartilage degradation by increasing chondrocyte autophagy and function[266].

Senolytics and senomorphics

Therapeutic approaches targeting the accumulation of senescent cells have emerged as alternatives for the prevention of rheumatoid disease progression. Currently, two classes of therapeutics are being developed: senolytics to induce apoptosis and removal of senescent cells and senomorphics to directly block SASP.

UBX0101. Local intra-articular injection of the senolytic drug UBX0101 (which inhibits the interaction between cellular tumour antigen p53 and E3 ubiquitin-protein ligase Mdm2) selectively clears senescent cells, limits proteoglycan loss and alleviates OA-related disease outcomes of pain and articular cartilage degradation in mice with post-traumatic OA[161]. UBX0101 is currently being investigated in several clinical trials for OA (NCT03513016, NCT04229225, NCT04349956 and NCT04129944).

Fisetin. The flavonoid senolytic fisetin activates sirtuins and inhibits IL-1β-induced inflammation in OA chondrocytes[267]. Fisetin and its family member quercetin can also activate NRF2, thereby promoting antioxidative and resilience pathways to reduce senescence[4]. Fisetin is currently being evaluated in clinical trials for efficacy in alleviation of OA symptoms by reduction of the senescence burden in cartilage (NCT04210986). In preclinical models, fisetin was able to inhibit production of inflammation-related cytokines and angiogenic factors in RA FLS, thereby considerably reducing incidence and severity in a collagen-induced arthritis model[268]. In addition, fisetin can effectively manage SLE by targeting chemokine (C-X-C motif) ligand (CXCL)1, CXCL2 and CXCL3 and chemokine receptor 2 signalling pathways and regulating T_H17 differentiation during development of lupus nephritis[269].

Anti-TNF and anti-IL-6. Senomorphic drugs such as monoclonal antibodies are effective in the treatment of rheumatic diseases. Notably, anti-TNF and anti-IL-6, two classes of biologic DMARDs, are already being used for treatment of various rheumatic diseases. Although these drugs are able to reduce inflammation, their effect on targeting senescence-induced inflammation directly or through indirect pathways remains to be explored.

Conclusions

Population ageing has become one of the most important social transformations of our times. Concomitantly, prolongation of lifespan has led to an increased number of people suffering from rheumatic diseases, and biological ageing processes clearly seem to be a key aspect to both the development and management of these diseases. Typically, rheumatic diseases, even when diagnosed at younger ages, continuously worsen with age, likely because of the cumulative effects of the exposome, cellular ageing and the loss of resilience, which are also implicated in the development of rheumatic disease-associated comorbidities.

As discussed in this Review, many rheumatic diseases share common ageing phenotypes, which enable their classification as premature or accelerated ageing diseases. The fact that rheumatic diseases can affect people ≤40 years old indicates that some individuals may be vulnerable at a young age, possibly as the result of premature ageing phenotypes that either exist prior to disease onset or are evoked by initial inflammatory stimuli. Long-term, prospective studies could enable assessment of characteristics of premature ageing that exist prior to the development of rheumatic diseases. However, currently available data suggest that inflammation and age-associated modifications in rheumatic disease perpetuate each other and thus add more fuel to the fire of rheumatic disease-associated inflammation (Fig. 2). In this regard, geroscience has provided us with several drugs that could be useful in treating rheumatic diseases in the future. Studying drugs that target age-related pathways in cohorts of patients with rheumatic disease (ideally early in the disease process) may facilitate better interventions, avoid disease-associated damage, maintain vitality and extend quality of life in these patients as they age.

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A comprehensive guide for managing the reproductive health of patients with vasculitis

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Abstract | Vasculitides and their therapies affect all areas of the reproductive life cycle. The ACR, EULAR and the Drugs and Lactation database offer guidance on the management of the reproductive health of patients with rheumatic diseases; however, these guidelines do not address patients with vasculitis specifically. This Review discusses the guidance from multiple expert panels and how these recommendations might apply to men and women with vasculitis, including the safety of contraception, use of assisted reproductive technology, preservation of fertility during cyclophosphamide therapy, disease management in pregnancy and the use of medications compatible with pregnancy and lactation. These discussions are augmented by the existing literature on vasculitis in pregnancy to enable physicians to provide comprehensive, precise and high quality care to patients with vasculitis. The contents of this Review, in conjunction with educational tools, serve to empower patients and physicians to participate in shared decision-making regarding pregnancy prevention, planning and management.

Vasculitides encompass a group of diseases defined by inflammation of the blood vessels that can clinically manifest as damage to single or multiple organ systems. The incidence of these diseases is 40 to 60 cases per 1 million persons[1]. Given the rarity of vasculitis, limited information is available to guide reproductive health care for women with these diseases. Despite the dearth of available data, women living with vasculitis still need to make decisions about contraception, pregnancy and lactation to live the full life they desire.

Specific forms of vasculitis cause distinct challenges in reproductive health care. Some types of vasculitis, including giant cell arteritis (GCA) and Kawasaki disease, do not typically occur in women of reproductive age, whereas others, such as the Takayasu arteritis and Behçet disease, are more common during this time period. IgA vasculitis is most commonly diagnosed during childhood but can affect women of reproductive age. Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is often diagnosed after the reproductive years, but can occur in younger women, and now that treatment has moved away from ovary-toxic cyclophosphamide, more women with vasculitis are able to conceive. Because each of these forms of vasculitis affects different blood vessels, their effects on pregnancy are very different (TABLE 1). The safety of contraceptives and medications, however, is relatively universal between these diseases.

The ACR[2], the American College of Obstetricians and Gynecologists (ACOG)[3], the European Board & College of Obstetrics and Gynaecology (EBCOG)[4] and EULAR[5] provide guidelines for managing the reproductive health care of patients with rheumatic diseases. However, none of the pre-existing recommendations specifically mentions vasculitis, and the most recent ACR vasculitis treatment guidelines do not mention reproductive health[6–8]. In this Review, we discuss how these guidelines apply to the specific medical needs of women with vasculitis to create a guide for women living with vasculitis and their providers as they make these challenging and life-changing decisions. Although various case series, observational studies and literature reviews of pregnancies in women with Takayasu arteritis, Behçet disease, IgA vasculitis and AAV are available, to fill in the gaps we have also extrapolated other available data and guidelines to these unique, rare populations. Men and women with vasculitis require reproductive management similar to patients with systemic lupus erythematosus (SLE) and anti-phospholipid (aPL) syndrome (APS) but additional considerations are needed for disease activity and pregnancy complications including structural vascular lesions (stenosis and aneurysms), renopulmonary syndrome, fetal loss and spontaneous abortion. Proactive pregnancy prevention and planning for women with vasculitis is important, and various resources to guide provider–patient discussions are available, including

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Key points

- Rheumatologists have the opportunity to initiate discussions with patients with vasculitis regarding family planning to make proactive decisions leading to improved pregnancy planning, management and outcomes.
- Birth control options and infertility interventions for women with vasculitis depend on their risk of thrombosis, serological profile and comorbid conditions.
- The majority of pregnancies in patients with vasculitis can be successful with the use of advanced family planning, medications compatible with pregnancy and lactation, and multidisciplinary collaboration among specialists.
- Vasculitis exacerbations and pregnancy complications can present with similar and overlapping clinical manifestations.
- Multiple expert panels provide guidelines and risk stratification regarding medication use in pregnancy and breastfeeding that can be applied to patients with vasculitis.

those from the Vasculitis Foundation (Supplementary Fig. 1). To improve our understanding of pregnancy in patients with vasculitis and to help inform and create data-driven approaches to clinical guidance and management, women with vasculitis can sign up to the Vasculitis Pregnancy Registry (VPREG).

Classification and epidemiology

Vasculitides are classified by the size of the inflamed blood vessels (small, medium and large). According to the 2012 revised Chapel Hill Consensus Conference[9], large vessel vasculitis (LVV) encompasses those vasculitides that affect the aorta and its major branches. The main types of LVV are Takayasu arteritis and GCA. Medium vessel vasculitides, such as polyarteritis nodosa (PAN) and Kawasaki disease, affect visceral arteries, veins and initial branches. The small vessel vasculitides, affecting the arterioles, capillaries, venules and veins, are further delineated into AAV (granulomatosis with polyangiitis (GPA), eosinophilic granulomatosis with polyangiitis (EGPA) and microscopic polyangiitis (MPA)) and immune complex vasculitides (that include IgA vasculitis, cryoglobulinaemic vasculitis and hypocomplementaemic urticaria vasculitis).

In 2022, the ACR and EULAR updated the classification criteria for AAV with weights assigned to specific clinical and serological characteristics to improve diagnostic sensitivity and specificity[2,5]. For GPA, sinonasal congestion and positivity for cytoplasmic ANCA (c-ANCA) and/or anti-proteinase 3 (PR3) antibodies are the highest weighted criteria[10]. For MPA, perinuclear ANCA (p-ANCA) and/or anti-myeloperoxidase (MPO) positivity are the highest weighted criteria, followed by pauci-immune glomerulonephritis and interstitial lung disease[11]. By contrast, the criteria for EGPA focus on eosinophilia, nasal polyps and obstructive airway disease, with ANCA positivity functioning as a negative item for classifying EGPA[10]. IgA vasculitis is an immune complex vasculitis that affects the small blood vessels and is characterized by palpable purpura, arthralgias, glomerulonephritis and enteritis[12]. Some vasculitides, such as Behçet disease, have variable vessel involvement and do not fit into a single category (labelled as variable vessel vasculitis). Recognizing the size and location of the inflamed vessels within each disease is essential for monitoring and predicting organ involvement and for predicting the effect of the vasculitis on pregnancy.

A patient's age and ancestry contribute to the likelihood of developing specific forms of vasculitis. The vasculitides that are most likely to affect young women include Takayasu arteritis, Behçet disease and PAN[13–15]. Takayasu arteritis is considered most common in patients of Asian ancestry with a typical age at onset ranging from ~20 to ~32 years[13]. Various HLA class I and II molecules are implicated in the susceptibility or development of Takayasu arteritis[16]. The age at onset of Behçet disease peaks between 20 and 30 years[14], and this disease is thought to be more prevalent in men than in women, with a male to female ratio that varies from 1.5:1 to 5:1 (REF.[17]). However, a Brazilian study revealed a female predominance[17]. The prevalence of Behçet disease is highest in areas that span the ancient Silk Road (including eastern Mediterranean regions and China)[18]. PAN, affecting those of European descent, is typically seen in the fifth to sixth decade of life and has a male to female ratio of 1.5:1 (REF.[15]). AAV most commonly occurs in white populations and the peak age of onset ranges between 60 and 70 years[19]; however, a notable number of young patients also develop this form of small vessel vasculitis. Other forms of vasculitis are less likely to affect young women, and hence to affect pregnancy. IgA vasculitis most commonly affects children between the ages of 4 and 7 years[20]. It remains rare in the adult population, with an annual incidence of 0.1–1.8 per 100,000 individuals[21]. Disease is more frequent in males, with a male to female ratio of 1.5:1 (REFS.[21,22]). GCA mainly affects patients who are aged 50 years or older, with the highest incidence occurring in Northern Europe, especially Scandinavian countries; hence, this disease is unlikely to affect pregnancy.

