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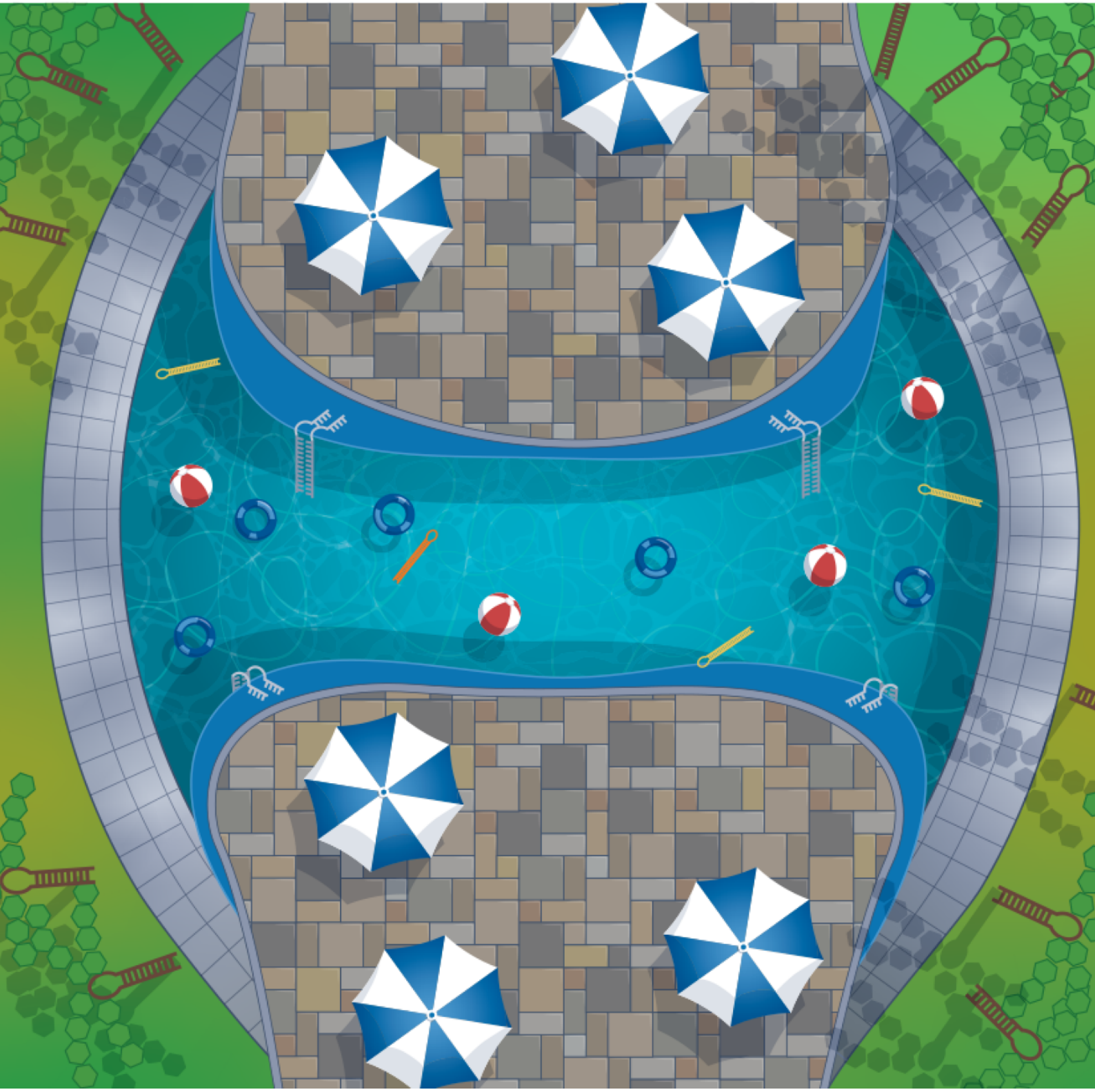

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Risks and benefits of immunosuppressant withdrawal in systemic lupus erythematosus

Catriona A. Wagner & Judith A. James

Withdrawing immunosuppressive treatment in systemic lupus erythematosus offers reduced toxicity and improved quality of life for patients in remission but carries a risk of disease reactivation. Emerging studies emphasize the importance of identifying patients who can safely discontinue therapy using clinical criteria and molecular profiling to guide personalized strategies.

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by heterogeneity in clinical presentation, disease course and response to therapy. Current SLE treatment approaches rely on glucocorticoids, immunosuppressants, targeted biologic therapeutics and antimalarials to achieve disease remission, prevent flares and mitigate long-term organ damage[1]. The treat-to-target strategy is a promising framework for guiding SLE management, emphasizing the attainment of validated endpoints, such as the Definition of Remission in SLE (DORIS) or Lupus Low Disease Activity State (LLDAS), which are associated with reduced flares, less organ damage accrual and improved health-related quality of life[2]. Although therapies have markedly improved outcomes for patients with SLE, prolonged use is associated with substantial adverse effects, including increased risks of infection, cancer, metabolic disorders, gastrointestinal upset and pregnancy complications. As a result, the 2024 EULAR recommendations for SLE treatment emphasize the importance of tapering or withdrawing medications in patients with quiescent disease[1]; however, the optimal timing, strategies and patient selection for treatment withdrawal are not clear and require further investigation, especially for glucocorticoids and immunosuppressants. Thus, in the past 5 years, numerous studies have focused on determining the feasibility and safety of immunosuppressant and glucocorticoid withdrawal in patients with stable SLE, with the aim of balancing the risks of continued treatment against the potential for disease reactivation (Box 1). This Comment examines studies of immunosuppressant withdrawal in SLE and explores potential strategies for identifying suitable candidates for withdrawal in the future.

Mycophenolate mofetil (MMF) is an immunosuppressant used to treat moderate-to-severe or organ-threatening SLE, including lupus nephritis; however, long-term MMF use is associated with increased infection risk, gastrointestinal issues, malignancies and teratogenicity. Thus, clinical trials have investigated the feasibility of MMF withdrawal in patients who have achieved disease quiescence. The randomized Weaning of Immunosuppression in Lupus (WIN-Lupus, NCT01284725) trial evaluated the non-inferiority of discontinuing maintenance immunosuppressants in 96 patients with SLE and proliferative lupus nephritis who achieved renal remission after 2–3 years of treatment with azathioprine or MMF while continuing hydroxychloroquine[3]. Patients who discontinued immunosuppressive treatment experienced a higher rate of renal relapse (27% versus 13%) and severe renal or extrarenal SLE flares (32% versus 13%; defined as disease activity that required the initiation or reintroduction of immunosuppressive therapy) within 2 years than those who did not. In addition, the time to severe SLE flare was shorter in the discontinuation group than in the group that continued treatment.

A multi-centre, open-label, randomized clinical trial (ALE06, NCT01946880) investigated MMF withdrawal in 100 patients with stable, quiescent SLE (which was defined as a clinical SLE Disease Activity Index (SLEDAI) score of less than 4) who had been on MMF for at least 2 years, with an average duration of 6.6 years[4]. Within 60 weeks, 18% of those who discontinued MMF and 10% of those who maintained MMF experienced clinically significant disease reactivation, defined as any SELENA-SLEDAI flare with a sustained increase in immunosuppressive therapy. A 7% increase in the estimated risk of clinically significant disease reactivation was reported when comparing MMF withdrawal with maintenance, but this increase was not statistically significant. Although this risk was somewhat higher for patients with SLE with a history of lupus nephritis ($n = 76$), similar trends were also observed in this group of patients[4]. In both studies, similar rates of adverse events occurred in the maintenance and withdrawal groups; however, in the ALE06 trial, infection rates were lower in the withdrawal group than the maintenance group (46% versus 64%), with 6-fold fewer severe infections[4].

A 2025 single-centre randomized non-inferiority trial by Gopal et al.[5] directly compared the feasibility of immunosuppressant withdrawal with that of glucocorticoid withdrawal in patients with SLE in remission for ≥1 year (defined as clinical SLEDAI score of 0, physician's global assessment score of 0 and stable treatment with ≤7.5 mg per day glucocorticoids and immunosuppressants for ≥1 year) and ≥3 years of stable therapy. Over 52 weeks, flare rates were comparable between the immunosuppressant withdrawal group and the glucocorticoid withdrawal group (20% versus 31%) with no risk difference. Non-inferiority was maintained at 2 years with a 32% flare rate in the immunosuppressant withdrawal group and 45% in the glucocorticoid withdrawal group. Thus, immunosuppressant withdrawal could be as feasible as glucocorticoid reduction when criteria for sustained remission are met.

Encouragingly, most patients (68–82%) maintained disease stability after immunosuppressant withdrawal in the aforementioned clinical trials, underscoring the feasibility of safely withdrawing treatment in many individuals. The 2024 EULAR recommendations propose some clinical parameters for safe withdrawal, including at least 3–5 years of therapy, 2 years of DORIS remission and hydroxychloroquine maintenance[1]. Thus, the higher flare rate in the WIN-Lupus trial might reflect its inclusion criteria, which required 2–3 years of maintenance treatment (with an average of 2.8 years, compared with 6.6 years in ALE06 and 4.8 years in the trial by Gopal et al.)[3–5]. Notably, none of these trials used DORIS remission as an inclusion requirement, and post hoc analysis of the ALE06 trial revealed that patients who met DORIS

Box 1 | Potential risks and benefits of immunosuppressant withdrawal in systemic lupus erythematosus

Benefits of stopping immunosuppressants

- Reduced risk of infections, including severe infections
- Avoidance of teratogenic effects in young women
- Reduced cumulative toxicity
- Avoidance of poorly tolerated adverse effects (such as nausea and fatigue)
- Improved quality of life
- Lower financial burden
- Avoidance of unknown long-term effects

Risks of stopping immunosuppressants

- Increased risk of flares in some patients (such as renal relapses and general systemic lupus erythematosus flares)
- Increased risk of hospitalization owing to disease reactivation
- Increased risk of organ damage from uncontrolled disease
- Potential for early mortality
- Possible need for initiation of other treatments with additional toxicities (such as glucocorticoids)
- When returning to immunosuppressant therapy, the prior medication might no longer work or could require substantial doses of bridging glucocorticoids with enhanced toxicities

criteria experienced fewer flares[4], demonstrating the potential use of these criteria in stratification.

