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# IL-17A and IL-17F in tissue homeostasis, inflammation and regeneration

Iannis E. Adamopoulos & Vijay Kuchroo

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IL-17 signalling regulates both protective and harmful immune responses; therefore, its complete inhibition can have adverse effects. Detailed consideration and fine-tuning of IL-17-inhibition strategies is needed to selectively regulate disease outcomes.

Rheumatic diseases often entail musculoskeletal, skin and neuropathological components that synthesize the cardinal signs of inflammation (redness, swelling, heat, pain and loss of function). Effective therapy is therefore considered the inhibition of all the active domains of the disease while restoring the loss of function by tissue repair after injury. The pleiotropic ability of cytokines such as IL-17A and IL-17F to regulate inflammatory processes and tissue repair is being unveiled at a fast pace and is of immense therapeutic importance.

The role of IL-17A in inflammation has been widely demonstrated in several mouse models in which mice deficient in IL-17A and/or IL-17F are protected against many autoimmune diseases. Similarly, mice that overexpress IL-17A show severe epidermal hyperplasia and psoriasis-like pathology concurrently with joint inflammation and an increase in osteoclastogenesis, exacerbating bone erosion and loss of joint function[1]. Thus, IL-17A modulates hallmark pathological features associated with a spectrum of rheumatic disorders. Recently, clinical trials of a monoclonal antibody that inhibits both IL-17A and IL-17F (bimekizumab) showed great efficacy across several active domains of psoriatic arthritis, confirming that IL-17A and IL-17F signalling is crucial in multiple pathways and systems in active disease[2].

IL-17A and IL-17F can exist as homodimers or IL-17A–IL-17F heterodimers and can signal through IL-17RA, IL-17RC and IL-17RD receptors (Fig. 1). IL-17A and IL-17F are mainly produced by αβ T (T helper 17, $T_H$17) and γδ T (γδT17) cells; however, an increasing number of innate immune cell types have been associated with their expression in various activation states, including innate lymphoid cells, activated monocytes and neutrophils. The activation and differentiation of cells that produce IL-17A, IL-17F and/or IL-17A–IL-17F are differentially regulated by other cytokines expressed in the microenvironment of each tissue. Thus, different populations of IL-17A- and IL-17F-producing cells might be present in the skin, joint or other tissues where they affect the clinical manifestations and course of the disease. Although $T_H$17 cells co-produce both IL-17A and IL-17F, in some tissue microenvironments IL-17A and IL-17F are produced by different cell types. Moreover, single-cell RNA sequencing demonstrated that even under optimal $T_H$17 cell differentiation conditions in vitro, more T cells produce IL-17F than IL-17A, but that the vast majority of pathogenic $T_H$17 cells produce both cytokines together[3]. Therefore, the first important consideration is that different subtypes of IL-17A, IL-17F or IL-17A–IL-17F double-producing cells are present at different tissues and hence IL-17 inhibition outcomes will vary.

Similarly, the expression of IL-17 receptors is also differentially regulated in different tissues. IL-17RD, which was previously considered an orphan receptor, is highly expressed in skin relative to other tissues. IL-17RD forms a heterodimer with IL-17RA that directly binds IL-17A but not IL-17F or the IL-17A–IL-17F heterodimer[4]. IL-17A-mediated gene expression is defective in *Il17rd*-deficient keratinocytes; however, IL-17F and/or IL-17A–IL-17F expression remain unaffected. Furthermore, *Il17rd* deficiency in non-haemopoietic cells attenuates imiquimod-induced psoriasis-like skin inflammation. Therefore, a second consideration for IL-17 inhibition strategies is that in addition to the complexities of IL-17A and IL-17F expression in various cells and microenvironments, the effects of these cytokines are also modulated at the receptor level. The differential regulation of IL-17 expression and IL-17RD signalling in the skin versus the joints could account for the discrepancy in the efficacy of IL-17A inhibition between individuals with psoriasis and those with psoriatic arthritis.

A third consideration is that IL-17 signalling is not strictly pathogenic but has beneficial roles in mediating tissue homeostasis, and contributes to regeneration after tissue injury including bone fracture and muscle damage. In homeostasis, $T_H$17 cells are present in vast numbers at mucosal surfaces, where IL-17 has been shown to have a crucial role in limiting microbial invasion and promoting barrier functions. Although IL-17A clearly contributes to bone destruction by increasing RANK expression in osteoclast precursors, IL-17A can also promote bone formation. Indeed, bone repair is impaired in IL-17A-deficient mice owing to a defect in osteoblastic bone formation, and IL-17A stimulation accelerates bone formation by enhancing osteoblast proliferation and differentiation[5]. Together with IL-22, another hallmark $T_H$17 cytokine, IL-17 has been shown to promote epithelial growth and thereby heal injured mucosal barriers. Other studies have shown that IL-17A also promotes muscle regeneration after acute injury[6]. This muscle regeneration process involves the accumulation of IL-17A-producing γδ T cells at the wound site, which orchestrates the early inflammatory events of the process, and specifically the recruitment of neutrophils that foster the proliferation of muscle stem and progenitor cells to accomplish regeneration[6]. Oligoclonal expansion of γδ T cells that favour myelopoiesis and neutrophil recruitment has also been described in infection and inflammatory arthritis, demonstrating similar patterns between inflammatory and regenerative events[7,8]. The exact molecular triggers that dictate when these cells become pathogenic or regenerative remains to be determined.

A fourth consideration is the importance of IL-17-producing innate immune cells, which have been largely overshadowed by $T_H$17 and γδT17 cells. Although the exact role of these IL-17-expressing innate immune cells is still under investigation, IL-17 released from

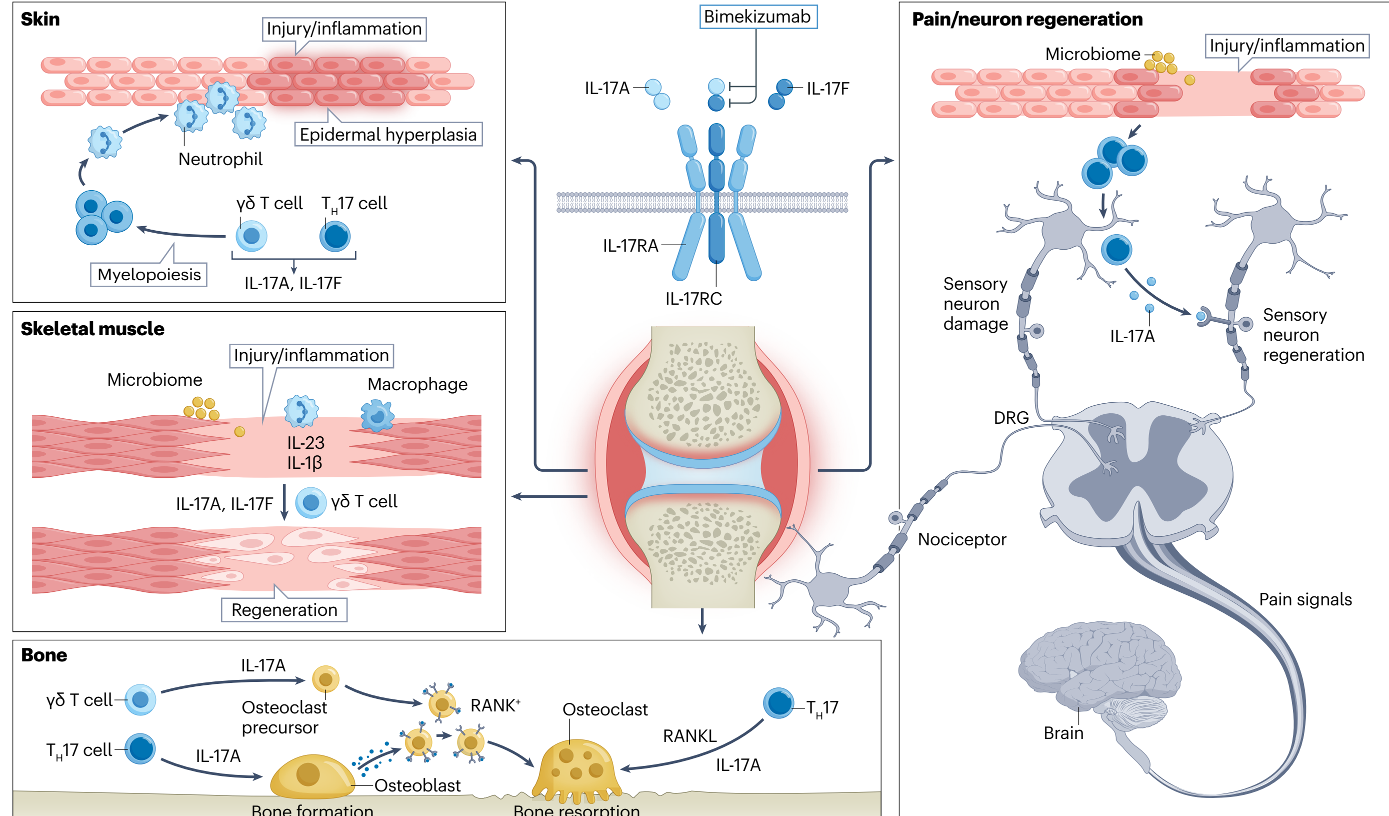


**Fig. 1 | IL-17 signalling in joint inflammation and regeneration.** IL-17A and IL-17F homodimers and heterodimers activate IL-17R downstream signalling through IL-17RA and IL-17RC receptor complexes. The differential expression of specific IL-17 receptors in each tissue, such as IL-17RD in the skin, dictates the tissue-specific potency of IL-17A and IL-17F. The effects of IL-17 signalling are also regulated by the presence of other inflammatory mediators, formulated by the unique tissue-specific resident cells and presence of commensal bacteria in homeostatic or inflammatory conditions. These inflammatory changes regulate tissue-destruction and also regulate tissue regeneration after injury.

macrophages is crucial in evoking mechanical pain via the activation of TRPV1-nociceptors leading to hyperalgesia in inflammatory arthritis[9]. Although IL-17-expressing macrophages are involved in the pain pathway, another report demonstrated that IL-17-expressing T cells promote neuronal axon growth and local nerve regeneration after injury. Injury induces the expression of IL-17RA in the dorsal root ganglion sensory neurons, which accept signals from IL-17A released by commensal-specific $T_H17$ cells. This IL-17A–IL-17RA-dependent signalling promotes the regeneration of peripheral sensory neurons[10]. These findings not only provide key insights into the cause of hyperalgesia in inflammatory arthritis but also indicate the need for further research to fully determine the proper strategy for blocking pathogenic IL-17A signalling while avoiding the inhibition of the homeostatic and/or regenerative capacity of IL-17.

The use of anti-IL-17 to treat inflammatory bowel disease exemplifies this phenomenon, as anti-IL-17 did not help inhibit tissue inflammation but made the disease worse. Clearly, the interplay between effectors and transducers that regulate the pleiotropic effects of IL-17A and IL-17F in tissue homeostasis and inflammation needs to be carefully examined to exploit the beneficial effects of IL-17 but inhibit its pro-inflammatory effects.

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## Competing interests

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**Vasculitis**

## Platelets promote cardiovascular complications in Kawasaki disease

Kawasaki disease is a childhood vasculitis characterized by inflammation of blood vessels, including coronary arteries, and is a leading cause of heart disease among children. New research provides mechanistic insight into how platelets contribute to the development of cardiovascular lesions in Kawasaki disease.

"Increased platelet count (thrombocytosis) is a common feature of Kawasaki disease and is typically reported in the second or third week after disease onset, usually when coronary artery aneurysms appear," explains Moshe Arditi, corresponding author on the study. "However, the role of platelets in the pathogenesis, development, progression and severity of cardiovascular lesions during Kawasaki disease is not well understood."

To better understand the underlying mechanisms, the researchers first analysed transcriptomic datasets, finding that the expression of various platelet-related genes in the blood was upregulated in patients with Kawasaki disease compared with healthy individuals, and expression was decreased during the convalescent phase of the disease (after intravenous immunoglobulin therapy).

In a mouse model of Kawasaki disease, induced by injection with *Lactobacillus casei* cell wall extract (LCWE), the number of platelets increased during disease progression, peaking at 2 weeks, similar to that observed in patients with Kawasaki disease. Notably, the platelet count correlated with the severity of cardiovascular lesions. Depletion of platelets, either by genetic depletion (using thrombocytopenic $Mpl^{-/-}$ mice) or treatment with an anti-CD42b antibody, reduced the severity of cardiovascular inflammation and lesions in the mice.

Platelets are best known as mediators of haemostasis and thrombosis but have an increasingly recognised role in inflammation and can secrete various pro-inflammatory mediators (such as calprotectin). Platelets can also form aggregates with monocytes (known as monocyte–platelet aggregates (MPAs)) to enhance their inflammatory functions, including the secretion of pro-inflammatory cytokines, such as IL-1β.

In the latest study, the expression of calprotectin and IL-1β, and the frequency of MPAs, were increased after LCWE injection in mice. These effects were diminished with platelet depletion, suggesting that platelets contribute to the development of Kawasaki disease-associated cardiovascular lesions by promoting the formation of MPAs and the release of IL-1β.

"Circulating levels of MPAs and calprotectin could have clinical utility as biomarkers of Kawasaki disease severity, and clinical studies are needed to assess the therapeutic value of blocking or inhibiting MPA formation and calprotectin to reduce the cardiovascular complications associated with this disease," says Arditi. "These mechanistic studies also emphasize the potential role of IL-1β in Kawasaki disease and should accelerate phase III clinical trials of IL-1R blockade (using anakinra)."

**Jessica McHugh**

**Original article:** Kocatürk, B. et al. Platelets exacerbate cardiovascular inflammation in a murine model of Kawasaki disease vasculitis. *JCI Insight* **8**, e169855 (2023)

**Osteoarthritis**

## Urchin-like nanoparticles for miRNA therapy of OA

The lack of disease-modifying therapies hinders the treatment of osteoarthritis (OA). In new research, the delivery and effectiveness of OA gene therapy was enhanced by the use of structurally modified nanoparticles.

Intra-articular microRNA (miRNA)-based gene therapy can potentially treat OA. However, effective therapy requires miRNA stability and cellular uptake, which can be achieved by complexing miRNA with suitable nanoparticles. In addition to acting as miRNA carriers, nanoparticles can have inherent properties that affect specific OA pathological features, such as oxidative stress.

Researchers have now found that the miRNA miR-224-5p is overexpressed in articular tissue of patients with OA relative to those without OA. Chondrocyte-specific deletion of this miRNA in mice enhanced joint-space narrowing and cartilage degradation in the OA model of destabilization of the medial meniscus (DMM), demonstrating a beneficial effect of miR-224-5p on OA pathology.

To deliver miR-224-5p, sea-urchin-like ceria nanoparticles were created by hydrothermal reaction between $Ce(NO_3)_3{\cdot}6H_2O$, $Na_3PO_4$ and deionized water. These nanoparticles had thorn-like projections that greatly increased their surface area relative to 'spherical' ceria nanoparticles generated by a modification of the hydrothermal reaction. Both types of nanoparticle scavenged reactive oxygen species, but although they had similar activity against superoxide anions, the urchin-like particles had greater activity against $H_2O_2$.

When nanoparticles were mixed with miR-224-5p, transfection into mouse chondrocytes was greater with the urchin-like nanoparticles than with spherical nanoparticles. In vivo, intra-articular injection of urchin-like nanoparticles had low toxicity and was followed by gradual clearance.

In vitro, complexes of miR-244-5p and urchin-like nanoparticles reduced oxidative stress, inhibited TNF-induced apoptosis and promoted autophagy in mouse chondrocytes. They also inhibited TNF-induced pathological changes in extracellular matrix production. In the DMM mouse model, a high dose of urchin-like ceria nanoparticles had beneficial effects on joint-space narrowing, osteophyte formation, subchondral bone microstructure, synovial hyperplasia and neovascularization, and these effects were enhanced by the addition of miR-224-5p.

"Our study provides a new option of non-viral delivery vectors for gene therapy of OA," notes corresponding author Dalong Ni. "Research on these vectors is still in its early stages, requiring further understanding of their mechanisms of action through in-depth studies."

**Robert Phillips**

**Original article:** Chen, H. et al. Urchin-like ceria nanoparticles for enhanced gene therapy of osteoarthritis. *Sci. Adv.* **9**, eadf0988 (2023)

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# Research highlights

## Inflammation

## Interosseous tendon inflammation associated with arthritis risk

Imaging studies have shown that inflammation in extra-articular tendons and bursae is an early feature of rheumatoid arthritis (RA). Research now indicates that inflammation around the hand interosseous tendons also manifests before the development of RA and is associated with the risk of developing the disease.

Interosseous tendon inflammation (ITI) was previously described in cohorts of patients with RA and in anti-citrullinated protein antibody (ACPA)-positive at-risk individuals, but its relationship to RA progression remained unclear. The latest study was undertaken to further investigate the presence of ITI in symptom-free healthy individuals and in ACPA-positive and ACPA-negative individuals with clinically suspect arthralgia (CSA), and to examine the association of ITI with RA development, symptoms and inflammation in other local tissues.

Among 667 individuals with CSA who underwent contrast-enhanced MRI evaluation of the hands, ITI was present in 67 (10%); by comparison, only 2 (1%) of 193 symptom-free healthy individuals had ITI on MRI. ITI was present more frequently in ACPA-positive patients with CSA than in those who were ACPA-negative (27% versus 7%).

ITI was independently associated with subclinical inflammation of other tissues at the metacarpophalangeal joints, including synovitis (odds ratio (OR) 2.2; 95% confidence interval (CI) 1.2–4.2) and tenosynovitis (OR 9.7; 95% CI 5.5–17.0). 3D MRI reconstruction suggested that inflammation of the interosseous tendons co-occurred with metacarpophalangeal-flexor tenosynovitis.

Clinical arthritis, defined as at least one swollen joint among the 66 examined, developed in 16% of individuals with CSA during follow-up (median 25.3 months). The risk of developing clinical arthritis was higher in those who had CSA with ITI than those without ITI (hazard ratio 4.5; 95% CI 2.8–7.2); this association did not differ significantly between ACPA-positive and ACPA-negative individuals.

**"The risk of developing clinical arthritis was higher in those who had CSA with ITI"**

Immunohistochemistry analysis showed that synovial tissue was absent around interosseous tendons, which indicates that ITI is not tenosynovitis but non-synovial peritendinous inflammation. The investigators suggest that ITI could therefore be considered as the first sign of tendon involvement in the development of RA. Further studies are needed to determine the sequence in which subclinical inflammation affects the various tissues in joints in the pre-arthritis stage and how these inflamed tissues interact as clinical RA develops.

**Sarah Onuora**

**Original article:** van Dijk, B. T. et al. Interosseous tendon inflammation in the hands of patients with clinically suspect arthralgia: analysis of MRI data from a prospective cohort study. *Lancet Rheumatol.* **5**, e401–e412 (2023)

## Rheumatoid arthritis

## Aminoacyl-tRNA synthetases function as alarmins in RA

Aminoacyl-tRNA synthetases (ARSs) are a highly conserved family of enzymes that are essential for protein synthesis; they also have non-canonical functions that induce innate immune responses, but whether they directly contribute to the pathogenesis of autoimmune diseases, including rheumatoid arthritis (RA), has been unclear. Findings from a new study reveal that extracellular ARSs function as potent alarmins in RA and could be a therapeutic target.

The researchers demonstrated that serum concentrations of several ARSs were higher in patients with RA than in healthy individuals, and for some ARSs, such as tyrosyl-tRNA synthetase, cysteinyl-tRNA synthetase and alanyl-tRNA synthetase, serum concentrations correlated with RA disease activity. Concentrations of several ARSs were likewise elevated in the synovial fluid of patients with RA in comparison with that of patients with osteoarthritis.

All 20 of the ARSs tested by the researchers were able to induce the production of pro-inflammatory cytokines, including IL-6 and TNF, in primary macrophages. Further investigations revealed that the ARSs induced cytokine production and nuclear factor-κB activation via Toll-like receptor 4 (TLR4), the TLR4 co-receptor MD2 (also known as lymphocyte antigen 96) and CD14. The immune responses induced by ARSs were more potent than those induced by other alarmins, including high-mobility group box 1 (HMGB1) and S100A8/A9 (calprotectin).

Stimulation of macrophages with human tyrosyl-tRNA synthetase led to the release of peptidyl arginine deaminase 4 (PAD4, an enzyme that mediates citrullination) via TLR4, but stimulation with HMGB1 or S100A8/A9 did not. Notably, as well as PAD4, citrullination of tyrosyl-tRNA synthetase was detected in the supernatant of the cultured macrophages, which suggests that the release of PAD4 by ARSs could induce citrullination of self proteins.

The researchers then evaluated the therapeutic potential of ARS inhibitory peptides in two mouse models of RA: collagen-induced arthritis (CIA) and collagen antibody-induced arthritis (CAIA). In CAIA mice, administration of the tyrosyl-tRNA synthetase inhibitory peptide YP51 ameliorated arthritis symptoms and YP51-treated CAIA mice had considerably reduced serum concentrations of cytokines and ARSs compared with control mice. In the CIA model, administration of YP51 prevented the development of arthritis and serum concentrations of IL-6 and PAD4 were decreased in YP51-treated mice compared with control mice.

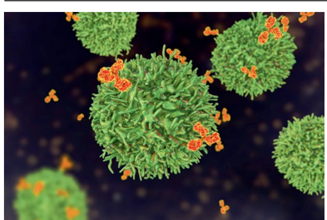
Together, the findings suggest that extracellular ARSs contribute to the pathogenesis of RA by functioning as alarmins, and that blockade of ARSs could offer a novel option for the treatment of this disease.

**Sarah Onuora**

**Original article:** Kimura, A. et al. Extracellular aaRSs drive autoimmune and inflammatory responses in rheumatoid arthritis via the release of cytokines and PAD4. *Ann. Rheum. Dis.* https://doi.org/10.1136/ard-2023-224055 (2023)

## Autoimmunity

# Non-depleting anti-CD19 B cell inhibition



B cell depletion therapy (BCDT) can be an effective approach to the treatment of autoimmune diseases, but incomplete depletion affects outcomes. A newly developed antibody therapy takes a non-depleting approach to B cell inhibition and has demonstrated greater efficacy than BCDT in pre-clinical models of autoimmune disease.

A common target in BCDT, CD20, is not expressed at all stages of B cell development. Whereas CD20 expression begins at the pre-B cell stage and diminishes at the plasmablast differentiation stage, CD19 is expressed earlier, at the late pro-B cell stage, and its expression continues at least until the plasma cell stage. Targeting CD19 can affect a wider range of B cells than anti-CD20 BCDT, including plasmablasts that are associated with autoimmune disease.

In new research, a CD19-specific antibody was identified and modified to optimize its biophysical properties, including hydrophobicity and affinity. The modified antibody (LY3541860) bound specifically to human B cells, but it did not induce antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity or B cell apoptosis.

LY3541860 did, however, inhibit the induction of B cell proliferation in vitro in a dose-dependent way. In whole blood, it also inhibited activation of B cells that was induced by TLR9 agonists (CpG oligonucleotides).

CD19 is expressed on antibody-secreting plasmablasts, and LY3541860 inhibited the differentiation of memory B cells into plasmablasts in vitro, demonstrating its potential to prevent pathogenetic autoantibody production.

CD19 is known to promote assembly of the B cell receptor (BCR) complex, which is necessary for amplification of BCR signalling. The researchers found that LY3541860 inhibited assembly of the BCR complex and prevented phosphorylation of downstream signalling components.

In animal models of the autoimmune diseases rheumatoid arthritis (RA), multiple sclerosis and type 1 diabetes, LY3541860 had greater efficacy than BCDT. For example, in the collagen-induced arthritis mouse model of RA, treatment with LY3541860 resulted in significantly greater reduction of clinical scores and joint-histology scores than did treatment with BCDT.

The researchers further demonstrated that suspending treatment of mice for 4 weeks resulted in complete washout of LY3541860, which enabled induction of B cell activation. Efficacy and reversibility are desirable characteristics for B cell–targeting therapies, and these results suggest that LY3541860 has great potential for the treatment of autoimmune disease.

**Robert Phillips**

**Original article:** Boyles, J. S. et al. A nondepleting anti-CD19 antibody impairs B cell function and inhibits autoimmune diseases. *JCI Insight* **8**, e166137 (2023)

## Paediatric rheumatology

# Oral JAK inhibitor baricitinib effective in JIA

Baricitinib, an oral inhibitor of Janus kinase 1 (JAK1) and JAK2, is safe and effective in the treatment of patients with juvenile idiopathic arthritis (JIA), according to the results of a new phase III, placebo-controlled, withdrawal trial published in *The Lancet*.

A previous phase III trial of tofacitinib had highlighted the efficacy of JAK inhibition in JIA. The latest JUVE-BASIS trial aimed to assess the safety and efficacy of baricitinib in 220 patients with various forms of JIA (polyarticular JIA, extended oligoarticular JIA, enthesitis-related arthritis or juvenile psoriatic arthritis) who had had an inadequate response to conventional synthetic or biologic DMARD therapy.

The trial consisted of three phases: a 2-week safety and pharmacokinetic phase for a subset of patients to verify age-based dosing, a 12-week open-label lead-in phase (or 10 weeks for the safety and pharmacokinetic assessment cohort), and a placebo-controlled double-blind withdrawal phase lasting up to 32 weeks. Patients who met specific response criteria during the open-label phase were randomly assigned to receive either placebo or baricitinib in the double-blind withdrawal phase.

Among the 219 patients who received baricitinib during the open-label phase, 163 (74%) met the response criteria and entered the double-blind withdrawal phase. The primary endpoint (time to disease flare during this phase) was shorter in the placebo group than in the baricitinib group (hazard ratio 0.241). The median time to flare was 27.14 weeks in the placebo group, but could not be evaluated in the baricitinib group, as less than 50% of patients experienced a flare event (17% of the patients versus 51% in the placebo group).

Baricitinib treatment led to clinically relevant improvements in disease activity, as evidenced by JIA–ACR30, JIA–ACR50 and JIA–ACR70 response rates, and also had positive effects on patient-reported outcomes, such as pain, physical function and health-related quality of life.

The safety profile of baricitinib in JIA was consistent with that observed in other indications for adults. No new safety concerns were identified, although the incidence of infections was higher in the baricitinib group. The oral suspension formulation of baricitinib was also well accepted and easy to administer.

"Children with arthritis and their families have long been asking for an effective oral option to injectable biologics," explains Athimalaipet Ramanan, corresponding author on the study. "Baricitinib, as an efficacious once-a-day oral agent, offers our patients and clinicians a more acceptable treatment option."

**Jessica McHugh**

**Original article:** Ramanan, A. V. et al. Baricitinib in juvenile idiopathic arthritis: an international, phase 3, randomised, double-blind, placebo-controlled, withdrawal, efficacy, and safety trial. *Lancet* https://doi.org/10.1016/S0140-6736(23)00921-2 (2023)

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Vasculitis

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# A basket genetic trial of the vasculitides

Elena Carnero-Montoro & Marta E. Alarcón-Riquelme

The rarity of various forms of vasculitis, as well as other rheumatic diseases, presents difficulties in studying the genetics of these diseases as well as for evaluating treatments. Might new approaches, such as joint genetic analyses and drug repurposing, provide opportunities to learn more about these diseases and identify new therapies and serve as a basis for basket clinical trials?

REFERS TO Ortiz-Fernandez, L. et al. Identification of new risk loci shared across systemic vasculitides points towards potential target genes for drug repurposing. *Ann. Rheum. Dis.* https://doi.org/10.1136/ard-2022-223697 (2023).

The vasculitides are a group of entities that affect small, medium and large blood vessels and that are caused by various different mechanisms. The rarity of these entities poses many challenges in research and clinical trials owing to the need for sufficient numbers of patients. Basket clinical trials are a new type of clinical trial, originating from the field of oncology, designed to investigate the efficacy of an intervention in multiple diseases of similar etiology (such as different types of tumours) simultaneously[1]. In a new study, Marquez and co-workers[2] have used a similar approach to a basket clinical trial to identify shared and novel loci genetically associated with diseases across the vasculitis spectrum, the findings of which could potentially pave the way for basket clinical trials in vasculitis. The researchers then used these results to perform a drug repurposing analysis. The findings of this latest study highlight the feasibility of using a basket clinical trial approach in the context of a genetic association study for diseases that are, in general, rare and for which genetic association studies for each single entity is difficult to perform.

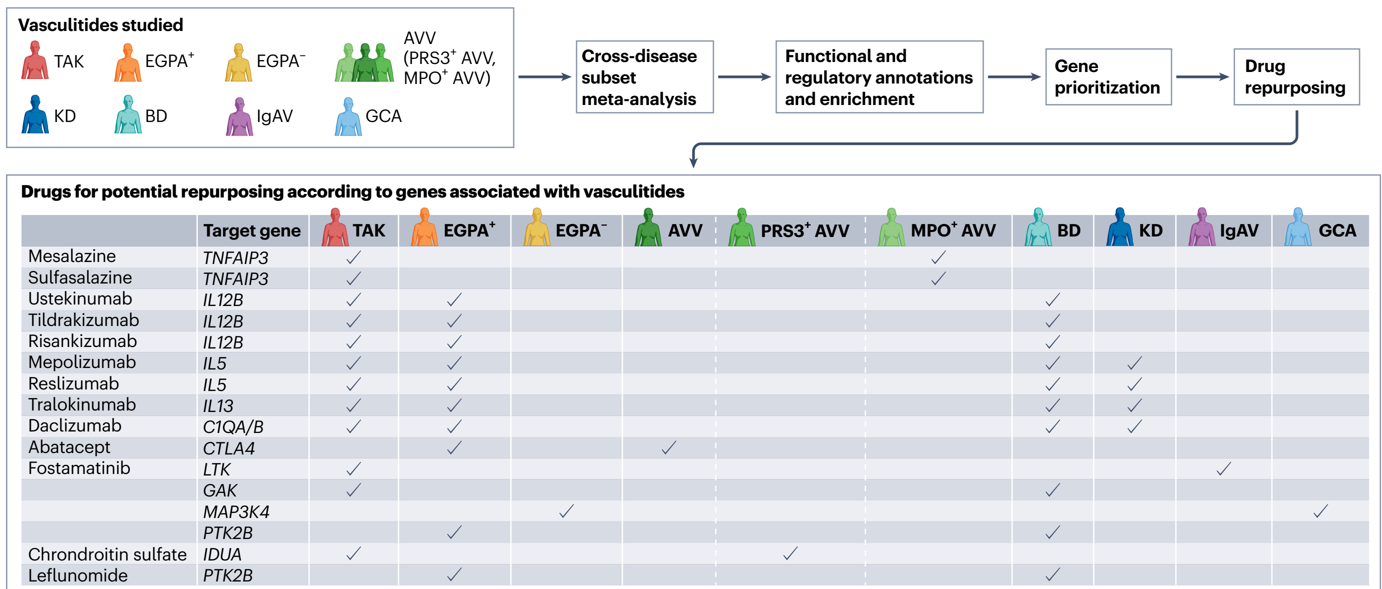
As a multi-centre collaborative effort, the study involved over 8,000 patients and nearly 30,000 healthy individuals. The analysis included patients with any of the main vasculitides, including various forms of large-vessel vasculitis (such as giant cell arteritis (GCA) and Takayasu arteritis), medium vessel arteritis (such as Kawasaki disease) and small vessel arteritis (subdivided into anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), namely granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA), ANCA–positive or ANCA-negative eosinophilic granulomatosis with polyangiitis (EGPA), and immune complex vasculitis, including IgA vasculitis (IgAV)). In addition, the analysis included patients with Behçet disease, a disease presenting with vasculitis affecting vessels of any size. With this approach, the researchers identified 12 new shared loci harbouring 14 independent genetic signals. Twelve of the loci had been previously identified in one of the diseases and two were completely novel for vasculitis. Two independent subgroups of patients with AAV were also considered on the basis of positivity for antibodies targeting myeloperoxidase (MPO) or proteinase 3 (PR3), which yielded three additional new loci.

The researchers assessed both genome-wide association datasets and immunochip datasets for genomic overlap and, as expected, most of the variants were localized to non-coding regions. Notably, the majority of shared genomic variants overlapped with predicted regulatory regions of immune cells, whole blood and/or arterial tissues, relevant for the diseases in question. Such regulatory regions were enriched in histone modifications specific for monocytes, B cells and $CD4^+$ T cells, cell types that are already implicated in vasculitis, as well as natural killer cells, highlighting a new target cell worth further investigation in this group of diseases.

Of the various shared loci identified, a few in particular stand out. For example, one potentially damaging missense variant was mapped to *BCL2L11*, encoding a BH3 domain-containing protein that is involved in apoptosis and B cell homeostasis. In cancer, so called BH3 mimetics are used to induce apoptosis of cancer cells in various B cell malignancies[3]; similar drugs could potentially be used to inhibit pathogenic B cells in vasculitis. A second non-synonymous variant was identified in *PLG*, encoding the inflammation and thrombosis regulator plasminogen; when activated, plasminogen converts to the serine protease plasmin and is involved in fibrin dissolution, as part of the terminal and homeostatic phases of the coagulation cascade. Mutations in *PLG* and plasminogen deficiencies are well-characterized and lead to severe thrombotic events[4]. Plasminogen is also believed to regulate macrophage migration towards inflammation sites[4]. In the latest study by Marquez and co-workers[2], the *PLG* variant was associated with both GCA and ANCA-negative EGPA and could explain the presence of thromboembolic events in these diseases. If this variant causes *PLG* deficiency, replacement therapy could be a potential therapeutic avenue of interest.

Another of the regions containing a shared risk locus, *CCR3* located in chromosome 3p21.31, has already been associated with severe COVID-19 (ref. 5) and Sjögren syndrome[6]. *CCR3* encodes the chemokine receptor CCR3 (also known as the Eotaxin receptor) that was originally found in eosinophils, but is also expressed by T helper cells in inflammatory infiltrates. Unexpectedly, this risk locus was associated with Behçet disease, IgAV and PR3 antibody-positive AVV, rather than eosinophilic vasculitides such as EGPA. However, other non-eosinophil mechanisms might explain this association and indeed the odds ratio indicates that this association has a protective effect. Finally, the researchers identified two genetic risk loci that had not previously been associated with any form of vasculitis (*CTLA4* and *CPLX1)*, supporting the utility of the basket strategy.