Reproductive health of female patients

Family planning

Taking a proactive approach to reproductive health can have a lasting, positive affect because the timing of pregnancy is a crucial variable in terms of optimizing pregnancy outcomes for mother and infant. The EULAR, EBCOG and ACR reproductive health guidelines strongly recommend considering pregnancy in the context of quiescent or low activity of the woman's rheumatic disease[2,4,5]. If a woman with vasculitis conceives when her vasculitis is active, she is at increased risk of miscarriage, intrauterine growth restriction, prematurity and pre-eclampsia[23]. When these risks are present, patients and providers might need to make difficult decisions about initiating therapy that would control disease but could potentially harm the fetus. According to the 2020 ACR reproductive health guidelines, some disease manifestations, such as pulmonary arterial hypertension, renal dysfunction and heart failure, could serve as contraindications to pregnancy[2]. Although these conditions are not listed as contraindications in the EULAR guidelines[5], they are recognized as notable threats to the health of the patient and pregnancy. EBCOG recommends counselling against pregnancy if the patient has had active lupus nephritis, severe renal impairment, severe pulmonary arterial hypertension, advanced heart failure or stroke within the prior 6 months[4]. If women with vasculitis have any of the listed

Table 1 | **Stratification of potential pregnancy and vasculitis complications by disease type**

Type of vasculitis	Potential pregnancy complications	Signs of active vasculitis	Characteristics of high risk patients	Evaluation for pregnancy risks	Intervention
Small vessel (GPA, MPA and EGPA)	Preterm delivery, pre-eclampsia, bleeding diathesis, spontaneous abortion, low birthweight, intrauterine growth restriction, respiration complications and decreased renal function	Renal insufficiency or failure, pulmonary haemorrhage, rash, joint swelling and fever	Active renal disease (proteinuria (>1 g per day) or active glomerulonephritis) and severe lung disease (recent pulmonary haemorrhage or severe decrease in lung function)	Urinalysis (microscopy and urine protein to creatinine ratio), serum creatinine, pulmonary function tests and chest imaging	Control active disease prior to conception and during pregnancy with pregnancy-compatible medications Daily low-dose aspirin to decrease the likelihood of pre-eclampsia
Medium vessel (PAN)	Hypertension, proteinuria, preterm birth and intrauterine growth restriction	Proteinuria, hypertension and abdominal pain	Uncontrolled hypertension and renal failure	Blood pressure monitoring and angiography to check the status of blood vessels	Control of hypertension with pregnancy-compatible medications Control active disease prior to conception and during pregnancy with pregnancy-compatible medications Daily low-dose aspirin to decrease the likelihood of pre-eclampsia
Large vessel (Takayasu arteritis)	Pre-eclampsia and low birthweight	Central occult hypertension, heart failure and renal insufficiency or failure	Renal artery and/or abdominal aorta involvement, aortic regurgitation and heart failure	Blood pressure monitoring and angiography to check status of the blood vessels	Control hypertension and active disease prior to conception and during pregnancy with pregnancy-compatible medications Daily low-dose aspirin to decrease the likelihood of pre-eclampsia
Behçet disease	Preterm delivery and spontaneous abortion	Worsening of oral ulceration, eye inflammation and arthralgias	Prior arterial or venous thrombosis	Evaluate for prior thrombosis	Anticoagulation therapy if the patient has a history of prior thrombosis Daily low-dose aspirin to decrease the likelihood of pre-eclampsia
IgA vasculitis	Preterm delivery, spontaneous abortion and gestational hypertension	Palpable purpura, abdominal pain, arthralgias and haematuria	Uncontrolled hypertension and renal failure	Blood pressure monitoring and urinalysis (microscopy and urine protein to creatinine ratio) and serum creatinine measurements	Control hypertension Active disease usually self resolves, but recalcitrant disease might require control with pregnancy-compatible medications

EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; PAN, polyarteritis nodosa.

clinical features or manifestations, discussions with their rheumatologist and obstetrician regarding maternal morbidity and mortality if pregnancy were pursued is warranted.

The ACR, EULAR, EBCOG and ACOG guidelines strongly suggest that medical professionals engage women in conversations about family planning on a regular basis[2–5]. Exploring the woman's desire, or lack thereof, to have children can help guide recommendations regarding contraception, infertility and medication regimens. Early initiation of these conversations also allows collaboration with specialists including specialists in maternal fetal medicine, obstetrics–gynaecology and reproductive endocrinology. A possible strategy to start these conversations is via the One Key Question online tool, beginning with the question "Would you like to become pregnant in the next year?". This data-driven tool gives patients the opportunity to discuss if, when and under what circumstances they want to get pregnant. Resources offered by this tool enable providers to undergo interactive training to understand the root causes of mistimed pregnancies, poor birth outcomes and disparities in maternal and infant health. The ACR reproductive health guidelines note that patients are appreciative of this discussion with their rheumatologist, who are viewed by the patients as "the doctors who know them and their medications best"[2].

Contraception

To make informed and personal decisions, patients should be made aware of the safe and effective contraception options available. Selection of contraception depends on both the safety of contraception and its efficacy (Supplementary Fig. 1). For an individual woman, the use of contraception depends heavily on her motivation to avoid pregnancy and her comfort with the form of contraception; these factors can be influenced by the provider but are often dependent on the lived experience of the woman outside the context of her vasculitis. Respecting the woman's desire, motivations and opinions about contraception is essential to effective and collaborative pregnancy planning. Comprehensive guidance

to contraception that can facilitate provider–patient discussions is available from the Vasculitis Foundation (Supplementary Fig. 1).

EULAR stresses the importance of considering disease-related risk factors (such as disease activity level and risk of thrombosis) and non-disease-related risk factors (such as hypertension, obesity, tobacco use and family history of hormone-related cancers) when counselling about contraception[4]. Effective birth control is particularly important for women taking teratogenic medications. Several medications used to treat vasculitis increase the risk of major birth defects, including methotrexate, mycophenolate mofetil and cyclophosphamide[2]. In women taking these medications, avoiding pregnancy will decrease the likelihood of suffering the emotionally challenging situations of deciding to continue or terminate a pregnancy, pregnancy loss or delivering an infant with a permanent birth defect.

The ACR strongly recommends the use of effective contraception (hormonal contraceptives or intrauterine devices) over less effective options or no contraception in fertile women with a rheumatic disease (that is, women of reproductive age without documented menopause), with special considerations for those patients who have SLE or those who are positive for aPL antibodies owing to the risk of thrombosis[2]. Although the ACR reproductive health guidelines and EULAR recommendations define women at high risk of thrombosis as those who are positive for aPL antibodies and/or have moderate to highly active SLE[2,5], this guideline might also be applied to women with vasculitis who are considered at high risk of thrombosis. The risk of thrombosis is highest during early and active vasculitic disease, but a procoagulant state might also be present in some patients with non-active AAV[24]. In the WeCLOT study, an observational cohort study that included 167 patients with GPA, the risk of venous thrombosis in patients with GPA was seven times higher than in patients with SLE[25]. Despite this increased risk of thrombosis in patients with vasculitis, no formal studies have assessed the risk associated with oestrogen-containing birth control in this population.

Emergency contraception (that is, progesterone-only contraceptives such as levonorgestrel) should also be discussed for patients with vasculitis because the risks associated with this contraceptive are lower than those associated with unplanned pregnancy. Levonorgestrel is widely available and has no medical contraindications[26], including for women with any form of vasculitis. Emergency contraception will not cause an abortion, which is a common misconception among patients that might limit its use[27]. Instead, this contraceptive prevents the sperm and egg from meeting and hence prevents fertilization[27]. Levonorgestrel is widely available in North America and Europe. The need for prescription to obtain this contraceptive is dependent on country-specific legislation.

Fertility preservation

High doses of cyclophosphamide can cause ovarian insufficiency, leading to infertility and/or premature menopause[28,29]. To avoid this adverse effect, EULAR recommends and ACR conditionally recommends monthly gonadotropin-releasing hormone agonist therapy (such as leuprolide) in women receiving monthly cyclophosphamide infusions[2,5]. Leuprolide is typically given as a 3.75 mg monthly dose, with the first dose being administered at least 10 days prior to the cyclophosphamide infusion to avoid cyclophosphamide exposure during the initial surge of oestrogen caused by leuprolide. This recommendation originates from studies in women undergoing treatment for breast cancer whose ovarian function remained stable with this intervention[30]. Fewer data are available regarding patients with rheumatic diseases, but the outcomes of the existing studies were positive[31–34]. Higher cumulative doses of cyclophosphamide and older age at the time of cyclophosphamide treatment both increase the risk of ovarian failure[28,29]. Although women who receive high dose oral or intravenous cyclophosphamide have a high risk of ovarian damage, women who receive a lower dose, such as the Euro-Lupus regimen of 500 mg intravenous cyclophosphamide every 2 weeks for six doses, have little ovarian damage[35].

Infertility and assisted reproductive technology

For women with infertility or women who wish to freeze eggs prior to cyclophosphamide therapy for later use, the ACR guidelines recommend avoiding assisted reproductive therapy (ART) when vasculitis is active[2]. Women considering ART should discuss options appropriate for their clinical situation with a reproductive endocrinologist. BOX 1 summarizes the various types of ART. For women with inactive vasculitis who have a limited risk of thrombosis, ART can proceed according to the standard of care. EULAR recommends low-dose aspirin or low molecular weight heparin (LMWH) during ART according to the patient's individual risk profile[5]. The ACR recommends LMWH during ART for women with aPL antibodies[2]. These recommendations can be extrapolated to women with vasculitis at high risk of thrombosis.

Box 1 | **Types of assisted reproductive technology**

- Egg retrieval for egg and/or embryo freezing: this procedure enables the possibility of a future pregnancy, which can then be delayed by months to years. Patients can continue their immunosuppressive and biologic therapies (including methotrexate and mycophenolate mofetil) during ovarian stimulation and cryopreservation without concern for teratogenesis. If a patient decides to freeze an embryo, the egg is fertilized prior to the freezing process. Low-dose aspirin should be stopped 3 days before egg retrieval and resumed the following day.
- Surrogate: if a woman (donor) chooses to have another woman (surrogate) carry her pregnancy, the donor can continue to take vasculitis medications while the eggs are being retrieved, then continue therapy while the pregnancy is carried safely without possible teratogen exposure.
- Embryo transfer: when an embryo is transferred into the uterus of a women with vasculitis, her vasculitis should be under control via the use of a pregnancy-compatible medication regimen before pursuing the transfer. Patients taking low molecular weight heparin (LMWH) to decrease the risk of thrombosis should stop this therapy at least 12 h prior to the procedure and resume LMWH the very same day as long as no bleeding occurs. All patients who are not taking low dose aspirin during the ovarian stimulation period should start low dose aspirin on the day of the embryo transfer as pre-eclampsia prophylaxis for the expectant pregnancy, usually in combination with LMWH (which should be continued during pregnancy).

Pregnancy and breastfeeding

Planning pregnancy is a crucial aspect of reproductive health care for women with rheumatic disease. Timing pregnancy to coincide with both disease quiescence and pregnancy-compatible medications increases the likelihood of pregnancy success. As such, rheumatologists have the opportunity at every clinic visit to address family planning goals to ensure an effective pregnancy planning process. Prior to and during pregnancy, rheumatologists should collaborate with other specialists to provide comprehensive care and close monitoring dictated by the patient's type of vasculitis and disease manifestations.

Pregnancy and vasculitis

The physiological changes that occur during pregnancy, such as an increase in blood volume, hormonal changes and fluid shifts, can affect the functionality of organs previously or currently affected by vasculitis[36]. For example, a woman with valvular disease or cardiac dysfunction from Takayasu arteritis might have difficulty adjusting to the increased blood volume that occurs during pregnancy. Quiescent and well-controlled vasculitis with thorough evaluation of all sequelae will aid physicians in anticipating complications and the need for close monitoring. Active vasculitic disease can complicate the ability to assess and stratify pregnancy risks, which further emphasizes the importance of planned pregnancies. Active vasculitis can worsen hypertension, especially in the setting of renal disease, and compromise critical blood flow to the placenta for fetal development, resulting in increased risk of maternal and fetal complications[23].

Most women with vasculitis can have a successful pregnancy, although they are all at higher risk of pregnancy complications owing to their disease than patients without vasculitis[34,37–44] (TABLE 1). Vasculitis complications during pregnancy are dependent on the type of vasculitis, but generally include preterm delivery, fetal loss, intrauterine growth restriction, severe hypertension and pre-eclampsia[37] (TABLE 2), and some diseases of pregnancy can mimic vasculitis activity (TABLE 3). The best approach to mitigating these risks is to ensure that a woman's vasculitis is under good control with pregnancy-compatible medications prior to and throughout pregnancy (TABLE 4).

Pregnancy planning and monitoring

Importance of a multidisciplinary approach. The pregnancy interests of a patient should be discussed often to ensure that pregnancy planning can begin several months to years prior to conception. This approach allows the patient to understand her risks in pregnancy and provides time to build a multidisciplinary team, make medication changes and ensure vasculitis activity is controlled prior to conception.

Given the complexities of pregnancy in women with vasculitis, all pregnant women with vasculitis should ideally consult with a maternal–fetal medicine physician (also known as a high-risk obstetrician or perinatologist). Although some patients can be followed by their local, low-risk obstetrician, the maternal–fetal medicine provider will give important guidance regarding medication use and pregnancy monitoring. Additionally, a patient's other organ-specific specialists should be involved in pregnancy planning and management.