Despite these advances, even stringent clinical targets, such as DORIS remission, might inadequately capture flare risk. Data from a 2023 cohort study show that the likelihood of flare in patients withdrawing therapy during DORIS remission is 1.85 times higher than in patients in complete remission, as indicated by the absence of clinical findings and normalized serology[6]. Furthermore, emerging molecular data suggest that some patients meeting DORIS or LLDAS criteria might still exhibit subclinical immune activation. Specifically, transcriptomics and clustering analysis that are based on differentially expressed pathways demonstrated the heterogeneity underlying remission and LLDAS in SLE. Most patients achieving DORIS remission or LLDAS were found in clusters characterized by reduced inflammatory activity[7]. However, some patients that met DORIS (7%) or LLDAS (17%) criteria were molecularly grouped into cluster 3, which was associated with an increase in the activation of inflammatory, Toll-like receptor and interferon pathways. Thus, despite these patients meeting clinical targets, residual immune activation might persist and could predispose them to disease flares after treatment withdrawal.

Emerging work has identified molecular dysfunction that precedes clinical flares, even in patients with clinically stable disease. Longitudinal studies reveal distinct immune activation patterns up to 12 weeks before symptom onset, including elevated pro-inflammatory mediators, interferon and inflammation transcriptional signatures and suppressed regulatory cytokines[8,9]. Activated immune cell subsets, including neutrophils, monocytes and naive B cells, are also elevated in patients with imminent flares[8]. A validated flare risk index that combines 11 plasma mediators has a 97% sensitivity and 98% specificity for predicting flare[10]. These findings highlight the potential of multi-omic profiling to identify subclinical immune activation that could predispose patients to flares after immunosuppressant or glucocorticoid withdrawal; however, prospective withdrawal trials are essential for validating the clinical utility of these multi-omic profiles and for refining patient selection strategies.

Findings demonstrate that immunosuppressant withdrawal can be safely performed in many patients with quiescent SLE, although a slight increase in flare risk exists. Despite this risk, the benefits of reduced treatment toxicity and improved quality of life could outweigh the risks for many patients. However, clinicians often hesitate to stop maintenance therapy owing to the lack of clear guidelines and the potential for disease reactivation.

Studies from the past 5 years demonstrate that most, but not all, patients maintain stable disease following immunosuppressant withdrawal, which underscores the need to identify suitable candidates both clinically and molecularly before treatment cessation. Although clinical criteria, such as DORIS or LLDAS, are important, they might not fully capture the biological remission necessary for safe immunosuppressant withdrawal. Molecular profiling offers a promising complement to clinical assessments, enabling the identification of patients with residual immune activity who might benefit from prolonged maintenance therapy. Future research should focus on defining and validating multiple molecular biomarkers for safe immunosuppressant withdrawal, as well as exploring treatment-specific pathways of remission. These biomarkers will facilitate the development of personalized withdrawal strategies tailored to individual patient profiles, moving towards precision medicine in SLE management. By integrating clinical, serological and molecular data, clinicians can optimize treatment cessation, balancing the risks of continued immunosuppression against the potential for disease reactivation. Ultimately, this approach will improve SLE outcomes by reducing treatment-related complications while maintaining disease control.

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Competing interests

J.A.J. has served as a consultant for GSK. C.A.W. declares no competing interests.

Clinical trials

Nerandomilast slows progression of pulmonary fibrosis

Interstitial lung disease — either associated with connective-tissue diseases, autoimmune, or metabolic conditions, or of unknown cause as in idiopathic pulmonary fibrosis (IPF) — involves irreversible fibrotic damage of the lungs. The anti-fibrotic drugs nintedanib and pirfenidone slow disease progression but since their approval ten years ago, no therapeutic alternatives have become available for patients with progressive pulmonary fibrosis (PFF), including patients with IPF. The results of the phase III FIBRONEER-IPF and FIBRONEER-ILD clinical trials now highlight the potential of the anti-fibrotic and anti-inflammatory agent nerandomilast to slow progression of IPF and PFF.

Nerandomilast is an orally administered inhibitor of phosphodiesterase 4B. Participants in the 52-week trials received nerandomilast 9 mg or 18 mg twice daily or placebo. Randomization of patients ($n = 1177$ in the IPF study and $n = 1176$ in the PFF study) was stratified on the basis of whether they had previously received antifibrotic treatment with nintedanib or pirfenidone.

Forced vital capacity (FVC) as measured by spirometry progressively declines in patients with interstitial lung disease as a result of lung fibrosis. Both doses of nerandomilast were associated with smaller FVC decreases in patients with IPF or PFF from baseline to 52 weeks. Indicatively, the adjusted difference in FVC values between patients with IPF receiving 18-mg nerandomilast twice daily and those who received placebo was 68.8 ml (95% CI, 30.3 to 107.4; $P < 0.001$), whereas in patients with PFF, this metric was 67.2 ml (95% CI, 31.9 to 102.5; $P < 0.001$). Statistically significant reduction in FVC decline was reported also in the subgroups of patients that had previously received nintedanib or pirfenidone. Diarrhoea was the most common adverse effect of treatment with nerandomilast, and serious adverse events were equally distributed across the three arms of each trial.

Nerandomilast did not improve patient-reported outcomes in either trial. In patients with IPF, nerandomilast did not affect risk of first acute exacerbation, hospitalization for a respiratory cause or death; however, in patients with PFF, the hazard ratio for these events for the nerandomilast 18-mg group compared with the placebo group was 0.77 (95% CI, 0.59 to 1.01; $P = 0.06$).

"Patients with pulmonary fibrosis associated with autoimmune diseases represented about 20% of the total study population and they did benefit from treatment," notes Luca Richeldi, corresponding author of the FIBRONEER-IPF study, adding that "real world [data] will further inform clinicians on … management strategies." Richeldi also highlights the need for "further studies [that] will hopefully provide data on patients with early, possibly preclinical, stages of disease and on specific subgroups, [such as] individuals with rheumatic disorders."

Maria Papatriantafyllou

Original articles: Richeldi, L. et al. Nerandomilast in patients with idiopathic pulmonary fibrosis. *N. Engl. J. Med.* **392**, 2193–2202 (2025); Maher, T. et al. Nerandomilast in patients with progressive pulmonary fibrosis. *N. Engl. J. Med.* **392**, 2203–2214 (2025)

Psoriatic arthritis

Tissue-resident memory CD8$^+$ T cells on the skin–joint route

A skin–joint axis has been implicated in psoriatic arthritis (PsA), which occurs in approximately 30% of individuals with psoriasis. Previous studies have identified shared CD8$^+$ T cell clones in skin and joint lesions of patients with PsA and have highlighted a role for IL-17 signalling in disease pathogenesis.

Single-cell RNA sequencing and spatial transcriptomic analyses of skin and synovial biopsies from six patients with PsA now help to better understand some aspects of the skin–joint cross-talk in PsA. Tissue-resident memory CD8$^+$ T cells (T_{RM} cells) with an IL-17 signature (type-17 T_{RM} cells) were found to be enriched in both the skin and joints of patients with PsA. The total frequency of T_{RM} cells was higher in skin than in synovial biopsies. Skin lesions were characterized by a strong IL-17-associated gene signature with both cytotoxic and non-cytotoxic type-17 T_{RM} cell subsets, whereas granzyme K$^+$ cytotoxic T_{RM} cells were specifically enriched in the inflamed joints. T_{RM} cells were found to interact with antigen-presenting cells in both the skin and joint, and ligand-receptor analysis suggested that Langerhans cells and macrophages in the skin had the potential to recruit T_{RM} cells and support differentiation into a type-17 phenotype.

Compared with the profiles of total CD8$^+$ T cells, phenotypic variability was less pronounced within the 155 CD8$^+$ T cell clones that were identified as being shared between the skin and joints in the six patients analysed. Shared CD8$^+$ T cell clones expressed cytotoxic molecules irrespective of tissue location and those in the joint expressed markers associated with tissue homing and residency.

The antigen-driven or tissue-homing mechanisms through which shared T_{RM} cell clones direct pathology across the skin and joints remain to be investigated. Although IL-17 targeting works for the treatment of psoriasis, consistent with the strong type-17 phenotype of skin T_{RM} cells reported here, it can be less effective at treating joint inflammation in PsA. Thus, on the basis of their findings, the authors propose that combination therapy targeting multiple pathways or T_{RM} cell-specific targets might hold promise in refractory PsA.

Maria Papatriantafyllou

Original article: Durham, L. E. et al. Clonal sharing of CD8$^+$ T-cells links skin and joint inflammation in psoriatic arthritis. *Arthritis Rheumatol.* https://doi.org/10.1002/art.43286 (2025)

Rheumatoid arthritis

Synovial fibroblast-mediated neovascularization in RA

Neovascularization is regulated by angiogenic factors such as vascular endothelial growth factor (VEGF) and angiopoietins (ANGPT1 and ANGPT2), which bind to TIE2. Interactions between rheumatoid arthritis synovial fibroblasts (RASFs) and endothelial cells promote pathogenic vascularization in the rheumatoid arthritis (RA) synovium. Understanding how RASF–endothelial cell interactions alter vascularization in RA might reveal therapeutic targets.