These latest findings are of high interest and relevance to the field, generating new knowledge and hypotheses about potential new targets for vasculitis. Nevertheless, as mentioned by the authors, caution must be taken when interpreting these findings and when considering whether the genes identified are truly causal. Genes were prioritized on the basis of in silico functional annotations or whether the variants had showed an association with gene expression in published expression quantitative trait loci (eQTL) studies. For this reason, pleiotropic




| | Target gene | TAK | EGPA+ | EGPA− | AVV | PRS3+ AVV | MPO+ AVV | BD | KD | IgAV | GCA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mesalazine | *TNFAIP3* | ✓ | | | | | ✓ | | | | |
| Sulfasalazine | *TNFAIP3* | ✓ | | | | | ✓ | | | | |
| Ustekinumab | *IL12B* | ✓ | ✓ | | | | | ✓ | | | |
| Tildrakizumab | *IL12B* | ✓ | ✓ | | | | | ✓ | | | |
| Risankizumab | *IL12B* | ✓ | ✓ | | | | | ✓ | | | |
| Mepolizumab | *IL5* | ✓ | ✓ | | | | | ✓ | ✓ | | |
| Reslizumab | *IL5* | ✓ | ✓ | | | | | ✓ | ✓ | | |
| Tralokinumab | *IL13* | ✓ | ✓ | | | | | ✓ | ✓ | | |
| Daclizumab | *C1QA/B* | ✓ | ✓ | | | | | ✓ | ✓ | | |
| Abatacept | *CTLA4* | | ✓ | | ✓ | | | | | | |
| Fostamatinib | *LTK* | ✓ | | | | | | | | ✓ | |
| | *GAK* | ✓ | | | | | | ✓ | | | |
| | *MAP3K4* | | | ✓ | | | | | | | ✓ |
| | *PTK2B* | | ✓ | | | | | ✓ | | | |
| Chrondroitin sulfate | *IDUA* | ✓ | | | | ✓ | | | | | |
| Leflunomide | *PTK2B* | | ✓ | | | | | ✓ | | | |

**Fig. 1 | Study design and novel genes and drug repurposing candidates identified.** The researchers performed a cross-disease subset meta-analysis, revealing many new shared loci across different groups of vasculitis forms, including anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV; a group consisting of granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) and further subdivided according to positivity for antibodies targeting proteinase 3 (PR3) or myeloperoxidase (MPO)), Behçet disease (BD), ANCA-positive eosinophilic granulomatosis with polyangiitis (EGPA+), ANCA-negative EGPA (EGPA−), giant cell arteritis (GCA), Kawasaki disease (KD), IgA vasculitis (IgAV) and Takayasu's arteritis (TAK). After the meta-analysis, the researchers assessed overlap between the associated loci and public functional and regulatory annotations and performed enrichment analyses. Genes were prioritized within associated loci if their expression showed genetic regulation or if the genes overlapped with regulatory elements. Using the prioritized genes, a drug repurposing analysis was performed to identify potential new targets for existing therapies.

relationships cannot be ruled out. Further experimental validation is needed to confirm the causal role and mechanisms of these genes in the vasculitides.

Results from this work combined with prior knowledge-based functional annotation led to the identification of over 100 putative causal genes. Importantly, many of these genes encoded proteins targeted by existing therapies, including drugs currently used as therapy for various autoimmune disorders, that could serve as drug-repurposing candidates in vasculitis. Notably, these drugs included abatacept (targeting *CTLA4*) and ustekinumab (targeting *IL21B*), which are both currently being assessed in clinical trials for the treatment of some forms of vasculitis, and fostamatinib, a tyrosine kinase inhibitor that targets several of the potential causal genes identified (Fig. 1).

Many of the genes identified by Marquez and co-workers[2] have been associated not only with vasculitis, but also with rheumatoid arthritis and systemic lupus erythematosus (such as *TNFAIP3*)[7,8]. This work provides support to the concept that vasculitides and other rheumatic diseases share various underlying genetic and molecular mechanisms, provides insight in the heterogeneity of these diseases and paves the way for basket trials of these diseases as the field moves towards precision medicine[9]. The idea of throwing diseases into a basket for assessment in clinical trials could be highly productive and could be accompanied by molecularly based classifications of rheumatic diseases[9]. Such basket clinical trials could benefit a larger number of patients with rheumatic diseases that are otherwise too rare for the development of new and expensive disease-specific therapies.

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## Competing interests

The authors declare no competing interests.

Myositis

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# Insights into juvenile myositis via engineered muscle

Hanna Kim

The pathogenesis of juvenile dermatomyositis (JDM) is complex and various evidence implicate a role for type I interferons. Could the use of a bioengineered paediatric skeletal muscle model provide insight into this disease and have potential for high throughput testing of therapeutic agents?

Refers to Covert, L. T. et al. Effect of type I interferon on engineered pediatric skeletal muscle: a promising model for juvenile dermatomyositis. *Rheumatology (Oxford)* https://doi.org/10.1093/rheumatology/kead186 (2023).

Juvenile dermatomyositis (JDM) is a rare systemic autoimmune vasculopathy characterized by notable morbidity including muscle weakness that can affect daily activities, skin rashes, and rarely, even death. Most individuals with JDM have chronic or polycyclic disease, and frequently experience medication-associated adverse effects, particularly from systemic corticosteroids[1,2]. Therapies that specifically target known pathogenic mechanisms could mitigate the adverse effects of treatment and provide more efficacious disease management. However, undercovering such pathogenic mechanisms is challenging partially owing to the lack of accurate disease models that can fully encapsulate the disease. In a new study, Covert et al.[3] have developed a bioengineered three-dimensional (3D) skeletal muscle model of JDM that might provide useful insights into the pathogenesis and treatment of this debilitating disease.

Several factors contribute to the pathogenesis of JDM, including genetic risk factors, environmental triggers, immune dysregulation and inflammatory responses[1]. In particular, interferons are implicated in the disease and are linked to hallmark features of JDM including perifascicular atrophy and upregulation of MHC class I in the muscle[1,4]. Endoplasmic reticulum (ER) stress pathways are also implicated in JDM[1]. Although myositis-specific autoantibodies define specific subgroups within JDM, their pathogenic role has not been fully determined[1]. Many studies have identified the presence of an interferon signature (associated with the activity of type I interferons such as interferon-α (IFNα) and IFNβ, and the type II interferon IFNγ) in the blood, muscle and skin of children or adults with dermatomyositis, characterized by upregulated expression of various interferon-regulated genes and proteins. These interferon or interferon-regulated markers correlate with disease activity, highlighting the importance of these pathways in JDM pathogenesis, although the exact mechanisms remain unclear[1,2,5]. Janus kinase (JAK) inhibitors are known to attenuate interferon signalling, and JAK inhibitor treatment in patients with refractory disease has resulted in marked clinical improvement and corresponded with decreased interferon signalling[1,2], highlighting the importance of interferon signalling in JDM.

A well-established animal model that simultaneously recapitulates the key features of JDM including the presence of autoantibodies, muscle weakness, vasculopathy and skin rashes is lacking. In vitro experiments can provide insights into the mechanisms of specific disease-related components. For example, in vitro treatment of healthy myoblasts and myotubes with type I interferon decreases their cell size, an effect that is reversed when the cells are pre-incubated with the JAK inhibitor ruxolitinib, anti-IFNAR antibodies or anti-interferon antibodies[6]. Similarly, incubation of human muscle stem cells with IFNβ reproduces various features of muscle cells in dermatomyositis and JDM such as reduced proliferative capacity and poor myotube formation, which is also rescued by anti-IFNAR antibody or ruxolitinib treatment[7]. However, although these in vitro studies provide mechanistic insights, they fail to establish a direct link with tissue-level disease features[6,7]. For example, changes in muscle strength with different exposures (such as exposures to interferons) cannot be assessed with typical in vitro cell-based or two-dimensional (2D) models, even though weakness is an important cause of morbidity in JDM. Biomechanical engineering has led to the development of 3D models of muscle with contractile properties that can be quantified[8]. Such models have the potential to study pathogenic factors in a more functionally realistic setting.

In the latest study, Covert et al.[3] used an engineered 3D muscle model to assess the effect of type I interferon and/or JAK inhibitor treatment on muscle structure and function, to better understand the role of interferon in juvenile myositis. Similar to the 2D muscle cell models[6], interferon treatment increased the expression of MHC class I, an effect that was more notable with IFNβ than with IFNα treatment. Unlike IFNα treatment, IFNβ also resulted in a sustained decrease in contractile tetanus force but had less of an effect on the transient twitch force. These findings are consistent with previous studies showing a more prominent role for IFNβ than IFNα in dermatomyositis[5]. As such, Covert et al.[3] focused only on IFNβ for their remaining assessments. Both the changes in MHC class I expression and tetanus force were reversed with JAK inhibitor (tofacitinib or baricitinib) treatment. The investigators used extended electric stimulation to assess the effects of interferon exposure on 'fatigue', defined as the difference between peak force versus force after 30 seconds of stimulation divided by the peak force versus baseline force, with calculations suggesting that interferon exposure paradoxically reduced fatigue in the myobundle. Interferon exposure had no effect on myofibre size or area in the 3D model. Furthermore, immunohistochemistry data suggested that IFNβ had no consistent effect on the expression of myositis-specific autoantigens (Mi2 and MDA5) or on ER stress as assessed by the ER stress marker GRP78.

The findings from Covert et al.[3] raise questions about the pathogenic role of interferon in JDM and how this model compares. The lack

of effect of interferon exposure on muscle cell size is unexpected[3], especially given that interferon exposure decreases the size of muscle cells in 2D models[1,4], which is consistent with the perifascicular atrophy that occurs in juvenile and adult dermatomyositis. This difference could relate to the specific interferon exposure, such as the concentration or duration, or the 3D muscle cells being more mature than those used in 2D models, but further investigation is warranted to assess the similarity of the 3D model to disease. The unchanged expression of MDA5 (as assessed by immunohistochemistry) after interferon stimulation is also surprising. MDA5 is encoded by the interferon-responsive gene *IFIH1*, and its expression would therefore be expected to increase with interferon exposure, but this lack of an effect might also be related to the specifics of interferon exposure. Simultaneously assessing other established interferon-responsive genes (such as *MX1* and *ISG15*[1,6,7]) might clarify whether this reflects a limitation of the immunohistochemistry assay used. Unlike MDA5, the expression of Mi2 and ER stress markers are not known to respond to interferon signalling and so the lack of effect on these markers is not unexpected and could indicate the existence of non-interferon-related pathways that regulate Mi2 and ER stress in JDM[3].

One caveat of the study is that the measurement of fatigue in this model, as described above, might not accurately reflect the clinical concept of fatigue regarding the effect of interferons. Notably, interferon exposure considerably lowered the maximum force of the myobundles and altered the kinetics, decreasing the potential fatigue possible by the 3D model calculation. Clinically, however, the demonstrated decrease in the maximum strength of muscle would likely lead to increased clinical fatigue and decreased potential or endurance for exercise. Hence, alternative assessments of fatigue for the 3D JDM model should be considered. Another limitation of the 3D model is the lack of extra-muscular aspects of disease such as haematopoietic immune responses and skin disease.

> **“the use of a bioengineered muscle model to help to understand JDM better is an exciting development”**

Despite these limitations, the use of a bioengineered muscle model to help to understand JDM better is an exciting development that raises other potential uses for engineered models. Comparing the functional aspects of a JDM muscle-derived 3D model to that of this healthy muscle-derived model could provide intriguing insights, as could exploring the involvement of other pathogenic mechanisms, such as other types of interferon and other cytokines. As has been done in other 3D muscle models[9], an important consideration is the incorporation and assessment of the vasculature in this model, given that vasculopathy and hypoxia are key components of JDM[1,4]. This model also has the potential for the testing of various therapies. For example, researchers have already used an in vitro engineered 2D myoblast-based model to assay more than 4,500 approved compounds to identify potential therapies, which included JAK inhibitors[10]. A 3D muscle model that is more clinically meaningful, similar to the one developed by Covert et al.[3], could be adopted for high-throughput testing of therapeutic agents. Hence, building on these latest findings, tremendous potential exists to gain a better understanding of JDM and pave the way for developing innovative treatments using biomimetic muscle models.

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## Competing interests

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Review article

# Heat of the night: sleep disturbance activates inflammatory mechanisms and induces pain in rheumatoid arthritis

Michael R. Irwin [1,2], Rainer H. Straub [3] & Michael T. Smith[4]

## Abstract

Sleep has a homeostatic role in the regulation of the immune system and serves to constrain activation of inflammatory signalling and expression of cellular inflammation. In patients with rheumatoid arthritis (RA), a misaligned inflammatory profile induces a dysregulation of sleep–wake activity, which leads to excessive inflammation and the induction of increased sensitivity to pain. Given that multiple biological mechanisms contribute to sleep disturbances (such as insomnia), and that the central nervous system communicates with the innate immune system via neuroendocrine and neural effector pathways, potential exists to develop prevention opportunities to mitigate the risk of insomnia in RA. Furthermore, understanding these risk mechanisms might inform additional insomnia treatment strategies directed towards steering and reducing the magnitude of the inflammatory response, which together could influence outcomes of pain and disease activity in RA.

## Sections

Introduction
Sleep characteristics and functions
Sleep disturbance in RA
Mechanisms contributing to sleep disturbance in RA
Sleep and regulation of inflammation
Sleep, neuroendocrine mechanisms and inflammation
Sleep and pain
Sleep, pain and inflammation
Interventions for insomnia
Insomnia treatment and reversal of inflammation
Conclusions

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## Key points

- Patients with rheumatoid arthritis (RA), a chronic inflammatory disorder, frequently complain of symptoms of insomnia and pain.
- Inflammation can induce sleep disturbance, and in turn sleep disturbance increases inflammation in a feedforward loop, which is sustained by sympathetic arousal mechanisms and a downregulation of glucocorticoid receptor sensitivity.
- Sleep disturbance can also increase pain sensitivity, as mediated by increases in inflammation; in patients with RA, sleep loss induces heightened pain responses and activation of arthritis-related joint pain.
- Interventions that treat insomnia have the potential to interrupt the effects of sleep disturbance on inflammation, pain sensitivity and symptomatic progression in patients with RA.

## Introduction

Rheumatoid arthritis (RA) is characterized by often symmetric, polyarticular disease involving the small joints of the hands and feet. As a genetically and environmentally determined disease, RA is a systemic inflammatory disorder that is chronic and progressive. Multiple potential pathogenic inflammatory pathways are thought to lead to joint inflammation and destruction that is pannus driven. In the synovial tissue, complex crosstalk exists between T cells, B cells, fibroblasts, macrophages, dendritic cells and others, which is facilitated by locally produced cytokines and chemokines, mainly IL-1β, TNF, receptor activator of nuclear factor (NF)-κB ligand (RANKL) and IL-6. In turn, IL-6 and other hormone-like inflammatory factors stimulate systemic disease sequelae outside the joints, leading to pain and alterations of sleep (such as insomnia), as reviewed elsewhere[1,2].

Insomnia and pain are two of the most prominent behavioural symptoms in individuals with RA. Inflammation is implicated in the dysregulation of sleep–wake activity and induction of pain. In turn, sleep disruption activates inflammation, which together suggests a positive feedback spiral between sleep and inflammation. Hence, a conceptual framework is needed to explain why some individuals with RA are at risk of insomnia. Moreover, an urgent need exists to understand the biological mechanisms that might be targeted to mitigate progression of pain symptoms and disease activity in individuals with RA who have comorbid sleep problems.

In this Review, we present evidence that inflammatory and autonomic arousal mechanisms, driven in part by social adversities (such as life stress or interpersonal difficulties), contribute to a misalignment of sleep–wake activity, such that sleep disturbance leads to excessive increases in inflammation, as coordinated by the central nervous system and multiple neural and endocrine effector mechanisms. In turn, inflammation can lead to further disturbances of sleep and this feedforward loop is hypothesized to serve as an accelerator of RA symptoms, with robust evidence that sleep disruption leads to increases in pain sensitivity and RA disease activity, likely mediated by activation of inflammatory pathways. This Review focuses on insomnia (that is, difficulties falling asleep or maintaining sleep) as it is the most frequent sleep disturbance in patients with RA, as noted below. Finally, this Review considers the ability of pharmacological and behavioural interventions to harness regulation of sleep and inflammation, in which treatment of insomnia reverses inflammation with implications for the progression of RA.

## Sleep characteristics and functions

Sleep is a behavioural state associated with reduced sensitivity to external stimuli and an increased arousal threshold[3], and characterized by reduced mental and physical activity, with decreases in muscle activity and interactions with the environment. Electroencephalography (EEG) and polysomnography (PSG) have demonstrated that brain activity varies along an arousal continuum (from fully awake to deep sleep) driven by two processes: a homeostatic process (Process S) that interacts with a process controlled by the circadian pacemaker (Process C). Process S drives sleep propensity and contributes to sleep onset, duration and length; process C modulates sleep timing by synchronizing sleep homeostatic mechanisms with the circadian system, as aligned with behaviours and physiological systems, along the 24-h sleep–wake cycle[4].

Assessment of sleep, including insomnia, can be performed using several methods, including PSG, actigraphy, diagnostic interview, sleep diary and self-report questionnaires. PSG uses EEG, electromyography and electro-oculography[5] to define three aspects of sleep: sleep continuity (that is, sleep latency, total sleep time, wake time after sleep onset, sleep efficiency), sleep architecture (amount of non-rapid eye movement (NREM) sleep, or sleep stages N1, N2, N3 or deep sleep), and amount of rapid eye movement (REM) sleep (including latency to the first REM period and REM density). Over the sleep period, sleep architecture dynamically changes, showing cyclic transition from NREM to REM sleep, with each cycle lasting about 100 min. NREM sleep predominates during the early part of the night, and REM sleep occurs mainly in the later night. Whereas sleep is dimensionally scored as a quantal behavioural state[6] with discrete scoring of the various stages of sleep, spectral analytic methods show a continuous shifting of mixed EEG frequencies to predominantly lower EEG frequencies in the transition from awake to NREM sleep and N3 sleep[7].

Sleep–wake activity can also be objectively estimated by sleep actigraphy (for example, by using an activity monitoring device worn on the wrist[5,8]), which, when coupled with a sleep diary, provides information on sleep duration and sleep maintenance (for example, waking after sleep onset and sleep efficiency). As noted below, few studies in patients with RA have used actigraphy to evaluate sleep.

Insomnia, the focus of this Review, is evaluated by questionnaire and interview assessment. The clinical diagnosis of insomnia disorder is defined by the Diagnostic and Statistical Manual of Mental Disorders, fifth edition, which characterizes the number, severity, frequency and duration of patient-reported insomnia complaints and daytime impairment[9]. The Insomnia Severity Index (a seven-item scale)[10] and the Pittsburgh Sleep Quality Index (a 19-item self-report questionnaire) are the two most widely used self-report instruments[5], both of which evaluate sleep difficulties (that is, quality, duration, night-time wakening and daytime dysfunction).

The varied functions of sleep include promoting homeostasis by regulating the immune system[11,12] and other physiological systems such as the hypothalamic–pituitary–adrenal axis (HPA) and the sympathetic nervous system (SNS). Data also show that sleep constrains inflammatory output[13], regulates haematopoiesis[14], preserves clonal diversity[13], and slows epigenetic ageing[15] and decay of the haematopoietic epigenome[13]. Additionally, sleep has a necessary role in supporting modifications in brain connectivity related to memory consolidation[16,17], cognitive resilience[18] and emotional regulation[19].

Thus, sleep protects against age-associated inflammatory disorders (including cardiovascular disease)[14], cancer and neurodegenerative disease (such as Alzheimer disease)[20,21].

## Sleep disturbance in RA

Insomnia symptoms affect nearly one-quarter of adults in the USA, and >10% of the population fulfil diagnostic criteria for insomnia or have a clinical diagnosis of insomnia[22,23]. Insomnia is often persistent, lasting up to 3 years in nearly 50% of patients[24]. Females have a two-fold greater risk of insomnia than males[23]; evidence shows that adults >60 years of age have a rate of insomnia that approaches 50%, which overlaps with age-related increases in chronic medical conditions such as RA[20]. Few studies have evaluated risk of insomnia in relation to ethnicity, although African American people are estimated to be twice as likely to report poor sleep quality as white people, which might be related to experiences of discrimination or due to disparities in socioeconomic status[25].

This Review focuses on insomnia in RA because self-reported insomnia complaints and insomnia disorder show a prevalence of 50–75% in patients with RA, more than twice the rate in the general population[26,27]; insomnia is also reported to be elevated in community-dwelling older adults with RA independent of depression[28]. Furthermore, among the various sleep disorders reported in patients with RA, insomnia is the most prevalent, with a prevalence that is reported to be more than twice that of obstructive sleep apnoea (OSA)[27]. Indeed, a matched comparison study of patients with RA versus controls found no difference in the prevalence of OSA[29], although prospective data indicate a greater risk of developing OSA in patients with RA than in those without RA[30]. Moreover, disturbances of sleep duration and sleep maintenance, two of the most common symptoms of insomnia, are linked to inflammation, pain sensitivity and disease activity in patients with RA[31] (reviewed below). By contrast, OSA and its severity are not reported to be associated with disease activity in RA[27,32].

Despite the high frequency of insomnia and insomnia complaints, few controlled studies have quantitatively assessed sleep (using EEG) in patients with RA, although evidence suggests that these patients have longer sleep latency, poorer sleep efficiency, more awakenings and more alpha-EEG arousals during N3 or slow wave sleep than healthy controls[33–35]. To our knowledge, only two pilot studies have used actigraphy to assess the number of nocturnal awakenings in patients with RA[36,37]. Moreover, the majority of research is limited by a lack of comparison controls, small sample sizes[38–40] and reliance on traditional sleep scoring methods, with a relative absence of studies that provide an integrated assessment of sleep and neurobiological mechanisms (such as inflammation) in RA.

## Mechanisms contributing to sleep disturbance in RA

### Psychosocial and psychological mechanisms

Pathophysiological models of chronic insomnia frame persistent poor sleep quality in terms of diathesis-stress, such that predisposing factors including genetics and adverse early life experiences[41–43] contribute to elevated neurobiological states of hyperarousal (Fig. 1). Indeed, early adversity alters emotional regulation and arousal circuits, including salience networks, that predispose individuals to heightened emotional and physiological arousal responses. Such arousal is further maintained by maladaptive behavioural factors (for example, irregular sleep–wake activity and self-medication with alcohol to decrease arousal level)[44,45]. In RA, a dose–response relationship exists between early life stress and arthritis incidence[46,47], especially in females[48].

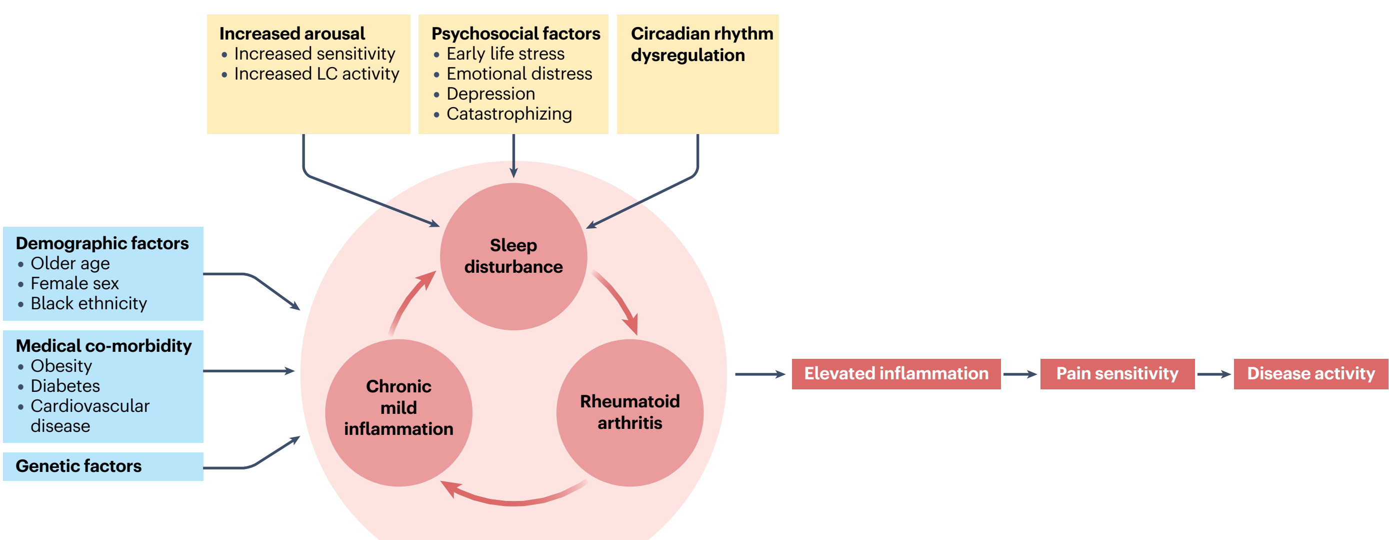


**Fig. 1 | Model depicting the impact of sleep disturbance and inflammation on pain and disease activity in patients with RA.** Predisposing factors such as demographic factors (older age, female sex and black ethnicity), medical co-morbidities (obesity, diabetes mellitus and cardiovascular disease) and genetic factors contribute to disturbances in sleep and chronic mild inflammation. Precipitating mechanisms including psychosocial factors (such as early life stress, depression and the cognitive style of catastrophizing) contribute to sleep disturbance directly and indirectly through increases in arousal, sensitivity to stimuli and activity of the locus coeruleus (LC), and through alterations in circadian rhythm. Together, these pre-disposing and precipitating factors drive a feedforward interaction between sleep disturbance and inflammation in patients with RA, which leads to further elevated levels of inflammation. When sleep disturbance and inflammation are perpetuated over time by the ongoing input from precipitating mechanisms, increases in symptoms of pain and pain sensitivity occur followed by progression of RA disease activity.

When exacerbated by stress, depression and pain catastrophizing reciprocally contribute to onset and perpetuation of sleep disturbance, which further drives pain, disease activity and disability in RA[49–51]. Pain catastrophizing involves pain-specific, cognitive-emotional processes that include the tendency to ruminate about pain, magnifying its threat, and related helpless feelings. Sleep disturbance, depression and pain catastrophizing reciprocally interact[52]. For example, poor sleep efficiency and high levels of pain catastrophizing predict heightened central pain processing and pain symptoms in patients with osteoarthritis[53] and RA[54], which suggests that these interacting constructs require routine assessment and/or targeted treatment.

### Arousal mechanisms

As noted above, PSG demonstrates frequent arousals, awakenings and extremity movements in patients with RA[35,55], which is thought to be due to increased sensitivity to stimuli as a consequence of early life stress, acute and chronic emotional distress, mood disorders, depression and pain[56]. Furthermore, inflammatory load (for example, high levels of TNF) can drive arousal and sleep fragmentation[38,57]. Increased sensitivity to external and internal stimuli is also influenced by the central sympathetic noradrenergic system. Indeed, the efferent SNS is centrally integrated in the insular cortex, medial prefrontal cortex and nuclei of the hypothalamus[58], which are tightly coupled to the HPA axis. The locus coeruleus, which is coupled to the amygdala[58,59], strongly influences these limbic elements[58], with effects on sleep; total locus coeruleus quietness is necessary for normal REM sleep[58]. Given that REM sleep promotes neuronal plasticity and memory consolidation, and is a prerequisite for overcoming emotional stimuli and experiences during the day[45], an active locus coeruleus during REM sleep leads to hyperarousal, insomnia and disturbed emotional regulation[45].

The mechanisms that account for increases in locus coeruleus activity in RA are not fully understood. However, release of corticotrophin-releasing hormone (CRH), endogenous opioids and inflammatory states (for example, high levels of IL-1β)[60] can induce locus coeruleus neuronal discharge and peripheral SNS activity[60]. Other factors present in RA, including angiotensin II, NF-κB, reactive oxygen species, IL-6 and Toll-like receptor (TLR)4 activation, might contribute to locus coeruleus dysfunction and might increase peripheral SNS activity[61]. Baroreceptor dysfunction is common in patients with RA[62] and might also drive arousal.

### Box 1

## Pathways between peripheral inflammation and the CNS

Inflammatory signals communicate with the central nervous system (CNS) via molecular pathways, which can regulate sleep (reviewed elsewhere[75,76]). The afferent vagus nerve expresses inflammatory cytokine receptors. When activated by inflammatory cytokines such as IL-1 (ref. 73), the vagus nerve signals multiple brain regions, such as the nucleus of the solitary tract, the ventrolateral medulla, the paraventricular and supraoptic nuclei of the hypothalamus and the amygdala, all of which are involved in the regulation of sleep. Inflammatory cytokines can induce sleep, which can be abrogated by vagotomy[221].

The circumventricular organs and the choroid plexus of the CNS contain macrophage-like cells. TLR activation of these macrophages by peripheral inflammatory signals leads to the production of inflammatory cytokines that enter the CNS by volume diffusion[222]. In addition, activation of endothelial cells leads to prostaglandin E2 production that induces inflammatory activation in the CNS.

Distinct from passive diffusion, inflammatory molecules can be actively transported across the blood–brain barrier[223]. CNS processes, including sleep and circadian mechanisms, modulate the transport of inflammatory cytokines such as IL-1, IL-6 and TNF, with evidence that sleep patterns dynamically increase transport across the blood–brain barrier[224].

Finally, activated monocytes and lymphocytes traffic to the brain vasculature and brain parenchyma when inflammatory cytokines stimulate brain microglia to produce CCL2 (also known as MCP1). Inflammatory activation of astrocytes can also induce these cells to produce CCL2, further facilitating CNS attraction of immune cells[225].

### Circadian rhythm dysregulation

Circadian rhythm is an important factor in the two-process model of sleep regulation[63]. Regulation of the circadian pacemaker in the superchiasmatic nucleus is altered[64] when elevated levels of inflammation occur[65,66], such as in RA. For example, higher peak values of IL-6 relative to cortisol in the early morning hours probably interfere with sleep quality[66–68]. Given that circadian rhythmicity is tightly linked to immune function[12], immune cell migration and immune cell precursor production in bone marrow[69,70], alterations in the circadian rhythm might stimulate a platform of disordered immunity[13,14,70]. Whether this new situation can stimulate autoimmunity in the first place is not yet known but has been discussed by some authors[71].

### Inflammatory mechanisms

During times of inflammatory activation, the central nervous system (CNS) receives inflammatory signals via multiple pathways including neural innervation (vagal afferents), humoral or circulating mediators, and active mechanisms of blood–brain barrier transport (Box 1). In turn, neurotransmitters (such as serotonin and dopamine) are altered, with resultant changes in behaviour, which are often characterized as 'sickness behaviours' involving social withdrawal and changes in sleep[72,73].

In experimental models of infectious challenge, animals typically show increases in NREM sleep and decreases in REM sleep[74–76], with evidence that viral activation of pro-inflammatory cytokines (such as IL-1, IL-6, TNF and type I interferon) induces increases in N3 sleep in a cytokine-receptor-specific manner[77,78]. Pharmacological administration of an inflammatory cytokine results in alterations in sleep architecture, although effects differ in animals versus humans: increases in NREM sleep are induced in animals[75], whereas decreases in NREM sleep, N3 sleep and REM sleep are reported in humans in response to exogenous doses of IL-2, for example[79].

Endogenous levels of cytokines have a homeostatic role in the regulation of sleep, in which inflammatory cytokines act to drive sleep propensity, with similar effects in animals and humans. During the sleep period in animals, brain levels of IL-1 and TNF mRNA and protein are higher than in the awake period[80], and elevated production of these cytokines in response to sleep deprivation drives increases in recovery sleep via cytokine-dependent receptor mechanisms[75]. Studies in

humans are limited, although TLR4-stimulated monocyte production of IL-6 and TNF prior to sleep onset correlates with increases in sleep efficiency and N3 sleep[55,76].

By contrast, in patient populations that show excessive increases in inflammatory cytokines, beyond typical endogenous levels, evidence indicates that abnormal increases in TNF, for example, have opposing effects on sleep and induce alterations in normal sleep architecture. In people with inflammatory disorders such as RA who typically show excessive levels of inflammation, antagonism of TNF with the monoclonal antibody infliximab induces increases, not decreases, in relative amounts of N3 sleep[38] but has no effect on REM sleep. TNF antagonists were also found to improve sleep continuity (that is, sleep latency and sleep efficiency)[38,81] as well as sleep quality[82–85] in patients with RA, with similar findings following administration of an IL-6 antagonist[86] and the Janus kinase inhibitor, tofacitinib[87]. Findings generalize to other patients who similarly have inflammation, low amounts of N3 sleep and high amounts of REM sleep. For example, pharmacological neutralization of TNF induced a short-term (that is, one night) decrease of REM sleep in alcohol-dependent persons[88]. Despite the promise of these clinical and experimental findings, a prospective study of patients with RA did not demonstrate a significant association between initiation or change of biologic therapies and improvement in sleep disorders including insomnia[89]. Hence, although evidence from some clinical trials suggests an improvement in patient-reported sleep quality[84,87,90,91], DMARD therapies might not be enough to prevent or treat insomnia in patients with RA; interventions that target insomnia are needed even when patients are treated with potent biologic therapies that reduce inflammation.

### Genetic factors

The heritability of insomnia ranges from 22 to 59%[92], with evidence that 956 gene variants are linked to insomnia, as found in a genome-wide association study (GWAS) of >1 million non-rheumatic adults[91]. Four overlapping genes are linked to RA and insomnia, each of which encodes proteins that have an immune-stimulating role: *PRRC2A* (also known as *BAT2;* involved in antigen presentation), *RASGRP* (activates the MAP kinase–ERK cascade and regulates T cell and B cell development), *LEMD2* (involved in cell signalling and differentiation) and *P2RX3* (encodes an ATP-receptor that has a role in pain perception).

## Sleep and regulation of inflammation

Sleep has a dynamic role in the regulation of innate immunity, and normal sleep is associated with nocturnal increases in markers of inflammation (Box 2). However, this phasic coupling of innate immunity and sleep becomes misaligned when sleep is disrupted, leading to a shift in inflammatory activation from the night-time to the day. When sleep disturbance becomes chronic, systemic inflammation increases during the night and day, as reviewed elsewhere[11].

Using experimental models of sleep disruption (such as sleep deprivation, multiple night sleep restriction and sleep fragmentation), the causal link between sleep disruption and increases in markers of inflammation has been demonstrated. For example, sleep disruption induces the following: increases in circulating levels of C-reactive protein (CRP), IL-6 and TNF[93]; increases in ex vivo mitogen-stimulated production of pro-inflammatory cytokines; and increases in TLR4-stimulated and resting monocyte production of IL-6 and TNF[94]. Additionally, sleep disruption affects upstream inflammatory mechanisms by inducing increases in mRNA levels of IL-6 and TNF[94], and inducing activation of inflammatory transcriptional pathways including NF-κB[95] and the signal transducer and activators of transcription (STAT) family of proteins[96]. Further findings demonstrate that partial night sleep deprivation, or disruption of sleep during the first or second part of the night, leads to upregulation of a gene ensemble that includes the master circadian regulator, several 'immediate early genes' involved in coordinating cellular signal transduction, and multiple inflammatory response genes[94]. Moreover, those with pre-existing sleep disturbance, as well as females in general, demonstrate a heightened sensitivity to the effects of sleep loss on cellular and transcriptional inflammatory activation[97]; notably, these subgroups also have a heightened risk of inflammatory disorders including RA[20].

Despite substantial experimental and observational evidence in healthy adults, findings linking sleep to inflammation in RA are limited. In patients with RA, one observational study found that sleep disturbance correlated with an overnight increase in tenderness in the peripheral joints, possibly due to increases in inflammation[39]. Further, because activation of stress arousal mechanisms is thought in part to drive disturbances of sleep, it is possible that observations linking stress to regulation of inflammation in RA might also be relevant to sleep disturbance in these patients. To this point, in an experimental model of psychological stress, patients with RA had an exaggerated inflammatory response, which was mitigated by treatment with anti-TNF medications (such as infliximab, etanercept or adalimumab)[98]. Given that acute psychological stress is known to induce NF-κB activation[99,100], it is possible

### Box 2

## Sleep and the nocturnal profile of innate immunity

Sleep induces increases in innate immunity, which is thought to support the host immune response and prepare for infectious exposure or injury that might occur the following day. Such priming of the immune system allows for a rapid, and possibly more robust, response to infectious threats[11,226,227]. These pathways, and related effector mechanisms, have been previously reviewed and illustrated elsewhere[11,12,228].