Differentiating active vasculitis from pregnancy complications. An important concern regarding vasculitis during pregnancy is differentiating active vasculitis from pregnancy complications. For example, glomerulonephritis from AAV can present similarly to pre-eclampsia with proteinuria, oedema and hypertension; furthermore, microangiopathic haemolytic anaemia can mimic haemolysis, elevated liver and low platelets (HELLP) syndrome[45–47] (TABLE 3). Physiological changes of pregnancy include an increase in intravascular volume and an increase in glomerular filtration rate, which can exacerbate pre-existing cardiac and renal abnormalities[36].

Women with vasculitis are at increased risk of hypercoagulability and thrombosis[24,25], as previously discussed. Pregnancy itself induces a state of hypercoagulability that results in a compounded risk of thrombosis for pregnant women with vasculitis[48]. The treating rheumatologists should be aware of presentation overlap between active vasculitis and pregnancy complications as anchoring on a diagnosis can increase the risk of morbidity and mortality for the mother and baby. Rheumatologists and maternal–fetal medicine physicians should collaborate to determine the aetiology of a presentation.

Monitoring rheumatic disease during pregnancy. The ACR reproductive health guidelines recommend that rheumatologists evaluate pregnant patients at least once per trimester and the frequency of rheumatological follow-up should be individualized according to the needs of the patient[2]. The primary role of the rheumatologist is to assess the level of vasculitis activity and adjust the anti-rheumatic medications accordingly; these tasks are typically outside the scope of practice for obstetricians or maternal–fetal medicine providers. EULAR recommends completing an evaluation of the patient by umbilical and uterine artery doppler ultrasonography at 20–24 weeks to assess the risk of placenta-associated pregnancy disorders (for example, pre-eclampsia and intrauterine growth restriction), which can affect the mode and timing of delivery[5]. Rheumatologists can stratify patients according to their risk of pregnancy complications by assessing for the presence of anti-Ro and aPL antibodies, which include IgM, IgA and IgG anti-cardiolipin antibodies, anti-β_2 glycoprotein antibodies and lupus anticoagulant. Although these autoantibodies are more commonly observed in patients with SLE than in patients with vasculitis, treating providers can screen to assess for additional risks if they have concerns regarding overlap syndrome[49,50]. If the patient is positive for anti-Ro antibodies, fetal echocardiograms are recommended by the ACR, EBCOG and EULAR between 18 and 24 weeks to assess for fetal atrioventricular block[2,4,5,51]. Hydroxychloroquine decreases the risks of neonatal lupus including atrioventricular block and is recommended by the ACR, EBCOG and EULAR for all women who are pregnant and are positive for

anti-Ro antibodies[2,4,5,52]. For aPL antibody-positive women, whether anticoagulation therapy is advisable will depend on whether the disease is classified as obstetric APS or thrombotic APS and whether the patient has a history of thrombosis, and can be discussed with a haematologist.

Medication management during pregnancy

Fortunately, multiple medications used to manage vasculitis are considered compatible with pregnancy (TABLE 4). These include azathioprine, colchicine, TNF inhibitors, cyclosporin, tacrolimus and NSAIDs. Glucocorticoids are considered safe during pregnancy, but their use

Table 2 | **Pregnancy outcomes in women with vasculitis**

Study	Number of pregnancies	Pregnancy losses, *n* (%)[a]	Preterm deliveries, *n* (%)[b]	IUGR or low birthweight, *n* (%)	Caesarean delivery, *n* (%)	Pre-eclampsia or gestational hypertension, *n* (%)	Other
ANCA-associated vasculitis							
Pagnoux et al. (2011)[34]	16	3 (18.8)	6 (37.5)	4 (25)	6 (37.5)	7 (43.8)	Acute maternal heart failure: 1 (6.25%) PROM: 2 (12.5%)
Gatto et al. (2012)[38]	79	10 (12.7)	25 (31.6)	14 (17.7)	22 (27.8)	8 (10.1)	Maternal death: 5 (6.3%)
Fredi et al. (2015)[39]	16	1 (6.3)	4 (25)	2 (12.5)	9 (56.3)	0 (0)	–
Nguyen et al. (2021)[37]	20	0	5 (25)	6 (30)	7 (35)	2 (10)	–
Behçet disease							
Gatto et al. (2012)[38]	229	21 (9.2)	3 (1.3)	2 (0.87)	12 (5.2)	3 (1.3)	–
Iskender et al. (2014)[40]	49	8 (16.3)	6 (14.6)	3 (7.3) with only low birthweight	17 (41.4)	8 (19.5) with gestational hypertension and IUGR	NICU admission: 5 (12.2%)
Fredi et al. (2015)[39]	31	3 (9.7)	6 (21.4)	3 (9.68)	10 (32.3)	8 (28.5)	1 pregnancy loss past 10 weeks
Clowse et al. (2013)[41]	6	3 (60)	0	NR	NR	NR	–
Orgul et al. (2018)[42]	66	18 (27.3)	12 (24)	12 (24)	NR	2 (4)	Higher rate of preterm labour and low birthweight in patients treated with colchicine
Barros et al. (2021)[43]	49	12 (24.5)	3 (9.1)	9 (18.4)	16 (43.2)	0 (0)	–
Takayasu arteritis							
Gatto et al. (2012)[38]	214	30 (14)	35 (16)	42 (20)	78 (36)	92 (43)	Maternal death: 2 (0.9%)
Tanaka et al. (2014)[44]	27	0	3 (11)	4 (15)	9 (33)	4 (15)	80% of pregnant women with chronic hypertension had a stricture of the renal artery
Alpay-Kanitez et al. (2015)[104]	84	5 (6)	3 (4)	4 (5)	15 (18)	7 (8.3)	No neonatal abnormalities observed
Assad et al. (2015)[105]	38	0	16 (45.7)	12 (34.2)	24 (68.5)	12 (31.5)	More pregnancy complications in women with hypertension
Comarmond et al. (2015)[68]	98	9 (9)	8 (8)	5 (5) reported in combination with fetal death	16 (16)	21 (21)	Neonatal deaths: 3 (3%) Maternal new onset or worsening hypertension: 26 (26%)
Fredi et al. (2015)[39]	8	2 (25)	3 (50)	0 (0)	5 (83)	2 (33.3)	–
Gupta et al. (2020)[106]	38	10 (26.3)	2 (5.2)	6 (15.8)	NR	15 (39.4)	Gestational diabetes: 2 (5.2%)
Nguyen et al. (2021)[37]	12	1 (8.3)	3 (37.5)	0 (0)	1 (14.3)	2 (25)	Gestational diabetes: 1 (12.5%)
Polyarteritis nodosa							
Pagnoux et al. (2011)[34]	4	1 (25)	2 (50)	0 (0)	1 (25)	3 (75)	PROM: 3 (75%)
Fredi et al. (2015)[39]	4	0 (0)	2 (50)	2 (50)	1 (25)	1 (25)	–
IgA vasculitis							
Nossent et al. (2019)[107]	247	25 (10.1)	17 (8.3)	NR	57 (26.9)	27 (5.6)	Gestational diabetes: 19 (6.4%)

ANCA, anti-neutrophil cytoplasmic antibody; IUGR, intrauterine growth restriction; NICU, neonatal intensive care unit; NR, not reported; PROM, premature rupture of membranes. [a]Excluding therapeutic abortions. [b]Prior to 37 weeks gestation.

Table 3 | **Distinguishing between active vasculitis and mimics during pregnancy**

	Active vasculitis[108,109]	Pre-eclampsia[110–113]	Chronic hypertension[110]	Gestational hypertension[110]	HELLP syndrome[45–47,114]
Traditional clinical features	Dependent on type of vasculitis	Headache, elevated blood pressure, vision changes and abdominal pain	Systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg	Systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg	Nausea, abdominal pain and elevated blood pressure
Timing	Any time	After 20 weeks gestation and postpartum	Onset before pregnancy or before 20 weeks gestation	Onset after 20 weeks gestation	Third trimester and postpartum
Proteinuria	Yes, if the vasculitis has renal involvement	Yes; ≥0.3 mg/mg (urine protein to creatinine ratio) or ≥300 mg/24 h (24-h urine protein test) or ≥1+ (dipstick)	Stable if present	No	Can be present, but not necessary for diagnosis
Platelets	High	<100,000/μl in severe cases	Normal	Normal	Low
AST to ALT ratio	Normal	High	Normal	Normal	High
LDH	Normal	High	Normal	Normal	High
CRP	High	High	Normal	Normal	High
Uric acid	Normal	High	Normal	Normal	Can be elevated
Intervention	Increased immunosuppression and glucocorticoids	Blood pressure control, magnesium, betamethasone <36 weeks and timely delivery	Blood pressure control	Blood pressure control	Blood pressure control, glucocorticoids and timely delivery

ALT, alanine aminotransferase; AST, aspartate transaminase; CRP, C-reactive protein; HELLP, haemolysis, elevated liver enzymes and low platelets; LDH, lactate dehydrogenase.

should be minimized to mitigate adverse effects. A vasculitis flare, particularly of internal organ disease, in pregnancy is probably riskier to the pregnancy than these medications. Nevertheless, some medications should be avoided, such as those medications known to cause major birth defects (teratogenic medications), particularly during the early stages of pregnancy. In this section, we discuss the best approach for managing patients with vasculitis during pregnancy and the safety of relevant drugs and medical interventions.

Discontinuation of teratogenic medications. Women should ideally discontinue medications known to cause major birth defects, including methotrexate, cyclophosphamide and mycophenolate mofetil, prior to conception[2] (TABLE 4). Despite this recommendation, some women will conceive on one of these medications; guidance for this situation is outlined in BOX 2.

The ACR guidelines recommend switching women from teratogenic to pregnancy-compatible immunosuppressant medications prior to conception[2]. This switch should be followed by a waiting period during which the stability of vasculitis on the new medication can be assessed. The duration of this waiting period depends on the patient's personal risk of a vasculitis flare following the change in medication. The benefit of quiescent disease prior to conception is exemplified in SLE, for which disease activity 6–12 months prior to conception increases the likelihood of disease activity and possible complications during pregnancy[53]. A 2013 study in 54 patients with SLE who were planning for pregnancy found that replacing mycophenolate mofetil with azathioprine in those with quiescent lupus nephritis rarely led to disease flare and was associated with favourable pregnancy outcomes[54]. Similar data are not available for women with vasculitis at this time; however, maintaining quiescent vasculitis by using pregnancy-compatible medications is the current best-practice approach[2].

Management of inactive vasculitis during pregnancy. Initiation or escalation of the dose of pregnancy-compatible immunosuppressants should be considered prior to pregnancy to obtain or maintain vasculitis remission. These medications can include azathioprine, tacrolimus, cyclosporin, colchicine or TNF inhibitors[2]. Given the periodic dosing of rituximab, which enables this drug to be effective for a prolonged period after a single dose, administering a dose of rituximab prior to conception can be an effective approach for controlling AAV disease activity.