Heck et al. provide insights into how RASFs alter neovascularization in RA. The authors use the severe combined immunodeficient (SCID) mouse model of RA, which involves implanting healthy human cartilage and RASFs into SCID mice. In this model, RASFs induced pathogenic helix-like vessel formation in both ipsilateral (site of RASF and cartilage implantation) and contralateral (distal cartilage implantation) sites. ANGPT2 expression on endothelial cells in RA synovial tissue and in SCID mouse implants was increased compared with control samples (osteoarthritis synovial tissue and control implants, respectively). In addition, culturing an endothelial cell line with RASFs upregulated ANGPT2 compared with untreated cells.

In a 2D model of tube formation (a technique used to study angiogenesis), adding RASFs to endothelial cells reduced the thickness of tubes compared with endothelial cells alone. In a 3D assay of endothelial cell sprouting, the addition of RASFs led to diffuse endothelial cell sprouting and subsequent disorganized tube formation. Furthermore, treating endothelial cells with RASF-conditioned media similarly altered tube formation, which was exacerbated by the addition of IL-1β to the media.

Interestingly, repeated stimulation of RASFs with IL-1β led to lower IL-6 expression than a single IL-1β stimulation, and repeated RASF stimulation with IL-1β restored tube formation. RNA sequencing indicated that repeated RASF stimulation with IL-1β did not affect VEGF expression but upregulated RA-associated molecules, such as TNF signalling components, and downregulated IL-11 and CXCL2. The addition of CXCL2 or IL-11 to the 2D tube formation assay showed reduced network area formation, which was restored by inhibiting either factor. Elena Neumann, the corresponding author of the article, comments that "there were other angiogenesis-associated factors and pathways in our RNA sequencing dataset that we plan to explore in the future."

The authors then assessed how canstatin (an angiogenic inhibitor that is released when RASFs degrade the extracellular matrix and that also blocks ANGPT1-induced proliferation of endothelial cells) might alter RASF–endothelial cell interactions. The level of canstatin was higher in serum from people with RA compared with healthy people and those with osteoarthritis. Long-term but not short-term treatment with canstatin in the 2D tube formation model reduced tube thickness. In the 3D model of endothelial cell sprouting, canstatin reduced the area of endothelial cell sprouting.

Adding canstatin to implants in the SCID model of RA led to a reduced number of helix-like vessels in ipsilateral implants but did not alter RASF invasiveness. ANGPT2 expression was also altered in a time-dependent manner in ipsilateral implants; ANGPT2 expression initially increased and then decreased to levels that were lower than those in control implants. The authors hypothesize that canstatin-mediated changes to ANGPT2, creates an imbalance between ANGPT2 and ANGPT1 and therefore alters ANGPT1–TIE2 signalling and subsequent RASF–endothelial cell interactions.

Neumann notes that "these findings might also be relevant to other diseases in which vessel formation is altered, such as psoriatic arthritis or systemic sclerosis."

Holly Webster

Original article: Heck, C. et al. Influence of canstatin on fibroblast-driven hypervascularisation in rheumatoid arthritis. *Ann. Rheum. Dis.* https://doi.org/10.1016/j.ard.2025.05.019 (2025)

Rheumatoid arthritis

https://doi.org/10.1038/s41584-025-01263-4

How JAK inhibitors tip the prothrombotic balance in rheumatoid arthritis

Vibeke Strand

The increased incidence of deep vein thromboses and pulmonary emboli has long been noted in rheumatoid arthritis and has been ascribed to the effects of chronic inflammation and disease activity, as well as to specific biologic DMARDs and JAK inhibitors. Reporting in *ACR Open Rheumatology*, Zavoriti and Miossec provide data that might explain the prothrombotic effects of the JAK inhibitor tofacitinib.

Refers to Zavoriti, A. & Miossec, P. Understanding cardiovascular events with JAK inhibitors: tofacitinib reduces synovial and vascular inflammation but not the prothrombotic effects of inflammatory cytokines on endothelium. *ACR Open Rheumatol*. https://doi.org/10.1002/acr2.11790 (2025).

Patients with rheumatoid arthritis (RA) have a 50–100% greater risk of developing venous thromboembolisms (VTEs) than that of individuals without RA[1]. For example, a nationwide cohort study from Sweden reported a 1-year cumulative incidence of VTEs of 0.71% for patients with RA, compared with 0.36% for the general population[2]. This risk has been ascribed to the effects of chronic inflammation and disease activity, as well as to the use of specific biologic DMARDs and targeted synthetic DMARDS[3,4].

The introduction of JAK inhibitors was received with enthusiasm, as they offered a convenient oral route of administration, but subsequent data have raised safety concerns. In the ORAL Surveillance clinical study, increased risks of major adverse cardiovascular events (MACEs) and malignancies were reported with both doses of tofacitinib (10 mg twice daily and 5 mg twice daily), and this led to a boxed warning label[5]. Before conclusion of the trial, an excess of thromboembolic events was noted by the data and safety monitoring board in the group of patients who received the higher dose (10 mg twice daily), and this led to a mandated decrease to 5 mg twice daily[6]. An earlier Arthritis Advisory Committee meeting for the US Food and Drug Administration that reviewed approval of another JAK inhibitor, baricitinib, for use in patients with RA noted an increased incidence of thromboembolic events in phase 3 randomized controlled trials, and a boxed warning to this effect was subsequently included in that label[6]. Following release of the ORAL Surveillance results, the US Food and Drug Administration and the European Medicines Agency added black box warnings to the labels of all approved JAK inhibitors (with exception of ruxolitinib), for MACEs, malignancies and thromboembolic events.

The increased risk of VTEs in RA was confirmed in a 2020 review of the randomized controlled trials of tofacitinib for RA, psoriasis and psoriatic arthritis[7]. In a nationwide register-based, active comparator, new user design cohort study in Sweden from 2010 to 2021, patients with RA who were treated with tofacitinib were found to have an increased risk for pulmonary emboli compared with that of patients receiving tumor necrosis factor (TNF) inhibitors[8]. A recent systematic review of MACEs and all-cause deaths with JAK inhibitors or the interleukin-6 (IL-6) inhibitor tocilizumab (intervention arm) versus control interventions (TNF inhibitors or placebo) that included 18 randomized controlled trials with 21,432 patients with RA and a total of 57,040 patient-years exposure linked all JAK inhibitors, but not the IL-6R inhibitor, to a non-significantly increased risk of MACEs and all-cause deaths[9].

Zavoriti and Miossec studied the specific effects of tofacitinib on synovial and endothelial cell function, vascular inflammation and coagulation[10]. In co-cultures of peripheral blood mononuclear cells with RA synoviocytes or endothelial cells, tofacitinib failed to reduce the prothrombotic effects of IL-17 and TNF on endothelial cells or tissue factor-initiated formation of thrombin, while it decreased the anticoagulant properties of thrombomodulin. Tofacitinib completely inhibited the expression of interferon-γ and decreased the production of TNF and IL-17A, as well as of the anti-inflammatory cytokine IL-10. Tofacitinib reduced endothelial cell activation, and stimulated the production of IL-6 and coagulation factors, specifically of tissue factor and thrombomodulin. However, tofacitinib failed to reduce the expression of not only the prothrombotic tissue factor but also IL-8 and E-selectin. Higher doses of tofacitinib further decreased the production of TNF, IL-17, IL-6 and the pro-coagulant adhesion molecule VCAM-1 but did not affect the levels of tissue factor.

Together these data demonstrate that despite improving synovial and vascular inflammation, tofacitinib does not reverse the prothrombotic effects of IL-17A- and TNF-activated endothelial cells. However, on the basis of pre-clinical data, these findings provide a plausible explanation for the increased cardiovascular risk observed with tofacitinib in RA. It is not known whether this effect is specific to tofacitinib or is shared by the JAK1–JAK3 inhibitors; this is an important topic for further study. Similar experiments with baricitinib and upadacitinib would be useful. Surveillance, as generated by the labels, will continue, and these data should help in further monitoring the prothrombotic effects of the JAK1–JAK3 inhibitors, particularly as new selective members of the class, such as deucravacitinib and zasocitinib (TAK-279), are introduced. Concurrent use of JAK1–JAK3 inhibitors and antithrombotic drugs is common and, in combination with TNF inhibitors, is expected to reduce the risk of thromboembolic events.

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Competing interests

The author declares no competing interests.

Clinical guidelines

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Region-specific, data-driven guidelines are needed for rheumatic diseases in LMICs

Amita Aggarwal

Separate guidelines are needed for the management and diagnosis of rheumatic diseases in low- and middle-income countries, especially with the advent of expensive biological therapies and monitoring techniques. The lack of robust data on the efficacy of low-cost drugs and biosimilars in these countries limits the development of data-driven guidelines.

Refers to Abu-Zaid M. H. et al. African guidelines for diagnosis and management of polyarticular juvenile idiopathic arthritis: PAFLAR initiative. *Pediatr. Rheumatol.* **23**, 27 (2015).

Consensus guidelines serve as a reference for many physicians and can help to guide disease management. These guidelines often include expensive biological therapies and the use of biomarkers and radiological techniques to assess responses to treatment that are not available in many low- and middle-income countries (LMICs). Thus, rheumatologists from the Paediatric Society of the African League Against Rheumatism (PAFLAR) have developed guidelines for the diagnosis and management of polyarticular juvenile idiopathic arthritis (JIA) with specific considerations for the challenges faced in Africa[1].