Briefly, sleep, as opposed to circadian factors, leads to nocturnal increases in the activity of natural killer (NK) cells[229,230], as well as markers of systemic inflammation including IL-6 and TNF. Although there are circadian-dependent effects on IL-6 (for example, with peaks at 7 p.m. and at 5 a.m.[231]), sleep deprivation in the early part of the night delays nocturnal increases of IL-6 until sleep onset, and night-time levels of IL-6 are decreased during total night sleep deprivation[231,232]. In addition, nocturnal sleep leads to increases in the levels of the trans-signalling molecule, soluble IL-6 receptor (sIL-6R)[233], which are pronounced during the late part of the night. Likewise, stimulated production of IL-6 is elevated during the late part of the night, owing in part to increases in REM sleep[232]; periods of REM sleep, compared with N3 sleep, are associated with higher levels of IL-6 (ref. 232). Finally, sleep leads to increases in the stimulated production of TNF[234], although neither circulating levels of TNF, soluble receptor (sTNF-R), nor resting or unstimulated levels of monocyte expression of TNF[235] are altered by sleep (depending probably on rapid internalization of this cytokine).

that TNF antagonists block activation of NF-κB. Indeed, IκBα and IκBγ, known inhibitors of NF-κB, are upregulated in vitro by infliximab[101]. Other studies have found that patients with RA with high disease activity show greater increases of CRP levels 30 min after exposure to acute psychological stress than those with low disease activity[102]; however, a differential increase in LPS-stimulated production of IL-6 was not found in patients with RA exposed to stress[103].

Similar to experimental findings, naturalistic, observational studies have also found that sleep disturbance is associated with increases in circulating markers of inflammation. Our meta-analytic review found that reported sleep disturbance is associated with higher levels of CRP and IL-6 (ref. 93); the number of studies was too small to detect an effect of sleep disturbance on TNF and other cytokines. Likewise, shorter sleep duration as evaluated by subjective or objective methods was linked to higher CRP levels, but not IL-6 levels. Larger effect sizes were found in younger and female subgroups, with exploratory results also suggesting an increased risk of inflammation in African American populations[104], and for those with poor social relationships[105]. Variability in sleep duration is also associated with increases in CRP levels[106]. Finally, poor sleep quality correlates with increases in TLR4-stimulated monocyte production of IL-6 and TNF as well as elevated inflammatory transcriptional factor activity that correlates with transcription markers of SNS and HPA axis function[107]. Importantly, the effect size linking sleep disturbances to inflammation is comparable with that demonstrated for multiple demographic (such as age and race) and biobehavioural (such as BMI and physical activity) factors[93,108]. Finally, prospective data show that poor sleep quality predicts increases in inflammatory cytokines in females[109]; self-reported sleep disturbance and short sleep duration predict subsequent increases in CRP[110,111]; and objective measures of short sleep duration (<5 h) and sleep fragmentation (nocturnal wakening for >90 min) predict an increase in a composite measure of inflammation, which taken together mediate mortality risk[111,112].

In addition to evidence that sleep disruption alters the expression of immune cell products such as IL-6 and TNF, poor sleep quality and insomnia are associated with increases in numbers of blood myeloid cells in humans[113,114], possibly due to increases in haematopoietic stem progenitor cell cycling in the bone marrow, as demonstrated in mouse models[14]. Furthermore, enhanced haematopoietic activity persists even during recovery from sleep disruption, which seems to contribute to a persistent heightened number of blood monocytes[13]. Consistent with human findings that chronic sleep disturbance accelerates epigenetic ageing of leukocytes[15], sleep disruption in mouse models also compromises the capacity for lymphoid differentiation. Together, it seems that sleep disturbance drives epigenetic re-programming, which primes immune cells towards the development of an exaggerated inflammatory response to challenge[13]. In patients with RA, we hypothesize that sleep disturbance might initiate changes in haematopoietic clonal diversity and the epigenome, contributing to accelerated epigenetic ageing, increases in inflammatory output, along with promotion of RA disease activity and related inflammatory co-morbidities such as atherosclerosis, other cardiovascular disease, diabetes mellitus and obesity[14].

## Sleep, neuroendocrine mechanisms and inflammation

### HPA axis

During normal sleep, N3 sleep predominates during the first part of the night coincident with an absolute 24-h minimum of HPA axis activity[11], which is thought to be permissive for immune activity necessary to prime anti-infectious and memory immunity[12]. By contrast, when REM sleep occurs (mainly in the late part of the night) HPA activity becomes dominant, reaching its absolute maximum shortly after wakening. In patients with RA, levels of cortisol are reported to be normal (or high) during the day but levels of adrenal androgens are low[115], which is thought to lead to glucocorticoid resistance and perpetuation of inflammation (and associated dysregulation of sleep[11]) (Fig. 2).

### Sympathetic nervous system

With sleep onset, efferent SNS activity is low[116,117], especially during N3 sleep[116,117]. During REM sleep, relative to N3 sleep, higher peripheral SNS activity is observed with increases in SNS neurotransmitters, sympathetic dominance in heart rate variability, and higher systolic and diastolic blood pressure[116,117]. In patients with RA, SNS activity is up to 30% higher[62,118], possibly because of baroreceptor dysfunction[62]. These findings suggest two major consequences[119]: sleep in patients with RA is disrupted owing to CNS hyperarousal, and peripheral inflammation is stimulated directly by sympathetic neuronal influences.

Several mechanisms are thought to underlie sympathetically stimulated peripheral inflammation: first, the SNS reaches primary and secondary lymphoid organs and similarly inflamed tissue through sympathetic nerve fibres[118]. Under conditions of high neurotransmitter concentrations of noradrenaline, some sympathetic influences are anti-inflammatory (for example, β2-adrenergically mediated inhibition of phagocytosis, neutrophils, NK cells, T helper 1 immunity, TNF, IL-2, IL-12, IFNγ), whereas other influences are proinflammatory (that is, immune cell mobilization, immune cell migration, chemotaxis, energy provision to an active immune system, T helper 2 immunity and others)[118]. Importantly, when inflammation is chronic, as in RA, anti-inflammatory pathways are blocked owing to a loss of sympathetic nerve fibres in inflamed secondary lymphoid organs and by a switch from anti-inflammatory to pro-inflammatory non-canonical receptor signalling of β-adrenoceptors[118]. Efferent SNS activity is also thought to promote metabolic activity (that is, the release of energy-rich fuels such as glucose, amino acids and lipids), which is necessary to support an inflammatory response for localized infections and wound healing.

### Gonadal and other hormonal systems

Low serum levels of testosterone are associated with severe OSA and other disturbed measures of sleep[120]. Given that sleep apnoea can be present in RA[121], the well-known loss of adrenal and gonadal androgens in RA might have a role in this and other sleep problems[122]. Loss of these androgens is linked to a proinflammatory status, which in turn might contribute to sleep disorders. Oestrogen levels have been linked to better sleep[120], but oestrogen levels are not typically changed in patients with RA.

### Consideration of age, sex and ethnic differences

During normal ageing, humans lose sex hormones around the age of 50, which is more striking in females than males, and serum levels of cortisol increase relative to sex hormones. The loss of oestrogen and progesterone in females can foster autoimmune diseases[123]. Similarly, the general loss of adrenal androgens supports inflammatory diseases in females and males[123], with effects on sleep as noted above.

Sexual dimorphism also applies to locus coeruleus function; females show increased locus coeruleus sensitivity to stressful events, which provides a molecular basis for a higher prevalence of

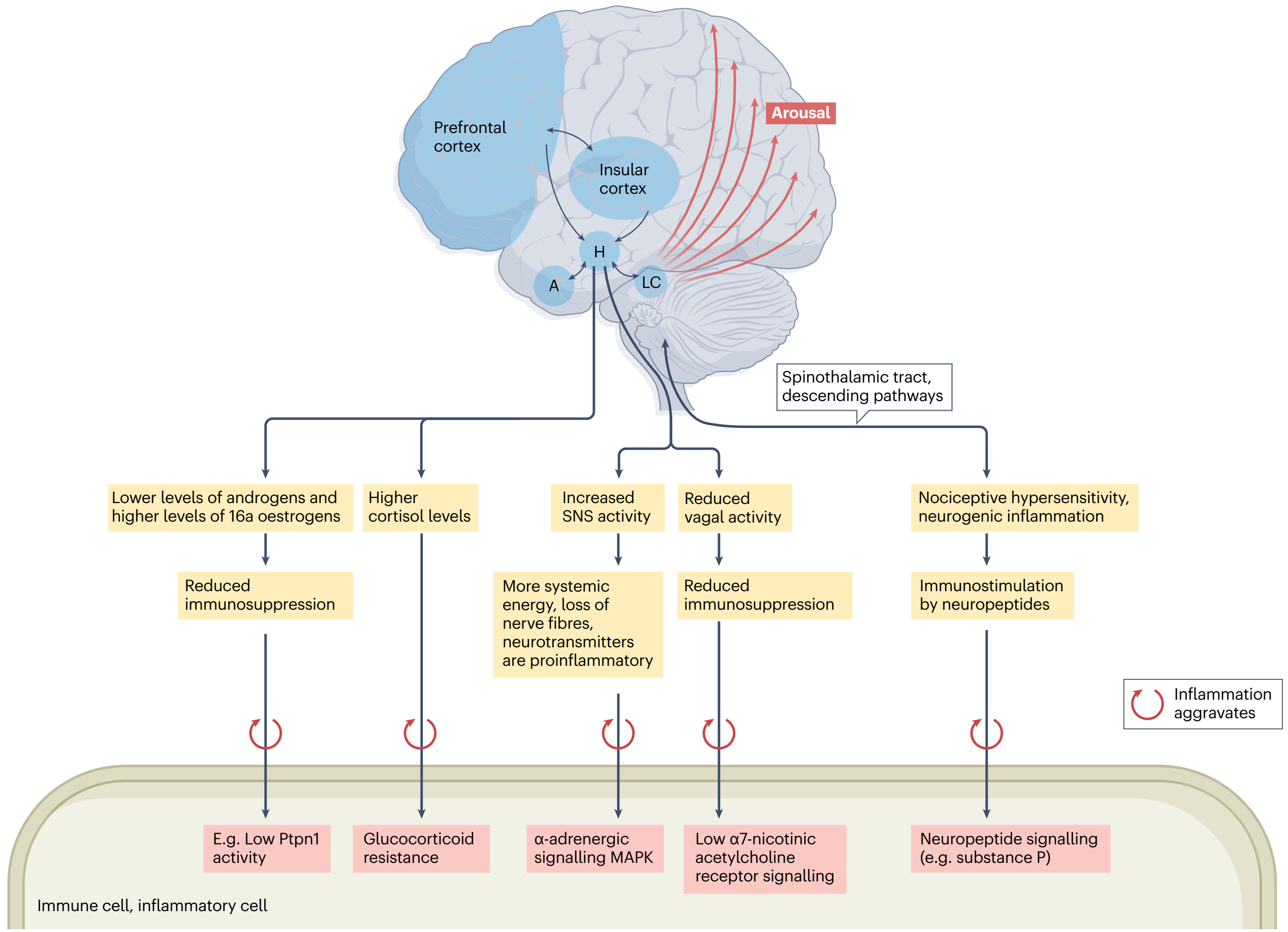


**Fig. 2 | Schematic model of neural sites and effector mechanisms involved in sleep regulation of inflammation.** Sleep disturbance and insomnia activate peripheral inflammation. Several brain centres are involved in coordinating effector mechanisms in response to sleep disturbance including the prefrontal cortex, hypothalamus (H), amygdala (A), insula and locus coeruleus (LC), which projects to the brain to alter arousal. Owing to the complex number of brain areas involved in arousal and sleep–wake activity, not all neural substrates are shown. Effector pathways from these various brain centres alter downstream neuroendocrine and neural pathways as shown, with changes in inflammatory activation as indicated for each pathway. Lower vagal activity has been described in RA[180]. Nociceptive hypersensitivity is typical in inflammatory arthritis as a consequence of peripheral, spinal and central nervous system alterations[140]. MAPK, MAP kinases; Ptpn1, protein tyrosine phosphatase non-receptor type 1; SNS, sympathetic nervous system.

stress-related diseases in females[58]. Locus coeruleus function has not been studied in RA, although females with RA might be more sensitive to arousal.

The bed nucleus of the stria terminalis, which is linked to anxiety and other social behaviours, also shows sexual dimorphism. Additionally, the bed nucleus of the stria terminalis is closely linked to hypothalamic and autonomic stress centres[124], and sleep stage transition (that is, from NREM sleep to wakefulness).

Finally, a large body of evidence suggests that sex differences exist at the molecular, cellular and systems levels of pain processing, which explains the female predominance in chronic pain disorders[125]. Given that pain is strongly linked to sleep disruption, sexual dimorphism in sleep quality might be based more on pain in females than in males. A study in osteoarthritis found associations between greater insomnia severity and lower pressure pain thresholds for non-Hispanic white patients but not for African American patients[126].

## Sleep and pain

The relationship between sleep and pain is reciprocal; daytime pain predicts poor sleep and in turn, poor sleep predicts elevations in pain the following day[127]. Moreover, self-reported poor sleep confers a two-to-threefold risk of developing new onset chronic pain, especially in females[128], and predicts emergence, progression and persistence of musculoskeletal pain[128,129]. Poor sleep also increases the risk of transitioning from acute to chronic pain, along with progression of localized regional pain to widespread pain[130].

Sleep difficulties and pain often cluster together with depressed mood in patients with RA[131]. Insomnia is not only comorbid with

**Table 1 | Quantitative sensory testing measures of pain sensitivity and central sensitization**

| Index | Assessment | Effects of experimental sleep loss/ disruption |
|---|---|---|
| Generalized hyperalgesia | Sensory testing extra-articular anatomical site | Not known |
| Heat pain threshold | Thermal stimulator with slow ramp in temperature from non-noxious warm temperature to noxious heat. Person indicates when stimuli (temperature) first feels painful | ↓; Mechanism might include elevations in cellular inflammation (TNF and IL-6) |
| Pressure pain threshold | Algometer applied to muscle belly at a constantly increasing rate of pressure. Person indicates when stimuli (applied pressure) first feels painful | ↓; Sleep-related mechanisms unknown |
| Temporal summation | Multiple (typically 10) brief (0.5-s) mechanical or thermal painful stimulations of equal intensity with a brief (2.5-s) constant inter-stimulus interval. Persons rate the intensity of the first stimulation and the peak intensity. The temporal summation index is expressed as a windup ratio, i.e. peak rating/first rating or as a difference score (peak-last) | ↑; Mechanisms involve upregulation of second-order dorsal horn neurons but sleep-related mechanisms are unclear. Females may be differentially impacted |
| Conditioned pain modulation | Involves a phasic measure of pain sensitivity (e.g. pressure-pain threshold at the trapezius) and a tonic pain conditioning stimulus (e.g. 20-s cold pressor task) applied to a distal contralateral anatomical region from phasic stimuli. The phasic measure of pain sensitivity is assessed before and immediately after the conditioning stimuli. Conditioned pain modulation is indexed as the change (percentage change or difference score) in sensitivity before and after the conditioning stimuli. Under normal circumstances, pain sensitivity is reduced by the condition stimuli | ↓; Sleep disruption impairs opioidergic analgesic systems but the mechanisms are unclear. Females are differentially impacted |

depressive disorder, but also serves as a potent risk factor for depression[132,133]. In turn, depression, including a past history of depressive disorder, is associated with greater pain overall and with more stress-reactive pain in patients with RA[134], with evidence that depression predicts increases in symptoms of pain, which in turn, worsen insomnia complaints and further increase reports of depression[135].

Few studies have evaluated the unique relationship between sleep and pain in patients with RA. One prospective study found that baseline sleep problems predicted heightened RA pain severity a year later[136]. In addition, a single night of experimental partial sleep loss was associated with heightened pain, fatigue and depression in RA compared with responses in controls[52]: patients with RA showed exaggerated increases in self-reported pain symptoms, increases in the number of painful joints and severity of associated joint pain, and increases in clinician-rated painful and tender joints compared with responses in controls[52]. Patients with RA also showed exaggerated increases in symptoms of depression, yet depression did not mediate increases in pain responses[52].

Pain in RA is shaped by a complex interaction between joint damage, peripheral sensitization of nociceptive afferents by inflammatory mediators (such as cytokines and other sensitizing molecules) and central pain-processing mechanisms involving bottom-up and top-down modulation of pain signals.

The mechanisms linking sleep disruption to pain are poorly understood. Even in patients with well-controlled RA, pain is often prevalent; this observation suggests a central sensitization component, which might be induced by sleep disturbance[137]. Central sensitization involves a reorganization of spinal cord circuitry and supraspinal neural pathways that modulate noxious peripheral input with dysregulated central pain-inhibitory and -facilitatory mechanisms that amplify and sustain pain[138]. Consistent with central sensitization, patients with RA display generalized hyperalgesia at extra-articular anatomical sites[139], which is thought to be a consequence of peripheral, spinal and CNS alterations[140].

Several pain measures, including heat pain threshold, pressure pain threshold, temporal summation and conditioned pain modulation (CPM) are used to quantify pain sensitivity and modulation (Table 1). Quantitative sensory testing measures of pain threshold can be applied to disease active joints to index peripheral sensitization and at unaffected sites to index generalized hyperalgesia (that is, heightened central pain processing). Dynamic quantitative sensory testing measures of pain include temporal summation and CPM, which indirectly measure central pain-facilitatory processes and descending pain-inhibitory capacity, respectively. Temporal summation, a common central sensitization measure of pain facilitatory processing, involves heightened pain responses to repetitive noxious stimulation of the same intensity, which predicts development of chronic pain[141]. Notably, the NMDA receptor is upregulated by sleep deprivation. Further, a deficit in CPM also predicts the development and trajectory of chronic pain[142], and enhanced clinical pain[143], as observed in chronic pain conditions (such as osteoarthritis)[144]. CPM can be modulated by monoaminergic and opioid-mediated supraspinal mechanisms[145].

In RA, emerging evidence suggests that aberrant temporal summation and CPM processes might contribute to pain. Elevated temporal summation was found in patients with RA with active disease[146], which correlated with RA disease activity[147] as well as increased clinical pain intensity[148]. Enhanced baseline temporal summation was also found to mediate the relationship between sleep and pain in RA in patients receiving DMARDs, suggesting that temporal summation might contribute to pain reports in patients with RA who have low levels of localized joint inflammation[149]. Deficient CPM is also reported in patients with RA[150,151], and low CPM predicts poorer response to DMARD therapy[152] and increases in tender joint counts[147].

Experimental sleep disruption induces generalized hyperalgesia (decreased pressure pain and heat pain thresholds) in healthy volunteers without RA, and leads to central sensitization-enhanced temporal summation of mechanical pain, especially in females[153], and increased temporal summation to cold stimuli[154]. Moreover, sleep deprivation also impairs CPM and pain inhibitory capacity in females[155–158], but not in males[156]. Observational findings indicate that poor sleep correlates with reduced CPM in several musculoskeletal pain populations[126,159]. Further, poor sleep quality partially mediates the relationship between CPM and RA, in which baseline CPM significantly accounts for the relationship between poor sleep and clinical pain[147]. These data, coupled with experimental disruption studies, indicate that sleep quality might drive clinical pain reports in RA by impairing endogenous pain

inhibitory capacity, even in patients taking DMARDs[149]. Interventions designed to enhance sleep might therefore have a role in long-term pain management. Together, multiple pathways, including central sensitization, have a role in the relationship between sleep disturbance and pain in RA.

## Sleep, pain and inflammation

Experimental research has found that sleep loss leads to aberrant increases in monocyte production of IL-6 and TNF[94], and that inflammation can heighten pain sensitivity[160–162]. However, research evaluating whether inflammation mediates the link between sleep disturbance and pain is limited, especially in humans. Moreover, as noted above, disturbances in sleep architecture such as loss of slow wave or N3 sleep seem to be uniquely associated with increases in inflammation[11,55], as well as with pain responses[163–165]. Furthermore, loss of N3 sleep, but not REM sleep, is associated with increases in markers of inflammation[11,55]. We interrogated the separate and joint contributions of N3 sleep and inflammation on pain sensitivity in healthy adults without RA. Using a multilevel approach, the effects of sleep disruption on hyperalgesia (heat pain threshold) were indirectly mediated by decreases in N3 sleep, which in turn led to increases in inflammation, which together served as double mediators to drive more than one-third of the pathway linking sleep disruption and pain sensitivity. Importantly, despite the effects of sleep disruption on total sleep time and REM sleep, neither of these sleep measures mediated changes in pain sensitivity[166]. Another experimental study found that sleep restriction led to elevations of circulating levels of IL-6, which in turn predicted elevated serum levels of IL-6, which in turn predicted increases in subjective reports of pain in healthy volunteers[167]. To the extent that loss of N3 sleep occurs in patients with RA who are experiencing sleep disruption, risk of heightened inflammation and pain sensitivity might be mitigated by interventions that target N3 sleep. The implications of such research would be substantial given the ubiquitous pattern of sleep fragmentation that occurs in association with pain and in patients with RA[34,35,168].

Several mechanisms might contribute to sleep- and inflammation-related hyperalgesia. Proinflammatory cytokines have receptor-mediated sensitizing effects on afferent nociceptive pathways[169,170] that mediate muscle and joint hyperalgesia[171,172], and sensitize nociceptors in peripheral nerve terminals[173,174]. Additionally, sleep disruption heightens SNS activity[11,116,175], which increases activity of NF-κB[99], upregulates inflammatory transcriptional profiles and increases expression of inflammatory mediators[176,177]. Alternatively, increases in the parasympathetic nervous system (that is, a shift towards vagal dominance) are associated with decreases of inflammation markers, with attendant benefit in animal models of arthritis[178,179]. In patients with RA, lower vagal activity is found along with increases in inflammation[180]. However, the potential links between sleep, parasympathetic activity and pain have not been evaluated.

## Interventions for insomnia

Given that sleep disturbance leads to increases in inflammation and pain sensitivity, and both inflammation and pain can have reciprocal effects to induce further disturbance of sleep in a feedforward loop, an urgent need exists to evaluate the efficacy of interventions that target insomnia and interrupt this cycle.

Sedative hypnotic medications are often used to treat insomnia. A systematic review of pharmacotherapy for insomnia identified only six studies (all from before 2000) testing the short-term (1 night to 4 weeks) effects of benzodiazepines, benzodiazepine receptor agonists, zopiclone and eszopiclone in RA[181]. Substantial heterogeneity precludes a formal meta-analysis, but improvements in patient-reported sleep continuity outcomes were observed in most studies, as confirmed in a controlled trial with eszopiclone[182]. However, benzodiazepine receptor agonists are not typically recommended for long-term use and can increase the risk of falls in older adults. Furthermore, given advances in DMARDs, which have powerful effects on inflammation and disease activity, there is a need to study the effects of various sedative hypnotics on sleep and disease activity in patients receiving these newer biologic agents. To date, new classes of sedative hypnotics, including melatonin receptor agonists and dual orexin receptor antagonists, are also yet to undergo systematic scientific investigation in RA. In addition, pharmacotherapy poses risks for daytime impairments in cognitive function and depressed mood, and can lead to physiological dependency and only temporary improvement in insomnia.

Sleep education therapy, a behavioural programme, is widely used to target day-to-day behavioural and environmental factors that contribute to poor sleep. Although this programme can improve sleep quality, these benefits are often not sustained to achieve remission of insomnia[183,184]. Cognitive behavioural therapy for insomnia (CBT-I) is currently recommended as the first-line treatment for insomnia; CBT-I is a multi-component non-pharmacological treatment that combines cognitive therapy, stimulus control, sleep restriction, sleep hygiene and relaxation[185–187], with demonstrated efficacy to produce sustained remission of insomnia for up to 3 years[188]. Evidence also suggests that mindfulness-based treatments (such as mindfulness meditation and tai-chi) are non-inferior in the treatment of insomnia[189,190], with durable maintenance of benefit >1 year[189–191]. Interestingly – given the links between sleep disturbance, inflammation and pain sensitivity – CBT and pharmacotherapy are reported to improve sleep and pain symptoms in various populations of patients with chronic pain[192–196].

Despite the robust efficacy of CBT-I in adults, older adults[197] and in those with chronic pain[198], studies in RA are limited, and have not systematically reported or examined whether pharmacological treatments directed at inflammatory pathways (that is, DMARDs) moderate these effects. One trial found that CBT-I improved patient-reported insomnia symptoms and standard self-reported sleep continuity parameters[199], but not PSG measures. However, CBT-I has been reported to improve insomnia symptoms and PSG measures in patients with osteoarthritis[200].

Given the evidence that exercise and mindfulness-based interventions can improve insomnia in adult populations, randomized controlled trials have begun to evaluate the efficacy of these approaches in patients with RA. One study found that intermittent aerobic exercise was associated with a significant improvement in PSG measure of sleep efficiency, but not in usual care control; self-reported measures of sleep continuity and sleep quality did not improve in either group[201]. Furthermore, a 28-session walking-based intervention over 8 weeks improved sleep quality more than an education control intervention in patients with RA, the majority of whom were taking DMARDS (75%)[202]. Finally, delivery of a progressive muscle relaxation programme improved sleep quality and fatigue more than usual care in patients with RA[203], although a pilot study of relaxation-based yoga had no effect on self-reported symptoms of pain or sleep in a small sample of patients with RA, the majority of whom were being treated with DMARDs (92%)[204]. Two of the trials above enrolled patients with clinically significant insomnia symptoms despite the predominant use of DMARDs. Even in patients receiving DMARDs, symptoms of insomnia are prominent; adjunctive treatments of insomnia might therefore be of benefit[202].

## Glossary

**C-reactive protein**
(CRP). An acute phase protein that is synthesized by the liver in response to the production of IL-6 by macrophages or T cells.

**Electroencephalography**
(EEG). A measure that tracks the electrical activity of the brain; one use is to graphically represent stages of sleep, which are defined by differences in waveform shape, frequency and amplitude.

**Electromyography**
A measure that tracks the electrical activity of muscle; it can be used, together with the EEG, to define stages of sleep, such as rapid eye movement sleep, in which low muscle tone or activity is accompanied by random and rapid eye movements.

**Epigenetic ageing**
An estimate of biological age given by evaluating changes in DNA methylation at particular genomic locations, which is found to be more predictive of mortality risk than chronological age.

**Experimental sleep disruption**
Imposing a loss of sleep during the night, for either part of the night (in other words, partial night sleep deprivation) or for the entire night (in other words, total night sleep deprivation).

**Glucocorticoid resistance**
A state of decreased sensitivity to the anti-inflammatory effects of glucocorticoids, which can be caused by ongoing increases in inflammation as well as by a genetic predisposition.

**Glucocorticoids**
Neuroendocrine hormones that belong to the steroid hormone class, which suppress inflammation and antiviral immune responses, in addition to having a role in the metabolism of protein, fat and glucose.

**Hypothalamic–pituitary–adrenal axis**
(HPA axis). A neuroendocrine system that links the hypothalamus, pituitary and adrenal glands and functions to regulate the immune system in response to circadian signalling, behavioural states such as sleep and peripheral inflammatory signals.

**Parasympathetic nervous system**
A component of the autonomic nervous system that comprises nerve fibres that innervate visceral tissues to regulate actions of the body when it is at rest, mainly through the release of the neurotransmitter acetylcholine.

**Rapid eye movement**
(REM). A stage of sleep, also known as paradoxical sleep, that is characterized by desynchronized electroencephalogram activity in a manner similar to waking, accompanied by random and rapid movement of the eyes together with low muscle tone. REM sleep is viewed as the sleep period in which there is a propensity to dream.

**REM density**
A measure specific to REM sleep and refers to the number of eye movements during REM sleep, which increases throughout the night along with a reduction in the drive to sleep. In other words, REM density is higher during the circadian or sleep period with arousal, and decreased in the night following sleep deprivation, which increases the drive to sleep.

**Sleep continuity**
The relative distribution of uninterrupted sleep, as opposed to wakefulness, during the night, as measured by sleep efficiency and wake time after sleep onset (the amount of time spent awake after turning off the lights and initiating sleep).

**Sleep duration**
The amount of time spent asleep during the night, measured either by subjective report or objectively, using polysomnography or actigraphy. Short sleep duration is defined as less than the reference amount of 7 h per night and is typically characterized as being less than 6 h of sleep per night. Long sleep duration is typically characterized as being more than 8 h of sleep per night.

**Sleep efficiency**
Time spent asleep as a percentage of the total time spent in bed.

**Sympathetic nervous system**
(SNS). A component of the autonomic nervous system that comprises nerve fibres that innervate lymphoid tissues, as well as nearly all other body tissues. The SNS regulates immune cell traffic and immune responses during sleep and in response to stress through the release of noradrenaline.

Given the experimental and longitudinal literature demonstrating a reciprocal relationship between pain and sleep, both the pharmacological and behavioural clinical trials literature have explored whether improving sleep translates into improved pain outcomes in RA. For example, pharmacotherapies are reported to improve pain[181], morning stiffness[196] and self-reported disease measures[199] in patients with RA and insomnia. Meta-analytic observations demonstrate that CBT-I improves clinical pain in non-RA samples[198,205] with chronic pain.

Finally, although evidence indicates that sleep disturbance can persist despite treatment with DMARDs[89], inflammatory cytokines are well recognized to alter sleep[75,206]. Given that these cytokines have a role in RA, further research is necessary to determine whether neutralization of these cytokines could effectively treat insomnia and associated pain symptoms in RA.

## Insomnia treatment and reversal of inflammation

In addition to the efficacy of CBT-I and other behavioural interventions for treating insomnia, evidence indicates that insomnia-related inflammatory activation profiles are reversed in association with insomnia remission, although findings are largely based on populations without chronic inflammatory disease such as RA. For example, in older adults, clinical trial data show that insomnia interventions (including CBT-I or mindfulness-based treatments) lead to the following: decreases in sympathetic activity (which is known to activate inflammatory mechanisms[207–209]); decreases in levels of systemic inflammation including CRP levels; decreases in cellular inflammation such as stimulated monocyte production of pro-inflammatory cytokines; and a reversal of the inflammatory transcriptional profile[210–212]. The effect of insomnia treatment on inflammation might be clinically important, as CBT-I leads to a decrease in the proportion of patients with insomnia who have elevated levels of CRP (as defined by the high risk threshold of >3 mg/dl CRP); the magnitude of this anti-inflammatory benefit is comparable with that found with vigorous physical activity[213] or weight loss[214]. Interestingly, patients with insomnia who received a mindfulness-based treatment demonstrated robust decreases in TLR4-stimulated monocyte production of proinflammatory cytokines and downregulation of inflammatory transcriptional profiles[212] even in the absence of complete remission of insomnia. These results suggest that the ability of these treatments to reduce stress arousal and related sympathetic activation might have additional physiological

benefits separate from those driven by insomnia[215,216]. Some data also indicate that behavioural interventions that target depression and sleep complaints might extend to patients with RA. For example, CBT for depression improves depressive symptoms and reduces stimulated production of IL-6 in patients with RA[217].

The HPA axis has an important role in normal sleep in which CRH increases light sleep and wake time after disease onset[218]. Low doses of glucocorticoids increase deep sleep and vice versa[219]. A situation with a chronic inflammatory load such as in patients with RA might support this problem. Patients with RA and high serum cortisol levels demonstrate less sleep efficiency and somewhat later wake time after sleep onset. However, anti-TNF therapy (etanercept) completely reverts this interrelation without apparent effects on cortisol levels[220]: patients demonstrated longer total sleep time, better sleep efficiency and shorter awake time after sleep onset[68]. In other words, anti-TNF therapy changed the influence of cortisol on sleep parameters in RA, even though levels of cortisol did not change.

## Conclusions

The CNS and the immune system reciprocally interact such that sleep enhances immune defences during the night, and in turn resting levels of cellular inflammation immediately prior to sleep can promote sleep continuity and depth in healthy adults. Sleep is a metabolically quiescent period, which means that energy sources are available during sleep to support innate as well as antiviral immune responses, which have a high metabolic demand. However, when activation of innate immune responses becomes chronic, for example, owing to an inflammatory disease such as RA, the homeostatic crosstalk between sleep and immunity becomes mis-aligned. For example, in patients with RA and co-morbidities, levels of inflammation become elevated, and these excessive inflammatory afferent signals lead to a disruption of sleep, which is further amplified by autonomic arousal mechanisms that are also activated in RA. Thus, sleep disruption activates SNS outflow, which steers the immune system towards greater increases in inflammation, which in turn magnifies sleep disruption in a positive feedback spiral. Maladaptive responses of the neuroendocrine system (for example, downregulation of glucocorticoid receptor sensitivity) further sustain this imbalance; in this setting, the inflammatory response is no longer effectively inhibited by cortisol. In RA, sleep disruption coupled with elevated inflammation induces hyperalgesia, increases pain sensitivity, and leads to progression of disease activity. The ability of pharmacological and behavioural interventions to treat insomnia and reverse insomnia-related inflammation reveals the potential to redirect mis-aligned inflammation. In other words, patients with RA might be able to accommodate the physiological insult of their inflammatory disorder more effectively if insomnia is treated.

To understand how distinct differences in RA map onto the risk of sleep disturbance and related immunological signatures, research on the environmental factors, medical co-morbidities, and psychosocial inputs that influence sleep and inflammatory biological mechanisms is needed. Multiple factors affect sleep quality, yet research in patients with RA remains limited. Improved understanding of the risk profile of sleep disturbance, including inflammatory and genomic differences, would substantially advance efforts to prevent the emergence of insomnia in RA, and provide insight into what specific aspects might be targeted to augment the therapeutic response of insomnia treatments.

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Review article

# Effect of DMARDs on the immunogenicity of vaccines

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## Abstract

Vaccines are important for protecting individuals at increased risk of severe infections, including patients undergoing DMARD therapy. However, DMARD therapy can also compromise the immune system, leading to impaired responses to vaccination. This Review focuses on the impact of DMARDs on influenza and SARS-CoV-2 vaccinations, as such vaccines have been investigated most thoroughly. Various data suggest that B cell depletion therapy, mycophenolate mofetil, cyclophosphamide, azathioprine and abatacept substantially reduce the immunogenicity of these vaccines. However, the effects of glucocorticoids, methotrexate, TNF inhibitors and JAK inhibitors on vaccine responses remain unclear and could depend on the dosage and type of vaccination. Vaccination is aimed at initiating robust humoral and cellular vaccine responses, which requires efficient interactions between antigen-presenting cells, T cells and B cells. DMARDs impair these cells in different ways and to different degrees, such as the prevention of antigen-presenting cell maturation, alteration of T cell differentiation and selective inhibition of B cell subsets, thus inhibiting processes that are necessary for an effective vaccine response. Innovative modified vaccination strategies are needed to improve vaccination responses in patients undergoing DMARD therapy and to protect these patients from the severe outcomes of infectious diseases.

## Sections

Introduction
Vaccine-evoked immunity
DMARD effects on vaccine immunogenicity
Influence of DMARDs on immune responses
Mechanisms of action of DMARDs
Implications for patient care
Conclusion

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## Key points

- Vaccines should ideally evoke efficient interactions between antigen-presenting cells and T cells and B cells; certain DMARDs disturb these interactions, leading to reduced vaccine responses and protection from infection.
- The immunogenicity of influenza and SARS-CoV-2 vaccines is often reduced in patients with rheumatic diseases, depending on the type of DMARD used during vaccination.
- A few DMARDs substantially inhibit responses to both vaccines (such as B cell depletion therapy or mycophenolate mofetil), whereas other DMARDs likely have no effect (including IL-6 inhibitors and hydroxychloroquine).
- The effect of some DMARDs (including TNF inhibitors, methotrexate and glucocorticoids) on vaccine responses could depend on the type of vaccine or DMARD dose used.
- The differential effects of DMARDs on vaccine responses are likely explained by the varying ways in which these drugs target disease and the functioning of antigen-presenting cells, T cells and B cells.
- Specific vaccine strategies, such as a drug holiday, should be considered for patients on each type of DMARD, depending on their effects on vaccine effectiveness and on controlling disease activity.

## Introduction

Infectious diseases have a high global burden and are one of the leading causes of mortality worldwide[1]. In the past decade, outbreaks of infectious diseases have increased, and because of the exponential growth of the human population and enhanced circulation of pathogens, the risk of novel infectious diseases is substantial[2]. Additionally, the increased contact between humans and wild animals augments the risk of zoonosis[3]. All these factors have probably contributed to the SARS-CoV-2 pandemic. Even though the peak of this pandemic seems to have passed, resurgence of the virus owing to new variants is expected to occur in the coming years. Moreover, the risk of new epidemics and pandemics in the future remains high.