The ACR recommends tapering glucocorticoids to an equivalent of less than 10 mg of prednisone daily, if possible, depending on the level of disease activity, to decrease the risk of adverse effects such as hypertension, intrauterine growth restriction and preterm birth[2,55]. The maintenance dose of prednisone (≥10–20 mg per day) is associated with an increased risk of preterm birth in women, with an odds ratio of 3.5 (REF.[5]). Additional pregnancy-compatible DMARD therapy is preferable to chronic high-dose glucocorticoid therapy and can improve disease control over the length of the pregnancy and postpartum period. Dexamethasone and betamethasone are fluorinated, synthetic glucocorticoids that readily cross the placenta[2]. Fetal uptake of prednisone, on the other hand, is limited due to its conversion to inactive metabolites by placental 11-β-dehydrogenase

Table 4 | **Medication recommendations during pre-conception, pregnancy and breastfeeding**

	ACR[2]	ACOG[3]	EULAR[a] (REF.[5])	EBCOG[b] (REF.[4])	LactMed[c]	Notes
Azathioprine						
Pregnancy	++	+	+ (3C)	+	NA	Check thiopurine S-methyltransferase levels in mothers before initiating azathioprine; increased risk of preterm birth and fetal growth restriction in pregnant patients taking azathioprine, although how this affect relates to medication use versus maternal disease is unclear[82–85]
Breastfeeding	+/–	+	NA	+	+	Compatible; consider monitoring complete blood count in infants as cases of mild, asymptomatic neutropenia have been reported[86]
Colchicine						
Pregnancy	++	NA	NA	NA	NA	–
Breastfeeding	++	NA	NA	NA	+	Compatible; avoid breastfeeding within 4 h of dose to minimize infant exposure
TNF inhibitors						
Pregnancy	+/–	+	NA	+/–	NA	The ACR recommends continuing TNF therapy in first and second trimesters but consider discontinuing in the third trimester (except for certolizumab) if disease is under control to decrease transplacental transfer; by contrast, the Society for Maternal-Fetal Medicine recommends continuing TNF inhibitors in the third trimester[3]
Breastfeeding	++	+	NA	+	+	Compatible; large protein molecules and IgG antibodies do not cross into breastmilk in high concentrations[87]
Cyclosporine and tacrolimus						
Pregnancy	+/–	+	+ (3C)	+	NA	Monitoring of blood pressure is recommended; these drugs are associated with an increased risk of preterm birth and growth restriction[88]
Breastfeeding	+/–	+	NA	NA	+/–	Compatible; consider monitoring infant drug levels if the infant shows signs of potential adverse effects
NSAIDs						
Pregnancy	+/–	NA	NA	+; contra-indicated beyond 32 weeks	NA	A FDA black box warning has been issued against NSAID use after 20 weeks due to oligohydramnios and closure of ductus arteriosus[89]; the ACR recommends NSAIDs over COX2-specific inhibitors
Breastfeeding	+/–	NA	NA	+	+/–	Compatible; ibuprofen is preferred over aspirin and naproxen owing to its extremely low levels in breastmilk, short half-life and safe use in infants at doses much higher than those transferred to breastmilk[90]
Rituximab						
Pregnancy	+	+	NA	–	NA	Discontinue when pregnancy is confirmed; can be used if organ-threatening or life-threatening disease occurs during pregnancy
Breastfeeding	++	+/–	NA	–	+/–	Compatible; large protein molecules and IgG antibodies do not cross into breastmilk in high concentrations[91]
Cyclophosphamide						
Pregnancy	+/–	+/–	–	–	NA	Discontinue cyclophosphamide 3 months prior to conception owing to the high risk of birth defects with first trimester exposure[2]; can be considered for life-threatening and organ-threatening disease during the second and third trimesters
Breastfeeding	––	+	NA	–	+/–	This drug enters breastmilk in potentially toxic amounts and has highly toxic active metabolites that add risk to the infant[92]; most sources consider breastfeeding to be contraindicated during cyclophosphamide treatment; the Academy of Breastfeeding Medicine recommends withholding breastfeeding for 72 h after a dose[93]
Methotrexate						
Pregnancy	––	–	––	–	NA	Stop 1–3 months prior to pregnancy; if a patient becomes pregnant while taking methotrexate, stop the methotrexate and start 5 mg folate daily
Breastfeeding	+/–	–	NA	–	+/–	Some evidence that breastmilk contains <1% of the maternal weight-adjusted methotrexate dose, which decreases within 24 h of weekly dosing[94,95]; this level of transfer is unlikely to harm an infant and monitoring the infant's complete blood count and differential can be considered

Table 4 (cont.) | **Medication recommendations during pre-conception, pregnancy and breastfeeding**

	ACR[2]	ACOG[3]	EULAR[a] (REF.[5])	EBCOG[b] (REF.[4])	LactMed[c]	Notes
Mycophenolate mofetil						
Pregnancy	––	–	––	–	NA	Associated with cleft lip and palate, micrognathia, microtia and auditory canal abnormalities[96,97]; expert opinion suggests that pregnancy should be delayed 6 weeks after discontinuing mycophenolate[98]
Breastfeeding	––	NA	NA	–	+/–	–
Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers						
Pregnancy	+/–	NA	NA	–	NA	Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers increase birth defects and should be avoided throughout pregnancy and especially in the second and third trimesters as they can cause severe, irreversible fetal renal injury[2]
Breastfeeding	NA	NA	NA	+	+/–	Compatible; captopril is transferred to the breastmilk at low levels and so the amount ingested by the infant would be small[99]; adverse effects are not expected
Glucocorticoids						
Pregnancy	+	+	+ (3C)	+	+	ACR conditionally recommends continuing low-dose (≤10 mg/day) prednisone during pregnancy if clinically indicated and tapering higher doses to <20 mg/day by adding pregnancy-compatible glucocorticoid-sparing agents if needed
Breastfeeding	+	+	NA	+	+	Only low levels of prednisone are transferred into breastmilk and no adverse effects have been reported

ACOG, American College of Obstetricians and Gynecologists; EBCOG, European Board & College of Obstetrics and Gynaecology; LactMed, Drugs and Lactation database; NA, not addressed. [a]For the EULAR recommendations: the information in parentheses refers to the level of evidence (1–5) and grade of recommendation (A–D); –– indicates that this medication should be avoided. [b]For the EBCOG recommendations: +, relatively safe, when absolutely necessary; –, to be avoided; +/–, not enough evidence. [c]For all other guidelines: ++, strongly recommend continuing; +, recommend continuing; +/–, conditionally recommend; –, recommend discontinuing; ––, strongly against continuing.

isoenzyme 2. Therefore, when using low to moderate doses of prednisone for vasculitis during pregnancy the majority of the drug will be metabolized prior to reaching the fetus[2].

Management of active vasculitis during pregnancy. If active vasculitis is suspected, especially pulmonary and/or renal vasculitis, initiation or increased doses of glucocorticoids might be warranted including pulse-dose glucocorticoid therapy in organ-threatening or life-threatening situations. A glucocorticoid-sparing agent should also be considered with plans to taper glucocorticoids if medically safe[2]. The ACR guidelines conditionally recommend the use of rituximab or cyclophosphamide if the woman has life-threatening or organ-threatening disease, such as glomerulonephritis or diffuse alveolar haemorrhage[2].

Although limited data are available on the safety of rituximab in pregnancy, this drug is being increasingly used prior to and early in pregnancy for diseases including vasculitis. However, rituximab dosing after 16 weeks gestation, when the medication is more likely to cross the placenta, puts the infant at risk of being born without B cells, a situation with unclear risks[56]. The ACOG notes that although limited data are available regarding rituximab in pregnancy, the existing data are encouraging from a safety aspect[57,58].

Although cyclophosphamide has known teratogenic effects during the first trimester, some data suggest that this drug can be safely used in the second and third trimesters once organ formation is complete[59]. In a 2005 case series of four pregnancies, fetal loss was 100% after the use of cyclophosphamide during the first or second trimester for the treatment of SLE, although discerning the role of cyclophosphamide from the role of severe disease in causing pregnancy loss is difficult[60]. Data on pregnant women with malignancies treated with chemotherapy, including cyclophosphamide, suggest that this drug can be used safely[61,62]. The risks and benefits of these medications should be discussed with the patient in conjunction with appropriate specialists on the treatment team.

New therapies with limited pregnancy data. Given the rapid development of treatment options for vasculitis, data regarding the safety of several medications during pregnancy and/or lactation are unavailable, including data on avacopan, abatacept, apremilast, belimumab, mepolizumab, tocilizumab and tofacitinib. A major concern is the potential effect of small molecules, including avacopan and tofacitinib, on the fetus and on the newborn, owing to likely transfer across the placenta and/or into breastmilk. Large protein molecules, including abatacept and mepolizumab, are unlikely to cross the placenta in the first half of pregnancy and only small amounts are expected in breastmilk[63,64]. A panel of experts from Europe, Australia and New Zealand concluded that mepolizumab is possibly acceptable during breastfeeding[65]. According to the ACR reproductive guidelines[2], all biologic drugs are expected to have minimal transfer owing to their large molecular size; therefore, continuation of these medications during breastfeeding is conditionally recommended.

Box 2 | Conception on a teratogenic medication

If conception occurs while the patient is taking a potentially teratogenic medication:

- Stop all teratogenic medications
- Start a prenatal vitamin
- If taking methotrexate during pregnancy: start folic acid 5 mg daily
- If taking leflunomide during pregnancy: start cholestyramine washout of 8 g three times daily for 11 days
- Estimate time of exposure: calculate based on timing of conception (estimated as 2 weeks after the first day of the patient's last menstrual period) and timing of teratogenic medication administration
- Assess vasculitis activity and transition to a pregnancy-compatible regimen (TABLE 4)
- Obstetrics: discuss the exposure with the patient and evaluate using ultrasonography
- Contact Mother To Baby or other country/region-specific teratogen resources for guidance

Risks relating to drug exposure during the first trimester:

- Mycophenolate mofetil: ~40% pregnancy loss; ~25% birth defects[115]
- Cyclophosphamide: ~50% pregnancy loss[116]; ~25% birth defects[117]
- Methotrexate: 40% pregnancy loss; ~7% birth defects[118]
- Leflunomide: no increase in pregnancy loss or birth defects with cholestyramine washout[119]

Management of hypertension in pregnancy. Hypertension during pregnancy increases the risk of poor placental development, which can lead to pregnancy loss, preterm birth, pre-eclampsia and fetal growth restriction[66]. According to the 2020 ACOG clinical management guidelines, labetalol, hydralazine and nifedipine can be used for blood pressure control during pregnancy[67].

The prevalence of hypertension might be underestimated in women with Takayasu arteritis as a small number of these patients present with stenoses of all four extremity vessels or the abdominal aorta, leading to a misleadingly low blood pressure recording in one to four extremities[68]. Blood pressure monitoring on all limbs, or limbs without stenosis, might help provide a more accurate assessment. At delivery, in the absence of a reliable approach to peripheral blood pressure monitoring, continuous arterial blood pressure assessments are required to avoid severe hypertension that could increase maternal morbidity.

Use of aspirin to prevent pre-eclampsia. The ACOG, ACR, EULAR and EBCOG recommend that women with one or more high-risk factors for pre-eclampsia (that is, a history of pre-eclampsia, multifetal gestation, renal disease, autoimmune disease, type 1 or type 2 diabetes mellitus and/or chronic hypertension) and women with more than one moderate-risk factor (that is, first pregnancy, age 35 years or older, a BMI of more than 30 kg/m^2 and/or a family history of pre-eclampsia) should receive low-dose aspirin (typically 81–162 mg) for pre-eclampsia prophylaxis[2–5]. This therapy should be initiated before 16 weeks gestation and continued until delivery; such an approach can reduce the risk of preterm pre-eclampsia by 62% in women at high risk[69]. The utilization of prophylactic aspirin to prevent pre-eclampsia has been extrapolated within the ACR guidelines to include women with SLE and APS[2]. Given that women with all forms of vasculitis are at increased risk of pre-eclampsia owing to placental dysfunction[70,71], this recommendation should also apply to them.

Use of regional anaesthesia. Anaesthesia during delivery is managed by an anaesthesiologist. For pain relief during delivery, regional anaesthesia prior to delivery in patients with vascular stenoses might control arterial pressure while also allowing neurological assessment in awake patients[72]. Neurological assessment during delivery is critical as a change in neurological status, such as altered mental status, could indicate a medical emergency. General anaesthesia can trigger a hypertensive response owing to inadequate anaesthetic depth prior to rapid sequence intubation[73], which might exacerbate pregnancy or vasculitic complications. In patients with difficult airways, such as patients with subglottic stenosis caused by GPA, regional anaesthesia permits the avoidance of airway manipulation.

Vaccination considerations

Chronic immunosuppression in patients with autoimmune conditions increases the risk of cervical dysplasia, vaginal cancers and vulvar cancers, all of which are associated with human papilloma virus (HPV) infection[74–76]. According to EULAR, the HPV vaccine should be offered to young patients with stable and/or inactive SLE and/or APS[5]; given that the level of immunosuppression is similar in patients with vasculitis, the HPV vaccine might also be advisable for these patients. Although very rare, venous thromboembolic events have occurred following administration of the quadrivalent HPV vaccine; of 31 patients with such a venous thromboembolic event, 90% had a known risk factor for thrombosis, including APS in two[77]. As vasculitis increases the risk of thrombosis, patients with vasculitis should be offered the vaccine following a discussion of their disease activity and risk of thrombosis with their rheumatologist. HPV vaccination during pregnancy is not recommended by the ACOG but can be administered in the pre-pregnancy or postpartum periods[78]. Women with SLE exposed to immunosuppression are at particularly high risk of these malignancies; however, limited data are available for women with vasculitis[76]. Papanicolaou (PAP) smear examination should be performed annually in heavily immunosuppressed patients (for example, patients taking cyclophosphamide) or in accordance with local screening guidelines for low-risk individuals[79].