Prior to a systematic literature search, 15 questions were formulated using the PICO (population, intervention, control and outcomes) framework and a Delphi process was used to reach an agreement. The guidelines contain four overarching principles, five diagnosis recommendations, and ten recommendations for disease management. The proposed recommendations for the diagnosis of polyarticular JIA addressed when to suspect disease, how to diagnose disease and which other diagnoses to consider. In addition, the recommendations also state that eye disease and the use of radiology should be considered. For disease management, the recommendations include setting treatment targets (such as remission or low disease activity), the use of methotrexate as a first-line agent along with NSAIDs and intraarticular steroids; second-line therapy, if required, should include escalating methotrexate dose and the addition of conventional synthetic DMARDs (including leflunomide or sulfasalazine) or biological DMARDs for those with a poor prognosis. Biologics are recommended as third-line agents along with tofacitinib. The use of biosimilars is suggested owing to their low cost[1].

Although these PAFLAR guidelines are a welcome step towards region-specific guidelines and could prompt other LMICs to develop similar guidelines, there are some limitations. The first and foremost is the lack of robust data concerning the efficacy and toxicity of different treatments in the local setting. The registry data from the PAFLAR society show that treatments being used for polyarticular JIA in Africa include NSAIDs (31.1%), synthetic DMARDs (18.1%), synthetic DMARDs combined with NSAIDs (17.5%) and glucocorticoids (9.6%); only a small number of patients received biological DMARDs at diagnosis[2]. In the absence of robust data, the evidence base is the same as for the guidelines from the developed world and in most multinational trials, patients from African countries are rarely included.

Most guidelines from professional societies have given recommendations for all major categories of JIA, whereas the PAFLAR guideline focus only on polyarticular JIA. Polyarticular JIA is defined as arthritis in children that involves five or more joints in the first 6 months. The recommendation is to refer all such patients to a paediatric rheumatologist as delays in referral to a rheumatologist can lead to a poor long-term outcome[3]. However, is this recommendation feasible? Early referral could be possible in the developed world, where there are fewer children and more paediatric rheumatologists. In 2021, there were 650 million children in Africa and the number of trained rheumatologists is limited[4,5]. Thus, this recommendation, despite being logical, is hard to implement in Africa and other LMICs. One possibility is to train paediatricians in LMICs to recognize polyarticular JIA and start first-line therapy prior to a rheumatology consultation to avoid delaying treatment.

The guidelines also suggest the use of musculoskeletal ultrasound (MSUS) both for diagnosis and follow-up to assess treatment response, disease progression and to document radiological remission. Although MSUS is cheap and available on the bedside, this technique has several issues, such as a steep learning curve for adequate expertise, interobserver variability, lack of equipment in rheumatology units and time needed for a detailed evaluation. For these reasons, MSUS is not included in the current guidelines for paediatric and adult joint diseases from developed regions such as those from the ACR or EULAR.

The guidelines include an important cautionary note against using specific diets and herbal supplements. In regions such as Africa and Asia, there is a general belief that certain kinds of food can aggravate arthritis, and herbal supplements and alternative systems of medicine are often used by patients to get relief. Inclusion of inflammatory ocular disease (such as uveitis) in the recommendations for diagnosis could have been avoided as this complication is rare in rheumatoid factor-positive polyarticular JIA and prevalence in rheumatoid factor-negative polyarticular JIA ranges from 1.8% to 21.1%[6]. In Africa and the Middle East, inflammatory ocular disease is observed in only 4.1% of children[6].

The suggestion of using intravenous methylprednisolone bolus for a maximum of 3 days in refractory polyarticular JIA has been adapted from Egyptian guidelines but does not have much scientific rationale. The use of glucocorticoids in children has considerable metabolic and infectious adverse effects, and this option should therefore be used rarely. In LMICs, the risk of tuberculosis is also increased with the use of

glucocorticoids[7]. Perhaps a single low-dose methylprednisolone bolus (3–5 mg kg^{-1}) to control severe joint disease activity could be considered.

Use of leflunomide or sulfasalazine as second-line agents in children that do not respond to methotrexate is a low-cost alternative to adding biologics, but the data that support this recommendation are limited. However, in clinical practice, a combination of methotrexate with leflunomide shows good response in polyarticular JIA[8]. Another cost-effective alternative is the use of subcutaneous methotrexate as this method increases bioavailability[9]; however, there are issues with feasibility and acceptability of subcutaneous methotrexate in LMICs, as nurses are not always available and parents are reluctant to personally administer the injection. Biosimilars have made access to biologics possible in LMICs, although it is still beyond the reach of the average person. In real life data, biosimilars have performed as well as their reference drugs, biosimilars of TNF therapies are widely available, but biosimilars of IL-6 inhibitors are not yet available in many LMICs[10].

Thus, in the future, local data on efficacy, toxicity and the cost effectiveness of low-cost therapies, such as the addition of leflunomide or sulfasalazine and the use of subcutaneous methotrexate, should be used to inform recommendations. Similarly, data on locally available biosimilars are needed as different biosimilars can vary widely both in regard to efficacy and toxicity. Studies that use generic JAK kinase inhibitors that cost less than US$10 a month in children with JIA could be transformative for LMICs; however, further safety studies specifically in these regions are needed. Another possible option is the repurposing of drugs that have immunomodulatory properties. It is an opportune time for LMICs to come together and perform these studies so that children in these countries can be effectively treated with low-cost effective therapies.

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Competing interests

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Review article

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Synovial fluid as a complex molecular pool contributing to knee osteoarthritis

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Abstract

The main homeostatic function of the synovial fluid is joint lubrication. However, during knee osteoarthritis (KOA), synovial fluid becomes modified with drivers of disease that contribute to symptoms (pain) and joint-related pathology. Acting as a sink of factors from both systemic circulation and local tissues, including articular cartilage, subchondral bone, synovium, and the infrapatellar fat pad, the synovial fluid enables bidirectional communication promoting KOA pathogenesis. Synovial fluid constituents might also be detected in circulation, functioning not only as accessible biomarkers but also as potential mediators of KOA-driven systemic effects. Factors deposited in synovial fluid have the ability to affect nervous system activity, acting at the neuronal projections that are integrated into joint tissues from dorsal root ganglia. Non-coding RNAs (microRNAs, long non-coding RNAs, circular RNAs), metabolites, cytokines and other secreted proteins of the synovial fluid in KOA have emerged as biomarkers of disease progression, therapeutic efficacy, and pain. These molecules might also function as molecular mediators of KOA, supporting them as candidates for therapeutic intervention. This review consolidates literature published primarily within the past 4 years, focussing on factors identified within synovial fluid as biomarkers and molecular mediators of KOA symptoms and pathology. Emerging therapeutic modalities to target synovial fluid molecular mediators are also discussed.

Sections

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Key points

- Synovial fluid bathes the entire joint, and the various joint tissues such as articular cartilage, subchondral bone, synovium and the infrapatellar fat pad contribute to the pool of molecular mediators in the synovial fluid.
- Molecules within synovial fluid, including non-coding RNAs, metabolites, cytokines, growth factors and other proteins, and cells have emerged as biomarkers of knee osteoarthritis (KOA).
- Molecules within synovial fluid might also act as molecular mediators of KOA pathologies and pain.
- Some synovial fluid-derived biomarkers and molecular mediators of KOA are also detected systemically, owing to direct transfer to and from the circulatory system, which might also be involved in painful nociceptive signalling through dorsal root ganglia neuron afferents.
- Intra-articular injection of therapeutics targeting synovial fluid components might help to mitigate specific KOA pathologies, including cartilage degeneration and inflammation, although further innovation is required to identify therapeutics that increase retention time in the joint and alleviate joint-wide pathologies.

Introduction

Osteoarthritis (OA) is a debilitating disease that diminishes the quality of life of those afflicted. Over the past 30 years, the prevalence of OA has increased by 132.2%, with the knee being the joint most impacted[1]. Pathologies associated with knee OA (KOA) include articular cartilage degradation, inflammation and fibrosis of the synovium and the infrapatellar fat pad, subchondral bone remodelling, and osteophyte formation[2,3]. Joint tissues, including meniscus, ligaments, tendons, synovium, the infrapatellar fat pad, and subchondral bone, contain sensory innervation from neurons whose cell bodies are in the dorsal root ganglia (DRGs) of the spine, which contribute to KOA pain[4,5]. Pain is the primary driver of patients seeking medical treatment for KOA. Although approved therapies might provide temporary pain relief, there are no disease-modifying drugs currently available.

Within the knee, synovial fluid fills the joint space, bathing all tissues including the synovium, infrapatellar fat pad and articular cartilage (Fig. 1). Synovial fluid is an ultrafiltrate of plasma containing additional factors produced primarily by synoviocytes in the synovium, including lubricin, hyaluronic acid, surface-active phospholipids and glycosaminoglycans, among others[6–8]. It functions to lubricate the knee joint, reducing friction and allowing for smooth articulation[9]. In addition, synovial fluid reduces the rate of articular cartilage failure after cyclic compressive loading[10]. However, during KOA, synovial fluid composition is altered, increasing joint friction and enhancing cartilage degradation and pain[9,11]. Pre-existing molecules in synovial fluid are also modified, such as non-coding RNAs, metabolites, cytokines and chemokines, growth factors, and other secreted proteins, and might contribute to KOA pathogenesis[12,13].