Vaccines provide the most efficient and safest interventions in the prevention and control of infectious diseases. All vaccines are based on the same basic principle: exposing the immune system to either an attenuated version or an immunogenic subunit of the pathogen, thereby generating an immune response that will protect the individual from becoming severely ill after infection[4]. Classical vaccines expose individuals to either whole inactivated or live-attenuated pathogens that have lost their virulence. More novel vaccine approaches include subunit vaccines, viral-vector vaccines, and, most recently, messenger RNA (mRNA) vaccines[5].

Vaccines are particularly important to protect individuals at increased risk of developing severe disease from infections, including individuals with underlying immune deficits. Immunodeficiency can be a consequence of various factors, including the use of immunosuppressive drugs[6]. DMARDs are prescribed for the treatment of various immunopathological conditions, including most autoimmune disorders[7]. A wide range of different DMARDs are currently being used, each targeting different parts of the immunological processes underlying these diseases[8]. DMARDs are selected on the basis of the type and severity of disease and other criteria such as age, the presence of comorbid conditions and the use of concomitant medication (Supplementary Boxes 1–3). Generally, DMARDs can be split into three types: conventional synthetic DMARDs that target a wide range of immunological processes, biological DMARDs that specifically target one protein (typically a cytokine, its receptor or a cell surface marker), and lastly targeted synthetic DMARDs, which mainly target the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway. Regardless of their therapeutic benefits, however, DMARDs are accompanied by the risk of a long list of severe adverse effects, including an increased susceptibility to infections such as influenza and SARS-CoV-2 (refs. 9–11). In addition, patients using certain immunosuppressive drugs (for example, high-dose glucocorticoids) are thought to have a delay in viral clearance that leads to prolonged SARS-CoV-2 infections[12].

Given the necessity for proper protection of these patients, the aim of this Review is to assess the effect of DMARDs on vaccine-induced immune responses. Given that influenza and SARS-CoV-2 vaccines have been investigated most rigorously, these vaccines are the main focus of this Review. In this Review, we first introduce the different vaccine platforms and the immunological responses after vaccination. Next, we provide an overview of clinical studies concerning the immunogenicity of influenza and SARS-CoV-2 vaccines in patients receiving DMARD treatment. Finally, we discuss the mechanisms that might underlie the effects of DMARDs on vaccine responses in patients with autoimmune disorders.

## Vaccine-evoked immunity

Immune responses to vaccinations are complex and involve the participation of various immune cell subsets and a wide range of cytokines. Even in healthy people these responses can be highly variable; however, a number of common denominators are required for an effective response to vaccination. In this section, we discuss immune responses to vaccination as they occur in non-immunocompromised individuals, and the different types of vaccine platforms that are used to initiate these immune responses.

### Immune response induction

The induction of immune responses, whether through infection or through vaccination, relies on an intricate interplay between innate and adaptive immune mechanisms[13]. Dendritic cells, the sentinels of the immune system, take up microbes or vaccine components in the periphery and transport them to nearby lymph nodes. Recognition of danger signals, such as pathogen-associated molecular patterns of the microorganism or adjuvants of the vaccine by pattern recognition receptors (PRRs), leads to the activation of dendritic cells, which in turn produce activation markers and cytokines.

The lymph nodes provide the microenvironment for the physiological interaction of dendritic cells and different subsets of lymphocytes that results in the induction of adaptive immune responses. Dendritic cells process internalized antigens to small peptides. Presentation of antigenic peptides on major histocompatibility complex (MHC) molecules of activated dendritic cells stimulates $CD4^+$ and $CD8^+$ T cells carrying the cognate T cell receptor (TCR) and induces their proliferation and differentiation to effector and memory T cells.

$CD4^+$ T cells are important for the activation of $CD8^+$ T cells and for the promotion of B cell maturation that is necessary for an effective

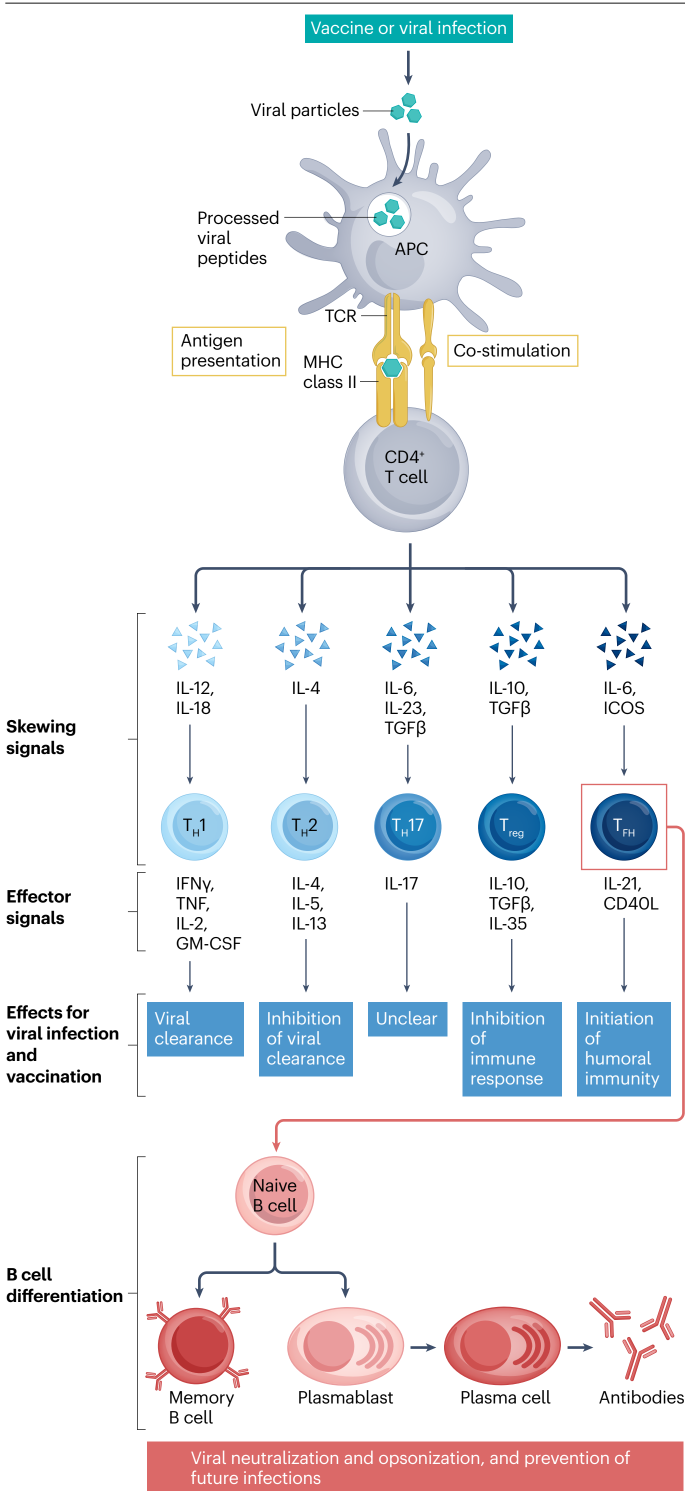



**Fig. 1 | Immune responses after vaccination.** Following vaccination, viral particles are taken up by antigen-presenting cells (APCs), which, upon activation, mature and migrate to secondary lymphoid organs to present the antigen. Antigen recognition by $CD4^+$ T cells occurs through the T cell receptor (TCR) and antigen-containing MHC class II complex. Costimulatory molecules, such as CD28 and CD80–CD86, initiate further differentiation of $CD4^+$ T cells into T helper 1 ($T_H1$) cells, $T_H2$ cells, $T_H17$ cells, regulatory T ($T_{reg}$) cells and follicular helper T ($T_{FH}$) cells. The lineage-specific differentiation is particularly dependent on the cytokine environment and the costimulatory molecules present. All subsets of $CD4^+$ T cells have their own repertoire of cytokine production, and each subset has specific effects on viral infection and vaccine responses. In the context of vaccinations against influenza and SARS-CoV-2, $CD4^+$ T cell skewing towards $T_H1$ and $T_{FH}$ cells is likely the preferred response, mediating viral clearance and the initiation of humoral immunity, respectively. Naive B cells, after antigen recognition and stimulation by $T_{FH}$ cells, differentiate into memory B cells and plasmablasts and subsequently into plasma cells. These plasma cells produce antibodies that are particularly important in providing protection against infection.

antibody response. $CD4^+$ T cells are activated through the interaction of their TCR with antigenic peptides presented by MHC class II molecules on the surface of activated dendritic cells. Cytokines are essential in determining the differentiation of $CD4^+$ T cells to certain subsets specialized in the defence of intracellular pathogens (T helper 1 ($T_H1$) cells), extracellular parasites (T helper 2 ($T_H2$) cells), and extracellular bacteria and fungi (T helper 17 ($T_H17$) cells)[14,15]. In addition, regulatory T ($T_{reg}$) cells are required to maintain self-tolerance[15,16]. Finally, follicular helper T ($T_{FH}$) cells are important cells for mediating humoral immunity. Efficient responses to viral pathogens, such as influenza virus or SARS-CoV-2, most likely require a particularly strong $T_H1$ and $T_{FH}$ response, whereas $T_H2$ and particularly $T_{reg}$ responses probably inhibit viral clearance[17] (Fig. 1). $CD8^+$ T cells are activated through dendritic cells, which present antigenic peptides on MHC class I molecules, after which these cells gain a cytotoxic function[18]. Even though $CD8^+$ T cells are important in killing virus-infected cells, only some vaccine platforms are potent activators of these cells[18,19].

B cells produce antibodies that are essential for protection against almost all pathogens[13]. B cell activation requires the interaction of the B cell receptor (BCR) with its cognate antigen, often displayed on the surface of follicular dendritic cells. Cross-linking of BCRs alone can trigger the production of antibodies, but T cell help is needed for the formation of memory B cells[15]. Upon interaction of an antigen with the BCR, the antigen is taken up, processed and presented on MHC class II molecules to $T_{FH}$ cells. Further interaction of B cells with $T_{FH}$ cells via CD40–CD40 ligand (CD40L) interactions and cytokines results in the full activation and proliferation of B cells. Some of the activated B cells differentiate into plasmablasts, which further develop into short-lived plasma cells that are characterized by a high proliferation rate but only produce antibodies for a short time span of 3–5 days. Other activated B cells enter the germinal centres, where they differentiate into long-lived plasma cells and memory B cells. In addition, the long-lived plasma cells undergo a process of maturation that results in the production of high-affinity antibodies. These plasma cells have a limited proliferative capacity but also have a very long lifespan, during which they keep secreting antibodies that can provide protection against infection[13]. The exact role of memory B cells in recall responses after vaccination remains unclear.

## Vaccines

Vaccination is aimed at inducing protective immune responses against pathogens without causing the damage associated with infection. Upon encounter with the respective pathogen, these immune responses can prevent infection and/or colonization, thereby avoiding or mitigating the symptoms of the disease. Classical vaccine platforms involve either attenuated or inactivated pathogens or consist of pathogen-specific antigens, which can be proteins or — in the case

of bacterial pathogens – polysaccharides. In the past decade, so-called nucleic acid vaccines, in particular, viral vector-based and mRNA-based vaccines, have become available. These vaccines do not contain the antigen of interest but rather the genetic information for its synthesis by cells that take up the vaccine[5]. Upon vaccination, the antigens – either present in the vaccine or produced by recipient cells of the vaccine – are taken up by dendritic cells and transported to the draining secondary lymphoid organ.

Current influenza vaccines for the adult population are typical examples of classical vaccines. These vaccines are produced from influenza virus grown in embryonated chicken eggs or cultured cells and further processed to generate split vaccines, containing all viral proteins, or subunit vaccines, consisting mainly of the viral surface protein haemagglutinin. Split and subunit influenza vaccines do not usually contain an adjuvant, although adjuvanted formulations for the elderly or immunocompromised are also available[20]. As most (adult) recipients of these vaccines have experienced several influenza infections during life, the vaccines evoke recall responses to conserved T cell and B cell epitopes, as well as primary responses to novel epitopes. Seasonal influenza vaccines are typically trivalent or quadrivalent, meaning that these vaccines contain antigens from three or four influenza virus strains, respectively.

Inactivated virus vaccines have also been used on a large scale for protection against SARS-CoV-2, mainly in China, Latin America and some African countries[21]. Moreover, a subunit vaccine consisting of the SARS-CoV-2 spike protein arranged on a nanoparticle is available[22]. Yet, few studies have assessed the effect of DMARDs on immune responses to these vaccines. Most SARS-CoV-2 vaccines used in Europe and the USA fall into the category of nucleic acid vaccines and consist either of viral vectors or mRNA encoding the spike protein of the SARS-CoV-2 (ref. 5). Viral vector vaccines make use of harmless viruses to deliver genetic information into human cells[20]. During the initial phase of the pandemic, adenovirus-based viral vector vaccines, Ad26.COV2.S and ChAdOx1, were developed and showed strong effectiveness against severe SARS-CoV-2 infections[23]. Even more recently, mRNA vaccines (that is, BNT162b2 and mRNA-1273) have been developed. The mRNA is packaged in lipid nanoparticles that are mainly taken up by antigen-presenting cells (APCs) such as dendritic cells. To avoid rapid degradation of the mRNA in the cytoplasm and overstimulation of the PRRs, both the BNT162b2 and mRNA-1273 contain modified uridine nucleotides (N1-Methylpseudouridine)[24]. mRNA vaccines have been widely distributed and administered, showing impressive effects on the prevention of infection and severe disease[23].

Before a vaccine is approved for use in the general population, adequate vaccine performance in terms of reducing rates of infection or severity of disease must be demonstrated in clinical trials. However, assessing the performance of a vaccine in specific populations, such as in patients using DMARDs, is difficult, as the recruitment of a sufficiently high number of patients would be both laborious and time consuming. Accordingly, studies of vaccine performance in these populations often look at correlates of protection, most importantly the capacity of the vaccine to induce presumably protective immune responses. Usually, such studies measure the amount and the neutralizing capacity of serum antibodies, as antibody responses are considered particularly important in the early elimination and neutralization of pathogens[25]. Generally, antibody concentrations correlate strongly with their neutralizing capacity, although this correlation depends on the extent to which the infecting pathogen deviates from the original. Indeed, the neutralization of novel SARS-CoV-2 variants requires much higher concentrations of vaccine-evoked antibodies than the neutralization of the original variant[26,27]. Additional, non-neutralizing antibody functions, such as their involvement in antibody-dependent cell-mediated cytotoxicity or complement activation, can be assessed by commercial assays, and should be considered when evaluating vaccine responsiveness.

Various forms of evidence emphasize the importance of cellular responses after vaccination[28]. The golden standard for measuring T cell responses is the ELISpot assay, which quantifies the frequency of antigen-specific T cells producing a certain cytokine, typically IFNγ; however, other robust methods such as an ex vivo IFNγ release assay, are also in use[29,30]. Monitoring cytokine production or activation markers in specific T cells after vaccination using flow cytometry could reveal other aspects of the quality of response[12].

Vaccine responses depend on efficient interactions between APCs and T cells in the secondary lymphoid organs, resulting in the formation of memory T cells, preferably $T_H1$ cells and $T_{FH}$ cells, and memory B cells and plasma cells[31]. These responses could differ among individuals and might be compromised for various reasons, including the use of immunosuppressive therapy. Dysfunctional regulation of T helper cell skewing is thought to be essential in the development of autoimmune diseases, which could also lead to a hampered vaccine response in these patients[14,32,33].

## DMARD effects on vaccine immunogenicity

DMARD use has been associated with more severe outcomes of influenza and SARS-CoV-2 infection in various large registry studies[10,34,35]; hence, patients undergoing DMARD therapy are among those individuals who would benefit the most from vaccination. However, the question remains whether vaccines work as effectively in this group as in the general population. A substantial number of studies have therefore investigated the immunogenicity and efficacy of influenza and SARS-CoV-2 vaccines in patients on DMARD therapy (Table. 1).

### Influenza vaccines

Several studies have investigated the effect of DMARDs on the induction of humoral immunity upon influenza vaccination. B cell depletion therapy (such as rituximab treatment) stands out as the most detrimental treatment for developing an adequate antibody response[36–40]. For patients on this therapy, the time since the last infusion seems particularly important in determining whether the influenza vaccine response is effective[38,40]. Ideally, vaccines should be administered at least 6 months after the last infusion, according to the European Alliance of Associations for Rheumatology (EULAR) recommendations[41]. Both the EULAR recommendations and the American College of Rheumatology (ACR) guideline, however, recommend no delay in influenza vaccination, owing to the seasonality of influenza[41,42]. If possible, the next rituximab infusion should be delayed for at least 2–4 weeks after vaccination. T cell responses are less affected by rituximab treatment, but responses in treated patients still seem to be less robust than responses in healthy individuals receiving no rituximab treatment[43,44]. Moreover, mycophenolate mofetil, azathioprine and abatacept also seem to affect humoral influenza vaccine responses in patients with autoimmune diseases[36–39,45–49]. The data on the effect of glucocorticoids, methotrexate, TNF inhibitors and JAK inhibitors on vaccine responses are conflicting.

Concerning glucocorticoids, evidence of a negative effect of these drugs on the immunogenicity of influenza vaccines is still under debate[38]. Possibly, the negative effect of glucocorticoid therapy on

**Table 1 | The effect of DMARDs on the immunogenicity of influenza and SARS-CoV-2 vaccines**

| DMARD therapy | Impact on influenza vaccine immunogenicity[37–39]? | Impact on SARS-CoV-2 vaccines immunogenicity[10,38,59]? |
|---|---|---|
| **Conventional synthetic DMARDs** | | |
| Azathioprine | Yes (humoral and cellular)[39,46,47] | Yes (humoral)[77,78] |
| Cyclophosphamide | Not enough information | Yes (humoral)[80] |
| Glucocorticoids | Unclear | Yes (humoral and cellular)[82,89,90][a] |
| Hydroxychloroquine | No[39,59][a] | No[75,104,105][a] |
| Methotrexate | Unclear | Yes (humoral and cellular)[82,92,99][a] |
| Mycophenolate mofetil | Yes (humoral)[39] | Yes (humoral)[74,77,114][a] |
| Sulfasalazine | No[39][a] | No[78,99,103][a] |
| **Biological DMARDs** | | |
| Abatacept | Yes (humoral and cellular)[45,48,49][a] | Yes (humoral and cellular)[74,76,77][a] |
| B cell depletion | Yes (humoral and cellular)[39,40,44][a] | Yes (humoral and cellular)[61,63,64][a] |
| IL-6 inhibitors | No[45,56] | No[75,76] |
| IL-17 inhibitors | No[230,231] | No[91,97] |
| IL-12–IL-23 inhibitors | No[232][a] | No[76,91,97][a] |
| TNF inhibitors | Unclear | Yes (humoral)[76,98,100] |
| **Targeted synthetic DMARDs** | | |
| JAK inhibitors | Not enough information | Yes (humoral and cellular)[71,72,74][a] |

[a]This effect has a high degree of certainty, on the basis of the number of studies showing the effect, agreeability amongst the studies and sample sizes assessed.

humoral vaccine responses is dose dependent, with more detrimental effects occurring with daily doses of 7.5–10 mg or more than with lower doses[39,42,46,50]. The studies that did not find reduced humoral responses in patients on glucocorticoid therapy tended to include patients receiving lower dosages and/or receiving a wider range of concomitant medication, which complicates the assessment of immunogenicity. In a systematic review of influenza immunogenicity studies in patients with RA and patients with systemic lupus erythematosus (SLE), the effect of glucocorticoids was only observed in patients with SLE, potentially owing to the typically higher glucocorticoid dosing used in patients with SLE than in patients with RA[51,52]. Only one study has investigated the effects of glucocorticoids on the induction of influenza-specific T cells upon influenza vaccination. The patients with SLE receiving treatment with prednisone and/or azathioprine had less influenza-specific IFNγ-producing T cells, as assessed by ELISpot, than the patients not receiving these drugs[47]. Furthermore, the patients on prednisone and/or azathioprine had fewer influenza-specific IFNγ-producing, TNF-producing and IL-2–producing $CD4^+$ T cells, as assessed by flow cytometry. No influenza-specific $CD8^+$ T cell responses were detected in any of the patients (irrespective of treatment) or in the healthy individuals. According to the ACR guideline, glucocorticoids should be tapered to <20 mg/day for most vaccinations, but not for influenza vaccination, owing to its seasonal nature[42].

Numerous studies have investigated the effect of methotrexate on the immunogenicity of influenza vaccines, with varying results[37,38]. Two large studies on responses to the 2009 pandemic H1N1 vaccine found an effect for methotrexate on antibody levels in a multivariate analysis[39,53]. However, a meta-analysis of responses in patients with RA showed that methotrexate had no effect on vaccine immunogenicity[36]. Additionally, another systematic review revealed a negative effect for methotrexate on vaccination responses, but only when assessing response rates to at least two influenza strains and not when assessing individual strains[54]. Potentially, these discrepancies could be explained by the pooling of data from influenza vaccines that invoke mainly primary immune responses (such as the 2009 H1N1 monovalent vaccine) and the seasonal trivalent or quadrivalent influenza vaccines that mainly induce memory responses. Despite the relatively weak evidence for impaired vaccine responses with methotrexate, some data show that delaying methotrexate therapy right after vaccination for 2 weeks can improve humoral vaccine responses[55,56]. The ACR guideline therefore recommends pausing methotrexate treatment for 2 weeks after vaccination[42].

The effects of TNF inhibitors on responses to the influenza vaccines also vary in studies, but most studies found no negative effect[37,38,54]. A 2018 meta-analysis of patients with RA concluded that TNF inhibitors probably do not reduce vaccine immunogenicity. However, a head-to-head comparison showed that patients on TNF inhibitor monotherapy had worse vaccine responses than patients on methotrexate therapy alone[57].

Influenza vaccination responses are probably not affected by hydroxychloroquine, sulfasalazine, IL-6 inhibitors, IL-12–IL-23 inhibitors or IL-17 inhibitors[36–39]. Data on the effect of JAK inhibitors remain limited; possibly these drugs only affect vaccine responses when used in combination with methotrexate[58]. Interestingly, one study found that hydroxychloroquine might counterbalance the negative effects of immunosuppressives on vaccine immunogenicity, although this finding requires further investigation and confirmation[59].

### Primary SARS-CoV-2 vaccination

Since the approval of the first SARS-CoV-2 vaccines in late 2020, an important question has been whether the immunogenicity of the vaccines is similar in immunosuppressed patients and the general population. The effect of DMARDs on the immune response to SARS-CoV-2 vaccination could differ from that on influenza vaccination responses, because of the use of different vaccination platforms or the fact that a primary response rather than a memory response is needed for protection against SARS-CoV-2. The number of studies investigating the immunogenicity of the SARS-CoV-2 vaccination in patients undergoing immunosuppressive therapy (such as DMARD therapy) has steadily increased, with the majority of studies focusing on the humoral immune responses of these patients. Whereas some of these studies have investigated the effects in relatively homogeneous populations of patients with one type of disease, many other studies have assessed a mixture of patients with a wide range of diseases. Most studies assessed antibody concentrations and seroconversion, but a few studies also assessed the neutralizing capacity of the patients or SARS-CoV-2-specific cellular responses. The majority of studies investigated effects on mRNA vaccine responses, whereas data on the effects of DMARDs on whole virus or viral vector vaccine responses are scarcer. Nevertheless, studies of different vaccine platforms tended to find uniform patterns in terms of the effects of DMARDs.

As also observed for influenza vaccination, the use of B cell depletion therapy stands out as the most impactful medication that affects not only the absolute SARS-CoV-2 antibody titre post vaccination, but also often prevents seroconversion, indicating a total lack of humoral protection[10,37,60–67]. In one study, a positive serological response after vaccination was associated with a lower total number of B cell depletion treatments, and an extended interval (more than 6–10 months) between the last treatment course and the vaccination[68]. In contrast to vaccinated patients, a humoral vaccine response can occur in some B cell-depleted patients after a SARS-CoV-2 infection[69]. Despite the substantial decrease in humoral responses, patients on B cell depletion therapy seem to have a relatively intact T cell response[63,64,70]. Other DMARDs, used in smaller patient populations, that clearly affect humoral and/or cellular vaccine responses include JAK inhibitors, mycophenolate mofetil, abatacept, azathioprine and cyclophosphamide[10,60,66,71–81]. Humoral responses are generally weakened but not completely lacking in these patients, indicating an increased risk of breakthrough infections.

In addition to the aforementioned therapies, most data suggest that humoral vaccine responses are also impaired in patients on glucocorticoid therapy[10,37,62,75,77,82–87]. In these studies, the seroconversion rates after two doses of vaccine were typically unaffected by glucocorticoid therapy, unlike that seen with B cell-depleting therapy, but the antibody concentrations were reduced when compared with other patients or healthy individuals. The effects of glucocorticoid seem to be dose dependent, with higher doses (>7.5 mg) having more notable effects than lower dosages[61,82,88,89]. Furthermore, a few studies showed that glucocorticoids had notable effects on the neutralizing capacity of the patients[84,87,89]. Also, treatment with (higher dosages of) glucocorticoids was associated with lower frequencies of IFNγ-producing antigen-specific T cells[77,82,85,86,90].

Methotrexate use is also associated with reduced SARS-CoV-2 vaccine immunogenicity. Although a systematic review from 2021 indicated that the evidence for a negative effect of methotrexate was not yet strong enough, more recent studies have indicated that methotrexate has a moderate effect. These findings were mostly based on assessments of humoral responses, whereas the effect of methotrexate on cellular responses is less clear. A number of studies have investigated T cell responses by flow cytometry or ELISpot, finding no evidence of impaired immunity with methotrexate therapy[82,90,91]. However, in two studies, methotrexate use was associated with impaired SARS-CoV-2-specific cytokine responses in T cells and a lack of increase in activation markers on $CD8^+$ T cells when compared with responses in healthy individuals[77,92]. As seen for influenza vaccination, pausing methotrexate therapy during vaccination seems to prevent impairment of immune response induction. This positive effect occurred across the different vaccine platforms, as primary immune responses to mRNA vaccines, viral vector vaccines and whole virus vaccines were all improved in patients who paused methotrexate during or directly after vaccination compared with those patients who remained on treatment[93–96]. However, pausing methotrexate might increase the risk of disease flares or disease activity in patients with rheumatic diseases, although so far the evidence suggests that this strategy only mildly increases the incidence and severity of flares[95,96].

TNF inhibitors might also affect SARS-CoV-2 vaccine responses. In initial studies, including in a meta-analysis, the data suggested that these drugs had no effect[37,60]. However, the meta-analysis was only based on seroconversion rates rather than on antibody concentrations. Furthermore, more recent studies that included larger groups of patients found that TNF inhibitors had a moderate effect on humoral immunity, including effects on both antibody concentrations and neutralizing capacity[66,74,76,97–100]. Interestingly, some data suggest that TNF inhibitor use is associated with a greater decay over time in antibody concentrations[97,100,101], as well as an increased occurrence of breakthrough infections[100]. By contrast, cellular immunity is likely less affected by TNF inhibitors[100,102].

Excluding TNF inhibitors, DMARDs that specifically target cytokines seem to have no effect on SARS-CoV-2 vaccine immunogenicity. Patients on inhibitors of IL-6, IL-17 or IL-12–IL-23 signalling have typical humoral and/or cellular vaccine responses that are similar to those of healthy individuals[10,60,71,77,91]. For both hydroxychloroquine and sulfasalazine, the available evidence suggests that not only is the vaccine response not impaired, but these responses are possibly even improved[78,83,89,99,103–105]. Indeed, vaccine responses were impaired in patients undergoing immunosuppressive therapy, but not in patients who used hydroxychloroquine or sulfasalazine in combination with the immunosuppressive drugs[99,104]. Finally, only one study has assessed the effect of leflunomide on humoral immunity after SARS-CoV-2 vaccination (in this case, vaccination with an inactivated whole virus vaccine) in a large group of patients, finding no evidence of a negative effect[89].

### SARS-CoV-2 booster vaccinations

Data are also emerging on the effect of DMARDs on humoral and/or cellular immunity after SARS-CoV-2 booster vaccinations. However, in some of these studies, whether DMARDs affect the immunogenicity of the booster vaccination is difficult to determine. This difficulty arises from the cross-sectional design of these studies, in which only immunity after the booster vaccination is measured, without information on prior immunity. Even though these studies often report lower humoral and/or cellular immunity after booster vaccination in patients receiving DMARD therapy, whether this effect is because of a weaker primary vaccine response or an impaired reaction to the booster vaccine is difficult to discern[106,107].

Other studies did have a longitudinal design, theoretically enabling a comparison between the primary and booster response; however, not all the studies assessed the fold change increase in booster response compared with the primary response or the antibody concentrations post booster adjusted for concentrations prior to the booster[71]. The findings from these studies exhibit a range of diverse outcomes. B cell depletion therapy, particularly when given shortly before booster vaccination, still prevented seroconversion in a substantial proportion of patients[66,69,108–110]. However, some of the patients still benefited from the booster vaccinations, particularly when there was a large gap between the last infusion and vaccination. Methotrexate also dampens the increase in humoral immunity after a booster vaccination, as assessed by studies of patients randomly assigned to pausing or not pausing methotrexate treatment during or after a booster vaccination[61,94,111]. In two longitudinal studies comparing the effect of a number of DMARDs on booster vaccination[61,110], patients receiving treatment with methotrexate, JAK inhibitors and/or cytokine inhibitors (including TNF inhibitors) had stronger humoral booster responses than patients receiving treatment with glucocorticoids, abatacept or B cell-depleting agents. Other studies, however, have shown that after a booster vaccination, TNF or JAK inhibitor therapy was associated with lower humoral responses, and lower cellular responses in the case of JAK inhibitors, compared with the responses in healthy individuals[71,112,113]. Finally, some data suggest that patients on DMARD

therapy who received the primary vaccination after a SARS-CoV-2 infection have a reduced boost of their humoral immunity compared with healthy individuals, although the power of these studies was too low to determine the effects of specific types of DMARDs[65,114].

### Effect on primary versus memory vaccine responses

The current literature, as discussed in the previous section, suggests that some types of DMARDs have a larger effect on the response to SARS-CoV-2 vaccination than on the response to influenza vaccination. Glucocorticoids, methotrexate, TNF inhibitors and JAK inhibitors clearly affect primary SARS-CoV-2 vaccine responses but seem to have a lower effect on influenza vaccine responses. There might be several explanations for these differences. The vast majority of individuals have some immunity to influenza virus infection prior to vaccination (owing to previous infections and/or vaccinations), including long-lived plasmablasts and memory T cells and B cells[115]. Hence, influenza vaccination could be viewed as a booster of an previously induced immune response. The extent of overlap between this prior immunity and the newly initiated immune response might differ each year, owing to the different compositions of the influenza vaccine in use. Nevertheless, these responses probably differ from the primary SARS-CoV-2 vaccination responses in immune-naive patients. In such circumstances, the complete immune response, including APC activation, antigen presentation, germinal centre formation and the differentiation of humoral and cellular immunity, has to develop from scratch, which introduces a wide range of processes open to influence by DMARDs. Interestingly, evidence showing reduced immunogenicity of the 2009 pandemic monovalent H1N1 influenza vaccine in patients using methotrexate support the idea that DMARDs affect primary vaccine responses more than memory vaccine responses[39,53]. Of course, other factors, such as the different platforms typically used for influenza vaccination (subunit or split vaccines) versus SARS-CoV-2 vaccination (viral vector or mRNA vaccines) might also contribute to these differences. Additionally, responses to influenza vaccines could be more difficult to quantify than responses to SARS-CoV-2 vaccines, owing to the variation in prior humoral and cellular immunity, and the multiple antigens included in the vaccine.

## Influence of DMARDs on immune responses

The typical immunosuppressive effects of DMARDs probably underlie the hampered vaccine response observed in patients on these therapies; however, the mechanisms do differ. Whereas some DMARDs have very specific effects, such as abatacept (CTLA4 co-stimulation blocker) or IL-6 inhibitors, other drugs rely on a wide range of mechanisms, such as glucocorticoids and methotrexate. Differences in these underlying mechanisms might also explain why some DMARDs impair vaccine immunogenicity, whereas others seem to have no effect. In this section, we discuss a number of routes in which DMARDs could affect the interaction between immune cells and thus interrupt an effective vaccine response.

### Effects on APC initiation of vaccine responses

The importance of APCs in the initiation of vaccine responses is often overlooked; nevertheless, the number and functionality of these cells might underlie the hampered vaccine responses of patients on DMARD therapy. DMARDs have variable effects on numbers of APCs, depending on the type of cell and type of DMARD. High-dose glucocorticoids, for example, are associated with reduced numbers of myeloid and plasmacytoid dendritic cells, and non-classical monocytes, but not with reductions in classical monocytes[116–119]. Enhanced apoptosis of these cells probably underlies these reduced counts, as observed after treatment with glucocorticoids, methotrexate or TNF inhibitors[118,120–126]. By contrast, higher frequencies of classical monocytes are predictive of reduced SARS-CoV-2 vaccine immunogenicity in patients with haematological malignancies[127], an association also seen for hepatitis B vaccination[128]. This finding implies that higher frequencies of classical monocytes prevent an effective vaccine response. This higher frequency of classical monocytes might reflect a chronic state of immune system activation, as seen in people with an aged immune system (inflammageing), although this association could potentially also be explained by other associated factors such as comorbidities or treatment.

An essential process for vaccine responses is the detection of pathogens by PRRs, including Toll-like receptors (TLRs), which initiate the activation and maturation of APCs. Whereas subunit vaccines often rely on TLR agonists in the form of adjuvants, the activation of these TLRs typically occurs naturally in the case of mRNA-based vaccines (particularly activation of TLR7 and TLR8)[129,130]. Activation of the PRRs initiates the maturation of APCs, a process resulting in the upregulation of MHC molecules, cytokines and other costimulatory molecules necessary for antigen presentation[131]. With aging, responsiveness to TLR stimulation in myeloid and plasmacytoid dendritic cells (such as reduced TLR-induced cytokine production) is typically decreased, and this decreased responsiveness is strongly associated with hampered humoral influenza vaccine responses in older individuals[132]. Similarly, reduced TLR responses are also observed in APCs following treatment with DMARDs in vitro[119,133,134], and various studies have reported increased numbers of dendritic cells with immature phenotypes in these patients[125,133,135–138]. The impaired maturation of dendritic cells might also impair their capacity to migrate to the secondary lymphoid organs[135,138,139].

Repression of dendritic cell maturation is a well-known mechanism in cancer and is associated with the formation of tolerogenic-like dendritic cells. Hence, dendritic cells with this phenotype are also likely to be less capable of initiating strong immune responses after vaccination[140]. Dendritic cells with a suppressive immune phenotype have also been associated directly with reduced vaccination response[141]. This suppressive phenotype of APCs in patients on DMARD treatment is characterized by defects in important vaccine response processes, such as the expression of MHC class II and costimulatory molecules, and the production of pro-inflammatory cytokines, particularly through inhibition of NF-κB[120,138,142–148]. The changes likely prevent efficient interaction of APCs with T cells. Indeed, tolerogenic-like dendritic cells can prevent the formation of $T_H1$ and $T_{FH}$ cells, and steer the T cell response towards a $T_{reg}$ phenotype[138,149–159].