According to the ACOG, all pregnant women should receive a tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (Tdap) vaccine during each pregnancy between 27 and 36 weeks of gestation, and an inactivated influenza vaccination during the influenza season[78]. Additional vaccinations can be considered in special populations. In women with lung disease or immunocompromising conditions including vasculitis, 23-valent pneumococcal polysaccharide vaccine (PPSV23) and 13-valent pneumococcal vaccine (PCV13) can be considered[78]. However, whether to administer these vaccines should be jointly discussed by the patient and the patient's obstetrician. The measles–mumps–rubella (MMR) vaccination is a live attenuated vaccine and is

contraindicated in pregnancy[78]; it should be administered in the pre-pregnancy or postpartum periods. In patients with vasculitis taking immunosuppressive medication, an MMR booster can be considered outside pregnancy after discussions with their rheumatologist[80]. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccinations are recommended in all pregnant and lactating individuals[81].

Breastfeeding

Breastfeeding should be discussed prior to delivery. The ACOG recommends exclusive breastfeeding for the first 6 months after delivery to optimize immunity and nutrition of the newborn, and recommends that, ideally, breastfeeding is continued until the child's first birthday[2]. The ACR reproductive health guidelines also encourage all women with rheumatic disease to breastfeed if they choose to do so[2]. Whether a woman breastfeeds is a personal choice, with some women not wishing to breastfeed or struggling to breastfeed owing to having a low milk supply, fatigue or illness. Regardless of how a woman feeds her infant, showing understanding and empathy will help the patient through this challenging period of her life.

Almost all medications used for vasculitis are considered compatible with breastfeeding[2–5,82–99] (TABLE 4). All medications that are pregnancy-compatible are also lactation-compatible, including azathioprine, colchicine, low-dose glucocorticoids (<20 mg prednisone equivalent), TNF inhibitors, NSAIDs, cyclosporine and tacrolimus[2]. For woman taking more than 20 mg prednisone equivalent daily, breastfeeding should be delayed for 4 hours after each dose. Biologic therapies, including anakinra, belimumab, abatacept, tocilizumab, secukinumab and ustekinumab, are also considered safe with breastfeeding as their very large molecular weight makes notable passage into breastmilk unlikely[2]. If breastfeeding patients require NSAIDs for pain control, ibuprofen is the drug of choice owing to the low amount of the drug transferred to the breastmilk[2]. Lactation information is not currently available for newly developed small-molecule medications (such as tofacitinib, baricitinib, upadacitinib and avacopan). Because of their small size, these drugs might transfer into breastmilk, so their use during breastfeeding is not currently advised. The Drugs and Lactation database (LactMed) is a free, online pharmaceutical database managed by the National Center for Biotechnology Information, that contains up-to-date information pertaining specifically to the safety and adverse effect profiles of medications during breastfeeding, and is a useful resource for patients and physicians.

Reproductive health of male patients

Family planning discussions involving men with vasculitis can be much simpler than for women. All anti-rheumatic medications are compatible with fathering a child except for cyclophosphamide and thalidomide, which should be stopped prior to conception[2]. The ACR strongly recommends sperm cryopreservation prior to cyclophosphamide therapy to protect a man's ability to conceive a child[2]. Cyclophosphamide is toxic to developing sperm and can lead to permanent azoospermia due to damage of the spermatogonial stem cells in the testes[100]. Sperm should be collected before treatment and even one frozen sperm sample can lead to a future pregnancy. Urologists can assist with acquiring sperm quickly in an acutely ill patient. Unfortunately, sperm that develop during cyclophosphamide therapy have a high degree of genetic damage[101], making sperm collected in the days and weeks following cyclophosphamide treatment the most likely to be abnormal. For this reason, urologists recommend waiting at least 3 months after completion of therapy with chemotherapeutic agents such as cyclophosphamide before sperm collection or attempts at conception[102]. However, male patients might develop infertility after treatment with cyclophosphamide[2]. The ACR strongly recommends against testosterone co-therapy for men receiving cyclophosphamide as evidence suggests that this approach does not help with preservation of fertility[2,103].

Conclusion

Although managing women and men with vasculitis throughout the reproductive cycle is complicated, most patients will have successful outcomes with the assistance of a multidisciplinary team and careful planning. Avoiding conception while taking teratogenic medications and/or during periods of active vasculitis can decrease the risks of suffering the emotional and medical tragedies associated with pregnancy loss, birth defects and preterm birth. Rheumatologists can help by having a proactive approach and addressing pregnancy prevention and/or planning at each visit. Guidance on birth control options is available from the Vasculitis Foundation (Supplementary Fig. 1), which can aid in such discussions.

Overall, for women with vasculitis who may want to become pregnant, performing disease-specific laboratory and imaging diagnostic tests prior to conception should enable anticipation of any potential complications, identification of additional interventions and monitoring requirements and the need for subspecialist collaboration throughout the pregnancy. Providers need to enquire about specific patient characteristics including history of thrombosis and the presence of anti-Ro autoantibodies as their presence might change the appropriate management approach during pregnancy. In patients with unexpected pregnancies, medications should be reviewed for teratogenicity, possible antidotal options should be sought, and medication-compatible and lactation-compatible alternatives should be discussed. Patients should be informed of the risk of miscarriage and fetal development abnormalities than can occur with exposure to the teratogenic medication. During pregnancy, rheumatologists should evaluate women with vasculitis at least once per trimester to assess for disease activity and the need for escalation of therapy. A reproductive endocrinologist should evaluate those men and women who are experiencing infertility issues and discuss the various fertility treatment options, which can be affected by vasculitis disease activity and treatment regimens.

To address the limited amount of data that are currently available on pregnancy management and outcomes among women with vasculitis, we encourage participation in the VPREG, which is an online, international, patient-driven registry in which pregnant women with all types of vasculitis can enrol and provide information about their vasculitis activity, medications and pregnancy outcomes.

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Author contributions
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Precision medicine: the precision gap in rheumatic disease

Chung M. A. Lin, Faye A. H. Cooles and John D. Isaacs

Abstract | For many oncological conditions, the application of timely and patient-tailored targeted therapies, or precision medicine, is a major therapeutic development that has provided considerable clinical benefit. However, despite the application of increasingly sophisticated technologies, alongside advanced bioinformatic and machine-learning algorithms, this success is yet to be replicated for the rheumatic diseases. In rheumatoid arthritis, for example, despite an array of targeted biologic and conventional therapeutics, treatment choice remains largely based on trial and error. The concept of the 'precision gap' for rheumatic disease can help us to identify factors that underpin the slow progress towards the discovery and adoption of precision-medicine approaches for rheumatic disease. In a rheumatic disease such as rheumatoid arthritis, it is possible to identify four themes that have slowed progress, solutions to which should help to close the precision gap. These themes relate to our fundamental understanding of disease pathogenesis, how we determine treatment response, confounders of treatment outcomes and trial design.

Precision medicine in the field of oncology has made considerable progress over recent decades, with the development and application of personalized treatments across a broad range of tumour types[1–3]. Advances in analytical technologies, including the 'omics' revolution, alongside advances in our understanding of oncogenesis at a molecular level, have led to the development of algorithms that enable patients' blood and tissue samples to be subjected to genotyping and screening for specific targetable pathogenic changes, such as *BRCA* mutations or gene translocations[4–6]. This analysis enables subsequent stratification of patients into appropriate treatment groups based on the presence of theragnostic biomarkers, ultimately leading to more favourable outcomes[5,6]. This progress has changed the landscape of cancer management, enabling the development of specific targeted therapies, such as small-molecule tyrosine-kinase inhibitors, with considerable clinical success[7–9]. Consequently, there is a huge desire to replicate this approach in other specialties, with the ultimate goal of personalizing therapy across a range of diseases[10].

In comparison with oncology, progress in rheumatic diseases has been much slower, despite an ongoing therapeutics revolution, and this lack of progress sits uncomfortably alongside the ongoing clinical need. For example, in rheumatoid arthritis (RA) there is an early therapeutic window of opportunity during which appropriate intervention can lead to disease remission whereas, by contrast, poor initial disease control predisposes to long-term morbidity and premature mortality[11]. The current therapeutic algorithm usually requires methotrexate as first-line therapy, despite notable intolerance and modest response rates[12–14]. Subsequent treatment choices are generally guided by clinician and patient preferences, which are informed by minimal scientific evidence, whereas the use of appropriate biomarkers could direct patient stratification into theragnostic subgroups, akin to current oncological paradigms. Rapid implementation of optimal personalized treatment would improve clinical outcomes and cost-effectiveness, illustrating the need for acceleration of progress towards a precision-medicine approach for rheumatic disease (FIG. 1).

A number of sizeable (inter)national consortia have evolved to develop precision-medicine approaches to rheumatic disease. For example, the UK-based RA-MAP Consortium is a multi-partner collaboration of academia and industry that is focused on RA. Created in 2012, its goal is to map clinical responses to immunological profiles, thereby enabling subsequent biomarker identification. The group enrolled a cohort of drug-naive patients in the early stages of RA and, by sampling blood and urine at various time points over the course of 18 months, generated 56 million individual data points using various technologies in association with deep clinical phenotyping[10]. Although novel and informative observations have emerged from RA-MAP, a variety of analytical tools and machine-learning algorithms have yet to identify robust and clinically useful stratification measures within this impressive dataset[15].

Using RA as our archetypal rheumatic disease, we hypothesize that there are four key domains that have contributed to the comparatively slow progress in the development of precision therapies in rheumatology, a phenomenon that we have termed the 'precision gap' (FIG. 2).

Domain 1: understanding pathogenesis

A major catalyst to the development of precision medicine in oncology was the availability of tissue from the site of disease, which has enabled deep characterization of tumour tissue, with tremendous insights into pathobiology, including associated genetic mutations and downstream immunobiology. Identification of fundamental oncogenic pathways is a major stimulus to the development (or occasionally repurposing) of matched targeted therapies. Examples of this development process include the use of poly(ADP-ribose) polymerase (PARP) inhibitors in patients with tumours with germline mutations in DNA homologous-repair genes, such as *BRCA1* and *BRCA2*. Similarly, trastuzumab is particularly effective against breast cancers that express its target, human epidermal growth factor receptor 2 (HER2)[16,17]. The fact that most tumours are sampled by biopsy has not only provided a wealth of information about oncogenic pathways, but also highlighted