As a molecular sink, synovial fluid is capable of mediating bi-directional communication between local joint tissues and the nervous system, as well as with systemic tissues through the transfer of factors to and from the circulation. For instance, cartilage degradation fragments are released into the synovial fluid, as demonstrated by N-terminomics, a subtype of proteomic analysis that identified 677 peptides originating from 153 proteins present in both KOA cartilage and synovial fluid[14]. Cartilage degradation fragments have the ability to promote inflammatory processes, including tissue vascularization, fibrosis and immune cell recruitment, changing the cellularity of the knee joint[15,16]. Owing to increased vascularization, metabolic disturbances occur in the joint through modification of nutrients and oxygen content, shifting cellular metabolic process and downstream products[17,18].

Additionally, soluble mediators of the synovial fluid stimulate distal nerve terminals to promote joint pain in KOA[19,20]. Increased nerve afferents infiltrating joint tissues create the potential for increased propagation of pain signals in KOA[21]. This is mediated, in part, by articular cartilage degeneration, which exposes sensory nerve endings inferior to the cartilage (such as in the subchondral bone) to additional molecules in the synovial fluid, increasing joint pain[22]. Most tissues in the knee, other than cartilage, are innervated with afferents from nociceptive neurons that detect pain signals[5]. Nociceptor cell bodies are found in DRGs of the spinal cord, which project to the somatosensory cortex of the brain, where painful nociceptive information is processed[5,23,24] (Fig. 1). During KOA, molecules in synovial fluid interact with afferents from DRG neurons, innervating joint tissues[23,24]. For example, when cultured neurons from mouse DRGs that innervate the knee were stimulated with human synovial fluid from individuals with KOA, they showed neuronal hyperactivity, as measured through resting membrane potentials, membrane channel activity, and intracellular $Ca2^{+}$ levels, as compared with cells stimulated with synovial fluid from healthy individuals[20]. This is indicative of changes to synovial fluid molecular mediators as part of KOA that can directly activate DRG neurons and contribute to pain.

In this Review, we discuss key biomarkers and molecular mediators of OA found in synovial fluid, including non-coding RNAs, metabolites, cytokines, growth factors, and other secreted proteins, that have potential as diagnostic tools for the presence or progression of KOA. The article focuses on primary KOA, concentrating on literature primarily from the past 4 years as an update of previous reviews, including both preclinical and clinical research. We discuss functional contributions of molecules within synovial fluid, while also considering synovial fluid-derived mediators detected in the circulation. In light of identified synovial fluid molecular changes, potential therapeutic targets and novel drug-delivery strategies to mitigate KOA-destructive mechanisms within the synovial fluid and joint microenvironment are visited.

Biomarkers of knee osteoarthritis within synovial fluid

Currently, total knee arthroplasty (TKA) remains the only strategy to alleviate pain and improve quality of life of those with KOA once conservative management strategies have been exhausted. However, delaying TKA remains crucial to alleviating health care and patient burdens created through multiple lifetime surgeries due to prosthetic wear and eventual failure[25]. Early diagnosis can help to ensure that treatments are delivered before progression to advanced disease stages that require TKA. Using biomarkers within synovial fluid in the detection of the early stages of KOA, as well as monitoring KOA progression, is an attractive option as it is relatively inexpensive and is directed to the affected joint. Table 1 provides a synopsis of studies describing major biomarkers identified within synovial fluid over the

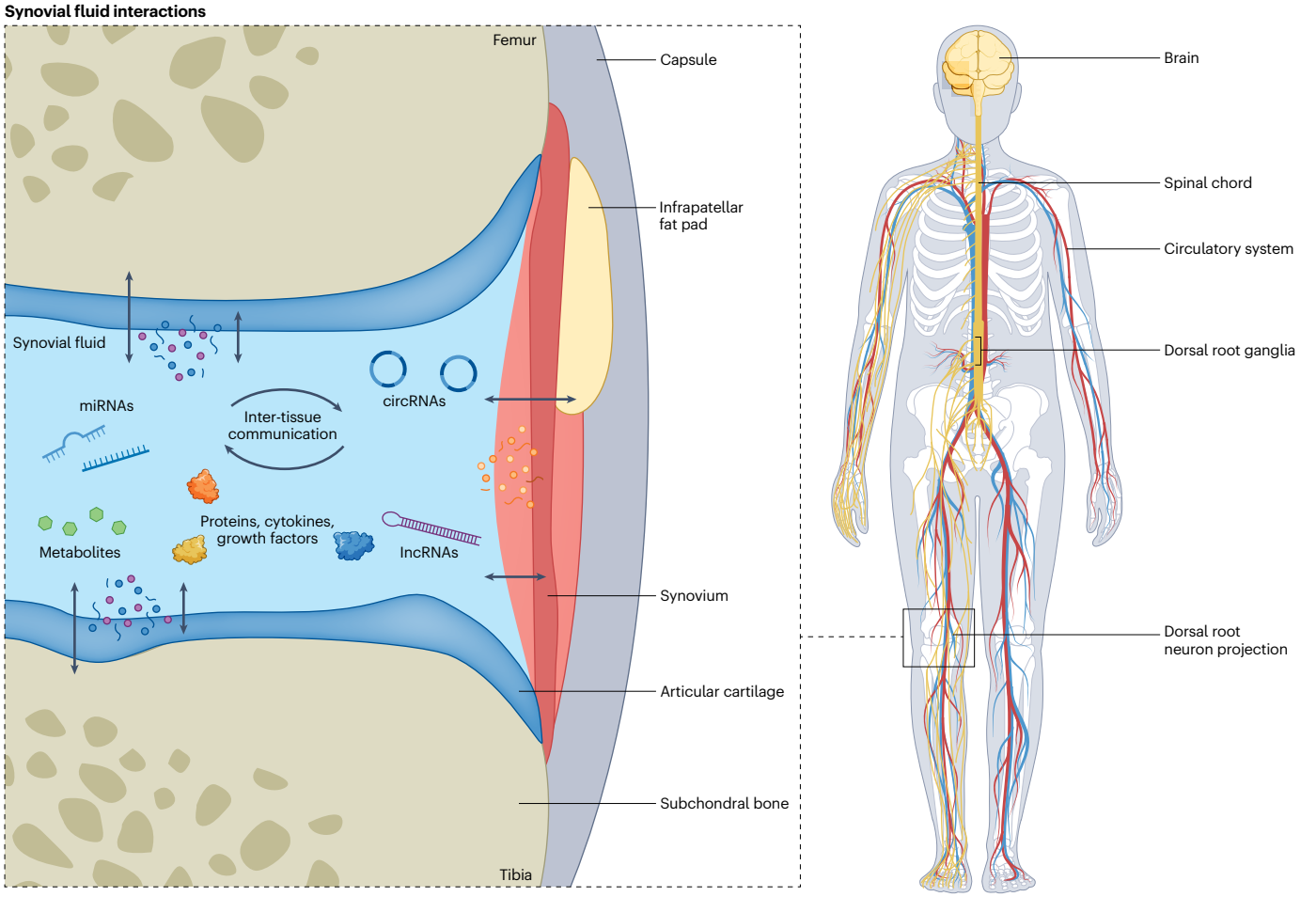

Fig. 1 | Local and systemic interactions of synovial fluid components during knee osteoarthritis. Within the knee joint, synovial fluid fills the joint space, bathing all tissues. Transfer of molecules detected in synovial fluid to and from joint tissues, including articular cartilage, subchondral bone, the infrapatellar fat pad and the synovium can act as biomarkers or molecular mediators of knee osteoarthritis (KOA). This may include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), metabolites and proteins (such as cytokines and growth factors). Molecules within synovial fluid may also be detected systemically in the circulation. Pain may be modified by actions of pain mediators in the circulation that can alter pain circuits, or locally in the knee by activating dorsal root ganglia sensory neurons of innervated joint tissues. Similar connectivity to the nervous and circulatory systems are also present within other synovial joints such as the hip, shoulder etc.

past 4 years, with some highlighted as also associated with other joint tissues or blood isolates.

Non-coding RNAs

Non-coding RNAs constitute a broad category of heterogeneous transcripts that are not translated and act as epigenetic regulators of gene expression, including microRNAs (miRNAs), circular RNAs (circRNAs), and long non-coding RNAs (lncRNAs). miRNAs target specific sets of mRNAs for degradation, or inhibit their translation[26]. circRNAs function as miRNA 'mops' that modify the availability of target miRNAs to bind mRNA targets[27]. lncRNAs regulate protein-coding gene expression through modifying chromatin architecture, enhancer activity, mRNA stability, and gene silencing[28]. Importantly, secreted non-coding RNAs are often packaged into extracellular vesicles (EVs) and have been shown to participate in paracrine and endocrine cellular communication processes in multiple diseases such as cancer and metabolic syndrome, altering gene expression and potentially the functions of neighbouring cells[29,30]. The biogenesis of non-coding RNAs has been extensively studied and recounted in other reviews[28,31–36]. During KOA, non-coding RNA levels within synovial fluid have been shown to be altered, contributing to KOA pathogenesis by modifying many cellular processes, including cell proliferation, propagating joint-tissue damage[12]. Owing to their ability to alter gene expression, non-coding RNAs can serve as potential biomarkers of KOA pathogenesis, able to detect KOA, track disease progression and potentially predict therapeutic outcomes.