### Effects on T cell differentiation

DMARDs might also directly affect T cells, thereby disturbing the formation of cellular and humoral vaccine responses. A reduced number of T cells is associated with impaired immunity after SARS-CoV-2 vaccination in patients with autoimmune conditions[82,90]. $T_H1$ cell responses after SARS-CoV-2 vaccination typically correlate with antibody concentrations in patients with rheumatic diseases[82,160,161]. As IFNγ-producing T cells are not required to initiate humoral responses (which instead require a functional $T_{FH}$ response), the observed impaired function in both $T_H1$ cell and humoral responses could be explained by a defect in their initiation by APCs. Nevertheless, APC-independent defects in T cells have been documented in in vitro experiments using

DMARDs. In sorted T cells, TNF inhibitors enhance the production of the anti-inflammatory cytokine IL-10 and delay their activation and proliferation[162]. Glucocorticoids and JAK inhibitors also have direct effects on T cells by preventing IL-12-induced and IFNγ-induced STAT phosphorylation as well as the expression of the $T_H1$ transcription factor T-bet[149,156,163,164]. Moreover, the presence of glucocorticoids in cultured T cells reduces the production of IL-21, an important $T_{FH}$ cytokine, which implies that the cells have a reduced capacity for stimulating humoral vaccine responses[157].

The induction of $T_{reg}$ cells by DMARDs could prevent efficient vaccine responses. A number of DMARDs, including glucocorticoids and methotrexate but not abatacept, promote T cell skewing towards a $T_{reg}$ cell phenotype[152,153,158,165,166]. DMARDs might promote $T_{reg}$ cell differentiation indirectly by affecting APCs, but could also have important effects on intrinsic T cell mechanisms. Glucocorticoids upregulate the expression of transforming growth factor-β (TGFß) receptor on T cells and methotrexate induces adenosine signalling in T cells[120,158]; both processes enhance $T_{reg}$ cell skewing. In congruence with their role in maintaining peripheral tolerance by suppressing immune responses directed against self-tissue, $T_{reg}$ cells also inhibit the development of vaccine responses. Inhibition of these processes is likely mediated via stimulation of inhibitory checkpoint molecules (such as programmed cell death 1 (PD1) and cytotoxic T lymphocyte antigen 4 (CTLA4)) and the release of anti-inflammatory cytokine, particularly IL-10 but also TGFß and IL-35 (refs. 167,168). These inhibitory signals affect a wide range of processes relevant for vaccine responses, such as downregulation of MHC class II and CD28 expression, interference in the formation of germinal centres and prevention of $T_{FH}$ differentiation[169].

### Effects on B cell subsets

Circulating numbers of B cells and/or plasmablasts correlate well with antibody concentrations after vaccination[73,82,90,170]. Indeed, the importance of these cells in mediating antibody responses is clearly evident from the lack of seroconversion after influenza or SARS-CoV-2 vaccination in patients undergoing B cell depletion therapy. In fact, only in those patients on B cell depletion therapies who still had measurable circulating B cells could a humoral vaccine response develop. Similarly, in patients on therapies that affect B cell numbers to a lesser extent, numbers of circulating plasmablasts or total B cells correlate with anti-SARS-CoV-2 antibody titres[73,82,90]. Similarly, TNF inhibition is associated with a reduced frequency of influenza-specific memory B cells and plasmablasts, and these frequencies correlate with reduced humoral vaccine responses[170].

In these studies, whether these reduced counts are caused by direct effects of DMARDs on B cells, or whether a defect in the immune response prior to the formation of plasmablasts and memory B cells is responsible, remains unclear. For instance, glucocorticoids modulate the interaction between helper T cells and B cells by inhibiting the expression of CD40L on T cells[171]. CD40L-mediated co-stimulation of CD40 on B cells is an essential step in initiating numerous immunological pathways, including germinal centre formation, immunoglobulin isotype switching and somatic hypermutation, required for an effective humoral response and the formation of long-lived memory B cells[172]. By contrast, JAK inhibitors can have T cell-independent effects on plasmablast formation and antibody secretion via the impairment of IL-21 signalling[173]. Furthermore, studies on glucocorticoid and methotrexate treatment showed that these DMARDs can induce the apoptosis of naïve or transitional B cells, but affect the transcriptional profile, rather than the apoptosis, of memory B cells[174–178]. By contrast, TNF inhibitor and abatacept therapy tend to reduce the number of memory B cells in particular[170,179,180].

## Mechanisms of action of DMARDs

Various routes and mechanisms can prevent an optimal immune response to vaccination. DMARDs are a highly heterogeneous group of drugs that can have either strong immunosuppressive or relatively mild effects, can have highly specific targets or a broad range of targets, and can have long-lasting effects or short-term effects (and hence must be administered daily). In this section, we discuss the mechanisms by which each type of DMARD affects the immune responses in such a way that vaccine effectiveness is impaired.

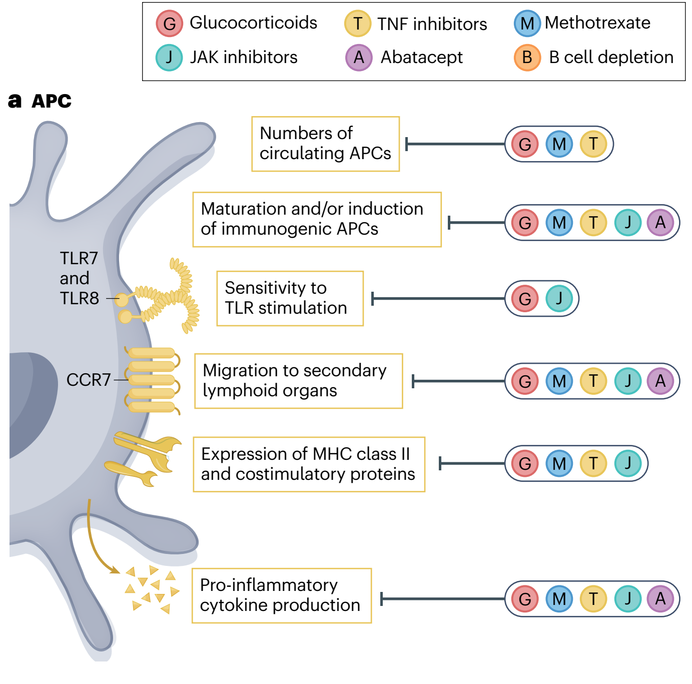
### B cell depletion therapy

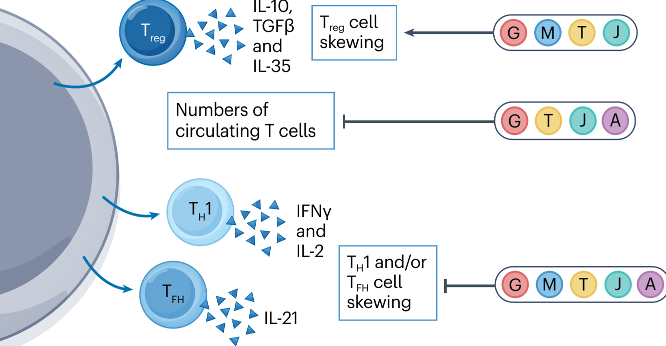
The main B cell depletion therapies employ antibodies that target CD20 (such as rituximab), although anti-CD19 and anti-BAFF therapies have also been developed[181]. Importantly, CD20 is expressed by all major circulating B cell populations, but not by long-lived plasmablasts[181], which has implications for vaccine responses. Recently administered anti-CD20 therapy typically prevents the formation of new humoral vaccine responses but does not eradicate prior humoral immunity, which is driven by the plasma cells. Studies have shown a strong association between B cell reconstitution and humoral vaccine response, and hence the timing of vaccination in these patients is particularly important[68]. The surprising finding that patients on rituximab treatment infected with SARS-CoV-2 often show humoral responses[69] indicates that B cell depletion therapy might spare some B cells that reside in protected niches, such as the bone marrow. These spared B cells do not seem to participate in humoral responses to SARS-CoV-2 vaccination but apparently do respond to an infection with SARS-CoV-2. $T_H1$ cell and $CD8^+$ T cell responses are not completely abrogated in these patients, stressing the fact that any vaccination is better than no vaccination in these patients[43,44,64]. Nevertheless, owing to a reduction in B cell–T cell interactions in B cell-depleted patients, and subsequent processes such as type I IFN production, $CD8^+$ T cell responses might also be impaired in these patients. Indeed, the expansion of influenza-specific and SARS-CoV-2-specific $CD8^+$ T cells is reduced in B cell-depleted patients compared with healthy individuals[44,64]. However, some data contrast with these findings, as in another study, some patients on B cell depletion therapy had higher $CD8^+$ T cell responses after SARS-CoV-2 vaccination than healthy individuals[70]. $T_H1$ responses might be less sensitive to B cell depletion than other T cell responses[64,70].

### Glucocorticoids

Glucocorticoid signalling is mediated by intracellular glucocorticoid receptors[182]. The activation of the glucocorticoid receptor results in numerous changes in the transcriptome, in particular via binding of the receptor to glucocorticoid response elements on the DNA. Additionally, non-genomic glucocorticoid signalling also occurs, via accessory proteins that detach from the activated glucocorticoid receptor[183]. Glucocorticoid signalling predominantly affects transcription factors, thereby altering the downstream signal transduction of inflammatory pathways including PRR signalling, suppressing the production and secretion of inflammatory mediators[142] (Fig. 2).

Glucocorticoids affect various aspects of the immune system, including small molecule secretion, immune cell populations and cell-mediated immunity[142]. Particularly important in the modulation of immune responses by glucocorticoids is the prevention of NF-κB and activator protein 1 (AP1) activation, which are essential for the



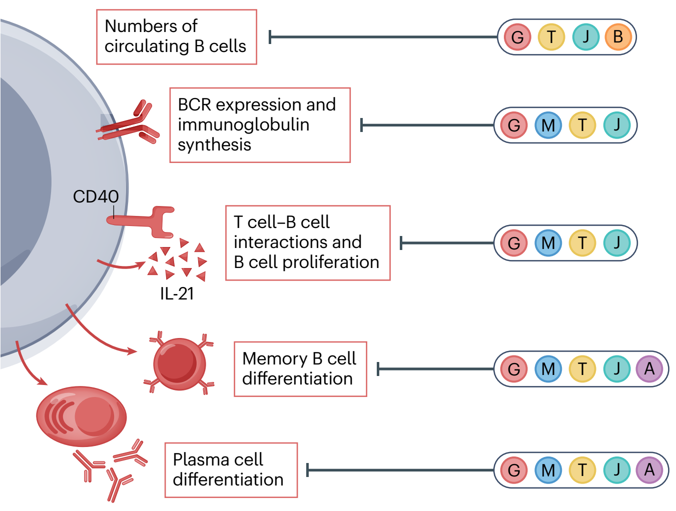




**Fig. 2 | The effect of DMARDs on immunological processes important for vaccine responses.** Interactions between antigen-presenting cells (APCs), T cells and B cells are essential for the development of robust humoral and cellular vaccine responses. Various data suggest that some DMARDs (including B cell depletion therapies, glucocorticoids, methotrexate, TNF inhibitors, JAK inhibitors, rituximab and abatacept) can disturb immunological processes involved in these responses, as summarized in this figure. For example, in APCs, some DMARDs can reduce the number of circulating cells, lower their sensitivity to stimulation, impair their maturation or migration, reduce the expression of important cell-surface proteins and suppress the production of cytokines. In T cells, various DMARDs can reduce T cell circulating numbers, inhibit skewing towards important T cell subsets (such as T helper 1 cells ($T_H1$) and follicular helper T ($T_{FH}$) cells) and promote the skewing towards others (such as regulatory T ($T_{reg}$) cells). Finally, in B cells, numerous DMARDs can reduce circulating B cell numbers, inhibit B cell interactions with T cells and subsequent B cell proliferation, downregulate immunoglobulin synthesis and disturb B cell differentiation into memory B cells and plasma cells. BCR, B cell receptor; TLR, Toll-like receptor.

maturation of APCs and the release of pro-inflammatory cytokines. In addition to reducing the number of dendritic cells, glucocorticoid treatment might also impair the migration of APCs towards secondary lymphoid tissues[184–187]. Therefore, antigen presentation could occur less frequently and efficiently, hampering crosstalk between the innate and adaptive immune cells. Glucocorticoid-mediated effects on the APC phenotype and on T cells directly impair the differentiation of T cells towards $T_H1$ and $T_{FH}$ cells, reducing the expression of CD40L and increasing $T_{reg}$ cell differentiation[146,154–157,163,171]. B cells, particularly transitional B cells, are sensitive to glucocorticoid-induced apoptosis[117,174,175,188,189]. In B cell cultures, glucocorticoids can downregulate components of the B cell receptor on B cells and reduce B cell synthesis of immunoglobulins[176,190]. The effects of glucocorticoids on short-lived and long-lived plasma cells have not been studied extensively, although initiation of glucocorticoid treatment reduces the circulating number of these cells[174].

## Methotrexate

The dose of methotrexate used for the treatment of rheumatic diseases is substantially lower than that used for the treatment of cancer, the initial indication for this drug[120]. Therefore, mechanisms of action could differ between these two settings. The main immunomodulatory effect of methotrexate at these lower doses likely occurs through enhancement of adenosine release, which has an effect on a wide range of immunological processes, including activation of NF-κB in APCs, T cells and B cells[191]. Methotrexate stimulates the release of adenosine in $T_{reg}$ cells in particular, but also in B cells[120]. Other mechanisms of action for methotrexate have been postulated, such as the promotion of apoptosis sensitivity in T cells through the uncoupling of nitric oxide synthase, and the inhibition of pro-inflammatory signalling via the JAK–STAT pathway[120].

Methotrexate is typically not associated with a reduced number of total T cells and B cells, but is associated with a reduction in APC numbers[120,192,193]. The latter effect is likely the result of both reduced haematopoiesis and maturation of APCs as well as enhanced apoptosis[120–122,194]. Methotrexate also affects the transcriptome of APCs, although this drug seems to favour the suppression of the more pro-inflammatory granulocyte-macrophage colony-stimulating factor (GM-CSF)-skewed macrophages rather than macrophage colony-stimulating factor (M-CSF)-skewed macrophages[195].

The underlying mechanisms of methotrexate might overlap with that of TNF inhibitors, as TNF is one of the cytokines most potently suppressed by methotrexate[195–197]. Methotrexate is postulated to inhibit $T_H1$ and $T_{FH}$ skewing, but evidence for this effect is mostly lacking[151,177]. Methotrexate can also directly affect B cells. For example, methotrexate use is associated with a reduction in frequency of transitional and naive B cells, cells of the early stages of B cell development in the blood, but not total memory B cells[170,177,178]. Vaccine-specific B cell responses are probably also impaired with methotrexate therapy, as the expansion of plasmablasts following influenza and pneumococcal vaccination is dampened in patients with RA undergoing methotrexate therapy when compared with the expansion seen in patients not undergoing DMARD therapy or healthy individuals[151,170]. Hence, methotrexate might preferably prevent humoral vaccine responses rather than T cell vaccine responses, and indeed studies in patients with GCA found that methotrexate use affected SARS-CoV-2 antibody responses, as assessed by antibody concentrations, but not T cell responses, as measured by ELISpot[82,90].

### TNF inhibitors

Five TNF inhibitors are currently approved for the treatment of various autoimmune diseases and all target the cytokine TNF, preventing pro-inflammatory signalling via TNF receptors. In addition to attaching to and blocking the action of soluble TNF, the therapeutic antibody attaches to membrane-bound TNF, leading to recognition by the immune system and lysis or apoptosis of cells via the complement system or through the activation of Fc receptors on innate immune cells[198]. Two TNF receptors exist – TNFR1 and TNFR2 – of which TNFR2 is particularly important for facilitating antiviral immune responses through the generation of $CD8^+$ T cells[199]. As TNF is such a pivotal cytokine, inhibition of TNF likely affects all the main players of vaccine immune responses. TNF signalling is particularly important for granuloma formation, suggesting that APCs such as macrophages are predominantly targeted by TNF inhibitors[198,200].

The important immunomodulatory effect of TNF inhibitors could be explained by TNF-mediated apoptosis of APCs[123–125]. However, some data suggest that this therapy prevents IL-12 and IL-23 production rather than instigating apoptosis of APCs[144]. Nevertheless, in patients on TNF inhibition therapy, immune interactions at the site of secondary lymphoid organs are disturbed, and the patients have substantially fewer and smaller germinal centres and follicular dendritic cell networks than healthy individuals[179,201]. A developmental defect in dendritic cells that leads to reduced costimulatory molecule expression and T cell stimulatory capacity might underlie these disturbed germinal centre responses[125,135,202,203]. Indeed, other studies have shown that T cell activation and subsequent cytokine production is reduced and anti-inflammatory T cell activity (such as IL-10 and TGFβ production) is enhanced in patients with RA on TNF inhibitors compared with patients with active RA or healthy individuals[153]. Surprisingly, and in contrast to other DMARDs, paediatric patients with rheumatic conditions on TNF inhibitors have higher $T_{FH}$ cell numbers than untreated patients[204] Total numbers of memory B cells are low in patients being treated with TNF inhibitors, and are lower than in those patients being treated with methotrexate[179]. TNF inhibition is also associated with a reduction in the number of influenza-specific memory B cells following vaccination, particularly 6 months later, resulting in reduced humoral influenza vaccine responses[170].

### JAK inhibitors

Various JAK inhibitors are approved for the treatment of different autoimmune and rheumatoid diseases, which vary in JAK protein specificity[205,206]. These therapies comprehensively block the JAK–STAT signalling downstream of a wide range of pro-inflammatory cytokines, including TNF, IFNγ, IL-21 and IL-6. Consequently, pinpointing the exact mechanism by which JAK inhibitors impair vaccine responses is difficult. JAK inhibition reduces the differentiation of plasmablasts and $T_H1$ cells, possibly by changing the phenotype of APCs, but also has direct effects on B cells[138,173,206,207]. JAK inhibitors seem to prevent the development of mature dendritic cells by steering the precursor cells towards a M1-like macrophage phenotype[133,138]. Germinal centre reactions are consequently also impaired[208]. Potentially, recall immune responses are less impaired than primary responses with JAK inhibition, as these inhibitors seem to have less of an effect on the immunogenicity of influenza vaccines than of SARS-CoV-2 vaccines. Indeed, JAK inhibition impairs $T_H1$ cell polarization in vitro and in vivo but does not impair the differentiation of antigen-experienced $T_H1$ cells[164]. Similarly, JAK inhibitors have a stronger inhibitory effect on the development of plasmablasts from naïve B cells than on their development from memory cells[173].

### Abatacept

An important costimulatory signal for T cells occurs via interaction of CD28 on T cells with CD80 or CD86 on APCs. CTLA4 is an inhibitory molecule, expressed mainly on T cells, that binds to CD80 and CD86 with greater affinity than CD28, thereby preventing CD28 costimulatory signalling and suppressing immune responses. Abatacept is a CTLA4–immunoglobin fusion protein that mimics this inhibitory process[209]. T cells that are stimulated through MHC molecules without proper co-stimulation enter a state of anergy[14]. Subsequently, the differentiation of T cells into $T_H1$ cells and $T_{FH}$ cells, but also $T_{reg}$ cells, is diminished following abatacept treatment[165,166,210]. This inhibition consequently affects germinal centre reactions, B cell processes and vaccine responses[180]. As abatacept directly binds to APCs, these cells might also be affected. Indeed, abatacept treatment of monocytes results in diminished production of pro-inflammatory cytokines; however, this treatment is also associated with increased frequencies of myeloid dendritic cells in patients[211,212].

### Azathioprine, cyclophosphamide and mycophenolate mofetil

Azathioprine, cyclophosphamide and mycophenolate mofetil are used for the treatment of various rare and severe autoimmune diseases. All three drugs have severe, immunosuppressive and cytotoxic effects that also prevent effective vaccine responses. Azathioprine impedes DNA and RNA synthesis and is therefore a strong inhibitor of leukocyte proliferation[213]. Prevention of leukocyte proliferation, in addition to induction of T cell apoptosis, likely explains how this drug inhibits the development of cellular and humoral vaccine responses. Cyclophosphamide therapy results in long-lived immunosuppression by inhibiting proliferation and instigating cell death in lymphocytes[214]. Patients on cyclophosphamide treatment have a long-lasting decrease in B cell numbers (including naïve and memory B cell numbers)[178]. Mycophenolate mofetil also prevents the proliferation of cells, and in particular lymphocytes, by inhibiting the formation of guanine nucleotides[213]. Furthermore, this drug downregulates the expression of CD40L on T cells. Compared with patients on azathioprine, patients on mycophenolate mofetil had relatively high frequencies of circulating transitional and naïve B cells, but much lower frequencies of plasmablasts[215]. As seen for other DMARDs, mycophenolate mofetil is also capable of modulating the polarization of dendritic cells, resulting in tolerogenic dendritic cells that inhibit $T_H1$ differentiation[216].

### Other DMARDs

Various other DMARDs, including IL-6 inhibitors, IL-17 inhibitors, IL-12–IL-23 inhibitors, hydroxychloroquine and sulfasalazine, are not associated with reduced immunogenicity of influenza and SARS-CoV-2 vaccines, despite the fact that these drugs modulate immune responses in such a way that they are effective in treating rheumatic and autoimmune diseases[217]. IL-6, IL-23 and IL-17 are all associated with $T_H17$ responses[14], which are important in these autoimmune diseases, but these T cells might not be needed for sufficient vaccine responses. IL-6, however, has additional roles, including the induction of $T_{FH}$ cells, and IL-6 inhibition is associated with reduced plasmablast and memory B cell frequencies[218]. IL-6 could be redundant for these processes, or local IL-6 production in germinal centres could be resistant to systemic IL-6 inhibition. Some studies have even reported that hydroxychloroquine and sulfasalazine have positive effects on vaccine responses[59,99,104]. Hydroxychloroquine has a wide range of effects on the immune system, one of which is the inhibition of TLR signalling, resulting in impaired APC maturation[219–221]. Potentially, this reduction of TLR-driven inflammation and clearance of viral material in the cytosol (that is, viral material from the vaccine), provides time for a broad immune response to develop. However, TLR-driven responses are also required for APC maturation, essential for the initiation of vaccine responses; therefore, the mechanisms behind these findings remain unclear.

## Implications for patient care

In general, patients with systemic diseases are at an increased risk of a hampered vaccine response owing to the effects of disease activity and ongoing treatment. As systemic diseases and the available treatment options are heterogeneous, the disease, the organ manifestations, the activity of the disease and the intensity of the treatment must be taken into account in the vaccination scheme. Active systemic disease (including new manifestations or relapse of disease), impairment of vital organs owing to illness, use of high-dose and multiple immunosuppressants, comorbidities, neutropenia and lymphopenia all increase the risk of a hampered immune response[10]. In particular, ongoing induction therapy, reflecting high disease activity, puts patients at risk of an impaired immune response. In contrast to variations in disease activity, the type of rheumatic disease seems to have less of an effect on vaccine responses, which is also supported by findings that patients with low disease activity and not receiving DMARD therapy have similar vaccine responses to healthy individuals[39,66,67,82,105]. Although age is a well-known risk factor for impaired vaccine responses[222,223], the effect of age on SARS-CoV-2 vaccination immune responses is relatively minor[23]. Whether age or sex also interferes with the effect (or lack of an effect) of DMARDs on vaccination is difficult to assess.

Other important aspects of vaccination under DMARD therapy that require further investigation include the optimal timing of a vaccine or booster during DMARD therapy, the dose and adjuvants used, as well as the durability of the primary response to a new vaccine (such as with initial SARS-Cov-2 vaccinations) and the recall response upon a 'booster' vaccination (such as with influenza vaccines and SARS-CoV-2 boosters). Future studies should also explore the benefit of combining different SARS-CoV-2 vaccines and test additional mitigation strategies to overcome waning immunity after primary vaccination in older patients with active disease and on induction treatment. The risk of moderate-to-severe SARS-CoV-2 infection in patients undergoing immunosuppressive therapy, such as B cell depletion, cyclophosphamide and mycophenolate mofetil therapy, should be balanced against the risk of under-treating patients with severe rheumatic and musculoskeletal diseases. The ACR recommends pausing methotrexate, JAK inhibitors, abatacept, mycophenolate mofetil and B cell depletion therapies during vaccination in certain patients with controlled disease; however, data to support this approach are scarce and more data are needed[41,42].

Certain DMARDs clearly affect the immunogenicity of vaccination. However, the effect of some DMARDs varies among different studies, particularly for glucocorticoids, methotrexate, TNF inhibitors and JAK inhibitors. The discrepancy among studies likely has several causes, such as the use of concomitant medication, the age of the patients, the different vaccine platforms used, the differences in timing of the assessment of vaccine responses, variations in outcome measures of humoral immunity (for example, seroconversion, antibody concentrations and neutralizing capacity) and variations in the type, duration and dosages of the DMARD used. Some studies showed only impaired immunogenicity for a combination of certain DMARDs, such as those involving TNF inhibitors, glucocorticoids and methotrexate[36,60,66,72,78,89]. The discrepancy between studies seems to be particularly high for influenza vaccination. In these studies, the degree of prior immunity probably differs substantially depending on the year and location, which likely impacts which and how much each DMARD reduces the vaccine's immunogenicity.

A few considerations remain concerning certain DMARDs. Evidence from various studies suggest that, unless used at doses ≥7.5 mg per day, glucocorticoids do not seem to increase the risk of a worse vaccine response. Doses above this cut-off of (7.5–10 mg/day) seems to have more apparent effects, which is biologically notable as this concentration is approximately similar to the daily amount of endogenous adrenal glucocorticoid produced in healthy adults[224]. Long-term glucocorticoid treatment can cause adrenal insufficiency, in which endogenous glucocorticoid production is reduced and replaced by the oral glucocorticoids[142]. Therefore, doses above the cut-off should lead to genuinely increased glucocorticoid levels in the circulation. However, lowering or temporarily stopping treatment at the time of vaccination, which is possible for other drugs such as methotrexate[93], is unsafe for glucocorticoid therapy owing to the risk of adrenal insufficiency and return of disease activity. The EULAR guideline, therefore, recommends against this strategy[41]. As discussed in an earlier section, B cell depletion therapies should be timed carefully with vaccination. Monitoring of the number of circulating B cell subsets might help to guide treatment decisions, as these cells are required for the humoral, and potentially even the cellular ($CD8^+$ T cell), vaccine responses. Finally, some evidence points at accelerated waning of (primary) vaccine responses in patients on certain DMARDs such as TNF inhibitors. Potentially, specific defects in developing memory responses, such as memory B cells and long-lived plasma cells, might underlie this defect. If more data confirm these findings, earlier timepoints might be considered for the administration of booster vaccinations in patients on these DMARDs.

Although this Review focuses on the effects of DMARDs on vaccine-induced immune responses, rather than real-life outcomes, various studies have also assessed the risk of (severe) breakthrough infections in patients on DMARDs. Interpreting the data of these studies, which mainly looked at SARS-CoV-2 breakthrough infections, is difficult owing to the possibility that the patients could have been more risk averse than the control population. Nevertheless, some DMARDs, particularly B cell depletion therapy, azathioprine and mycophenolate mofetil, were associated with higher hospitalization rates[225,226]. However, even though the immunosuppressed patients were at a higher risk of break-through infection and hospitalization, the results varied in terms of the effect by immunosuppressant type:

one study found no differences in SARS-CoV-2 infection among the various types of DMARDs, whereas another found a specific effect for TNF inhibitors[100,227]. Notably, a lower humoral immunity after primary vaccination is strongly associated with the risk of breakthrough infections in the general population[228,229]. Therefore, vaccination strategies should be aimed at inducing strong humoral responses, as assessed by high antibody titres, and the effect of specific DMARD therapies on antibody titres is an important aspect to consider when determining the best strategy in particular groups of patients.

New and innovative studies are required to obtain more knowledge on if and how DMARDs affect different aspects of vaccination responses. Currently, most human studies have investigated DMARD effects by in vitro stimulation of DMARD-naive immune cells with certain DMARDs to measure changes in cell function. Although this setting allows for controlled manipulation of immune cells, this approach potentially overlooks the extensive interactions that occur in vivo with circulating cytokines and with other cells such as endothelial cells. Also, these setups typically only allow the study of the short-term effects of DMARDs, which might be different from the effects in most patients on systemic DMARD therapy. The latter issue is likely also problematic in studies in which healthy participants are given short-term DMARDs to evaluate their effects. Finally, the interpretation of data from cross-sectional studies in patients requiring immunosuppressive medication could also be challenging. In these studies, associations between DMARD use and immunological changes might be obscured by the timing and route of DMARD administration, concomitant medication, differences in disease activity and other confounders. Potentially, long-term longitudinal studies in patients using DMARD monotherapy at different dosages and in treatment-free remission might provide more reliable data on the effects of each DMARD on different aspects of the immune system. However, even these types of studies have confounding factors such as changes in disease activity and ageing of the immune system.

## Conclusion

Some, but not all, DMARDs influence immune responses in such a way that protective features of vaccine responses (such as humoral and cellular immunity) are impaired. Hence, vaccination, although still providing a certain level of protection in patients on DMARDs, is less efficient at preventing serious outcomes of infections in these patients compared with healthy individuals. Strong evidence points to impaired vaccine responses in patients on B cell depletion therapies, cyclophosphamide, azathioprine, mycophenolate mofetil and abatacept. Whether glucocorticoids, methotrexate, TNF inhibitors and JAK inhibitors impair vaccine responses could depend on their dosing, timing, vaccine platforms and whether the vaccine evokes a memory response rather than a primary vaccine response. Effective immune responses after vaccination require efficient interactions between activated, mature APCs and T cells and B cells, which then respectively develop into $T_H1$ and $T_{FH}$ cells and memory B cells and plasma cells. DMARDs employ a plethora of mechanisms to interact with and disturb these processes, leading to impaired humoral and cellular protection after vaccination. New vaccination strategies, such as the combination of different types of vaccination, accelerated booster vaccination and vaccination during a so-called ‘drug holiday’, have been and will be developed to improve protection after vaccination in patients with autoimmune diseases on DMARD treatment.

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## Author contributions

Y.v.S. researched data for the article and wrote the article. All authors contributed substantially to discussions of content and reviewed and/or edited the manuscript before submission.

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Review article

# TNF and TNF receptors as therapeutic targets for rheumatic diseases and beyond

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## Abstract

The cytokine TNF signals via two distinct receptors, TNF receptor 1 (TNFR1) and TNFR2, and is a central mediator of various immune-mediated diseases. Indeed, TNF-neutralizing biologic drugs have been in clinical use for the treatment of many inflammatory pathological conditions, including various rheumatic diseases, for decades. TNF has pleiotropic effects and can both promote and inhibit pro-inflammatory processes. The integrated net effect of TNF in vivo is a result of cytotoxic TNFR1 signalling and the stimulation of pro-inflammatory processes mediated by TNFR1 and TNFR2 and also TNFR2-mediated anti-inflammatory and tissue-protective activities. Inhibition of the beneficial activities of TNFR2 might explain why TNF-neutralizing drugs, although highly effective in some diseases, have limited benefit in the treatment of other TNF-associated pathological conditions (such as graft-versus-host disease) or even worsen the pathological condition (such as multiple sclerosis). Receptor-specific biologic drugs have the potential to tip the balance from TNFR1-mediated activities to TNFR2-mediated activities and enable the treatment of diseases that do not respond to current TNF inhibitors. Accordingly, a variety of reagents have been developed that either selectively inhibit TNFR1 or selectively activate TNFR2. Several of these reagents have shown promise in preclinical studies and are now in, or approaching, clinical trials.

## Sections

Introduction

TNF receptor activation

Approved TNF-neutralizing drugs

Emerging TNFR1-targeting drugs

Emerging TNFR2-targeting drugs

Conclusions

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## Key points

- TNF is an important pleotropic cytokine that triggers complex immune-regulatory circuits of crucial relevance in tissue homeostasis as well as in many inflammatory diseases and pathological conditions.
- The clinically approved TNF blockers inhibit activation of both the pro-inflammatory and cytotoxic TNF receptor 1 (TNFR1) and the largely anti-inflammatory and tissue-protective TNF receptor 2 (TNFR2).
- TNFR2 promotes the expansion of regulatory T cells and enhances the suppressive activity of these cells, and is thus a promising therapeutic target in the treatment of autoimmunity.
- Biologic drugs that selectively and/or preferentially interfere with TNFR1 activation are in clinical trials and are effective in various preclinical disease models, including models of autoimmune disease.
- Various ligand-based and antibody-based TNFR2 agonists are therapeutically effective in preclinical disease models including collagen-induced arthritis.
- TNF receptor-targeting reagents with conditional and/or local activity are in early preclinical development and have the potential to expand the spectrum of applications of TNF receptor-regulating biologics in the future.

## Introduction

Excessive chronic production of TNF is an important contributor to autoimmune diseases, fibrosis and cardiac dysfunction, and this cytokine has further complex context-dependent activities in tumorigenesis and cancer growth[1–5]. As such, various TNF inhibitory biologic drugs have been developed and evaluated in clinical trials for the treatment of immune-related pathological conditions. Although TNF-blocking biologic drugs are ineffective in the treatment of certain indications (such as multiple sclerosis and heart infarction)[3,5], these drugs are quite effective in treating other TNF-driven diseases, such as rheumatoid arthritis, Crohn disease and psoriasis[4]. Indeed, various TNF inhibitors have now been in use for decades for the treatment of autoimmune diseases and every year these drugs are listed among the top-selling drugs worldwide, only being surpassed by the mRNA vaccine BNT162b2 (Pfizer/BioNTech) in 2021 (ref. 6). However, in addition to having deleterious pro-inflammatory effects, emerging data suggest that TNF can also elicit strong immunosuppressive activities through the stimulation of regulatory T ($T_{reg}$) cells, myeloid-derived suppressor cells (MDSCs) and IL-10-producing regulatory B cells[2]. Moreover, TNF promotes the survival of certain cell types, including oligodendrocytes, cardiomyocytes and keratinocytes[2].

TNF signals via two structurally related receptors — TNF receptor 1 (TNFR1) and TNFR2 — which are the eponymous members of the TNF receptor superfamily (TNFRSF). Although TNFR1 is ubiquitously expressed, the expression of TNFR2 is more restricted. TNFR2 is constitutively expressed at high levels in myeloid cells, $T_{reg}$ cells and B cells but is also expressed at low levels in resting T cells, type 2 innate lymphoid cells (ILC2) and some non-haematopoietic cells, including mesenchymal stem cells, endothelial cells and epithelial cells[2,7]. Intriguingly, various pro-inflammatory cytokines, such as IL-33, IFNγ, IL-1, TL1A and TNF itself, can further promote the expression of TNFR2 in immune and non-immune cells including fibroblast-like synoviocytes in rheumatoid arthritis[2,7–11]. TNFR1 has strong pro-inflammatory signalling capabilities but can also trigger context-dependent cell death by stimulating apoptosis and necroptosis and also by promoting the generation of reactive oxygen species (ROS) or activating acidic SMase[12–14].

The overwhelming clinical success of TNF inhibitors in the treatment of autoimmune diseases is in large part due to their ability to inhibit the pro-inflammatory activity of TNFR1 (refs. 4,15). However, the less well investigated receptor TNFR2 also elicits pro-inflammatory effects. For example, TNFR2 co-stimulates $CD8^+$ T cells, sustains survival and persistence of dendritic cells and promotes endothelial transmigration of leukocytes by inducing the expression of E-selectin, VCAM1 and ICAM1 on endothelial cells[16–19]. Nevertheless, TNFR2 also has a variety of anti-inflammatory and protective effects, particularly on $T_{reg}$ cells, MDSCs, IL-10-producing B cells and cardiac myocytes[2]. The differing relative importance of the cytotoxic and pro-inflammatory effects of TNFR1 and the relatively strong anti-inflammatory and tissue-protective activities of TNFR2 in certain pathological conditions or in certain patient subgroups might explain, at least partly, why TNF inhibitors fail in some TNF-driven diseases and why some patients do not respond to TNF inhibitor treatment, despite having a disease for which the inhibitors are approved.