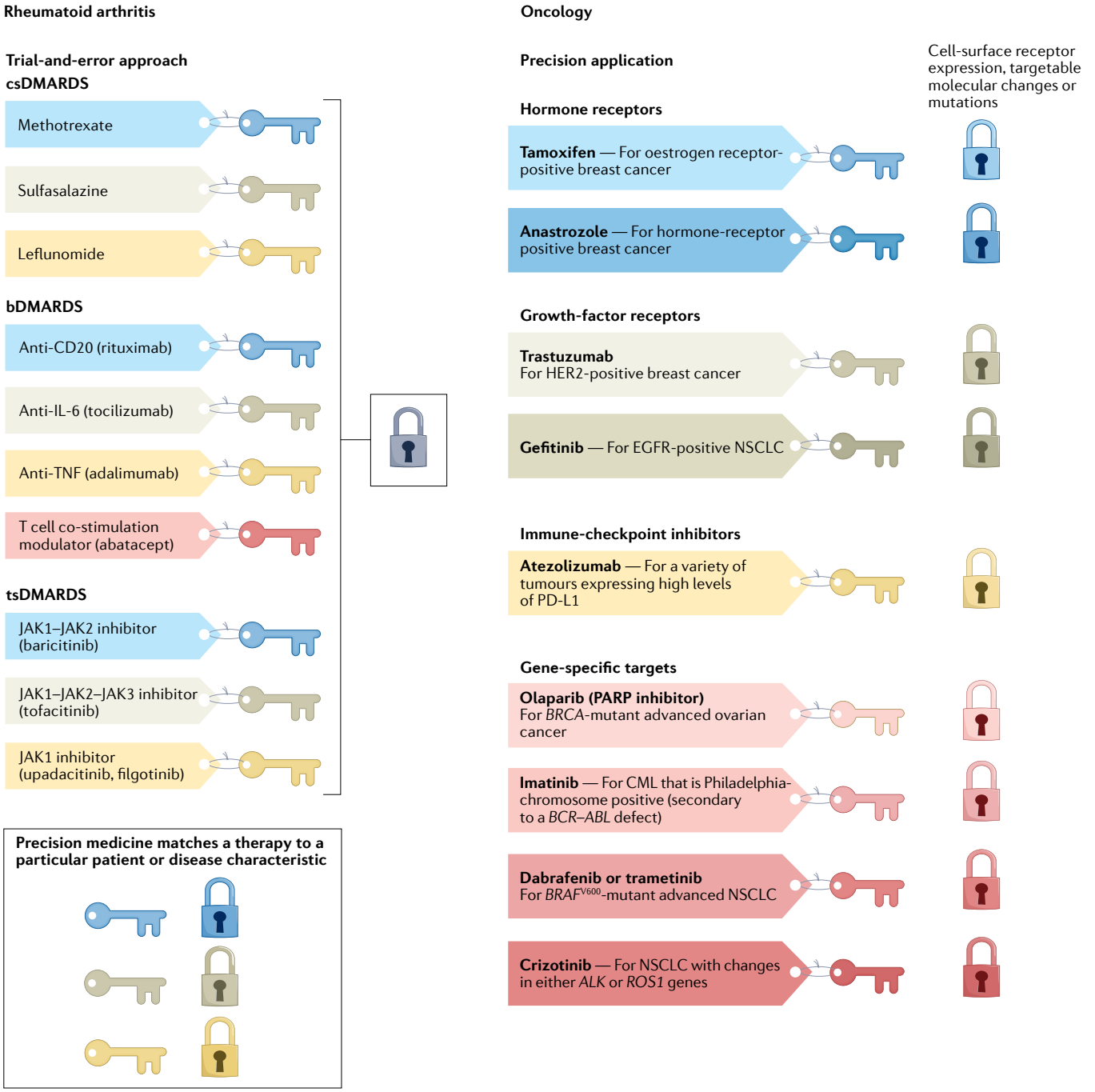

Fig. 1 | **The 'precision gap' between treatments for rheumatoid arthritis and for cancer.** For rheumatoid arthritis, increasingly sophisticated treatments (conventional synthetic DMARDs (csDMARDs), biologic DMARDs (bDMARDs) and targeted synthetic DMARDs (tsDMARDs)) have been developed without a means of targeting specific patient subsets, with a trial-and-error approach to treatment. By contrast, in oncology, a number of precision approaches have been developed whereby drugs targeting specific cell-surface receptors and molecular changes or mutations can be matched to tumour characteristics; some examples are illustrated. CML, chronic myeloid leukaemia; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; JAK, Janus kinase; NSCLC, non-small-cell lung cancer; PARP, polyADP ribose polymerase, PD-L1, programmed cell death 1 ligand 1.

relevant heterogeneity, further catalysing development of precision therapies. By contrast, synovial biopsies are not part of the standard of care for patients with RA, and our knowledge of RA pathobiology, and therapy-relevant heterogeneity, remains rudimentary and largely focussed on peripheral blood. For example, we remain uncertain which cell type drives the complex pathobiology of RA, and it seems quite remarkable that, more than two decades into the approval of TNF inhibitors, we have yet to discover a robust biomarker for responsiveness to TNF blockade.

The Pathobiology of Early Arthritis Cohort was established partly in response to the rheumatic disease knowledge gap[12,15]. Established in 2008, this international consortium links detailed pathobiological synovial data to established clinical phenotypes of patients with early inflammatory arthritis, with the goal of identifying theragnostic biomarkers. To date, Pathobiology of Early Arthritis

Cohort studies have associated disease outcomes and treatment responses with specific patterns of synovial inflammation. For example, the consortium identified three major types of synovial biology, termed lymphomyeloid, diffuse myeloid and pauci-immune. Myeloid and lymphoid features correlated best with disease activity, and lymphocytes with joint damage[18]. The consortium has also pioneered synovial biopsy-driven clinical trials. Results of a phase IV clinical trial comparing rituximab and tocilizumab in patients following an inadequate response to a TNF inhibitor were reported in 2021 (REF.[19]). The hypothesis that inspired the trial was that a low number or absence of synovial B cells would favour responsiveness to tocilizumab. Analysis of histological features did not meet the primary end point of the trial, but an equivalent stratification on the basis of the results of RNA sequencing supported the hypothesis, suggesting that automated analysis of sequencing data is more discriminatory than histological categorization by the human eye. Although the trial results suggested that synovial data could be used for assignment of almost two thirds of patients to 'correct' treatment, only 50% of patients with B cell-rich synovium responded to either therapy, suggesting the existence of additional pathobiology that was not being targeted.

Cytokines and chemokines are very important factors in RA pathogenesis as downstream mediators of inflammation, cellular differentiation and immune-response regulation[20,21]. Relevant factors include TNF and IL-6, which have important roles in RA pathogenesis that specific and potent targeted therapies have been developed to target[22]. However, it is precisely these targeted therapies that epitomize the precision gap, because they result in remission in only a minority of patients, and there are no biomarkers to identify individuals who will respond[23,24]. Whether the limited response reflects multiplex pathology that is not amenable to intervention against a single cytokine target, or pathobiology that is not targeted by any existing therapies, remains uncertain; notably, other pro-inflammatory cytokines are also present in RA, such as IL-17, whose targeting provides even less benefit than targeting of TNF and IL-6 (REF.[25]). Furthermore, Janus kinase (JAK) inhibitors, which target (but do not completely inhibit) multiple cytokine pathways, are associated with efficacy that is only slightly better than that of the complete inhibition provided by TNF blockade[26].

Most precision-medicine studies in RA have focussed on immune and inflammatory mechanisms. However, the normal synovium contains only fibroblasts and macrophages, which leads us to ask which cell really is the ultimate 'effector' that is analogous to the malignant cell in oncology. Although the genetics of RA strongly point to an immune pathogenesis, to date there is limited evidence, from preclinical models, to support an immuno-inflammatory mechanism as the ultimate effector of damage[27]. By contrast, for example, autoantibodies are known to be involved in the pathogenesis of Grave's disease or myasthenia gravis. Immune dysregulation might yet be identified as an enabling factor in RA, catalysing the transformation of a resident cell that ultimately triggers joint damage and destruction.

Single-cell technologies provide more detail than conventional histology or transcriptomic profiling of whole tissue, often revealing previously unsuspected cell types and disease heterogeneity. The Accelerating Medicines Partnership is another international consortium focussed on the study of disease-relevant tissues, but applying single-cell analyses such as cytometry by time-of-flight and single-cell RNA sequencing. An elegant study of disaggregated RA synovial biopsy samples identified four novel subsets of fibroblasts that contribute to RA pathogenesis by promoting intra-articular inflammation and causing RA-associated joint damage[28,29]. These results suggest that fibroblast subsets

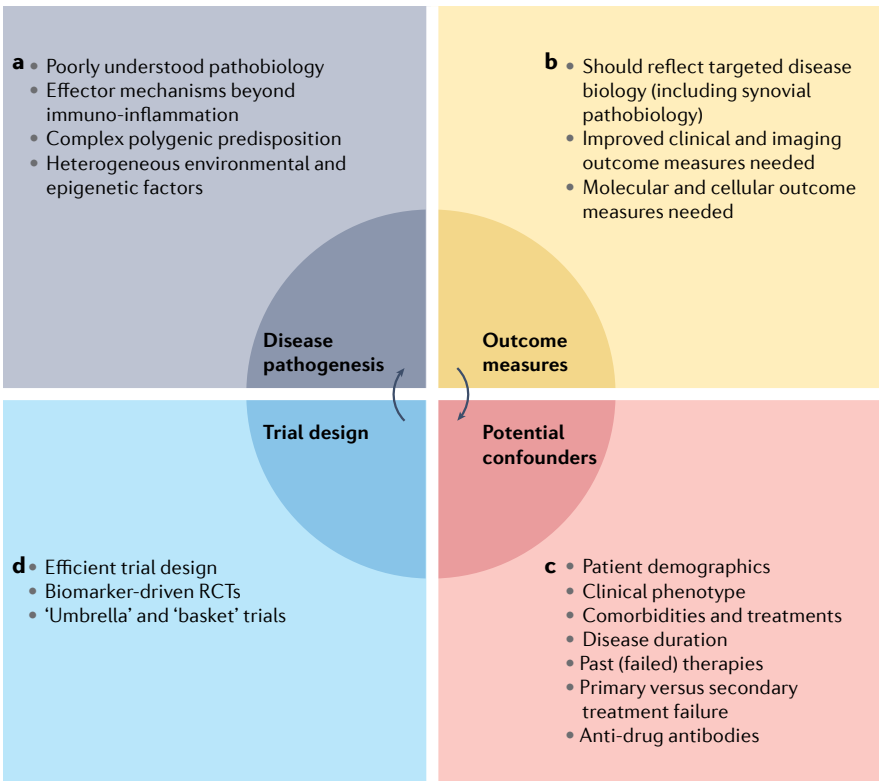

Fig. 2 | **The four main drivers of the precision gap that hinder theragnostic stratification in rheumatoid arthritis. a** | Current anti-rheumatic drugs target the immunoinflammatory cascade, but it is increasingly recognized that additional cell types and pathways have critical roles in the sophisticated pathobiology and effector mechanisms of rheumatoid arthritis (RA), reflecting a highly complex genetic, environmental and epigenetic predisposition. A better understanding of disease pathogenesis is essential, to enable us to target appropriate pathways with our therapies. **b** | Improved understanding will also ensure that outcome measures reflect the specific biology that is being targeted. In the future, molecular and/or cellular outcome measures may supplant or complement improved clinical and imaging outcomes. **c** | RA is heterogeneous, not only in terms of clinical phenotype, but also with regard to patient characteristics such as demographics, comorbidities, disease duration and previous failed treatments. It is also important to distinguish primary versus secondary treatment failure, including the possible emergence of anti-drug antibodies. **d** | Potential confounders should be considered in trial design, either by stratification at randomization or during analysis of data. Efficient trial designs are rarely applied in rheumatology, but can increase the likelihood of identifying response differences in patient subgroups. Biomarker-driven randomized controlled trials (RCTs) will ultimately help to identify precision therapeutics, whereas umbrella and basket trials should facilitate comparisons of distinct therapeutic approaches and accelerate precision medicine across immune-mediated inflammatory diseases as a group.

are potential therapeutic targets in RA, but currently, no approved anti-rheumatic drugs target these cells directly, although a phase Ib/IIa trial is currently underway[30]. Results from other studies have identified fundamental pathways that underpin synovial architecture, such as NOTCH3 signalling, which has a crucial role in RA synovial-fibroblast differentiation and topography[31]. The latest data from the Accelerating Medicines Partnership consortium, thus far available only as a preprint, suggest that there are at least six subtypes of RA, on the basis of synovial cell-type abundance, although these findings require validation[32].

Macrophages have long been recognized as key players in RA pathogenesis[33]. Single-cell analyses in RA have identified the potential relevance of macrophage subsets and heterogeneity. For example, results from an elegant set of experiments identified two remission-associated synovial macrophage subpopulations (MerTK^{pos}TREM2^{high} and MerTK^{pos}LYVE1^{pos}) that produced inflammation-resolving lipid mediators and induced repair responses in synovial fibroblasts[34]. Having a low proportion of these subpopulations during remission was associated with a heightened risk of subsequent disease flare if treatment was discontinued. Similarly, sophisticated murine studies, including spatiotemporal analyses, revealed the presence of a locally generated macrophage subpopulation lining the synovial cavity, forming an immunological barrier that protected intra-articular structures[35]. Additional cell types revealed by advanced technologies include eosinophils with a regulatory function and PRIME cells (circulating fibroblasts of uncertain provenance) that anticipate the onset of RA flare[36,37]. The pathobiological sophistication revealed by these cutting-edge technologies suggests that our current targeted therapeutic tools, though impressive in range, are perhaps not as 'sharp' as they need to be to truly dissect and separate different subtypes of RA.