Detection of knee osteoarthritis. Non-coding RNAs, specifically miRNAs, as candidate biomarkers for the early detection of KOA have been a popular topic of research. For example, a donkey model with monosodium iodoacetate-induced KOA pathologies, including joint pain and cartilage degradation[37], showed increased synovial fluid levels

Table 1 | Potential biomarkers of knee osteoarthritis in synovial fluid, blood or joints (as reported between 2021 and 2024)

Biomarker	Associated fluid or tissue	Biomarker activity	Refs.
Non-coding RNAs			
miR-146b and miR-27b	Synovial fluid and serum	KL-0/I graded KOA in humans	38
miR-335-3p and miR-335-5p	Synovial fluid	KL-0/I graded KOA in humans	39,40
miR-542 and miR-543	Synovial fluid	Detection of KOA in canines	41
Profile of 809 EV miRNAs	Synovial fluid	Detection of post-traumatic OA in horses	44
Profile of 658 EV miRNAs	Plasma	Fibrosis and inflammation in post-traumatic OA in horses	44
Exosome content profile of 52 mRNAs, 196 lncRNAs and 98 circRNAs	Synovial fluid	Detection of KOA in humans	45
miR-27b-3p	Synovial fluid and synovium	Extracellular matrix dysregulation and synovial fibrosis in humans	47,48
miRNA-34a-5p	Synovial fluid, plasma, articular cartilage	KOA in individuals with obesity	47,100
FER1L4 (lncRNA)	Synovial fluid and plasma	Detection of KOA in humans	49
miR-126-3p	Synovial fluid and plasma	KOA progression in humans and rats	101,102
Metabolites			
Profile of 19 metabolites	Synovial fluid	Detection of metacarpophalangeal OA in horses	53
Profile of 28 metabolites	Synovial fluid	Distinguish KOA from RA in humans	54
Mannose, betaine, isoleucine	Synovial fluid	Distinguish KL-I versus KL-II graded KOA in canines	57
Lactate	Synovial fluid	Detection of late-stage KOA in canines	57
2-Hydroxyisobutyrate	Synovial fluid	Progression of KOA in canines	57
Lysophosphatidylcholine 16:0	Synovial fluid	KOA-associated joint pain in mice	58
Proteins or peptides			
Profile of 677 peptides	Synovial fluid and articular cartilage	Cartilage degeneration in humans	14
COL10A1	Synovial fluid	Detection of KOA in humans	38
IL-40	Synovial fluid	Detection of joint Inflammation and cartilage degeneration in humans	65
COMP and PIICP	Synovial fluid and serum	Detection of KOA progression, disease severity, cartilage metabolism and joint pain in humans	77–79
RETN and CRP	Serum	Detection of KOA and joint inflammation in humans	71
IL-8	Synovial fluid	Detection of joint inflammation and clinical severity in humans	72
TDO2	Synovial fluid	Rating of KOA severity	73
IL-34	Synovial fluid and plasma	Detection of KOA progression and synovitis in humans	74
sVCAM-1, MMP-3, sICAM-1, TIMP-1, VEGF and MCP-1	Synovial fluid	Detection of inflammation and macrophage or neutrophil activation, and prediction of radiographic and clinical severity in humans	75
Profile of 786 EV proteins	Synovial fluid	Detection of KOA severity in humans	76
IL-1β, IL-10, IL-12 and GM-CSF	Synovial fluid and serum	Associated with decreased joint pain and stiffness, and improved joint function in individuals with KOA	83,85
IL-10, IL-1β, VEGF and IL-12–IL-23p40	Synovial fluid and plasma	Associated with chronic joint pain post-total knee arthroplasty	83
ADIPOQ	Synovial fluid	Associated with clinical severity and joint pain in women with KOA and a normal BMI	86
PROK2	Synovial fluid	Associated with knee joint pain in humans	87,88
NGF	Synovial fluid and plasma	Detection of KOA and associated with joint pain post-total knee arthroplasty	90
C2C-HUSA	Synovial fluid and urine	Predictor of joint pain post-total knee arthroplasty	92

Table 1 (continued) | Potential biomarkers of knee osteoarthritis in synovial fluid, blood or joints (as reported between 2021 and 2024)

Biomarker	Associated fluid or tissue	Biomarker activity	Refs.
Proteins or peptides (continued)			
Plasma EV protein complement	Plasma	Detection of carpal joint OA progression in horses	103
Profile of 199 EV proteins	Synovial fluid and plasma	Detection of joint inflammation in humans	104
Surface EV expression of FGA, FGB, FGG, TLN1 and AMBP	Plasma	Predictors of KOA progression	104
Profile of 82 proteins	Plasma	Correlating with poor joint function and PROMs	105
HABP2, HRG, ZPI AND PLF4	Synovial fluid and plasma	Predictive of KOA progression in humans	106
Panel of 11 proteins	Serum	Predictive of KOA progression in humans	107
CRAC1	Synovial fluid and serum	Predictive of KOA progression and pain in humans	108,110
Panel of 5 proteins	Plasma	Detection of bone marrow lesions in humans	109
T2CM	Synovial fluid and serum	Detection of KOA in canines (synovial fluid) and distinguishing late-stage versus KL-II graded KOA in humans (serum)	111,112
IL-6	Synovial fluid, articular cartilage and synovium	Detection of KOA in humans	128

ADIPOQ, adiponectin; AMBP, α-1-microglobulin/bikunin precursor protein; C2C-HUSA, type 2 collagen C terminal cleavage peptide assay; circRNA, circular RNA; COL10X1, alpha chain of type X collagen; COMP, cartilage oligomeric matrix protein; CRAC1, cartilage acidic protein 1; CRP, c-reactive protein; EV, extracellular vesicle; FER1L4, fer-1-like family member 4; FGA, fibrinogen alpha chain; FGB, fibrinogen beta chain; FGG, fibrinogen gamma chain; GM-CSF, granulocyte–macrophage colony-stimulating factor; HABP2, hyaluronan-binding protein 2; HRG, histidine-rich glycoprotein; KL, Kellgren–Lawrence; KOA, knee osteoarthritis; lncRNA, long non-coding RNA; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; NGF, nerve growth factor; OA, osteoarthritis; PLF4, platelet factor 4; PROMs, patient-reported outcome measures; PIICP, procollagen type II C-terminal propeptide; PROK2, prokineticin 2; RETN, resistin; RA, rheumatoid arthritis; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; T2CM, type II collagen neo-epitope; TDO2, tryptophan 2,3-dioxygenase; TIMP-1, tissue inhibitor of metalloproteinases-1; TLN1, talin-1; VEGF, vascular endothelial growth factor; ZPI, protein Z-dependent protease inhibitor.

of miR-27b, miR-146b and collagen type X alpha chain (COL10A1) at early timepoints post-monosodium iodoacetate injection, with levels decreasing as the model progressed[38], suggesting that these molecules might be useful biomarkers for early KOA detection. miR-335-5p and miR-335-3p were first found to be increased in the plasma of individuals with early radiographic KOA (Kellgren–Lawrence grade (KL) 0/I) compared with patients having late radiographic KOA (KL grade III/IV)[39]. Subsequently, miR-335-5p levels were increased in the synovial fluid of individuals with KL 0/I-graded KOA compared with late-stage KOA, whereas miR-335-3p showed no statistically significant variation[40]. These findings suggest that miR-335-5p might be a promising synovial fluid biomarker of early stages of KOA in humans.

Non-coding RNAs within synovial fluid could also be used as biomarkers for detecting more advanced stages of KOA. One preclinical study investigating differentially expressed miRNAs in the synovial fluid of canines with spontaneous KOA determined that levels of miR-542 and miR-543 were significantly higher in synovial fluid of dogs with KOA compared with that of healthy dogs, indicating that these miRNAs are promising biomarkers of KOA in canines[41]. Recently, there has been a focus on the use of exosome and EV contents as indicators of KOA. Exosomes and EVs are secreted by almost all cell types into biofluids, potentially supporting intercellular communication[42,43]. In a preclinical study of horses with and without injury-induced post-traumatic OA, 805 miRNAs were differentially expressed in synovial fluid EVs from horses with versus without post-traumatic OA[44]. These miRNAs were associated with a variety of pathogenic mechanisms including, but not limited to, fibrosis, angiogenesis and inflammation[44]. Additional preclinical studies using animal models of spontaneous KOA should be investigated to identify common elements of this miRNA profile consistent across species and KOA phenotypes, and to associate miRNA features with human primary or secondary KOA. Furthermore, analysis of the RNA content of synovial fluid exosomes from patients with KOA and non-OA knee injury controls, reported 52 mRNAs, 196 lncRNAs, and 98 circRNAs as being differentially expressed between the two groups, with a subset of these RNAs having putative roles in the PI3K–Akt and autophagy pathways[45]. Thus, comparisons of non-coding RNA changes in humans and animal models of KOA can help understanding of putative biomarker consistency across species and KOA phenotypes. Such putative biomarkers might also represent disease-mediating factors, which can be investigated further as therapeutic targets for KOA.

Taken together, these studies demonstrate the utility of non-coding RNAs, specifically miRNAs, within synovial fluid as biomarkers for KOA detection within early or later stages of the disease. Going forward, the focus should be shifted onto identifying profiles of biomarkers in synovial fluid that can reliably detect early stages of KOA in human cohorts. By identifying early biomarkers of KOA, therapeutics can be administered sooner, hopefully relieving pathological and clinical symptoms of the disease before they progress to more advanced stages where current therapeutics are only palliative until TKA becomes necessary.

Monitoring disease progression. Alterations in the expression of non-coding RNAs within synovial fluid might be indicative of disease progression in individuals with KOA. In a horse model of experimentally induced carpal OA that mimics human KOA, expression of miRNAs in synovial fluid changed as OA progressed[46], demonstrating that miRNA signatures can correlate with disease stage. In humans, miR-27b-3p has been detected at higher levels in the synovial fluid[47] and synovium[48] of late-stage (KL III/IV) versus KL I/II-graded KOA, indicating that as KOA progresses, synovial fluid miR-27b-3p expression increases. A separate study of lncRNAs revealed that *FER1L4* expression was downregulated in the synovial fluid and plasma of individuals with KL IV compared with

KL III and healthy controls[49], highlighting the potential of *FER1L4* as a disease-staging diagnostic marker.