Against this background, TNFR1-specific inhibitors and TNFR2-specific agonists have the potential to tip the balance in TNF-driven immune-mediated diseases away from the 'detrimental' activities of TNFR1 towards the 'beneficial' activities of TNFR2. Such drugs could find success in the treatment of patients with diseases that are refractory to current TNF inhibitors. In this Review, we give a brief description of the current knowledge on TNF receptor activation as a basis for a comprehensive discussion of the mode of action of these novel TNF receptor-specific compounds. We also summarize the preclinical experience of these drugs and debate their advantages compared with conventional TNF inhibitors and their potential limitations.

## TNF receptor activation

Research over the past 20 years has provided detailed insights into the molecular mechanisms by which TNF and antibodies activate TNFR1 and TNFR2 and has also resulted in a profound knowledge about the signalling pathways stimulated by TNF, as discussed in this next section. In particular, these research activities have revealed the crucial relevance of clustering of liganded TNF receptors for initiation of signalling and culminated in the rational development of several TNF mutants and TNF receptor-targeting antibodies that selectively inhibit TNFR1 or selectively activate TNFR2.

### TNF receptor ligand binding and clustering

TNF is the prototypic and name-giving member of the TNF superfamily (TNFSF). This cytokine is initially expressed as a type II transmembrane molecule in which the homotrimer forming and receptor-interacting C-terminal TNF homology domain (THD) is separated from the transmembrane and intracellular domain by a stalk region[2,4]. Soluble TNF (sTNF) is released from the membrane-bound form of TNF (memTNF) after cleavage in the stalk region by disintegrin and metalloproteinase domain-containing 17 (ADAM17, also known as TACE), a membrane-bound protease that sheds the ectodomains of >80 membrane proteins, including TNFR1 and TNFR2 (ref. 20). Both TNF receptors bind sTNF with sub-nanomolar affinity but only TNFR1 signalling is strongly and comprehensively induced by sTNF[21,22]. By contrast,

memTNF efficiently stimulates both TNFR1 and TNFR2 signalling[21] (Fig. 1). Thus, the sole ligand occupation of TNFR2 is not sufficient for receptor activation. The differential efficacy of sTNF and memTNF molecules to activate TNFR2 largely also applies to anti-TNFR2 antibodies. Although, 'free' anti-TNFR2-IgG antibodies typically fail to efficiently stimulate TNFR2 signalling, these antibodies can anchor to the cell surface, for example, via interactions with Fcγ receptors (FcγRs), bestowing the antibodies with TNFR2-stimulating activities[23].

As well as the two forms of TNF, other ligands of the TNFSF can interact with TNFR1 and TNFR2, including homotrimeric lymphotoxin α (LTα, also called TNFβ) and soluble and membrane-bound $LT\alpha_2$-LTβ heteromers[24,25]. Cells that co-express LTα and LTβ not only express the $LT\alpha_2$-LTβ heteromers but also express membrane-bound and soluble LTα-$LT\beta_2$ (refs. 24–26). The LTα-$LT\beta_2$ heterotrimer does not interact with TNFR1 or TNFR2 and instead interacts with the so-called LTβ receptor (LTβR, another member of the TNFRSF) and weakly interacts with the TNFRSF receptor (TNFR) herpesvirus entry mediator (HVEM)[25,27]. Unlike TNF, LTα also interacts with HVEM[25,27]. There is currently no evidence that homotrimeric LTα occurs in a membrane-bound form, but the soluble LTα trimer seems to engage with TNFR1 in a similar fashion to soluble TNF. LTα and TNF have a threefold symmetry and interact in a symmetric fashion with three TNFR1 or TNFR2 molecules[28–30]. By contrast, the inevitably asymmetrical nature of the $LT\alpha_2\beta$ heterotrimers means that despite the trimer having three different potential receptor binding surfaces, only one can efficiently interact with TNFR1 or TNFR2. Despite this limitation, ectopically expressed membrane $LT\alpha_2\beta$ heterotrimers are able to stimulate TNFR1 and TNFR2 signalling[25]. The extent to which LTα homotrimers and/or $LT\alpha_2$-LTβ contribute to the physiological and pathophysiological functions of TNFR1 and/or TNFR2 is, however, largely unknown. Separating the effects of LTα–TNFR1 or $LT\alpha_2\beta$–TNFR1 interactions from the activities of the $LT\alpha\beta_2$-LTβR signalling axis or the LTα-HVEM system is experimentally challenging. Finally, progranulin has been implicated as a factor that competes with TNF for TNFR1 and TNFR2 binding, but several groups have failed to find evidence for a direct progranulin–TNFR1 or progranulin–TNFR2 interaction[31–34].

The well-established fact that enforced physical linkage of two or more soluble TNF trimers (or anti-TNFR2 antibodies) enable the resulting oligomeric TNF trimers (or anti-TNFR2 antibody oligomers) to potently stimulate TNFR2 signalling suggests that the limited capacity of sTNF to stimulate TNFR2 signalling reflects the poor capacity of sTNF-liganded TNFR2 trimers to cluster on the plasma membrane[21,35–37] (Fig. 1). In accordance with this idea, studies have shown that plasma membrane-anchored trimeric sTNF fusion proteins that mimic memTNF trigger microscopically detectable clustering of TNFR2 molecules and potently activate TNFR2 signalling[38]. Furthermore, single-molecule super-resolution microscopy data have shown that sTNF-bound TNFR1 molecules not only assemble into trimers but can

**Fig. 1 | Model of TNFR1 and TNFR2 activation. a**, The first step of activation of TNF receptor 1 (TNFR1) and TNFR2 involves ligand-induced receptor trimerization. Even in the absence of a ligand, TNFR1 and TNFR2 molecules transiently self-associate via their N-terminal preligand binding assembly domains (PLADs; shown in red) into dimers and trimers. TNF trimers preferentially interact with these inactive unstable receptor dimers or receptor trimers, resulting in stabilized liganded TNFR1 or TNRF2 complexes. However, the stabilized, liganded TNFR1 and TNRFR2 trimers are not sufficient to trigger efficient signalling. **b**,**c**, An important step of TNFR1 and TNFR2 activation is clustering of the liganded receptor trimers. Membrane-bound TNF (mTNF)-liganded TNFR1 and TNFR2 trimers spontaneously cluster via their PLAD in the extracellular space of the cell-to-cell contact zone owing to the high local molecule concentrations in this context; this process occurs largely irrespective of the affinity of the PLAD–PLAD interaction ($KD_P$) (**b**). For soluble TNF (sTNF)-liganded TNFR1, the relatively high autoaffinity of the PLAD enables spontaneous secondary clustering of the TNFR1 trimers (**c**). However, for sTNF-liganded TNFR2, the autoaffinity of the PLAD is too low to promote efficient receptor clustering.

also form clusters on the plasma membrane[39]. Replacement of the extracellular domain of the TNFRSF receptor CD95 with those of TNFR1 and TNFR2 results in receptor chimeras that respond to sTNF and memTNF in a manner similar to the two TNFRs[40]. Therefore, the differential capacity of sTNF to stimulate TNFR1 and TNFR2 signalling seems to reflect the differential capacity of the sTNF-liganded TNFR1 and TNFR2 trimers to cluster spontaneously via their ectodomains rather than the different mechanisms by which the two TNFRs engage in intracellular signalling. Notably, various data suggest that 'free' TNRF1 molecules can self-associate before ligand binding[39,41]. Self-assembly of 'free' TNFR1 is mediated by the N-terminal part of the molecule, the so-called preligand binding assembly domain (PLAD)[41]. The PLAD largely spans cysteine-rich domain 1 (CRD1) of the extracellular portion of the receptor, in a region that does not interact with TNF, and various evidence suggests that PLAD also mediates the clustering of liganded TNFR1 trimers[39,42]. TNFR2, as with many other TNFRs, also possesses a PLAD[41] but the TNFR2 PLAD has a lower capacity to self-associate than the TNFR1 PLAD. The low auto-affinity of the TNFR2 PLAD might mean that the PLAD interactions are too weak to promote clustering of sTNF-TNFR2 complexes but are strong enough to promote clustering of memTNF-TNFR2 complexes in the cell-to-cell contact zone (Fig. 1). The PLAD not only contributes to TNFR1 and TNFR2 activity by promoting the clustering of liganded receptor trimers but also facilitates the initial binding of ligands to TNFR1 and TNFR2 by promoting transient formations of receptor dimers or trimers, which have a higher affinity for TNF than receptor monomers[43].

### TNF-induced signalling pathways

Despite the structural similarity of the ectodomains of TNRF1 and TNFR2, the two TNF receptors belong to two distinct, structurally and functionally related subgroups of the TNFRSF. The intracellular part of TNFR1 contains a protein-interaction domain, the so-called death domain (DD), which enables liganded TNFR1 to bind by homotypic interactions to the DD-containing adapter protein TRADD and the DD-containing kinase RIPK1 (refs. 13,14) (Fig. 2). Both TNFR1-associated TRADD and TNFR1-associated RIPK1 secondarily recruit TNF receptor-associated factor 2 (TRAF2) and the TRAF2-interacting E3 ligases cellular inhibitor of apoptosis 1 (cIAP1) and cIAP2, resulting in K63-ubiquitination of TNFR1 signalling complex components, including RIPK1 and the cIAPs[13]. This process is followed by K63-polyubiquitin chain-assisted recruitment of the TGFβ activating kinase 1 (TAK1)–TAK-associated binding protein (TAB) complex and the linear ubiquitin chain assembly complex (LUBAC), formation of linear polyubiquitin chains by the LUBAC and the recruitment of the nuclear factor-κB (NF-κB) essential modulator (NEMO) and IκBα-kinase-2 (IKK2)-containing IKK complex that phosphorylates IκBα and an NEMO–NAP1–TANK–TBK1–IKKε complex that prevents cytotoxic RIPK1 signalling[44,45]. Prior to phosphorylation, IκBα interacts with dimeric transcription factors of the NF-κB family and prevents their nuclear translocation[46]. Phosphorylation of IκBα by the IKK complex triggers proteasomal degradation of this inhibitory protein and enables nuclear translocation of the NF-κB transcription factors and the transcription of NF-κB-regulated genes[46]. This IKK complex-mediated IκBα degradation-dependent mode of NF-κB activation is generally called classical or canonical NF-κB signalling pathway[44,46] (Fig. 2). TRADD, RIPK1 and TRAF2 have also been implicated in TNFR1-induced activation of MAP kinases, such as p38 and JNK, but the corresponding signalling mechanisms are poorly understood and perhaps involve different redundantly acting pathways[12,47]. After release from the TNFR1-associated signalling complex (called complex I), TRADD and RIPK1 can instruct the assembly of apoptosis-inducing and necroptosis-inducing cytosolic protein complexes (called complex IIa, complex IIb or the necrosome, depending on the complex composition)[48,49]. TRADD and RIPK1 but also riboflavin kinase (RFK) might furthermore link TNFR1 to activation of the plasma membrane-associated NADPH oxidase 1 (NOX1) complex and the generation of ROS[12]. TNFR1 can also trigger mitochondrial ROS production by complex JNK-mediated mechanisms[12]. Dependent on the strength and context, TNFR1-induced ROS generation can elicit prolonged ASK1-mediated JNK signalling, cytotoxicity and pro-inflammatory gene induction[12]. Notably, the cytotoxic activities of the apoptotic, necroptotic and ROS-generating complexes induced by this pathway are held in check by TRAF2 and the cIAPs, but also by kinases of the NF-κB signalling pathway, such as IKK2 and TAK1, and genes transcriptionally upregulated by this pathway such as those encoding cell survival proteins, including caspase 8 and FADD-like apoptosis regulator (CFLAR, also known as FLIP), anti-apoptotic BCL2 family members, such as BFL1 (also known as BCL2A1) and BCL-XL, but also cIAP2, A20 and H-ferritin[12,14].

TNFR2 has no DD, does not interact with DD-containing proteins and instead recruits TRAF2 and the TRAF2-interacting cIAPs via a short TRAF2 binding motif[49]. TNFR2 also activates the classical NF-κB pathway via TRAF2 and the cIAPs, but activates this pathway less efficiently than TNFR1 (ref. 43). Recruitment of TRAF2 and the cIAPs to TNFR2, but not to TNFR1, typically reduces the cytosolic pool of these molecules and can therefore inhibit other activities requiring TRAF2 and/or the cIAPs. One of these activities is the constitutive proteasomal degradation of the NF-κB-inducing kinase (NIK); via interaction with the NIK-binding TRAF3 protein, TRAF2 recruits the cIAPs into a complex with NIK to mediate K48-ubiquitination of NIK, marking this molecule for proteasomal degradation[50]. In the absence of TRAF2, TRAF3 or the cIAPs, NIK molecules accumulate and activate IKK1, leading to phosphorylation and proteolytic processing of the p100 NF-κB precursor protein to p52 and nuclear translocation of p52-containing NF-κB transcription factors, including the p52–RelB heterodimer[50]. TNFR2 can trigger this chain of events (known as the alternative or non-canonical NF-κB signalling pathway) by limiting the availability of TRAF2 and the cIAPs[49] (Fig. 2). TNFR2 activation also limits the availability of TRAF2 and the cIAPs for TNFR1-induced NF-κB signalling, thereby sensitizing cells to TNFR1-induced cell death[49]. TNFR2 activation might even reduce the net effect of TNFR1 and TNFR2 engagement on classical NF-κB signalling[49] (Fig. 2). Intriguingly, TNFR2-triggered sensitization to TNFR1-induced cell death can cooperate with TNFR2-induced TNF production and result in scenarios where exclusive stimulation of TNFR2 is sufficient to trigger cell death in an eventually TNFR1-dependent manner[49]. Using TRAF2 and the cIAPs, TNFR2 also recruits the LUBAC and engages the classical NF-κB pathway but is less potent in inducing this pathway than TNFR2 (refs. 43,51). Some evidence suggests that TNFR2 can also signal in a TRAF2-independent manner. In endothelial cells, TNFR2 associates with the cytoplasmic tyrosine-protein kinase BMX (also known as ETK) and promotes TNF-induced cell migration and angiogenesis through TNFR2/BMX-mediated transactivation of vascular endothelial growth factor 2 (VEGFR2) and TNFR2/BMX-mediated activation of the PI3K–AKT signalling pathway[52,53]. TNFR2-mediated activation of the PI3K–AKT pathway might explain the neuroprotective properties of this receptor[54]. Finally, in endothelial cells, TNFR2 can induce JNK activation in an internalization-associated, TRAF2-independent and BMX-independent manner, but the relevance of this pathway to the physiology and pathophysiology of TNFR2 is unclear[55].

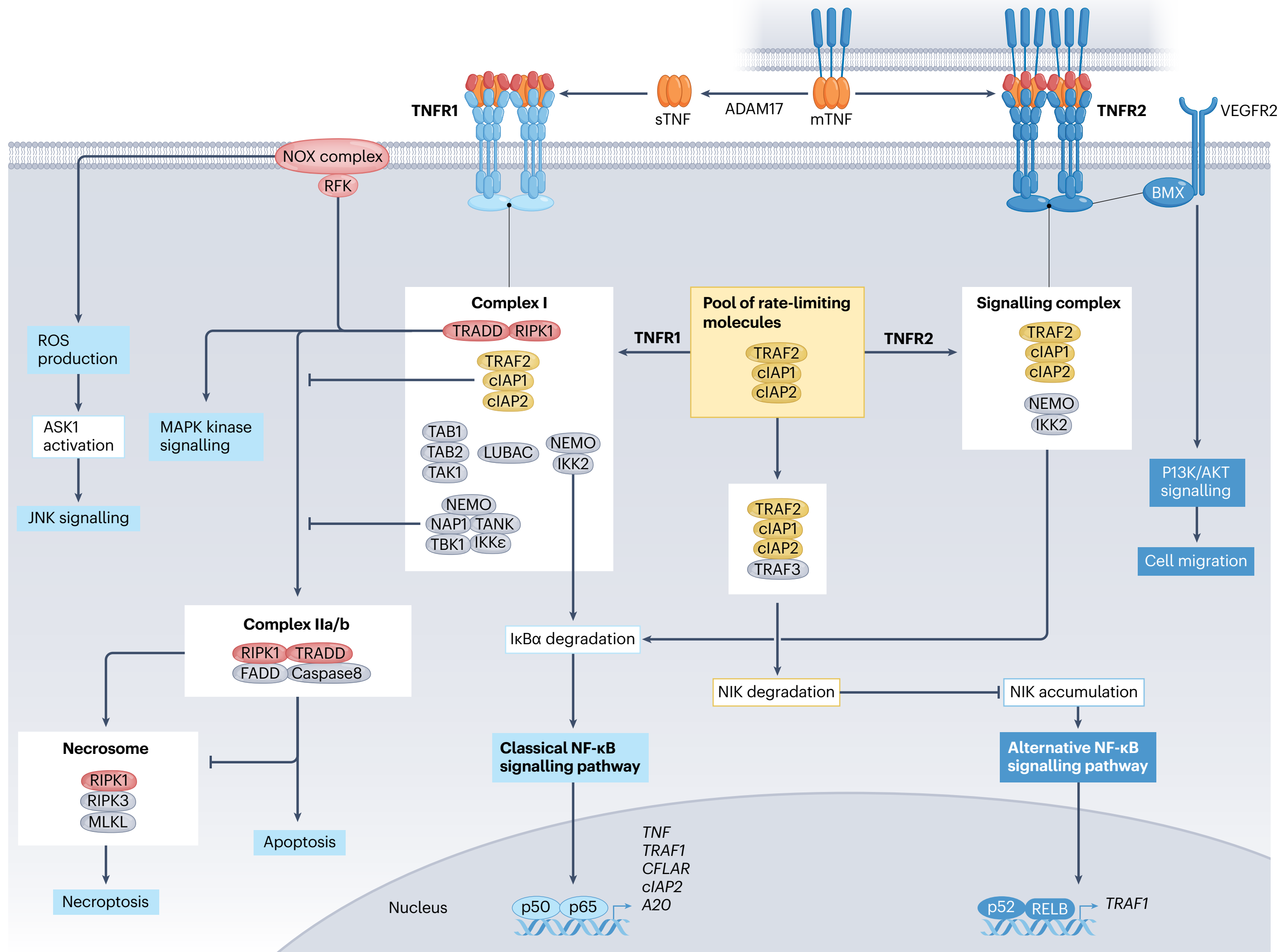



**Fig. 2 | The TNF–TNF receptor signalling network.** The complexity of TNF receptor 1 (TNFR1) and TNFR2 signalling arises from the differential capability of the two receptors to respond to soluble TNF (sTNF; as well as lymphotoxin α (LTα)) and membrane-bound TNF (memTNF) and the fact that signal transduction by TNFR1 and TNFR2 are connected by various feedback loops and different crosstalk mechanisms. For example, TNFR1-mediated stimulation of various kinases (such as IκBα-kinase-2 (IKK2), TBK1 and IKKε) results in the activation of the classical nuclear factor-κB (NF-κB) pathway and MAP kinases and inhibition of the cytotoxic complexes IIa and IIb[49]. TNFR2-mediated recruitment of TNF receptor-associated factor 2 (TRAF2)–cellular inhibitor of apoptosis 1 (cIAP1) and TRAF2–cIAP2 deplete the cytosolic pool of these molecules, triggering the alternative NF-κB pathway (by preventing NF-κB-inducing kinase (NIK) degradation) and sensitizing the cell to TNFR1-induced cell death (by preventing TNFR1-mediated survival signalling)[49]. Various factors are induced downstream of TNFR1 and TNRF2 that modify TNFR1 signalling (such as *CFLAR*, *cIAP2* and *A20* (ref. 14)). TRAF1 is also induced downstream of TNFR1 and TNFR2 and forms heterotrimeric complexes with TRAF2, improving its cell death-inhibiting activities in the context of TNFR1 signalling. Finally, both TNF receptors can induce the production of TNF, helping to amplify TNF signalling[49]. The two TNF receptors might also trigger receptor-selective sets of signalling pathways (for example, TNFR1 triggers pathways involved in cytotoxicity and riboflavin kinase (RFK)-mediated reactive oxygen species (ROS) generation, whereas TNFR2 triggers alternative NF-κB signalling and BMX-mediated vascular endothelial growth factor receptor 2 (VEGFR2) transactivation). Notably, TNFR1 is a more potent activator of classical NF-κB signalling than TNFR2. Furthermore, the classical and alternative NF-κB pathway have different, partly overlapping target genes. Please note that the figure is a simplified depiction of the TNF signalling network and that the actual signalling network is even more complex.

## Approved TNF-neutralizing drugs

Currently, six biologic drugs that inhibit the TNF-TNFR1-TNFR2 system are approved for clinical use: the three IgG1 antibodies infliximab, adalimumab and golimumab, the pegylated $Fab_2$-fragment certolizumab, the trivalent nanobody construct ozoralizumab (comprising two TNF-specific VHH domains and a human serum albumin-specific VHH domain) and the fusion protein etanercept (comprising the ectodomain of TNFR2 and the Fc protein of a human IgG1 antibody) (Table 1 and Fig. 3). All five antibody variants bind TNF and prevent its binding to the two TNF receptors but do not interfere with receptor binding of LTα and

LTα$_2$β heterotrimers. Thus, these reagents still allow residual TNF receptor activation via LTα (TNFR1) and possibly via membrane-bound LTα$_2$β heterotrimers, but whether this continued activity limits the clinical efficacy of the TNF-blocking antibodies in rheumatic diseases and/or other TNFR1-driven diseases is poorly investigated and largely unclear. In one study, LTα was detected in the serum of approximately 22% of patients with rheumatoid arthritis (RA) but was not present in the serum of healthy individuals; furthermore, synovial fibroblasts from patients with RA respond in a similar manner to TNF and LTα in vitro[56,57]. These observations suggest that LTα has a role in RA pathogenesis alongside TNF. However, in a phase II randomized head-to-head study of adalimumab and the anti-LTα antibody pateclizumab, pateclizumab failed to meet the primary end point (4-variable 28-joint disease activity score–erythrocyte sedimentation rate response at 12 weeks) when compared with placebo treatment, and only met 2 out of 8 secondary endpoints[58]. Unfortunately, no studies have assessed the effects of LTα inhibitor therapy in combination with TNF inhibitor treatment. In a mouse model of graft versus host disease, grafts deficient in TNF or LTα, but not those deficient in LTβ, had an attenuated capacity to induce disease, indicating that TNF and LTα can function redundantly to promote disease in this inflammatory scenario[59]. Etanercept binds TNF, LTα and LTα$_2$β heterotrimers and is therefore currently the only TNF inhibitor that can in principle completely block both TNFR1 and TNFR2 signalling. Various studies of patients with RA have shown that etanercept treatment can be beneficial in patients with disease that is refractory to infliximab or adalimumab treatment or for whom the initial response to these reagents is declining[60–63]. This effect might reflect, at least partly, an involvement of LTα in the disease, but other less obvious mechanisms might also contribute. Indeed, some patient with disease that is non-responsive to etanercept therapy can also be efficiently treated with infliximab, even though this drug does not block LTα[64]. The three anti-TNF IgG1 antibodies, and also the IgG1 Fc fusion protein etanercept, bind with low affinity to FcγRI, FcγRIIa, FcγRIIIa and FcγRIIb[65–67]. Binding of TNF increases the affinity of the anti-TNF antibodies, but not of etanercept, for FcγRIIa/b and FcγRIII, enabling the induction of antibody-dependent cellular cytotoxicity (ADCC) in memTNF-expressing cells[65–67]. Similarly, the binding of the three anti-TNF IgG1 antibodies to memTNF triggers complement-dependent cytotoxicity (CDC)[65–67]. Reverse signalling of memTNF after anti-TNF antibody binding might also promote cell death of memTNF-expressing cells[66,67]. However, the extent to which the induction of ADCC, CDC and reverse memTNF signalling contributes to the therapeutic efficacy of the various TNF neutralizing biologic drugs is unclear.

## Emerging TNFR1-targeting drugs

A broad range of evidence from animal models highlights the detrimental effects of TNF in tissue damage and during autoimmune diseases, dominantly arising from activities downstream of TNFR1. For example, TNFR1-knockout mice are largely protected from collagen-induced arthritis (CIA)[68], myelin oligodendrocyte glycoprotein-induced experimental autoimmune enzephalomyelitis (EAE)[69], JunB/cJun double deficiency-induced and imiquimod-induced psoriasis[70,71], APP23 transgene-induced Alzheimer disease[72], ischaemia–reperfusion-induced retinal damage[73], angiotensin II-induced and bile duct ligation-induced fibrosis[74] and myocardial infarction[75]. Furthermore, TNFR1 signalling is mainly responsible for the life-threatening effects of TNF in sepsis[76–79]. Moreover, the lethality of knocking out genes that encode NF-κB signalling components (such as *Rela*) or inhibitors of apoptotic or necroptotic signalling (for example, *Cflar*, cIAP-encoding genes and *Casp8*) is often rescued or delayed upon knocking out TNFR1 (refs. 80–83). Currently, three distinct types of biologic drugs are under clinical investigation that preferentially or selectively interfere with the TNF-TNFR1 signalling axis (Table 2): dominant-negative mutants of sTNF, antagonistic TNFR1-specific antibodies and PLAD variants (Fig. 3).

### Dominant-negative sTNF mutants

Pegipanermin (formally known as XPro1595, XENP1595, INB03 or XPro™) is a TNF mutant that is unable to interact with TNFR1 and TNFR2 (owing to the mutations Y87H and A145R) and that can exchange protomers with soluble wild type TNF, resulting in dominant-negative TNF trimers with a mixed wild type–mutant protomer composition[84]. Pegipanermin carries additional mutations (C69V, C101A and R31C) that enable specific PEGylation at C31 to improve its half-life. Intriguingly, XPro1595 fails to inhibit memTNF-induced caspase activation in U937 cells[85], suggesting that pegipanermin has a limited capacity to exchange

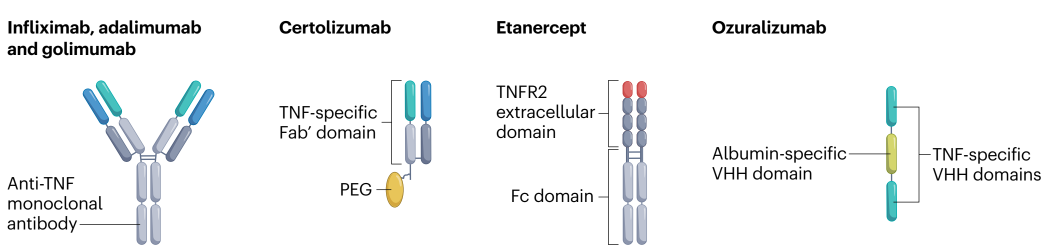
**Table 1 | Approved TNF-neutralizing biologic drugs**

| Biologic drug | Molecule type | Mode of action(s) | Limitation(s) | Indication(s) | Ref. |
|---|---|---|---|---|---|
| Etanercept | TNFR2–Fc fusion protein | TNF and LTα neutralizing | - | RA, PsA, AS, plaque psoriasis and JIA | 4 |
| Infliximab | Chimeric IgG1 antibody | TNF neutralizing | No LTα neutralization | RA, PsA, AS, plaque psoriasis, adult Crohn disease, paediatric Crohn disease and ulcerative colitis | 157 |
| Adalimumab | Human IgG1 antibody | TNF neutralizing | No LTα neutralization | RA, PsA, AS, plaque psoriasis, adult Crohn disease, paediatric Crohn disease, ulcerative colitis, JIA, hidradenitis suppurativa and uveitis | 157 |
| Golimumab | Human IgG1 antibody | TNF neutralizing | No LTα neutralization | RA, PsA, AS and ulcerative colitis | 157 |
| Certolizumab | PEGylated Fab′ fragment | TNF neutralizing | No LTα neutralization | RA, PsA, AS and plaque psoriasis, adult Crohn disease and axSpA | 157 |
| Ozoralizumab | Trivalent nanobody construct (two TNF-specific VHH domains and a human serum albumin-specific VHH domain) | TNF neutralizing | No LTα neutralization | RA (Japan) | 158 |

AS, ankylosing spondylitis; axSpA, axial spondyloarthritis; JIA, juvenile idiopathic arthritis; LTα, lymphotoxin α; PsA, psoriatic arthritis; RA, rheumatoid arthritis.

protomers with memTNF and accordingly elicits its effects specifically by preventing sTNF-induced TNFR1 activation. Thus, memTNF–TNFR1 signalling remains unaffected by pegipanermin. Pegipanermin has therapeutic effects in two mouse models of arthritis (CIA and collagen antibody-induced arthritis)[85], as well as a variety of other disease models including several models of neurological diseases[86–99]. In view of the latter and initial evidence showing that patients being treated with TNF inhibitors have a reduced risk of Alzheimer disease[100,101], pegipanermin is currently under investigation in an open-label extension phase II clinical study to evaluate its safety and efficacy in patients with mild Alzheimer disease or in patients with mild cognitive impairment[102,103].

R1antTNF is another dominant-negative variant of TNF that is under preclinical investigation[104], but this variant has a different mode of action to pegipanermin. R1antTNF was obtained by screening a phage display library of receptor binding-site mutants of TNF for TNFR1 binding and subsequent evaluation of the selected TNF mutants for a lack of cytotoxicity. Using this approach, a high-affinity TNFR1-interacting TNF mutant was identified that had low cytotoxic activity and low affinity for TNFR2. This TNF variant inhibits TNF–TNFR1 interactions by competing for TNFR1 binding[104]. Why R1antTNF fails to trigger TNFR1 signalling despite binding TNFR1 with a high affinity is poorly understood. Both the association rate constant and the dissociation rate constant of the R1antTNF–TNFR1 interaction are higher than that of the TNF–TNFR1 interaction, indicating that this ligand–receptor complex assembles more rapidly but, more importantly, also dissociates more rapidly[104]. Thus, the half-lifetime of the R1antTNF–TNFR1 complex might be too short to enable intracellular assembly of the TNFR1-associated signalling complex. R1antTNF has therapeutic effects in a mouse model of acute hepatitis[104] and a pegylated version of R1antTNF is effective in mouse models of CIA and EAE[105,106]. The competitive mode of action of R1antTNF suggests



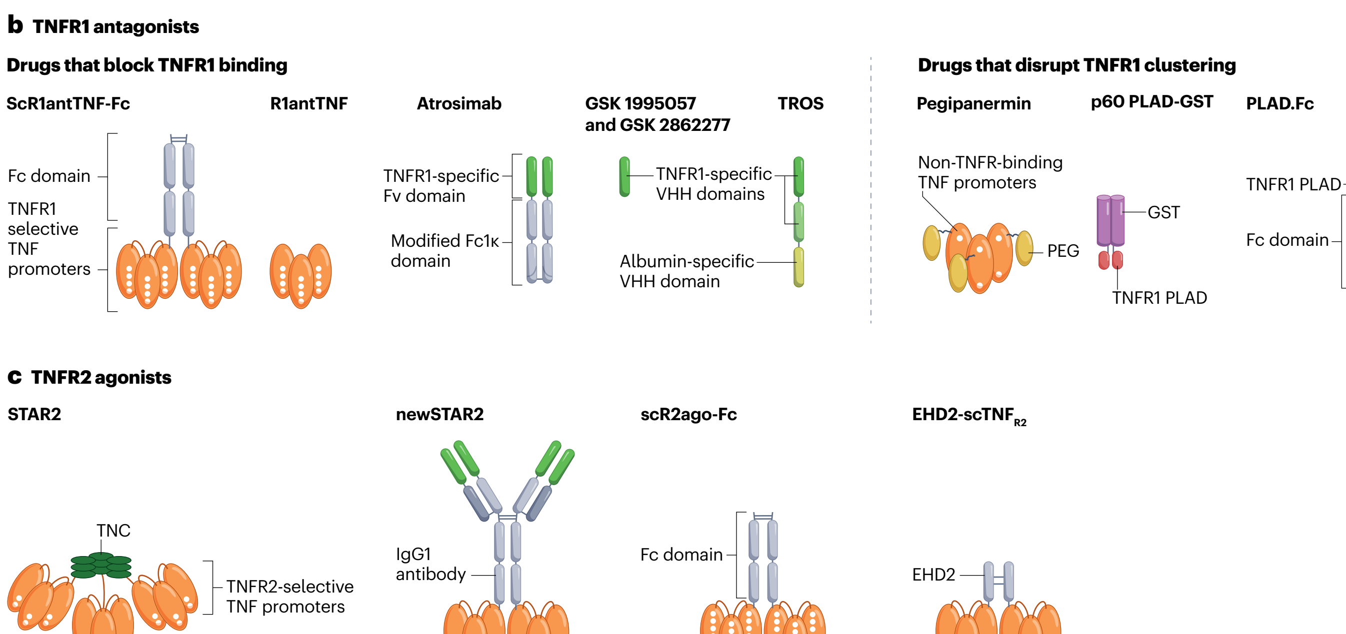




**Fig. 3 | Domain architecture of selected approved and experimental biologic drugs that target TNFR1 or TNFR2. a**, All approved TNF inhibitors (consisting of various antibodies and a soluble TNFR2–Fc fusion protein) bind soluble TNF (sTNF) and membrane-bound TNF (mTNF) and prevent their binding to TNFR1 and TNFR2. **b**, The antagonistic TNFR1 biologics under preclinical and clinical development either contain dominant-negative TNF variants or antibody fragments that block access of wild type TNF to TNFR1 or interfere with TNFR1–TNFR1 clustering. **c**, The major TNFR2 agonists in preclinical development contain two or more single-chain encoded TNFR2-specific TNF trimers. White circles in the TNF protomers indicate mutations conferring TNFR2 specificity. EHD2, EH-domain–containing protein 2; GST, glutathione S-transferase; PEG, polyethylene glycols; PLAD, preligand binding assembly domain; TNC, tenascin C domain.