In summary, evidence suggests that we have, to date, severely underestimated the complexity of RA pathobiology and its heterogeneity. Importantly, immuno-inflammation could be a facilitator rather than an effector of destruction akin to the malignant cell in cancer. In this context, relatively few studies have focussed on disease-relevant tissue, and single-cell sequencing of synovium has implicated cell types that are resident in healthy synovium and that current therapies do not directly target, such as fibroblasts and macrophages and, importantly, subsets thereof. A secondary analysis of data from the R4RA clinical trial suggests that synovium from treatment-refractory patients, who have synovitis despite exposure to three targeted therapies, contains an excess of stromal tissue[38]. This finding is important, but it does not exclude the possibility that this tissue was present at the outset of the disease, and that immuno-inflammatory pathways have, in fact, been controlled by the 'failed' prior therapies. Furthermore, even if the downstream 'effector' cell of RA lies beyond traditional immuno-inflammatory pathways, results from genome-wide association studies and polygenic-risk studies tell us that there will still be distinct mechanisms of immune dysregulation that underpin disease in different patients, with differential but overlapping susceptibility to existing (and future) targeted therapies — as we witness in clinical practice[39]. All of this newfound knowledge should stimulate considerable drug-discovery efforts, helping to close the precision gap. Once relevant biomarkers are discovered and validated, efforts will also be needed to identify peripheral-blood correlates for routine practice (FIG. 3).

Domain 2: outcome measures

In cancer, outcomes are dichotomous, such as actual or tumour-free survival versus non-survival, or tumour shrinkage versus progression on imaging. This clarity facilitates interpretation of clinical trials, including those testing precision therapies. Even a survival advantage of a few months can make an important difference to a patient with cancer and to their family, and an adequately powered study will detect such a benefit if present. Furthermore, these outcome measures clearly link disease biology and outcome.

In RA we use composite measures such as the 28-joint disease activity scale (DAS28), simplified disease activity index, clinical disease activity index or ACR responses. At one level, these holistic outcome measures address relevant aspects of disease such as inflammation (swollen-joint count and acute-phase response) and patient-reported outcome measures (patient global assessment, tender-joint count, pain and function). Indeed, they are sometimes criticized for omitting additional important symptoms such as fatigue and sleep quality. At another level, however, the link between biology and outcome is less clear, particularly for more subjective measures. Tender joints, for example, are not always inflamed when imaged, which does not mean that they are less relevant to the patient, or a less important element of the disease, but simply that a therapy that targets inflammation rather than pain is less likely to improve them. Similarly, damage that can be determined by X-radiography takes time to accumulate and is likely to be a poor outcome measure in short-term trials. Although the presence of inflammation on MRI scans is a more sensitive imaging outcome measure than the development of damage on X-radiography, as with C-reactive protein (CRP) and swollen joints it largely reflects inflammation, and remains considerably downstream from the fundamental immune dysregulation that drives RA. On the other hand, joint damage is the ultimate manifestation of RA; it underpins functional deterioration and may represent a robust and quantifiable integrator of multiple pathways.

In an attempt to define molecular fingerprints of response in RA, experiments were conducted to compare the blood transcriptome, blood immunophenotype and serum proteome in healthy individuals, patients with treatment-naive RA, and responders and non-responders to the DMARDs methotrexate, infliximab and tocilizumab[40]. The underpinning hypothesis was that responsiveness can be measured at the molecular level, and that treatment can modulate RA signatures towards those found in health. This sophisticated analysis demonstrated clear differences between the molecular effects of the different drugs, but it also reinforced the complexity of RA pathobiology. For example, responders were better distinguished from non-responders by immunophenotyping and serum proteomic analysis than by blood transcriptomics[40]. Surprisingly, tocilizumab modulated the blood transcriptome towards health, whereas infliximab modulated many transcripts in the opposite direction. Methotrexate had a lesser overall effect on the transcriptome, but a more profound effect on the proteome than tocilizumab, although many proteins were modulated away from health by methotrexate, in contrast to tocilizumab. Curiously, proteomic molecular remission (that is, normalization of the serum proteome) was associated with DAS28 response but not with responses in the clinical disease activity index or health assessment questionnaire disability index. Overall, these findings suggest that it is not currently possible to define a consensus molecular outcome measure in patients with RA (FIG. 3).

Given the complex pathobiology of RA, perhaps we should again turn to the

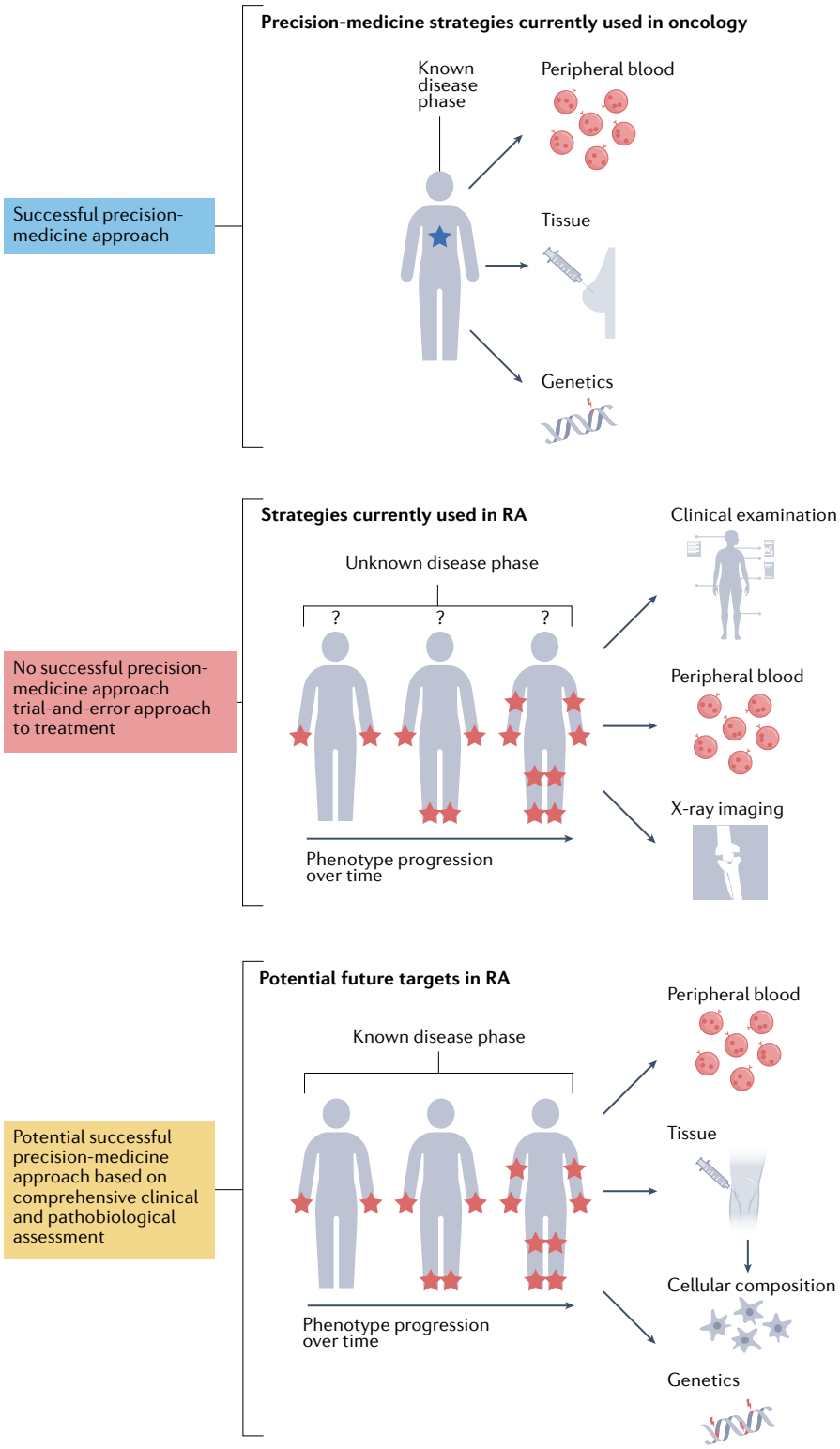

Fig. 3 | **Disease assessment and precision biomarkers.** In oncology, careful analysis of clinical material, especially the tumour itself, can identify precision biomarkers such as single-gene mutations or the presence of cell-surface receptors to guide specific treatments. Patients are also screened for oncogenic mutations, for example, in *APC*, to direct patient stratification and management. Disease phase is carefully documented and considered in terms of tumour stage and previous therapies. By comparison, assessment of rheumatoid arthritis (RA) currently relies on counts of swollen and/or tender joints, measurements of C-reactive protein and/or erythrocyte sedimentation rate, and patient-reported measures such as patient global assessment, pain and functional capacity, which do not facilitate a precision-medicine approach. Autoantibody assessment and imaging may also be performed, but do not usually guide specific treatment approaches. Disease phases such as early, established and refractory RA are informally recognized, but generally do not influence choice of treatment apart from the use of conventional synthetic DMARDs for early RA. Several approaches are being investigated for the attainment of precision medicine in RA. These include increasingly in-depth analyses of peripheral blood and synovium, including various 'omics' approaches, as potential sources of precision biomarkers[10,19,38]. Single-cell analyses and increasingly sophisticated molecular imaging are also being applied to synovium, identifying new cell subtypes (fibroblast and myeloid subtypes) and their interactions, for which targeted therapies do not currently exist. Polygenic risk scores may also guide personalized treatment in the future[28,29,31,32,34,35,39].

synovium to assess treatment effects. In a commentary in 2021, emphasis was placed on the potential importance of single-cell analyses of disaggregated tissue to gain an understanding of the complex immunobiology of immune-mediated inflammatory diseases (IMIDs) that, unlike cancer, largely reflect subtly dysregulated gene expression rather than critical single gene mutations[41]. Such analyses could identify heterogeneous targetable mechanisms involved in these diseases, as well as potentially providing the 'ultimate' molecular outcome at the single-cell level. However, a 'tissue outcome' only makes sense in the context of a better understanding of integrated disease pathobiology. Logically, the identification of a circulating biomarker (or biomarkers) of synovial inflammation and/or damage could provide a tractable and highly relevant outcome marker.

In the absence of specific biomarkers, we continue to utilize composite outcome measures to assess RA. A short-term fix, for assessing treatments that target the immuno-inflammatory component of the disease, might be to reduce such measures to components that best reflect inflammation. The DAS28 score integrates tender-joint count, swollen-joint count, acute-phase markers and the patient's global symptom score[42]. The tender-joint count and global symptom score are subjective measures, and a two-component DAS28 score excluding these measures has been proposed for use in precision-medicine studies. Indeed, this modified score showed a stronger correlation with ultrasonography-detected inflammation and structural damage than the DAS28, and it has now been adopted as an outcome