The above synovial fluid non-coding RNAs that are dysregulated and potentially alter KOA pathogenesis have emerged as biomarkers for the development and progression of KOA. Although steps have been taken to improve characterization of synovial fluid non-coding RNA profiles, additional validation and association studies in other biofluids, such as urine, are necessary to determine efficacy, while also considering the least invasive options for detection. Further analysis is also needed to determine relationships of non-coding RNA biomarkers to disease trajectories and therapeutic efficacy, to shift from a detection-of-presence to prognostic and predictive utility. Unfortunately, limited research within the past 4 years has focused on circRNAs within KOA synovial fluid, suggesting a crucial gap in the current literature that needs to be filled. Future studies should focus on circRNAs as putative biomarkers in KOA synovial fluid, which might contribute to disease symptoms and pathologies.

Metabolites

Metabolites are a broad category of small molecules generated through dietary ingestion and enzymatic reactions associated with cellular processes. Metabolite categories include carbohydrates, lipids, nucleotides, amino acids, and organic acids, among others. Concentrations of metabolites are typically maintained in physiological ranges indicative of homeostatic regulation of upstream metabolic process and downstream cellular functions[50]. During diseases such as KOA, levels of metabolites are modified, indicative of metabolic disturbances that influence downstream cellular mechanisms[51,52]. Thus, similar to non-coding RNAs, metabolite levels within synovial fluid emerge as useful biomarkers of KOA.

Detection of knee osteoarthritis. Recent studies have associated altered profiles of metabolites within synovial fluid with OA, including KOA, and shown their potential as biomarkers to detect the disease. In horses, 19 metabolites were differentially detected in synovial fluid of animals with metacarpo-phalangeal OA compared with controls: the levels of 1,3-dihydroxyacetone were increased in horses with OA, whereas the levels of tryptophan, phenylalanine, tyrosine, uridine, creatinine, creatine, glycine, choline, asparagine, glutamine, arginine, 3-hydroxybutyrate, valine, 2-hydroxyisovalerate, α-ketoisovaleric acid, 3-methyl-2-oxovalerate, and methionine were increased in control horses[53]. In addition, differential metabolite signatures have been detected in human synovial fluid. For instance, 28 differentially secreted metabolites were found within the synovial fluid of individuals with KOA when compared with that from individuals with rheumatoid arthritis (RA)[54], highlighting the potential of synovial fluid metabolite profiles to distinguish OA from RA. Furthermore, in synovial fluid of individuals with knee injuries, metabolite profiles correlated with injury type, sex, or both factors[55], suggesting that clinical parameters and patient characteristics influence the role of synovial fluid during the pathogenesis of post-traumatic KOA and, potentially, of primary KOA. In addition, analysis of the infrapatellar fat pad by single-nucleus RNA sequencing and metabolomics identified obesity-specific differences in transcriptomic and metabolite profiles in fibroblasts from individuals with KOA based on obesity status[56]. However, more comprehensive studies are necessary to identify the contribution of metabolites secreted by all joint tissues to KOA synovial fluid profiles and how cross-tissue signalling via synovial fluid metabolites promotes KOA joint pathologies.

Monitoring disease progression and pain. Progression of KOA has been associated with altered metabolite profiles within synovial fluid in animal models of KOA. For instance, metabolite profiles of canine synovial fluid have been found to change during the progression of KOA, whereby increased levels of mannose and betaine alongside decreased levels of isoleucine were detected in synovial fluid of KL I-graded KOA compared with KL II-graded KOA, and decreased levels of 2-hydroxyisobutyrate were identified in KL II/III compared with KL I-graded OA, whereas increased levels of lactate were detected in synovial fluid of KL III-graded KOA when compared with KL I/II-graded KOA[57]. As a next step, similar studies using human samples should be conducted to identify how metabolites change in synovial fluid over time during KOA.

Metabolite biomarkers in synovial fluid have also been associated with pain in KOA. For example, the metabolite lysophosphatidylcholine (LysoPC) in synovial fluid has been shown to contribute to acute cutaneous pain[58]. Specifically, increased LysoPC16:0 within human KOA synovial fluid was correlated with higher pain, whereas, in mouse models, LysoPC16:0 appeared to increase knee pain responses through the acid-sensing ion channel 3 (ASIC3) on DRG neurons[58]. These data highlight LysoPC16:0 as a metabolite biomarker with the potential to monitor the efficacy of therapeutics targeting pain as well as a target for modifying pain directly.

Although changes in synovial fluid metabolite levels during primary KOA appear to be useful as biomarkers of disease, several factors are likely to influence metabolite levels in synovial fluid, including sex, joint-tissue involvement and, in the case of post-traumatic OA, time post-injury. Thus, differences in synovial fluid metabolites in individuals with KOA are possibly impacted by patient demographics and clinical factors, such as sex, body mass index (BMI), and primary versus secondary KOA, and further studies should be completed to parse out profiles of metabolites that can distinguish similar patient subgroups considering patient characteristics[55,59]. By subgrouping heterogeneous individuals with KOA into endotypes by metabolite profiles, personalized therapeutics can be generated for improved treatment of KOA. Additional studies should also investigate early changes in metabolites within KOA synovial fluid to improve early detection of disease, similar to studies conducted for disease staging, as discussed above. Synovial fluid metabolite ratios might also be useful as biomarkers of disease[51,60], and should be considered in future studies.

Proteins

Within synovial fluid, proteins associated with progression and pathological features of KOA have been widely studied, including cytokines, growth factors, and cartilage degradation products, among others. Dysregulation of synovial fluid proteins appear to be associated with KOA[61]. Conversely, growth factors might be important protective factors and are being investigated as potential treatments for repairing tissue damage[62–64]. Thus, detection of protein biomarkers in synovial fluid has the potential to assist diagnosis of disease, determine pathological stage, follow the trajectory of disease progression, and predict therapeutic potential.

Detection of knee osteoarthritis. Protein biomarkers have long been used for the detection of KOA and associated pathologies. For instance, the levels of the cytokine IL-40 were found to be increased in the synovial fluid of individuals with KOA compared with that from individuals without OA, and IL-40 was expressed in articular cartilage with KOA[65]. Cytokines detected within synovial fluid from individuals with KOA, RA,

or joint injury have also been found to exhibit differential expression profiles that can be used to discriminate joint pathologies, although common features are also shared[66–68]. Similar to cytokines, adipokines produced primarily by adipocytes[69], as well as by other joint cells such as chondrocytes[70], promote inflammation. Local adipokines have potential as biomarkers of KOA-associated inflammation — for example, high levels of the adipokine resistin (RETN) correlated with high levels of C-reactive protein (CRP) in synovial fluid in individuals with KOA[71].

Monitoring disease progression and pain. Protein expression changes over time within the synovial fluid of individuals with KOA and might therefore be useful for monitoring disease progression. For instance, the levels of IL-8 in the synovial fluid of women with KOA-related joint effusion were significantly associated with clinical severity, as well as with synovial fluid levels of tumour necrosis factor (TNF), IL-6, visfatin (NAMPT), and osteopontin (OPN) after corrections for confounding variables, but not resistin[72]. These correlations were not reproduced with levels of IL-8 in the serum[72], indicating that the pro-inflammatory effects of IL-8 are likely localized to the joint. Furthermore, tryptophan 2,3-dioxygenase (TDO2), a primary enzyme associated with the production of the inflammatory mediator kynurenine, was detected in synovial fluid of individuals with KOA and correlated positively with KOA severity and with levels of pro-inflammatory cytokines in synovial fluid[73]. In addition, synovial fluid levels of IL-34 were associated with increasing disease severity and synovitis in individuals with KOA[74]. A set of six secreted proteins in the synovial fluid — soluble vascular cell adhesion molecule-1 (sVCAM-1), matrix metalloproteinase 3 (MMP-3), soluble intercellular adhesion molecule-1 (sICAM-1), tissue inhibitor of metalloproteinase-1 (TIMP-1), vascular endothelial growth factor (VEGF), and monocyte chemoattractant protein-1 (MCP-1) — were also associated with synovial inflammation, radiographic severity, symptom severity, and secreted activation markers of synovial fluid macrophages or neutrophils[75], suggesting that activated immune cells might mediate synovial fluid changes contributing to KOA (Box 1). An additional study identified 786 EV peptides within synovial fluid that correlated with KOA severity[76]. Thus, pro-inflammatory molecules of the synovial fluid, many of which are typically associated with inflammatory arthritis (such as RA), emerge as biomarkers of disease progression in individuals with KOA and might reflect disease-specific mechanisms.

Protein biomarkers within synovial fluid might also be used to monitor KOA symptoms such as pain. For instance, cartilage oligomeric matrix protein (COMP) and procollagen type II C-terminal propeptide (PIICP) have previously been detected within the synovial fluid from individuals with KOA, and the serum and synovial fluid levels of COMP and PIICP were associated with the development and progression of OA, respectively[77,78]. These molecules, as well as other serum proteins or peptides associated with cartilage metabolism, have also been associated with KOA severity and pain[79]. Thus, COMP and PIICP have emerged as potential local and systemic biomarkers of OA pathology and symptom progression.

Although cytokines are most commonly associated with inflammation, they have also been linked to pain responses experienced by individuals with KOA. Receptors for specific cytokines are expressed on nociceptive nerve endings, and thus cytokines stimulate DRG neurons, altering pain responses[80–82]. IL-1β, IL-10, and IL-12 have previously been detected in synovial fluid from individuals with KOA[83]. Moreover, the levels of these cytokines, as well as that of granulocyte–macrophage colony-stimulating factor (GM-CSF) in the serum correlated with improvements in self-reported Western Ontario and McMaster Universities Arthritis Index (WOMAC)[84] scores of pain, stiffness, and functional disability[85]. Adipokines might also have a role in pain responses.