**Table 2 | Selected preclinical and/or experimental drugs that target TNF and TNF receptors**

| Biologic drug | Molecule type | Mode of action(s) | Limitation(s) | Translational stage | Refs. |
|---|---|---|---|---|---|
| **TNF inhibitors** | | | | | |
| TNF kinoid | TNF–KLH conjugate | Active vaccination | No LTα neutralization | Phase II for RA and Crohn disease | 159–163 |
| AVX-470 | Oral polyclonal bovine anti-TNF antibody | TNF neutralizing | No LTα neutralization | Phase I for ulcerative colitis | 159,164 |
| **TNFR1 antagonists** | | | | | |
| Atrosab | Humanized anti-TNFR1 antibody containing an effector-dead IgG1 Fc region | TNFR1 blocking | Residual agonistic effects | Preclinical | 109 |
| Atrosimab | Fv–Fc fusion protein (monovalent derivative of atrosab) | TNFR1 blocking | – | Phase I in healthy volunteers | 108,111 |
| TROS | A heterotrimeric nanobody fusion protein composed of two different TNRF1-specific VHH domains and an albumin-specific VHH domain | TNFR1 blocking | – | Preclinical | 118 |
| GSK 1995057 and GSK 2862277 | Single domain antibody (comprising one VHH domain) against TNFR1 | TNFR1 blocking | Agonistic effects; development of autoantibody complexes | Phase II for respiratory disorders | 112–116,165 |
| Pegipanermin | PEGylated TNF mutant | sTNF 'destructive' | memTNF-TNFR1 axis remains intact | Phase II for Alzheimer disease or mild cognitive impairment; Phase III for COVID-19 but terminated owing to futility. | 84,102,103, 166–168 |
| R1antTNF | TNFR1-selective, antagonistic TNF mutant | TNFR1 blocking (competitor) | – | Preclinical | 104 |
| scR1antTNF–Fc | Fc fusion protein containing six TNFR1 competitive TNF protomers | TNFR1 blocking (competitor); no TNFR2 binding | – | Preclinical | 142 |
| PLAD.Fc | Fc fusion protein containing PLAD of TNFR1 | Inhibition of TNFR1 clustering | – | Preclinical | 122 |
| p60 PLAD–GST | GST fusion protein containing PLAD of TNFR1 | Inhibition of TNFR1 clustering | Modest half-life in serum (approximately 10 hours) | Preclinical | 121 |
| **Potential TNFR2 agonists** | | | | | |
| STAR2 | TNC fusion protein containing nine TNFR2-specific TNF protomers | TNFR2 agonist | Modest serum retention | Preclinical | 35,143 |
| NewSTAR2 | IgG1 fusion protein containing six TNFR2-specific TNF protomers | TNFR2 agonist | – | Preclinical | 143 |
| scR2ago–Fc | Fc fusion protein containing six TNFR2-specific TNF protomers | TNFR2 agonist | – | Preclinical | 169 |
| EHD2–$scTNF_{R2}$ | EHD2 fusion protein containing 6 TNFR2-specific TNF protomers | TNFR2 agonist | – | Preclinical | 140 |
| SIM0235 (or SIM1811–03) | Anti-TNFR2 IgG1 antibody | Ligand blocking FcγR-dependent agonism; ADCC and ADCP | Complex, qualitatively contrasting effects, including $T_{reg}$ cell depletion | Phase I in cancer | 150,170 |
| HFB 200301 | Anti-TNFR2 IgG1 antibody | Synergistic agonism with sTNF; FcγR-dependent agonism; ADCC and ADCP | Complex, qualitatively contrasting effects, including $T_{reg}$ cell depletion | Phase I in cancer | 151 |
| BI-1808 | Anti-TNFR2 IgG1 antibody | Ligand blocking FcγR-dependent agonism; ADCC and ADCP | Complex, qualitatively contrasting effects, including $T_{reg}$ cell depletion | Phase I–II in cancer in combination with pembrolizumab | 149 |

ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; EHD2, EH-domain-containing protein 2; GST, glutathione-S-transferase; KLH, Keyhole limpet hemocyanin; LTα, lymphotoxin α; memTNF, membrane-bound TNF; PLAD, pre-ligand binding assembly domain; RA, rheumatoid arthritis; sTNF, soluble TNF; TNC, tenascin C domain; TNFR, TNF receptor; $T_{reg}$ cell, regulatory T cell.

that this mutant inhibits TNFR1 stimulation by all its natural ligands (that is, sTNF, memTNF, LTα and $LT\alpha_2\beta$), but this effect has not yet been explicitly analysed.

### TNFR1 inhibitors

At first glance, the most straightforward way to selectively prevent TNFR1 engagement while leaving the potentially anti-inflammatory signalling of the TNFR2 axis intact is the use of ligand blocking anti-TNFR1 antibodies. In view of the high systemic toxicity of TNFR1 engagement and the high auto-affinity of TNFR1, however, the key challenge is in preventing residual weak TNFR1 agonism that could be triggered by the binding of the blocking antibody. This challenge is nicely exemplified by the experience in the development of the TNFR1-blocking antibody variant atrosimab. Atrosimab essentially evolved from the murine IgG2a anti-TNFR1 antibody H398 that effectively blocks TNF–TNFR1 interactions[107] but still has a weak agonistic activity[23]. This residual activity remained even after humanization of the antibody and introduction of an effector-dead IgG1 Fc region, resulting in the atrosimab precursor atrosab[108,109]. The problem of the remaining agonism of atrosab presumably led to the termination of its phase I evaluation and was solved by affinity maturation of the variable domains of atrosab and their fusion to a Fc heterodimerizing scaffold, resulting in the monovalent TNFR1 blocker atrosimab (Fig. 3). Indeed, atrosimab has shown clinical efficacy in various disease models of human TNFR1 knock-in mice[108,110]. The pharmacokinetics and safety of atrosimab in healthy individuals has now been investigated in a phase I study[111] (Table 2).

A second monovalent blocking TNFR1 antibody construct under clinical investigation is the TNFR1-specific nanobody (VHH domain) GSK1995057 (Fig. 3). In a first clinical dose escalation study[112] of intravenous infusion of GSK1995057, signs of cytokine release, and thus potential TNFR1 agonistic activity, became evident in some volunteers with pre-existing drug-reactive autoantibodies[113]. In a second clinical study of GSK2862077, a variant of GSK1995057 selected for reduced binding affinity to pre-existing autoantibodies, the majority of volunteers, with one exception, showed no signs of TNFR1 agonism[114]. One volunteer, however, showed signs of mild cytokine release owing to having autoantibodies that recognized GSK1995057 but not GSK2862077 (ref. 115). Pretreatment of non-human primates with aerosolized GSK1995057 showed protective activity against neutrophil-driven lung injury induced by nebulized LPS and also reduced pulmonary inflammation in a related phase I study of healthy volunteers pre-screened for the absence of autoantibodies[116,117]. Another TNF-blocking TNFR1-specific antibody construct is TNF receptor one silencer (TROS) (Fig. 3), a fusion protein comprising a N-terminal VHH domain specific for serum albumin to enhance its serum retention, a blocking TNFR1-specific VHH domain and a non-blocking TNFR1-specific VHH domain that strongly enhance the affinity of the construct to TNFR1 (ref. 118). TROS protects against the development of EAE in TNFR1 transgenic mice[119].

### PLAD constructs and small molecules

The PLADs of TNFR1 and TNFR2 facilitate TNF binding by transient formation of ligand-free receptor trimer or dimer complexes that have a higher affinity for TNF than receptor monomers and by promoting the clustering of liganded receptor trimers (Fig. 1). As the PLAD is not directly involved in ligand binding, soluble PLAD-containing variants should bind to and lower the apparent affinity of intact TNFR molecules for TNF and should furthermore bind to and reduce the clustering of liganded TNFR trimers (Fig. 3). Therefore, fusion proteins of the TNFR1 PLAD and the human IgG1 Fc domain or glutathione S-transferase (GST) have been evaluated for their therapeutic efficacy in models of autoimmune diseases. The TNFR1 PLAD–GST fusion protein can ameliorate CIA in DBA/1 J mice and prevent skin injury in the MRL/lpr model of systemic lupus erythematosus[120,121]. The TNFR1 PLAD–Fc fusion protein, furthermore, can reduce the severity of diabetes in NOD mice and ameliorate disease in mice with EAE[122]. Ultimately, despite their different mode of action at the molecular level, TNFR1 PLAD constructs and TNFR1-blocking antibodies are expected to result in selective TNFR1 inhibition. Notably, in contrast to the majority of approved TNF inhibitors, all these novel reagents, with the exception of PLAD.Fc, do not engage FcγRs. The future awaits on whether such novel types of biologic drugs can compete with the well-established antibody-based biologic drugs and find their way into the clinic.

Various attempts have been made to identify TNFR1-binding small molecule inhibitors. The small molecule F002 was identified by virtual screening as a compound that interacts with a loop in the extracellular domain of TNFR1 that is crucially involved in ligand binding[123]. F002 and its next-generation analogues C7 and SGT11 show therapeutic activity in mouse models of CIA and traumatic brain injury[123,124]. R1, another small molecule, has also been identified by in silico studies as an inhibitory TNFR1 binding compound but this molecule only partly blocks TNF-induced killing of L929 cells, even at a high concentration of 200 μM (ref. 125). However, all these TNFR1-inhibitory small molecules identified by in silico studies are poorly characterized with respect to their selectivity for TNFR1 and their effects on TNF–TNFR1 interactions and TNFR1 signalling complex formation. Another series of inhibitory TNFR1-binding compounds has been identified using high-throughput screening of small molecules that affect TNFR1 dimerization in the absence of a ligand[125–127]. These efforts resulted in TNFR1-inhibitory compounds that fail to inhibit ligand binding but that disrupt or perturb TNFR1 dimerization. These small molecules are poorly characterized with respect to their selectivity for TNFR1 and have not been studied with respect to modulation of TNFR1 activity in vivo. None of the small molecule inhibitors of TNFR1 has yet been tested for its capability to interfere with memTNF-induced TNFR1 signalling.

## Emerging TNFR2-targeting drugs

TNFR2 might be targeted for different purposes, depending on the disease and pathological condition considered. Targeted activation of TNFR2, with the aim of exploiting the anti-inflammatory activities and tissue-protective functions of this receptor, is a promising strategy for the treatment of immune-mediated diseases. Indeed, an array of strong evidence from independent groups using different types of TNFR2 agonists suggests that therapeutic activation of TNFR2 in vivo promotes the proliferation, survival, suppressive activity and stability of $T_{reg}$ cells[2,4]. Targeted activation of TNFR2 could also be an option to boost antitumour immunity via the costimulation of $CD8^+$ T cells, but the prospects of success are highly dependent on the net effect of the TNFR2 agonist on $T_{reg}$ cells and on effector T cells. Likewise, targeted inhibition of TNFR2, either through the use of TNFR2 blocking antibodies or through depletion of TNFR2-expressing $T_{reg}$ cells, might have an overall anti-tumoural effect when the TNFR2-driven activity of suppressive immune cells is of sufficient relevance to the underlying tumour processes.

Mechanistically, the beneficial effects of TNFR2 stimulation on $T_{reg}$ cells are likely caused by TNRF2-mediated activation of RelA (also known as transcription factor p65) and cRel-containing NF-κB dimers via the classical NF-κB pathway, a switch from oxidative to glycolytic metabolism and inhibition of DNA methylation of the Foxp3 promoter,

## Glossary

**Antibody-dependent cellular cytotoxicity**
An antibody effector function by which antibody-opsonized cells are recognized and killed or lysed by immune cells that express appropriate Fcγ receptors (such as natural killers).

**Antibody-dependent cellular phagocytosis**
An antibody effector function by which antibody-opsonized cells are recognized and phagocytosed by macrophages.

**Association rate constant**
This constant describes the kinetics by which two components (such as a ligand and receptor) form a complex.

**Auto-affinity**
Affinity of proteins for self-assembly.

**Complement-dependent cytotoxicity**
An antibody effector function triggered by cell-bound IgG and IgM antibodies, which results in cell killing or lysis by the so-called membrane attack complex of the complement system.

**Dissociation rate constant**
This constant describes the kinetics of the dissociation of a two-component complex (such as a ligand–receptor complex) into the individual components.

reducing the conversion of $T_{reg}$ cells to T helper 17 ($T_H17$) cells[128–131]. The idea that NF-κB signalling is of central relevance to the activity of TNFR2 in $T_{reg}$ cells is in accordance with the broadly documented role of the NF-κB system in the development and maintenance of $T_{reg}$ cells[132]. Intriguingly, other NF-κB-stimulating receptors of the TNFRSF such as 41BB, GITR and CD27, which, similar to TNFR2, directly interact with TRAF2, and DR3, which, similar to TNFR1, signals via TRADD and RIPK1, also promote the expansion of $T_{reg}$ cells[129,133]. Moreover, TNFR2, GITR and OX40 redundantly promote thymic development of $T_{reg}$ cells in mice[134]. Some evidence also suggests that TNFR2-induced NF-κB signalling in MDSCs is crucial for their survival and immune suppressive activity[135,136]. The NF-κB system has a ubiquitous cellular distribution. Therefore, its central relevance to the immune suppressive activities of TNFR2 suggests that the nature of the TNFR2-responsible cell types rather than specialized TNFR2-associated signalling mechanisms is the major mechanism determining the anti-inflammatory effects of TNFR2. This notion concurs with the high expression of TNFR2 on $T_{reg}$ cells, MDSCs and regulatory B cells. Agonistic TNFR2 targeting could also improve the tissue-regenerative capacity of mesenchymal stem cells and their ability to induce conversion of conventional T cells into $T_{reg}$ cells[137,138].

Therapeutic activation of TNFR2 could principally be achieved using TNF (or LTα) derivative or antibody constructs. The development of both types of agonists has to overcome the distinct limitations associated with sTNF and conventional anti-TNF antibodies.

### Ligand-based TNFR2 agonists

Three limitations must be addressed in the development of TNF-based TNFR2-specific agonists: the inefficient secondary clustering of sTNF-TNFR2 complexes; co-stimulation of TNFR1; and the short serum half-life of sTNF. The 'activating' secondary clustering of sTNF-TNFR2 complexes can be enforced by linking two or more sTNF trimers via genetic engineering. Mutations in TNF that prevent TNFR1 binding but preserve its interaction with TNFR2 can ensure selectivity for TNFR2. Finally, the serum half-life of sTNF can be improved by fusing it with a protein or protein domain that has a long serum half-life. Several highly active TNFR2-specific TNF fusion proteins have been rationally designed, taking these considerations into account (Fig. 3). Practically all the TNF-based TNFR2 agonists developed so far use stabilized forms of TNFR2-specific TNF mutants as building blocks, in which the three protomers of the TNF trimer have been genetically connected via short linker peptides (Table 2 and Fig. 3). Genetic fusion of these TNFR2-specific single-chain encoded TNF (scTNF) building blocks with oligomerizing protein domains have resulted in fusion proteins with highly agonistic properties. STAR2 (also known as TNC–scTNF80) is a fusion protein of scTNF80 and the small trimerization domain of tenascin C and thus comprises three scTNF80 trimers[35,139]. This fusion protein was the first stoichiometrically defined TNF-based fusion protein that showed high, memTNF-like activity in vitro and in vivo[35,139]. Several other TNFR2-specific scTNF fusion proteins have since been developed with two TNFR2-specific single-chain trimer domains (including NewSTAR2 (also known as irrelevant IgG1(N297A)–scTNF80), EHD2–$scTNF_{R2}$ and scR2ago–Fc) or four TNFR2-specific single-chain trimer domains (including p53–sc(mu)$TNF_{R2}$ and GCN4–sc(mu)$TNF_{R2}$)[140–143]. With the exception of scR2ago–Fc, all of these fusion proteins utilize the same two mutations (D143N and A145R[144]) on two conserved positions in human and mouse TNF to prevent TNFR1 binding. All these TNF-based agonists can induce efficient expansion of $T_{reg}$ cells in vitro (Table 3). The various TNFR2 agonists, however, differ in their pharmacokinetic properties. The serum retention of the neonatal Fc receptor (FcRn)-interacting IgG1(N297A)-based NewSTAR2 variant is considerably higher than that of STAR2, which does not interact with the FcRn[139,143]. Serum retention of the other ligand-based TNFR2 agonists has not yet been studied side by side. However, comparative analysis of EDH2–scTRAIL and Fc–scTRAIL, two fusion proteins with domain architectures similar to those of EHD2–$scTNF_{R2}$ and scR2ago–Fc, revealed that the Fc fusion protein had a superior half-life[145]. Furthermore, comparative analysis of Fc–scTRAIL and IgG1–scTRAIL, which has the same domain architecture as NewSTAR2, revealed that the half-life of the IgG1 fusion protein format was superior to that of the Fc fusion protein format[146]. The ligand-based TNFR2 agonists have been tested in various preclinical disease models, showing promising therapeutic activity in EAE, CIA, graft versus host disease, T cell transfer-induced colitis, myocardial infarction, spinal cord injury and Alzheimer disease (Table 3). In particular, in accordance with the high TNFR2 specificity conferred by the mutations in the building blocks scTNF80 and $scTNF_{R2}$ (ref. 144), the various agonistic fusion proteins showed no toxicity in mice, even when the mice were treated repeatedly with high doses.

### Antibody-based TNFR2 agonists

Agonistic antibody targeting of TNFRs in general and of TNFR2 in particular have garnered increasing interest. Comprehensive and steadily growing work in this field have revealed an unexpected complexity in how anti-TNFR antibodies function in vivo. This complexity mainly results from the interaction of the anti-TNFR antibodies with FcγRs, the availability of FcγR-expressing cells in vivo and the effect an antibody has on TNF–TNFR interactions (Fig. 4). Anti-TNFR2 IgG antibodies, and also IgG antibodies that target several other types of TNFRs, do not, or only poorly, activate receptor signalling as free molecules but often display strong agonistic activity upon binding to FcγRs[29] (Fig. 4). Intriguingly, the agonism of FcγR-bound anti-TNFR2 antibodies is independent from the epitope the antibody recognizes. Thus, ligand-blocking anti-TNFR2 antibodies that can bind FcγRs can elicit both antagonistic or agonistic activities, depending on whether the

antibodies are functioning as free or FcγR-bound molecules[23]. Furthermore, the agonistic activity of anti-TNFR2 antibodies is independent of the IgG subclass and the type of FcγR as long as the strength of the interaction between these two types of protein molecules is sufficient[23]. These observations suggest that the sole presentation of anti-TNFR2 IgG antibodies in a plasma membrane-associated form is enough to bestow the molecule with agonistic activity, resembling the situation of the differential effects of sTNF and memTNF on TNFR2.

The development of FcγR-binding agonistic anti-TNFR2 IgG antibodies has to consider several issues beyond TNFR2 activation. For example, in the case of interactions with FcγRI1, FcγRIIA or FcγRIIIA, an anti-TNFR2 antibody not only functions as a TNFR2 agonist but also as a FcγR agonist capable of killing the TNFR2-expressing cell via antibody-dependent cellular phagocytosis (ADCP) and/or ADCC (Fig. 4). Similarly, anti-TNFR2-IgG3 and anti-TNFR2-IgG1 antibodies might kill TNFR2-expressing cells via CDC (Fig. 4). The availability of FcγR-expressing cells might be too low to ensure occupation of all TNFR2 molecules by FcγR-bound anti-TNFR2 antibodies. Hence, in the case of ligand-blocking anti-TNFR2 antibodies, this low availability can result in the 'unproductive' situation in which the FcγR-bound fraction of the anti-TNFR2 antibody molecules activates TNFR2 while the free fraction of the same antibody inhibits TNFR2 signalling by blocking TNFR2 engagement by endogenous memTNF. The use of non-blocking anti-TNFR2 antibodies can prevent this interference. Indeed, some non-blocking anti-TNFR2 antibodies, such as 80M2, can elicit FcγR-independent agonistic effects in synergy with sTNF, presumably by cross-linking inactive sTNF-liganded TNFR2 trimers[21] (Fig. 4). Overall, the surrounding microenvironment of TNFR2-expressing cells, with respect to the number and type of FcγR-expressing cells, likely shapes the quality of the anti-TNFR2 antibody net response. As such, the agonistic effects of FcγR-binding anti-TNFR2 IgG antibodies must be considered as a 'fluid' property that occurs in concert with other

**Table 3 | Therapeutic activity of TNFR2-specific agonists in vivo**

| TNFR2 agonist | Disease model | Dosing | Effect(s) | Ref. |
|---|---|---|---|---|
| STAR2 | Graft versus host disease; graft versus leukaemia (FVB/N donor mice and B6 recipient mice) | 6×75 µg i.p. injection (12, 9, 7, 4, 2 and 0 days prior to disease induction) | 50–100% increase in total $T_{reg}$ cell number; reduced graft versus host disease lethality (by 60–80%); intact graft versus leukaemia | 139 |
| | BCG-induced inflammation | 1–2×75 µg i.p. injection before last BCG challenge | 100% increase in $T_{reg}$ cell frequency; enhanced IL-10 production | 171 |
| | CIA | 6×75 µg i.p. (0, 2, 4, 6, 8 and 10 days after reaching a clinical score of 10) | Reduced clinical score | 172 |
| | T cell transfer-induced colitis | $T_{reg}$ cells incubated with 48 ng/ml for 2 h prior to transfer alongside conventional T cells | Reduced clinical score | 129 |
| | Myocardial infarction | 6×75 µg i.p. (0, 2, 4, 7, 9, 12 days after disease induction) | Improved left ventricular function, but also increased myocardial infarction-associated mortality | 173 |
| | EAE | 75 µg every second day (days 4–18) | Reduced clinical score | 174 |
| NewSTAR2 | Healthy $Foxp3^{+}$ Luci mice | 1×140 µg i.p. | Approximately 300% increase in $T_{reg}$ cell numbers 5 days post- injection | 143 |
| | Graft versus host disease (FVB/N donor mice and B6 recipient mice) | 1×140 µg i.p. injection (5 days prior to disease induction) | Reduced graft versus host disease lethality (by 60–80%) | |
| | Alzheimer disease (J20 mice) | 2.5 µg/g i.p. injection, twice weekly for up to 6 weeks | Partly improved cognitive function; reduced Aß plaque load and BACE expression | 175 |
| EHD2–$scTNF_{R2}$ | Constriction injury-induced Pain | 10 µg/g i.p. injection (7, 10 and 13 days post-trauma) | Reduced neuropathic pain (by 50%); 100% increase in $T_{reg}$ cell density in the sciatic nerve | 176 |
| | EAE | 10 µg/g i.p. injection (6, 9 and 12 days after disease induction) | Reduced clinical score | 176 |
| | Spinal cord Injury | 1 µg/h continuous infusion (osmotic pump), immediately after injury (days 3–28) | Improved locomotion score | 177 |
| | CIA | 10 µg/g i.p. injection twice weekly starting 4 days post-immunization or after onset of disease | Reduced clinical score when treatment started 4 days post-immunization; no effect after onset of disease | 178 |
| | Nucleus basalis magnocellularis lesion | 1×540 ng local injection alongside NMDA injection | Reduced microglia activation; reduced NMDA-induced memory impairment | 140 |
| scR2ago–Fc | DNFB-induced contact hypersensitivity | 2×5 or 50 µg i.p., 1 and 2 days prior to DNFB injection | 80–100% increase in $T_{reg}$ cell frequency; reduced hypersensitivity (by 50%) | 169 |

BCG, Bacillus Calmette-Guérin; CIA, collagen-induced arthritis; EAE, experimental autoimmune encephalomyelitis; DNFB, 2,4-dinitrofluorobenzene; i.p., intraperitoneal; NDMA, N-nitrosodimethylamine; $T_{reg}$ cell, regulatory T cell.

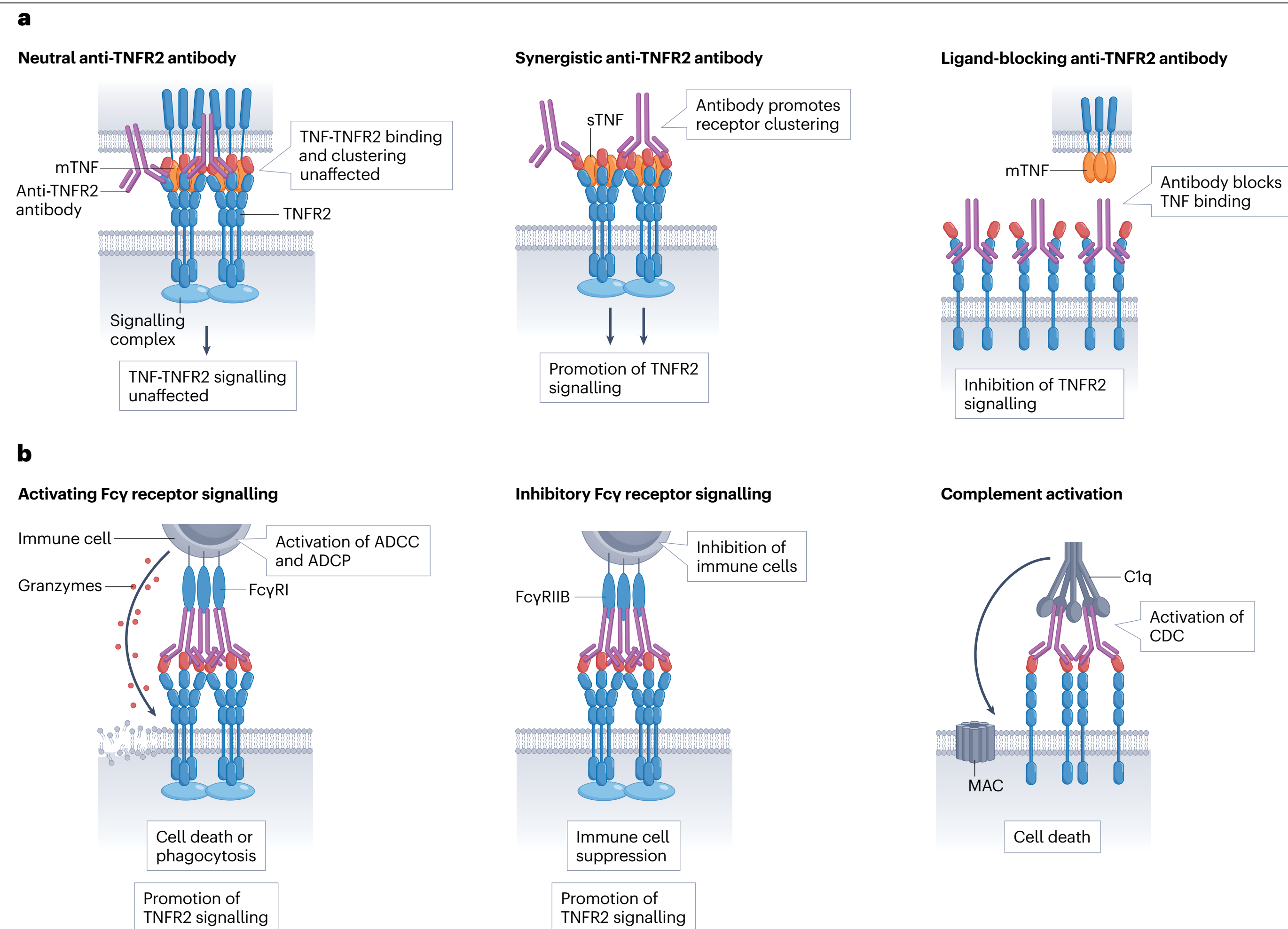



**Fig. 4 | Possible modes of action of anti-TNFR2-specific IgG antibodies.** The in vivo activities of anti-TNF receptor 2 (TNFR2) IgG antibodies depend on the antibody subclass and their effect on ligand binding and TNFR2 clustering. **a**, Anti-TNFR2 antibodies, irrespective of their IgG subclass and FcγR-binding or C1q-binding capacity, have three potential modes of action. First, the antibody does not affect ligand binding and has no effect on the ligand–TNFR2 complexes formed (neutral anti-TNFR2 antibodies). Second, the antibody does not affect ligand binding but synergistically promotes the activity of TNFR2 in combination with soluble TNF (sTNF) or LTα, for example, by facilitating secondary clustering of liganded TNFR2 trimers (synergistic anti-TNFR2 antibodies). Third, the antibody blocks ligand binding and prevents membrane-bound TNF (memTNF)-induced TNFR2 activation (ligand-blocking anti-TNFR2 antibodies). **b**, Anti-TNFR2 antibodies, dependent on their subclass, can interact with inhibitory or activating Fcγ receptors (FcγRs), triggering antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP) of TNFR2-expressing cells or inhibition of immune cell activities. Anti-TNFR2 antibodies might also trigger complement-dependent cytotoxicity (CDC) lysis by binding to C1q. Please note, the modes of action shown in **a**, and **b**, are not necessarily exclusive. MAC, membrane attack complex.

TNFR2-independent activities. In terms of other strategies, similar to oligomerization of sTNF trimers, oligomerization of anti-TNFR2 antibodies results in FcγR-independent agonism, presenting the possibility of obtaining anti-TNFR2 antibody variants with intrinsic agonistic activity[147].

Several anti-TNFR2 antibodies have so far been described in the literature[1,148], and three of these antibodies (SIM0235, HFB 200301 and BI-1808) are currently under investigation in phase I trials in patients with cancer[149–151]. All three antibodies are of the IgG1 subclass and are accordingly able to deplete TNFR2-expressing cells, particularly $T_{reg}$ cells (Table 2) but might also function as agonists in FcγR-bound form[152–154]. SIM0235 and BI-1808 are blocking antibodies and prevent ligand-induced TNFR2 activation, whereas HFB 200301 is a non-competing antibody, despite binding to CRD2 (a ligand-interacting domain) of TNFR2 (refs. 152–154). Notably, HFB 200301 has agonistic effects on T cells in vitro that are enhanced in the presence of sTNF[154]. Whether this synergism is fully TNFR2-mediated or involves sTNF-stimulated TNFR1, however, is not clear. All three antibodies have antitumour effects in preclinical models[152–154]. None of the 'agonistic' anti-TNFR2 antibodies has yet been evaluated for its therapeutic efficacy in autoimmune diseases and inflammatory diseases.

## Conclusions

TNF inhibitors are of overwhelming relevance in the treatment of rheumatoid arthritis and several other immune diseases. Hence, unsurprisingly, six different TNF-neutralizing drugs are approved for clinical use and several more are in clinical development (Tables 1 and 2). However, in addition to interfering with the detrimental activities of TNFR1, TNF-neutralizing biologic drugs also often inhibit the anti-inflammatory and tissue-protective activities of TNFR2. Hence, compounds that selectively inhibit TNFR1 or selectively activate TNFR2 could have therapeutic potential. Against this background, a variety of companies and research groups have developed TNFR1-specific or TNFR2-specific biologic drugs, including genetically engineered antibodies, nanobodies and TNF mutants. These reagents have shown good efficacy and safety in various preclinical models and some of them have entered clinical trials. In the coming years, clinical studies must now investigate to what extent TNFR1-specific and/or TNFR2-specific reagents show therapeutic benefit in scenarios in which TNF-blockers have failed and whether these reagents can have adverse effects, such as those related to immune suppression that occur with TNF inhibitors that have already been approved. The fact that exogenous stimulation of TNFR2 has therapeutic benefits in TNF-driven diseases implies that endogenous memTNF-induced TNFR2 activation is insufficient in this context to fully maximize the potential benefits of the TNFR2 response. Hence, blockade of TNFR1 activation in combination with exogenous stimulation of TNFR2 might elicit superior therapeutic effects compared with the corresponding individual treatments. Thus, if TNFR1-inhibitory compounds and TNFR2 agonists should find their way into the clinic, an obvious next step is to evaluate regimes that combine these two types of drugs.

In view of the broad expression of the two TNF receptors and the pleiotropic activities of these receptors, modulation of this system (either through TNF or TNFR1 blockers or TNFR2 agonists) will inevitably not only have therapeutic effects on disease-relevant sites and cell types but will also have therapy-limiting adverse effects. Thus, next-generation biologic drugs might utilize bispecific reagents that are constructed to achieve localized activity. Indeed, TNFR1 inhibitors and TNFR2 agonists with conditional targeting-dependent activity are already under preclinical development[23,155]. For example, myeloid cell-specific TNF inhibitors (MYSTIs) have been developed in which a neutralizing TNF-specific nanobody is fused with a myeloid cell-specific nanobody to enable myeloid cell targeting; the resulting inhibitors can limit the activity of macrophage-derived TNF and show therapeutic activity in CIA[155,156]. Likewise, the fact that poorly agonistic or non-agonistic anti-TNFR2 antibodies engage TNFR2 signalling when bound to FcγRs can be exploited to construct anti-TNFR2 antibody fusion proteins with a similar mode of conditional agonism. Thus, anti-TNFR2 antibodies equipped with an anchoring domain that recognizes a plasma membrane-exposed target are empowered to elicit target-dependent agonism[23].

An obvious question is which of the numerous reagents discussed in this Review will find their way into clinical practice in the next few years. Various factors such as CMC (chemistry, manufacturing and controls) aspects, choice of indication and the development of competing products will influence the clinical development of these reagents, as well as business policy considerations. However, in view of the large number of possible areas of application and their different modes of action, the likelihood is that several of these reagents will achieve clinical approval in the future.

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## Author contributions

The authors contributed equally to all aspects of the article.

## Competing interests

H.W. declares that he received consultancy fees from Dualyx NV regarding the targeting of $T_{reg}$ cells via TNF receptor 2 (TNFR2). D.S. declares no competing interests. The University of Würzburg has filed patent applications for “Novel TNFR2 binding molecules” and “Tumour necrosis factor (TNF) receptor superfamily (TNFRSF) receptor-activating antibody fusion proteins with FcγR-independent agonistic activity (TRAAFFIA)”, with H.W. as one of the inventors.

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Consensus statement

# Towards a novel clinical outcome assessment for systemic lupus erythematosus: first outcomes of an international taskforce

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## Abstract

Systemic lupus erythematosus (SLE) is a disease of high unmet therapeutic need. The challenge of accurately measuring clinically meaningful responses to treatment has hindered progress towards positive outcomes in SLE trials, impeding the approval of potential new therapies. Current primary end points used in SLE trials are based on legacy disease activity measures that were neither specifically designed for the clinical trial context, nor developed according to contemporary recommendations for clinical outcome assessments (COAs), such as that substantial patient input should be incorporated into their design. The Treatment Response Measure for SLE (TRM-SLE) Taskforce is a global collaboration of SLE clinician–academics, patients and patient representatives, industry partners and regulatory experts, established to realize the goal of developing a new COA for SLE clinical trials. The aim of this project is a novel COA designed specifically to measure treatment effects that are clinically meaningful to patients and clinicians, and intended for implementation in a trial end point that supports regulatory approval of novel therapeutic agents in SLE. This Consensus Statement reports the first outcomes of the TRM-SLE project, including a structured process for TRM-SLE development.

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## Sections

Introduction

Methods

Rationale for development of TRM-SLE

Protocol for TRM-SLE instrument development

Future stages of TRM-SLE development

Conclusions

## Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by multi-system involvement and unpredictable fluctuations in disease activity. For a substantial proportion of affected patients, current therapeutic strategies are insufficient[1]. Uncontrolled disease activity driven by autoimmunity combines with unwanted consequences of therapy to contribute to irreversible organ damage, the accumulation of comorbidities, and negative effects on patients' lives. Individuals with SLE can experience numerous severe symptoms, impaired quality of life and reduced function[2], and the disease is one of the leading causes of death in young women[3].

Despite recognition of the unmet therapeutic need and the identification of many promising drug targets in SLE, late-phase clinical trial successes and regulatory approvals of novel treatments have been few and far between. Although critical review of the contributory factors has led to some evolution in trial design[4], concerns about the inconsistent performance of SLE trial end points and how best to establish the efficacy of new therapies remain unresolved. In particular, the most common efficacy end points in current use, the SLE Responder Index (SRI) and the British Isles Lupus Assessment Group (BILAG)-based Composite Lupus Assessment (BICLA) both have well-recognized limitations[5]. Consequently, the development of new, evidence-based and discriminatory outcome measures to determine treatment effect in clinical trials is a major research priority in SLE.

The Treatment Response Measure for SLE (TRM-SLE) Taskforce was established to execute a project to develop and subsequently validate a novel clinical outcome assessment (COA) specifically intended for implementation as a primary outcome measure in SLE clinical trials that support regulatory approval of therapeutic agents. In this Consensus Statement we describe the first consensus outcome of the project that defines the high-level measurement goals that will underpin the development of the TRM-SLE COA, as well as a consensus on the research methods that will lead to an operational COA that can be incorporated into and validated in future clinical trials.

## Methods

### Composition of the TRM-SLE Taskforce

The TRM-SLE Taskforce (also referred to herein as the TRM-SLE Consortium) was established in January 2022 explicitly to fulfil the goal of developing a new COA for use in SLE clinical trials. Acknowledging the need for input from multiple stakeholders, the taskforce consists of four governance committees (a Steering Committee, Scientific Advisory Board, Patient Advisory Panel and Industry Advisory Board). Potential taskforce members were nominated by the core research team and industry partners on the basis of the following criteria: clinical expertise in SLE, demonstrable experience in SLE clinical trials (for example as a principal investigator), expertise in outcome measurement and/or other relevant methodological expertise. Experts were directly approached and invited to participate. Patient and patient representative members were suggested by SLE patient organizations, including the Lupus Foundation of America and Lupus Europe, with the intention to recruit patients with clinical research and prior advocacy experience. We endeavoured to achieve representation of all major geographical regions for both clinician and patient representatives. Industry representatives were nominated by each of the collaborating pharmaceutical companies, and all companies known to the investigators to be active in SLE drug development were approached. The taskforce currently consists of 78 members in total. The TRM-SLE governance structure and committee roles are summarized in Table 1.