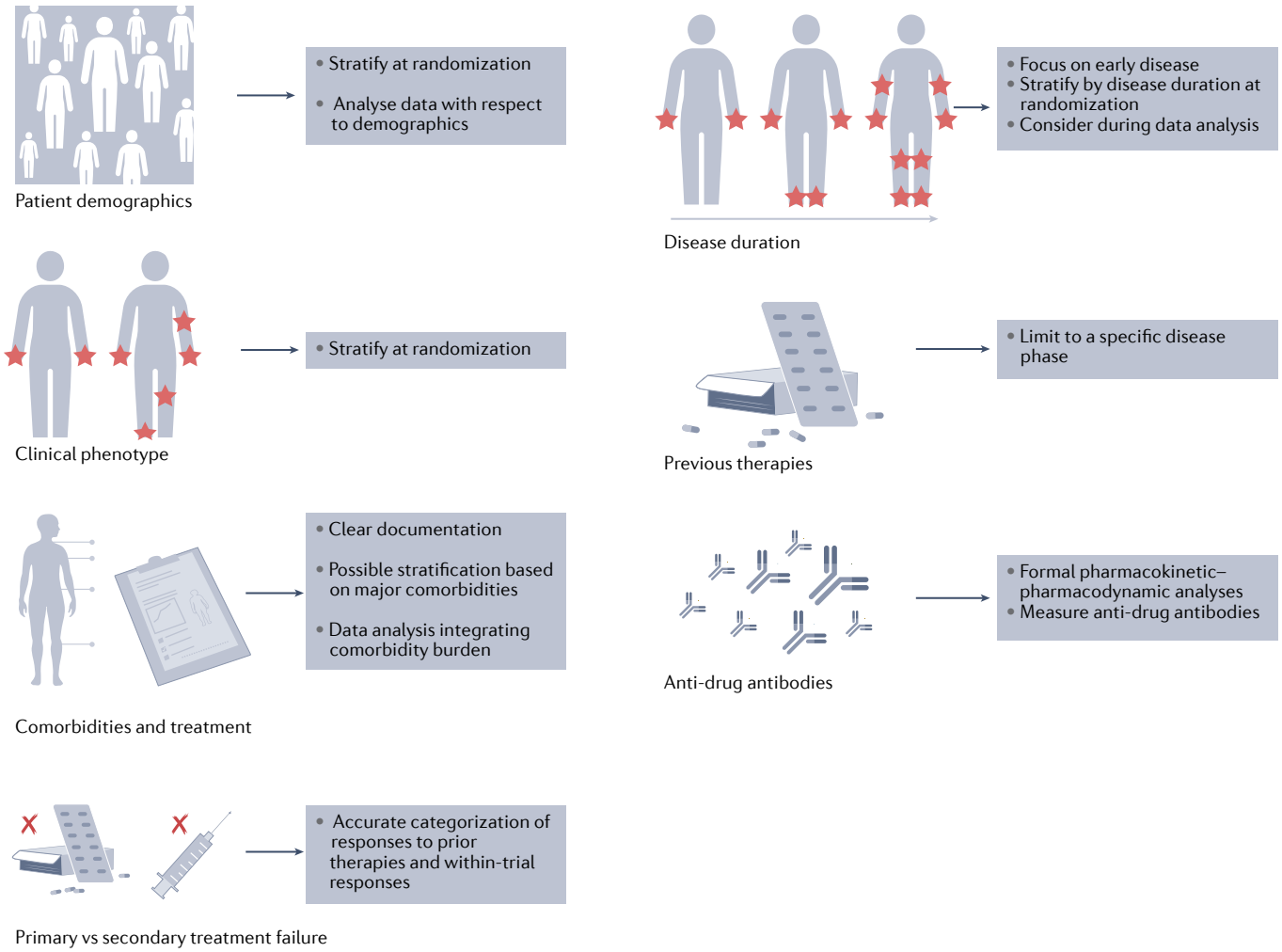

Fig. 4 | **Potential confounders in rheumatoid arthritis clinical trials and how to minimize them.** A number of factors can influence the response of an individual patient to anti-rheumatic therapies. These include demographic factors such as age, sex and socio-economic status, the clinical phenotype (best demonstrated in Sjögren syndrome[51]), the presence and type of comorbidities, disease duration and previous drugs received. Pharmacokinetic factors are also important, such as the concentrations of drugs in the circulation and, for biologics, development of anti-drug antibodies, which is one factor that can lead to secondary treatment failure. These factors should be considered and minimized during trial design and analysis, for example, by stratification at randomization, or via statistical modelling.

measure in some precision-medicine studies[42]. However, it is important to note that CRP and related outcome measures are pharmacodynamically modulated by specific drugs such as IL-6-pathway blockers, so they can provide potentially 'biased' assessments.

In summary, if the 'wrong' biology is assessed in a sub-optimal tissue, using a partially relevant or noisy clinical outcome, it will be hard to identify differences between treatments, particularly when seeking precision therapies. Composite outcomes might be needed for the assessment of RA, on the one hand to confirm control of inflammation and on the other to confirm control of the fundamental downstream pathological condition. Molecular assessments of relevant cells from the site of disease may provide the most robust outcome measures for the latter, but in the meantime we must be as certain as we can that our precision-medicine assessments reflect synovial pathobiology, with a strong link between measured biology and disease outcome. We increasingly apply sophisticated algorithms and artificial-intelligence techniques to large datasets to attempt to identify disease subtypes, but even machine learning can only work when there is something to learn!

Domain 3: confounders

Most RA clinical trials enrol patients with established disease, thereby introducing a number of confounders into the picture. In particular, patients with established RA often have multiple comorbidities (which affect treatment outcomes)[43], such as cardiovascular disease, osteoporosis and anaemia. Fibromyalgia often coexists with RA, and can strongly influence outcome measures with a pain component, such as tender-joint count, and obesity is associated with reduced response to some therapies. Furthermore, drugs prescribed for comorbidities can interfere with outcome measures, as is seen with the anti-inflammatory effects of statins and, possibly, metformin[44,45]. In this way, comorbidities can influence outcome measures directly, for example, by reducing pain thresholds, or by reducing quality of life in general, indirectly reducing efficacy. Patients with a longer disease duration overall tend to have a poorer response to therapy, particularly if several previous therapies have failed[46]. Previous drug failures may even alter disease biology. Evidence suggests, for example, that anti-TNF therapy can 'deviate' pathobiology in some patients to a type 17 T helper cell subtype,

resulting in treatment failure[47,48]. Last, the complex immunobiology of RA means that even 'precision' therapies such as TNF blockade will have indirect effects on other pathways. For example, TNF blockade may indirectly suppress IL-6 (REF.[49]), potentially complicating the analysis of precision-medicine trials.

To avoid some of the confounders associated with RA clinical trials, one approach would be to perform stratification analyses at disease presentation, accepting that sub-clinical disease has likely been present for several years at that juncture. In the future, all individuals might have biological material stored at birth, and although analysis of this material could provide a useful template on which to base treatment decisions, the immune system at that stage would have had minimal environmental exposure and, epigenetically, would be very different to that of the patient with early arthritis. By contrast, we should be able to learn more about the potentially progressive nature of RA from longitudinal studies of pathobiology[34].

Causes of treatment failure vary, and we sometimes refer to primary or secondary failure, depending on the timing of non-response. Primary failure is when a patient fails to respond to a therapy from the outset, whereas a patient with secondary failure responds initially, but subsequently loses therapeutic benefit (a common scenario in RA). This difference is particularly important with biologic therapies (in relation to which immunogenicity can presage secondary failure), yet many precision-medicine studies have failed to distinguish primary from secondary treatment failure, or to consider the presence of anti-drug antibodies as a confounder. Although secondary treatment failure is an important phenomenon, studies of precision medicine in RA should perhaps focus initially on patients with primary treatment failure, for whom there has been no benefit of a recently prescribed therapy.

As in many diseases, treatment response in RA varies according to patient demographics, such as age, sex, educational status and indices of deprivation. It is well recognized, even in trials involving late-phase RA, that response rates can vary in different parts of the world. Clinical phenotype can also influence the response to treatment. RA is clinically heterogeneous, in terms of both joint involvement and mode of onset and associated features. For example, compared with seropositive RA, seronegative RA causes less joint damage but responds less well to therapies[50]. For some rheumatic conditions, disease phenotype may be a stratifier in its own right. For example, in primary Sjögren syndrome, patients can be stratified into specific phenotypes according to their symptom profiles, and each phenotype can subsequently be shown to have a biological basis that underpins it. Furthermore, retrospective application of these phenotypes to clinical-trial data suggests that they have possible theragnostic relevance[51].

In summary, response to treatment in RA reflects a multitude of factors, which need to be considered when assessing therapeutic efficacy (FIG. 4). Attempts to characterize patients theragnostically generally focus on established disease, where it is essential to consider these multiple confounders when designing and interpreting trials.

Domain 4: trial design

When designing clinical trials in RA there are some common pitfalls to avoid. For example, depending on the outcome measure, baseline disease activity can influence response: an ACR70 response or DAS28 improvement is easier to achieve from a position of high initial disease activity than from a position of low disease activity. Conversely, any form of remission is easier to achieve from a position of low initial disease activity. Such factors must be considered during trial design. More fundamentally, however, we believe that the rheumatology community, including the regulators, should embrace contemporary clinical-trial design to facilitate the identification of effective theragnostic approaches. The oncology community has been progressive in the use of 'efficient' trial designs, including Bayesian approaches, but such designs remain rare in rheumatology. These design strategies have the potential to greatly accelerate the identification of novel treatment paradigms compared with the use of conventional double-blind, randomized controlled trials[52]. The novel trial strategies include emergent biomarker-driven randomized controlled trials, umbrella and basket designs, as reviewed previously[53]. These designs incorporate a more patient-centric approach, for example, matching biomarker positivity with specific therapies, thereby accelerating the development of precision-therapy algorithms. Outcome measures and potential confounders remain relevant regardless of trial design, and inclusion and exclusion criteria remain important.

The time may come when multiple IMIDs are studied together in basket trials, utilizing a common tissue-based outcome at the single-cell level[41]. For example, the appearance of a particular macrophage subset in disease tissue could represent an objective outcome that underpins disease remission independently from clinical diagnosis and potential confounding factors[34]. We are entering a new era in our understanding of IMID biology, driven by cutting-edge technologies, which will revolutionize the conceptualization of diseases such as RA, providing opportunities to close the precision gap.

Other rheumatic diseases

The focus of this Perspective is RA, but important insights can also be obtained from consideration of other rheumatic diseases. In systemic sclerosis, the pathogenic cell is clearly fibroblastic, despite the presence of underlying immune dysregulation as in RA. In systemic sclerosis, analysis of samples derived from skin biopsy enables classification of patients into distinct subtypes, with machine-learning algorithms accurately classifying individual patients[54]. In some ways the pathobiological classification of systemic sclerosis is analogous to our previous discussion of RA, with both immune and stromal dysregulation leading to inflammatory and fibro-proliferative subtypes. Notably,

Box 1 | **Key research questions relating to the development of precision medicine for rheumatoid arthritis**

- What is the fundamental pathobiology of rheumatoid arthritis (RA)? Is dysregulated immunobiology the ultimate effector or an enabler of downstream mechanisms that cause tissue destruction?
- What is the optimal clinical outcome (or outcomes) for RA precision-medicine trials?
- How do these clinical outcomes relate to distinct aspects of pathobiology?
- Does synovium provide the most robust outcome measure for RA precision-medicine trials, or are there elements best assessed in blood or by imaging?
- Are there signals in the blood that reflect synovial pathobiology, or should we always biopsy synovium when developing novel precision therapies?
- What are the optimal synovial theragnostic markers? Are there specific cell subtypes or molecular pathways that signify successful treatment?
- How do potential confounders affect outcomes and how do we account for them?
- How can modern trial designs expedite precision therapies for RA?

by studying samples from the Scleroderma: Cyclophosphamide or Transplantation clinical trial[55], it was possible, retrospectively, to identify a particular scleroderma subtype that was associated with response to autologous stem cell transplantation. Furthermore, this could be achieved by studying peripheral blood rather than skin. Although this approach requires validation, it demonstrates how artificial intelligence can be applied to inform theragnostic algorithms and, importantly, how peripheral blood signals may map to pathobiological processes in the tissue.

Many of the arguments applied to RA apply equally well to psoriatic arthritis or spondyloarthritis, in which some 'obvious' targets have not been validated or found to deliver benefit[56,57]. Added complexities with these conditions can be the difficulty of making a definitive diagnosis, for example, in non-radiographic axial spondyloarthritis, and the influence of associated features or conditions (such as inflammatory bowel disease or uveitis) on treatment decisions.

Conclusions

A precision gap exists between oncology and the rheumatic diseases, hindering the development of precision therapies for diseases such as RA. The evolution of our understanding of disease pathobiology, leading to more relevant outcome measures that are less influenced by confounding factors, alongside adoption of contemporary trial designs, has the potential to close the gap in the coming years (BOX 1). We may need to accept more complexity moving forward, with different disease outcomes reflecting distinct elements of underlying pathobiology, and each pathobiological element containing intrinsic heterogeneity and precision-medicine opportunities. The important message is that, for each new therapy, we will need to be clear which aspect of pathobiology is being targeted and select appropriate outcome measures, stratifying for likely confounders. Progress will require collaboration between rheumatologists, patients and regulators, to ensure unanimous support for a step change that will ultimately remove the trial and error from rheumatic-disease therapy.

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In the version of this article initially published, the affiliation for Maurizio Cutolo was incorrectly given as "Department of Rheumatology and Clinical Immunology, Charité University Medicine Berlin, Berlin, Germany" instead of "Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, IRCCS Polyclinic Hospital San Martino, University of Genoa, Genoa, Italy." The change has been made to the HTML and PDF versions of the article.

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In the version of this article initially published, the footnote to Table 1 incorrectly defined IBD as "irritable bowel syndrome" instead of "inflammatory bowel disease." The change has been made to the HTML and PDF versions of the article.

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