### Generation of the study protocol for instrument development (Stage 1.1)

The overall TRM-SLE project is divided into stages of instrument development (Stage 1) followed by instrument validation (Stages 2 and 3), as illustrated in Fig. 1. Instrument development, which is the focus of this Consensus Statement, is divided into five sub-stages (Stages 1.1–1.5). In Stage 1.1, a detailed protocol was developed describing the specific methods to define the high-level measurement goals and context of use for the TRM-SLE instrument (Stage 1.2), to select the domains to be included in the TRM-SLE instrument (Stage 1.3), to determine how they will be measured (Stage 1.4), and to determine how domains will be incorporated into an overall definition of treatment response (Stage 1.5).

The study protocol was initially drafted by the core research team on the TRM-SLE Steering Committee (K. Connelly, L.E., R.K., D.A., V.G., R.K-.R. and E.M.). Protocol development was informed by an extensive review of the literature[5], focusing on understanding the characteristics and limitations of current SLE clinical trial end points, the complexities of outcome measurement in SLE and potential strategies to overcome these limitations via new approaches. Stages in TRM-SLE instrument development were also designed to align with the steps recommended by the relevant FDA Patient-Focussed Drug Development Guidance and related guidance from the Professional Society for Health Economics and Outcomes Research[6,7].

The draft protocol was circulated electronically to all members of the TRM-SLE Taskforce and initial feedback provided by e-mail correspondence. A Protocol Working Group was then established, consisting of a subset of 22 volunteering members of the TRM-SLE Taskforce, to

**Table 1 | Governance committees comprising the TRM-SLE Taskforce**

| Committee | Members | Role |
|---|---|---|
| Steering Committee | 35 members:<br>Core research team (principal investigator, clinician–researchers and fellows, methodology experts)<br>Patient and/or patient-organization representatives<br>Industry representatives<br>Metrology and regulatory experts<br>Additional clinician–researchers with relevant expertise | Responsible for the scientific direction and delivery of the project |
| Scientific Advisory Board | 29 members:<br>Clinician–researchers with relevant expertise<br>Patient and/or patient-organization representatives<br>Industry representatives | Provide additional scientific input and oversight |
| Patient Advisory Panel | 16 members:<br>Patients with SLE<br>Patient-organization representatives (Lupus Foundation of America and Lupus Europe) | Provide guidance regarding the patient experience of SLE via direct involvement and oversight of instrument development |
| Industry Advisory Board | 28 members:<br>Representatives from 10 industry partners with relevant expertise | Advise on industry-specific matters including leading regulatory engagement |

SLE, systemic lupus erythematosus; TRM-SLE, treatment response measure for SLE.

**a TRM-SLE instrument development (Stage 1)**

| Stage 1.1 | Stage 1.2 | Stage 1.3 | Stage 1.4 | Stage 1.5 |
|---|---|---|---|---|
| **Literature review and protocol development** | **Conceptual definition of measurement goals and context of use** | **Domain-level concepts for measurements** | **Domain measures and response definitions** | **Multi-domain TRM-SLE instrument** |
| Define unmet need and establish the aim and scientific approach for TRM-SLE development | Consensus to define the high-level measurement goals for TRM-SLE and define the context of use | Consensus on the domain-level concepts of interest to be measured, which are associated with meaningful health aspects and align with the TRM-SLE high-level measurement goals and context of use | Consensus on:<br>A An outcome assessment for each domain using existing instruments and/or identifying gaps for instrument modification or development<br>B Response definition (entry and improvement thresholds) for each domain outcome assessment based on instrument-specific evidence of meaningful within-person change | Consensus on the integration of domain measures into a multi-domain clinical outcome assessment for use in a trial end point |

**b TRM-SLE instrument validation (Stages 2 and 3)**

**Stage 2**

**Evaluation in clinical trial datasets**

Prospective incorporation of TRM-SLE in future clinical trials, and where possible in existing trial datasets

**Stage 3**

**Evaluation of additional measurement properties**

Prospective cohort study evaluating TRM-SLE attainment against long-term clinical outcomes and nested sub-studies of additional measurement properties

**Fig. 1 | Steps in the development and validation of a novel treatment response measure for SLE. a**, Stage 1 (instrument development) in the definition of a novel clinical outcome assessment via expert consensus informed by available data comprises five sub-stages that will lead to an operational, multi-domain, clinical outcome assessment that defines treatment response at both a global and a domain-specific level. The outcomes of Stages 1.1–1.2, and consensus methods agreed upon by the taskforce for Stages 1.3–1.5, are described in this Consensus Statement. **b**, The provisional instrument will be validated in trial datasets (Stage 2) as well as concurrently undergoing additional testing of its measurement properties (Stage 3). Key measurement properties that will be evaluated include construct validity, reliability, ability to detect change, discrimination of treatment effects, interpretability and feasibility.

work through proposed changes to the draft protocol. The Protocol Working Group (K. Connelly, L.E., R.K., D.A., H.B., L.B., L.A., A.A., C.A., E.V., G.P.-E., K.D., Y.S., Y.T., L.S., Y.L., A.F., K.K., Q.Z., V.W., S. Garces and E.M.) included experts in COA development, consensus methodology and regulatory affairs, industry partners, patient representatives and SLE clinician–researchers. The Protocol Working Group revised the methodological steps via group discussion over three virtual meetings and e-mail correspondence. During protocol revision, the proposed methods were also presented at a meeting of the Patient Advisory Panel, and patient feedback was collected and incorporated into changes made by the Protocol Working Group. A revised protocol document was prepared by the core research team, circulated electronically and individually approved by TRM-SLE Taskforce members.

### Methods to define high-level measurement goals for TRM-SLE development (Stage 1.2)

Following protocol development, the first major scientific step towards instrument development was to achieve consensus on the high-level goals of measurement underpinning the TRM-SLE project (Stage 1.2). This process occurred in two parts: first, the measurement goals of TRM-SLE were conceptually defined using the Patients/Population, Intervention, Comparator/Control, Outcome/Objective, Context (PICO-C) framework; and second, consensus was established on the context of use for the TRM-SLE instrument. From a regulatory perspective, the context of use is a specific statement describing the manner and purpose of use of an instrument, including a description of the targeted disease and study population, study design and setting for its intended use[6,7]. Defining the context of use is a key step outlined in regulatory guidance pertaining to COA development, to help to ensure that any new instrument is developed and validated for its intended scope of implementation, so that results can be appropriately interpreted and applied[6,7].

Members of the TRM-SLE Steering Committee and Scientific Advisory Boards, encompassing representation of key stakeholder groups, were invited to participate in the definition of the high-level measurement goals and context of use for TRM-SLE. Participants (A.A., A.C., A.F., C.A., C.B., C. Sibley., C. Stach., D.A., E.V., E.M., E.Z., G.S., G.P.-E., H.A., H.B., J.A., J.B., J. Merrill, J. Maller, J.R.T., K. Costenbader, K.

Connelly, K.D., K.G., K.K., L.E., L.A., L.B., M.D., M.S., M.M., N.D., P.M., Q.Z., R.K., R.K.-R., R.F., R.v.V., S. B., S. Garces, V.G., V.W., Y.S., Y.T. and Y.L.) met over a series of four virtual meetings (three meetings addressing the high-level measurement goals and one meeting addressing the context of use). The participation in each meeting is indicated in Supplementary Table 1. Item generation was facilitated using a web-based application (MURAL; https://www.mural.co/), which enables participants to post ideas via 'sticky-notes' onto a common virtual whiteboard in real time. Suggested items were then grouped and refined via moderated discussion in an interactive fashion. Online polling integrated into the virtual meeting platform was used for voting on the proposed final wording of each element of the conceptual definition and context of use, with a pre-defined consensus threshold set at 70% agreement. Only members present in the meetings participated in voting, and voting was not compulsory (to enable committee members without SLE-specific expertise to opt out of voting at their discretion). Consensus statements and percentage agreement were recorded for each element of the final conceptual definition and context of use. The consensus outcomes were then circulated electronically and presented at virtual meetings of each TRM-SLE governance committee (Table 1), for final approval.

## Rationale for development of TRM-SLE

Although some recent positive outcomes have been achieved in SLE clinical trials, including the approval of anifrolumab (an antibody that targets the type I interferon receptor), inconsistent end point performance continues to affect the accurate interpretation of the treatment effects of novel agents. This inconsistency is demonstrated by positive phase II trial results of drug candidates that fail to be replicated in subsequent phase III trials[8–12], phase III trials with identical study protocols that produce conflicting results with regard to their primary end points[11–14], and discrepancies between the outcomes of primary study end points and other clinically relevant measures, such as steroid-sparing effects[15]. Recent examples of these challenges include the phase III clinical trials of anifrolumab (TULIP studies), the Janus kinase inhibitor baricitinib (BRAVE studies) and ustekinumab, a monoclonal antibody that blocks the p40 subunit shared by IL-12 and IL-23, each of which followed on from successful phase II studies. In the TULIP-1 and TULIP-2 phase III trials of anifrolumab that used the same eligibility criteria, TULIP-1 found no significant difference between anifrolumab and placebo using the SRI-4 as the primary end point, but differences favouring anifrolumab were detected using BICLA as an alternative measure of overall efficacy[13]. By contrast, TULIP-2 detected a significant treatment effect of anifrolumab using BICLA as a primary end point, as well as SRI-4 as a secondary end point[14]. Both studies also met other key secondary efficacy end points, ultimately resulting in regulatory approval of anifrolumab in several countries. Similar to the TULIP studies, the recent SLE-BRAVE-I and SLE-BRAVE-II phase III studies of baricitinib also produced conflicting results, meeting the SRI-4 primary end point in BRAVE-I, but failing to meet the same primary end point in BRAVE-II[11,12]. Meanwhile, phase III trials of ustekinumab were abandoned because of negative results in an interim analysis, again following promising results in phase II testing[10]. Inconsistency between results with different end points within the same population are also characteristic of some of these trials[16], and of a trial of belimumab in childhood SLE[17].

The COAs currently used for determination of the treatment response in SLE trials are imperfect; we have previously reviewed the factors behind their inconsistent performance[5], and summarize their key limitations in Box 1. Many issues stem from the fact that COAs incorporated in current trial end points, such as the SLE Disease Activity Index (SLEDAI) and BILAG were primarily developed as disease activity measures and were repurposed for the measurement of treatment response in the absence of better alternatives. As a consequence, the concepts measured and thresholds used for defining meaningful improvement were not grounded in the context of clinical trial use, and importantly, lacked substantial patient input. Current recommendations for COAs intended for use in clinical trials highlight the importance of these instruments being sufficiently validated for their specific scope of intended use, and the vital role that the patient perspective has in ensuring that the interpretation of outcomes reflects meaningful health aspects[12,13]. Therefore, COAs used in current SLE trial primary end points do not meet contemporary measurement standards, which in combination with their history of unreliable performance, underpins the major need for new instruments for this purpose. Such new instruments should specifically seek to avoid replicating the liabilities of legacy measures and follow modern recommendations for instrument development, including guidance documents published by regulatory bodies such as the FDA and EMA[6,7].

## Protocol for TRM-SLE instrument development

The first consensus outcomes relating to TRM-SLE instrument development are described in detail below. The first two stages (Stages 1.1 and 1.2) have been completed, yielding consensus on the high-level measurement goals and context of use for TRM-SLE and a detailed protocol describing specific methods for future stages of instrument development (Stages 1.3–1.5). Terminology related to COA development used

### Box 1

#### Key limitations of outcome measures currently used to determine treatment response in SLE clinical trials

- Lack of patient input in determining which concepts are important to be measured.
- Use of discrete thresholds introducing floor and ceiling effects and limiting the ability to capture variations in the severity of manifestations.
- Numerical thresholds defining improvement and fixed weightings applied to manifestations that are not based on empirical evidence of meaningful within-patient change.
- Developed using post hoc analysis of trial data, risking bias to specific drug mechanisms or specific study designs.
- Some included manifestations are poorly defined and/or rarely appear in clinical trials.
- Adoption in clinical trials prior to extensive testing of measurement properties.
- Can be complex and non-intuitive to complete and interpret.

in FDA and Professional Society for Health Economics and Outcomes Research guidance documents[6,7] was adopted in the study protocol and throughout this Consensus Statement.

### Conceptual framework for TRM-SLE

The recommended steps towards a fit-for-purpose COA can be visually depicted in the form of a conceptual framework (Fig. 2) that summarizes the following key elements: relevant health outcomes (symptoms, signs and effects of the disease) in the target population; specific concepts of interest targeted for assessment; COAs proposed to measure each concept of interest, potentially including existing, modified and novel COAs; and the use of these COAs to generate a score for each concept and to define treatment response[10].

### Patient involvement in TRM-SLE development

A key goal of TRM-SLE development is to ensure that the resultant COA captures aspects of health that are meaningful to both the patient and clinician. For this reason, the study protocol has been designed to incorporate the perspectives of both of these stakeholder groups, as illustrated in Fig. 3.

### Consensus high-level measurement goals and context of use for TRM-SLE (Stage 1.2)

A panel of 45 taskforce members representative of the key stakeholder groups generated a conceptual definition for the high-level measurement goals of TRM-SLE using the PICO-C (Patients/Population, Intervention, Comparator/Control, Outcome, Context) framework. Over the course of three virtual meetings, multiple rounds of item generation, moderated discussion and real-time voting resulted in the conceptual definition for TRM-SLE, which overall achieved predefined levels of agreement (Table 2). Dissenting opinions were mostly with regard to a desire to expand beyond the traditional study design of SLE clinical trials, for example, by consideration of the patient populations to whom TRM-SLE should apply. Although most participants expressed a preference to focus on active immune-mediated disease manifestations for the purpose of a clinical trial, others suggested additional

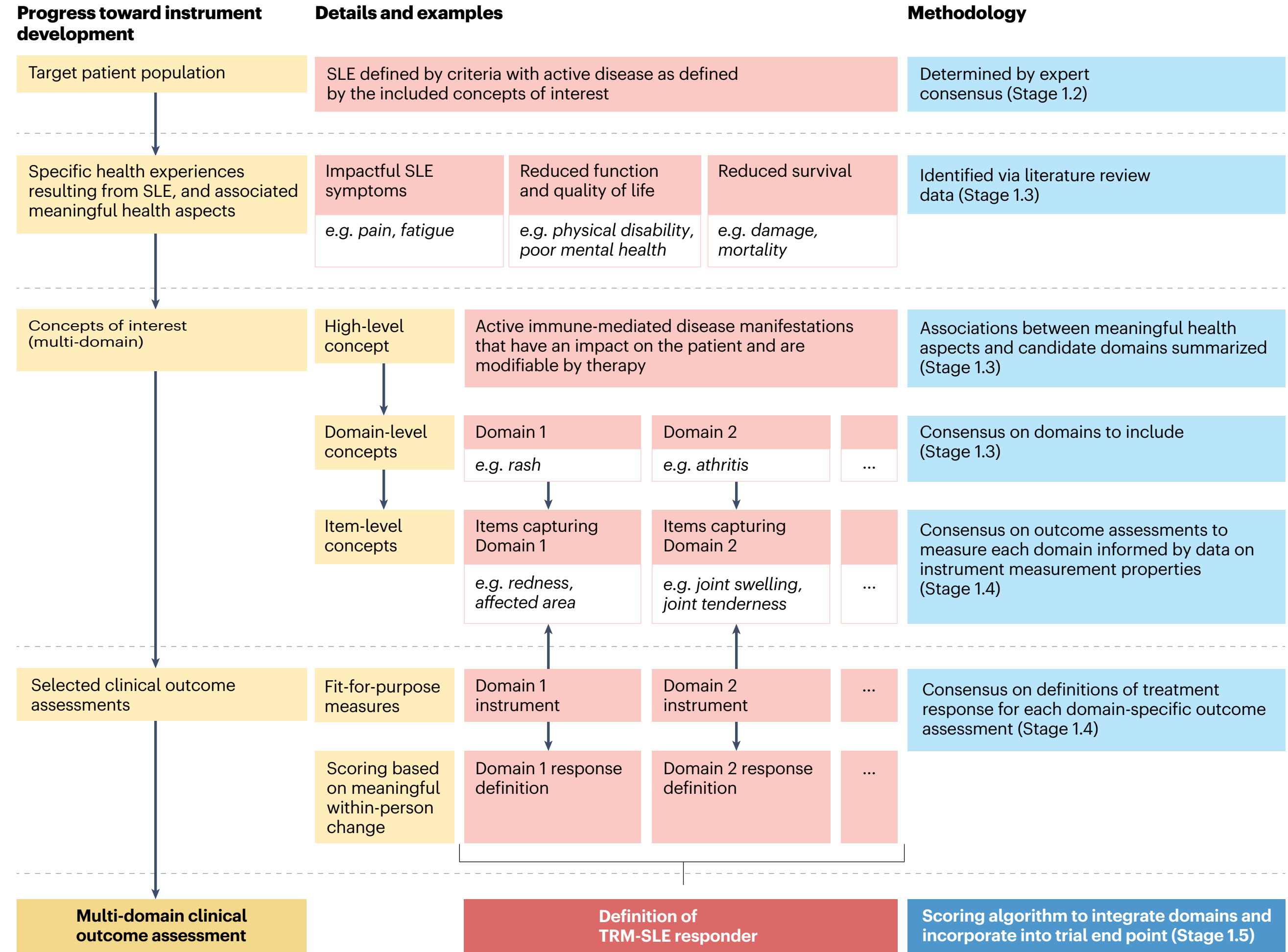


**Fig. 2 | Conceptual framework for TRM-SLE.** Illustration of the conceptual framework that will be established using the proposed methods for development of the treatment response measure for systemic lupus erythematosus (TRM-SLE) instrument. As recommended by regulatory guidance, this framework includes the concepts of interest (including domain-level and item-level concepts) targeted for assessment and how these relate to the patient experience, along with consideration of how the concepts will be measured and scored. In this figure, italic text represent theoretical examples; the specific elements that will fill the framework be determined by the completion of Stages 1.3–1.5.

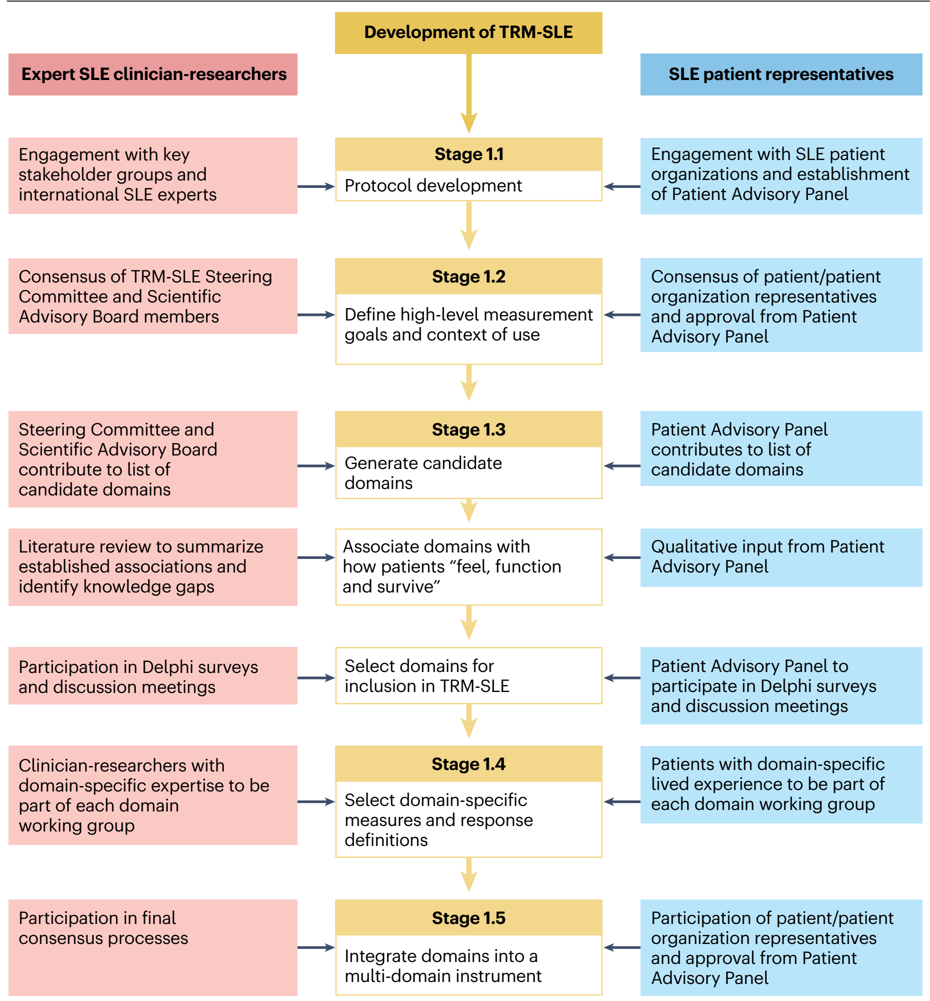


**Fig. 3 | Input of SLE patient representatives and expert clinician–researchers in TRM-SLE development.** An illustration of the planned involvement of patient partners and expert systemic lupus erythematosus (SLE) clinician–researchers in the development process for the treatment response measure for systemic lupus erythematosus (TRM-SLE).

populations with unmet therapeutic needs in SLE, or the ability to assess interventions targeting non-inflammatory disease features, or use in settings beyond randomized controlled trials. These suggestions were balanced against the regulatory requirement for COA approval to have a pre-specified and very specific context of use. With reference to the consensus PICO-C framework, a statement was derived describing the high-level concept that should be captured by the TRM-SLE instrument as "active immune-mediated disease manifestations that impact the patient and are modifiable by therapy to reduce or control disease activity" (Box 2).

Expanding upon this high-level conceptual definition, consensus on the primary context of use for which TRM-SLE will be developed was sought. Over the course of a single virtual meeting, moderated discussion and voting focussed on defining the target disease and study subpopulation, study setting and trial design for which the TRM-SLE instrument will be primarily developed. This resulted in the context of use detailed in Table 3, which achieved 100% consensus among 30 participating taskforce members. We anticipate that the described primary context of use may be refined in an iterative manner based on the outcomes of the subsequent steps in instrument development, which includes the incorporation of TRM-SLE into an end point, and its associated analysis plan.

## Future stages of TRM-SLE development

The methods described below represent an approach that has both similarities to and differences from processes adopted in rheumatic disease outcome measurement more generally. Given the goal of developing an outcome measure suitable for supporting regulatory approval of new agents in clinical trials, particular attention has been paid to ensuring that methods conform to published recommendations that apply to this specific context[6,7], while accounting for disease-specific measurement challenges and the intention to avoid replicating known limitations of current SLE trial outcome measures.

### Selection of domains to be measured in TRM-SLE (Stage 1.3)

The next stage of TRM-SLE development will select domain-level concepts to be measured by the instrument. A concept (also known as a concept of interest), for regulatory purposes, is a health aspect that is intended to be captured by a COA. Current guidance emphasizes that these aspects of health should be meaningful for patients, defined as having an effect on how the patient "feels, functions or survives"[6]. As illustrated in the pyramid within Fig. 2, concepts can be considered at multiple levels. The high-level concept to be measured by TRM-SLE will be captured by consideration of multiple sub-concepts or 'domains'. These domains might include organ-based and/or system-based

manifestations (such as lupus nephritis), symptoms of SLE (such as rash), or in some cases manifestations measured by laboratory tests or other investigations (such as thrombocytopenia). Each domain will then be defined by one or more 'items', which are the specific concepts measured and scored to produce a representation of each domain. For example, a hypothetical domain that falls under the high-level concept for TRM-SLE could be 'arthritis'. The items assessed to capture this domain potentially include concepts such as joint swelling, tenderness or pain. The focus of Stage 1.3 is the selection of domains to be captured in the TRM-SLE instrument. The specific COA(s) with which each domain is measured, and the associated item-level concepts, are addressed in Stage 1.4.

**Generation of candidate domains and associations with meaningful health aspects.** Clinician–researchers with lupus expertise, industry representatives with SLE trial experience, and patients will nominate candidate domains. These nominations will be grouped and refined to produce a core list of domains to be rated for inclusion in TRM-SLE.

The TRM-SLE instrument is intended to be a patient-centred measure, so the domains considered in the determination of response to therapy must be associated with health effects that are meaningful from the patient perspective (meaningful health aspects), including symptoms and functional effects identified to be important by patients themselves, and associations with outcomes of prognostic importance such as damage and mortality. Evidence of associations of the core list of candidate domains with meaningful health aspects will be evaluated by a targeted review of the literature, and summarized to inform domain selection during a modified Delphi process, as described below. Complementary to the literature review, the Patient Advisory Panel will also provide patient perspectives on the candidate domains and their associations with meaningful health aspects, and help to prioritize future research agendas where evidence gaps are identified.

**Selection of domains for inclusion in TRM-SLE: modified Delphi process.** Consensus on which of the candidate domains will be included in the TRM-SLE instrument will be achieved by a two-part modified Delphi process. In the first part, domains will be rated on their 'importance', defined as the extent to which a domain is associated with meaningful health aspects (impact on how a person with SLE "feels, functions or survives"). Clinicians with expertise in SLE clinical care and trials, including experts external to the TRM-SLE project, and patient representatives via the Patient Advisory Panel, will participate in two to three Delphi survey rounds, with discussion meetings between voting rounds. We plan to recruit a total of 50–100 participants, with international representation. Participants will rate each domain for 'importance' on a nine-point scale, with ratings assigned to three categories: ≥7 (critically important to include), 4–6 (important but not critical) and ≤3 (not important)[18]. A summary of evidence from the literature associating candidate domains with meaningful health aspects will be provided to inform participant ratings and support panel discussions.

Consensus on domain 'importance' will be defined as ≥70% of total participants scoring 7–9 (ref. 14) with the additional requirement that the consensus threshold is met in both expert clinician and patient groups. Domains achieving consensus on 'importance' will proceed to a second set of ratings, where participants will rate domains on three additional characteristics relevant to their inclusion. The first characteristic is 'appropriateness', defined as whether the domain is an active immune-mediated-disease manifestation that is modifiable by therapy to reduce or control disease activity in an SLE clinical trial (as defined by the measurement goals and context of use for TRM-SLE arising from Stage 1.2). The second characteristic is 'representation', defined as whether domain activity occurs with sufficient frequency in patients

## Glossary

**Clinical outcome assessment**
Assessment of a clinical outcome that describes or reflects an aspect of health, and that can be made through a report by a clinician (clinician-reported outcome measures), a patient (patient-reported outcome), a non-clinician observer (observer-reported outcome), or through a performance-based assessment (performance outcome).

**Concepts of interest**
In a regulatory context, the aspect of an individual's clinical, biological, physical or functional state or experience that the assessment is intended to capture (or reflect).

**Context of use**
A statement that fully and clearly describes the way the outcome assessment is to be used and the regulated product-development purpose.

**Domain**
A sub-concept represented by a score of an instrument that measures a larger concept comprising multiple domains.

**End points**
Precisely defined variables intended to reflect an outcome of interest that is statistically analysed to address a particular research question, including the type and timing of assessments, the assessment tools used, and other details, as applicable, such as how multiple assessments within an individual are to be combined.

**Fit-for-purpose**
A conclusion that the level of validation associated with a clinical outcome assessment is sufficient to support its context of use.

**Meaningful health aspects**
Aspects of health (feelings, functions or survival) adversely affected by the disease, which the patient cares about and has a preference that they do not become worse, or that they improve, or that they are prevented.

**Table 2 | Conceptual definition of the high-level goals of measurement for TRM-SLE**

| PICO-C | Consensus definition | Number of contributors to definition | Agreement[a] |
|---|---|---|---|
| Patients or population | SLE defined by criteria, with active immune-mediated disease manifestations modifiable by therapy | 41 | 81% |
| Intervention | Treatment to reduce or control disease activity | 33 | 92% |
| Comparator or control | Placebo and/or active comparator | 33 | 96% |
| Outcome or objective | The impact of an intervention on the patient, as measured by change in the concepts of interest | 33 | 100% |
| Context | Clinical trials assessing efficacy and satisfying requirements for registration | 33 | 96% |

[a]Percentage agreement for each consensus definition amongst voting taskforce members. PICO-C, Patients/Population, Intervention, Comparator/Control, Outcome, Context.

with SLE and active disease to warrant inclusion in TRM-SLE. The third characteristic is 'measurability', which is defined as whether the domain can be clearly defined and treatment response accurately quantified. Domains must achieve ≥70% of participants scoring ≥7 on all three of these additional characteristics to meet consensus for inclusion in the TRM-SLE instrument.

### Domain measures and response definitions (Stage 1.4)

Once consensus has been achieved on TRM-SLE domains, individual working groups will be established for each domain to achieve consensus on selection of an outcome assessment to measure the domain and/or outline a necessary research agenda if a fit-for-purpose measure is not available, and on how to numerically define domain-specific responses using the chosen outcome assessment, based on entry and improvement thresholds that are anchored to evidence of meaningful within-person change.

**Domain-specific working groups.** Each domain working group will comprise 6–12 individuals with expertise relevant to their specific allocated domain. The composition of each working group may vary depending on the nature of the domain, but will include clinician–researchers with domain-specific clinical, trial and/or measurement expertise and patient representatives with lived experience of the affected SLE domain.

**Selection of domain measures.** Members of each working group will nominate ideas for suitable candidate instruments to measure their target domain. A nominal group technique will then be used to rank and achieve consensus on a preferred domain-specific outcome assessment, which will be followed by a systematic literature review of the measurement properties of the selected outcome assessment for each domain, particularly as they pertain to the TRM-SLE context of use, to determine whether the instrument is fit for purpose or whether additional studies are required. At the discretion of the working group, more than one candidate instrument could proceed to the review step, with a final decision incorporating evidence of the measurement properties of the candidate instruments. Evaluation of measurement properties will include consideration of face validity (working group expert opinion), content validity (evidence that the included items adequately capture the domain, and that the instrument score represents the intended measurement concept), feasibility (working group expert opinion and evidence from use in previous SLE clinical trials), reliability (evidence of test–retest, intra-rater and inter-rater reliability), construct validity (evidence of associations with other relevant measures, through analysis of cohort and clinical trial datasets), ability to detect change (evidence of responsiveness to change in the concept being measured), meaningful thresholds and interpretability (evidence of thresholds of meaningful disease activity and improvement anchored to appropriate patient-centred outcomes), and discrimination of a treatment effect (evidence from previous SLE clinical trials). If further instrument validation is required, it will be planned in subsequent validation stages using existing trial datasets or as an exploratory end point in the setting of a new trial.

### Definition of domain-specific response

Once an outcome assessment has been identified for the measurement of a particular domain, the working group will establish consensus on the numerical definition of response for that domain. For each domain-specific outcome assessment, a response definition will have two components. The first will be the entry threshold: the minimum level of domain-specific disease activity at study entry, from which improvement can be measured (reflecting a level of severity associated with a meaningful clinical effect and permitting sufficient room for improvement). The second component will be the improvement threshold: the minimum level of improvement (from baseline) required to be defined as a responder in a specific domain (reflecting a clinically important within-person change anchored to appropriate patient-centred outcomes, and sufficiently stringent to discriminate a treatment effect between arms in a clinical trial). Similar to the process to select domain measures, members of the domain working group will nominate candidate response definitions. This process will be informed by available empirical data using anchor-based methods supporting specific thresholds of activity and improvement associated with meaningful clinical effect and clinical benefit, respectively. A nominal group technique will then be used to rank and achieve consensus on a particular response definition to be included in the final TRM-SLE instrument, subject to further validation in subsequent trial datasets where required.

> **Box 2**
>
> **High-level concept to be measured by the TRM-SLE**
>
> "Active immune-mediated disease manifestations that impact on the patient and are modifiable by therapy to reduce or control disease activity".

### Integration of domain measures into a multi-domain COA (Stage 1.5)

Once consensus is achieved on which concepts will be measured by the TRM-SLE instrument, the outcome assessments that will be used for measurement of these concepts, and the thresholds that define meaningful within-patient change for each domain, the final stage of instrument development will establish consensus on how to integrate these measures into a multi-domain COA that can be interpreted to define treatment responders when deployed as part of a trial end point in validation studies. Specific points for consensus at this stage will include defining the scoring algorithm that specifies an overall responder (including possible weighting of different domains), and determining methods to capture worsening or new activity that develops over the course of a trial (such as in domains that are not specifically measured in the TRM-SLE instrument).

### Validation of the TRM-SLE instrument (Stages 2 and 3)

Following instrument development, the taskforce plans an extensive validation programme to evaluate the performance of the multi-domain TRM-SLE measure and to ensure that scoring and definition of treatment response within a trial end point reflect meaningful within-patient change. Validation will include prospective incorporation of TRM-SLE as an exploratory end point in future clinical trials, as well as evaluation of TRM-SLE in available existing trial datasets, where possible. Key measurement properties, including construct validity, the ability to detect change and the ability to discriminate a treatment effect (for example, between treatment arms in a clinical trial) will be assessed,

**Table 3 | Primary context of use for the TRM-SLE instrument**

| Context of use | Description | Agreement[b] |
|---|---|---|
| Targeted disease and study subpopulation | Adult and adolescent patients with criteria-defined SLE, with active disease in one or more included domains[a] despite standard-of-care therapy | 100% |
| Targeted study design and study setting | Randomized controlled trial conducted in an outpatient setting, with the TRM-SLE instrument being the main outcome assessment within a primary efficacy end point with landmark analysis comparing meaningful response between treatment arms | 100% |

[a]Expanded definition: active disease in one or more included domains with sufficient domain-specific activity to have a meaningful impact on the patient (domains confirmed in Stage 1.3 and sufficient domain-specific activity numerically defined in Stage 1.4 using chosen domain measurement instrument). [b]Percentage agreement for each component of the context of use amongst voting members of the 30 taskforce members present.

including comparison with legacy disease activity measures used for trial eligibility (such as SLEDAI and BILAG) and with currently used responder indices (such as SRI and BICLA). This process will also enable evaluation of operational considerations affecting implementation of TRM-SLE as a trial end point in its defined context of use. Concurrently, it is intended to conduct a prospective cohort study to validate attainment of TRM-SLE response against long-term clinical outcomes, including patient-reported outcomes, damage accrual, flare and attainment of target disease activity states such as Lupus Low Disease Activity State[19] and remission[20]. Studies addressing other measurement properties, including reliability, are planned via sub-studies nested within the prospective cohort study, along with additional case-based studies.

## Conclusions

This Consensus Statement reports the first consensus outcomes and agreed study protocol of an international taskforce specifically established to develop a novel COA for SLE trials. This new outcome measure will be specifically designed for the clinical trial context, will learn from the limitations of legacy trial outcome measures, will follow updated regulatory and instrument development guidance, and will incorporate input from all key stakeholder groups, importantly including the patient voice. The new outcome measure has the potential to be used as a composite measure, but also as a source of individual domain measures, and in contexts additional to the defined context of use. Although a challenging endeavour, it is the ambition of the TRM-SLE project to develop an outcome measure in SLE that is accepted by key stakeholders (including regulators), that is successfully incorporated into future registration trials, and that leads to clearer interpretation of the efficacy of new treatments that reflect both patient and clinician health priorities.

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### Author contributions

K. Connelly, L.E. and E.M. researched data for the article. K. Connelly, L.E., R.K., D.A., V.G., R.K.-R., K.G., H.B. L.B., L.A., A.A., C.A., E.V., G.P.-E., K.D., J.A., A.C., J.B., Y.S., Y.T., L.S., Y.L., A.F., K.K., Q.Z., V.W., S. Garces and E.M. made a substantial contribution to discussion of the content. K. Connelly and E.M. wrote the article. All authors reviewed and/or edited the manuscript before submission.

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