Box 1 | Cellular components of synovial fluid in knee osteoarthritis

Immune cells and other inflammation-promoting cells in the synovial fluid

- Macrophages, T cells and neutrophils are major immune cell populations identified within synovial fluid[195].
- Neutrophils and macrophages primarily contribute to the synovial fluid levels of transforming growth factor-β1 (TGFβ1) and elastase, respectively, and increases in the levels of both proteins have been associated with progression of knee osteoarthritis (KOA)[195].
- In early-stage KOA (as defined in Luyten et al.[196]), T helper 1 (T_H1) $CD4^+$ T cells are present in both the synovium and synovial fluid, where they promote a pro-inflammatory environment coincident with increased levels of IL-6 in synovial fluid[197].
- $CD14^+$ monocytes found in synovial fluid have also been associated with synovial inflammation and the levels of soluble CD14, interleukin-6 (IL-6), complement component 3 (CC3), IL-1β and tumour necrosis factor (TNF) in synovial fluid[198].
- The distribution profiles of T cells, monocytes and macrophages, natural killer cells, and activated $CD8^+$ T cells within synovial fluid were able to distinguish 'activated', 'lymphoid progressive', 'myeloid progressive', and 'aggressive' phenotypes of KOA[199].
- Increased abundance of neutrophils in synovial fluid correlated with increased synovial fluid protein levels of TNF, IL-1RA, matrix metalloproteinase 9 (MMP-9), soluble triggering receptor expressed on myeloid cells 1 (sTREM-1), and visinin-like protein 1 (VILIP-1), as well as with decreased surface expression of CD54, CD64, Toll-like receptor 2 (TLR2), and TLR4 on neutrophils[200].
- Increased synovial fluid monocyte-to-leukocyte ratio was indicative of poor response to intra-articular corticosteroid injections[201].
- Mesenchymal stromal cells originating from the synovium infiltrate synovial fluid during KOA, with potential anti-inflammatory and cartilage reparative effects[202,203].

Injectable cellular therapeutics to attenuate knee osteoarthritis

- Adipose- or bone marrow-derived stromal cells injected into joints of animals and humans with KOA can improve symptoms and joint function[204–206], while modifying the proteome of synovial fluid-derived extracellular vesicles[207] and reducing the inflammatory profile of synovial fluid[208,209] (Fig. 2).
- A phase I/II clinical trial showed that intra-articular injection of adipose-derived stromal cells was safe, reduced pain, and improved joint function[210].
- Combining a suicide gene transcriptionally linked to a cell division locus to prevent cell division[211] with additional modifications masking transplanted cells from the immune system[212] might help to provide long-term cell therapy without rejection or tumour growth (Fig. 2).

For example, the levels of adiponectin (ADIPOQ) in synovial fluid have been associated with clinical severity, including pain, in women with KOA and a non-obese BMI[86]. Furthermore, prokineticin 2 (PROK2) was detected within the synovial fluid of individuals with KOA and has been associated with increased nociceptive sensitization[87,88]. Thus, cytokines and adipokines detected in synovial fluid might influence nociceptive stimulation, resulting in alterations to KOA pain.

Predicting surgical outcomes. TKA remains the final option for the treatment of advanced-stage KOA, although not all patients respond well. Up to 34% of patients undergoing TKA do not show improvements in pain[89]. Studies show that synovial fluid levels of nerve growth factor (NGF) are pre- or intra-operatively lower in individuals with KOA than in individuals with RA or systemic lupus erythematosus, but increase post-operatively in KOA in a positive correlation with postoperative pain, as measured by visual analogue scale scoring[90]. Furthermore, higher pre-operative synovial fluid concentrations of pro-inflammatory cytokines, such as TNF, are associated with less pain reduction 2 years post-TKA[91]. A phase II clinical trial that examined 345 individuals with painful KOA that was completed in 2023 (NCT04675034) demonstrated that subcutaneous injection of MEDI7352, an anti-NGF–TNF antibody, moderately improved pain, as measured by the WOMAC pain scale and the Numeric Rating Scale pain score. These findings suggest that binding of this antibody to NGF and TNF reduced pain; however, no definitive results have been published to date. Utilizing a therapeutic, such as MEDI73352, post-operatively might be useful for individuals with post-operative pain, but investigations on the bioavailability of MEDI73352 in synovial fluid are needed to determine if pain-alleviating effects are locally mediated. Local delivery of MEDI73352 into the synovial fluid might be a strategy worth investigating for further mitigation of pain. Increased post-surgery plasma levels of IL-1β, VEGF, and IL-12–IL-23p40, and decreased synovial fluid levels of IL-10 have also been associated with chronic pain post-TKA, as indicated by a numerical rating score of ≥4 at 6 months post-surgery[83].

Cartilage breakdown fragments might also contribute to pain. The baseline synovial fluid-to-urinary ratio of type 2 collagen C terminal cleavage peptide assay (C2C-HUSA) correlated inversely with the likelihood of reductions in pain 1 year after TKA[92]. Going forward, additional biomarkers associated with chronic pain after TKA should be validated preoperatively to determine usefulness as predictive biomarkers of surgical pain responses. This will aid in joint patient–clinician decision making for optimal treatment to improve KOA-related pain, including considering pre-surgical interventions to potentially modify post-surgical outcomes.

Current studies continue to point to proteins in the joint, particularly inflammatory mediators, as putative biomarkers of KOA. These proteins might also help to define mechanisms mediating disease in individual patients, leading to improved precision medicine approaches[93,94]. As with other molecular features, further studies should focus on identifying associations between proteins secreted locally in synovial fluid and their detection in other biofluids, such as blood and urine, for potential translation of biomarker studies using less invasive methods. Overall, studies suggest that non-coding RNAs, metabolites and secreted proteins, such as cytokines and growth factors, can be detected within synovial fluid, emerging as biomarkers for the detection of KOA, disease progression, and associated pathologies such as pain.

In addition to RNA, metabolite and protein biomarkers, microbial components that are not local to the joint space but have been identified within synovial fluid have been implicated in the onset or progression of KOA. Intra-articular injection of lipopolysaccharide (LPS), an outer membrane component of Gram-negative bacteria that activates the innate immune system, is often used in animal models to induce joint inflammation and synovitis. LPS has been identified within both synovial fluid and serum of individuals with KOA, and LPS levels were positively associated with the presence of activated macrophages and increased total WOMAC score in these patients[95]. Bacterial nucleic acids have also been detected within the synovial fluid of individuals with KOA or RA[96]. Microbial profiles of the synovial fluid show alterations based on KOA status as well as in individuals with KOA who did or did not require revision surgery because of non-infectious or infectious causes[97]. This finding indicated that the presence of a joint microbiome is independent of other organ systems and has the potential to influence KOA. Thus, the microbiome of the synovial fluid of the knee might interact with the microbiome in other locations and influence local joint tissues or KOA pathogenesis. Additional studies are required to further understand the influence of the joint microbiome on the presence and progression of KOA and whether antibacterial therapeutics have the potential to reduce KOA pathologies.

Future studies should focus on identifying biomarkers capable of detecting early stages of KOA, so that interventions can be applied to modify symptoms and slow disease progression. Additionally, combinations of biomarkers potentially consisting of non-coding RNAs, metabolites, secreted proteins, and microbiome-related factors should be consolidated and tested in a clinical setting to improve diagnostics available for the detection of KOA, associated pathologies, and therapeutic outcomes. Integration of biomarker types may improve detection of endotypes of KOA[98], allowing for personalized therapeutics to be developed.

Circulating synovial fluid-derived molecules

The local joint synovial fluid has direct contact with the circulatory system; thus, locally derived molecules might also be detected in circulation (Fig. 1). Furthermore, during KOA, increased joint-tissue angiogenesis allows for greater exchange of mediators between synovial fluid and circulation[17]. Circulating molecules that originate from the synovial fluid of the affected joint might provide both a source of disease biomarkers and a range of potential therapeutic targets.

Synovial fluid-derived biomarkers in circulation

Using circulating molecules as biomarkers for the detection of KOA is advantageous in diagnostics, prognostics, and precision medicine approaches, owing to less invasive sample accessibility and improved cost effectiveness. Circulating biomarkers have the potential to be used as proxies for joint pathological changes, in part because of the connections between joint structures, synovial fluid, and circulation. However, circulating biomarkers might originate from other joints not affected by KOA, or from other tissues in the body. Previous investigations have associated biomarkers, such as some non-coding RNAs and proteins, that are found in both circulation and synovial fluid with KOA pathologies[99].

miR-34a-5p was initially shown to be increased within synovial fluid of individuals with late-stage (KL III/IV) KOA versus individuals with KL I/II-graded KOA[47], but was later reported to also be increased in the plasma, cartilage, and synovium of individuals with late-stage KOA (KL III/IV) compared with individuals without KOA or who had KL 0/I-graded OA[100]. In addition, miR-34a-5p was expressed at even higher levels in the plasma of individuals with late-stage KOA and obesity,

as determined by a high BMI, compared with individuals with late-stage KOA and a low BMI. miR-34a-5p levels were also found to be increased in the plasma, cartilage and synovium of mice fed a high-fat diet for 18 weeks compared with those fed a lean diet[100]. Thus, miR-34a-5p levels within synovial fluid appear to be associated with KOA, whereas circulating levels of miR-34a-5p are likely to be associated with both KOA and obesity. The levels of another miRNA, miR-126-3p, were found to be decreased in synovial fluid EVs from individuals with KOA compared with individuals who had no OA[101], but were increased in the plasma of individuals with severe KOA compared with individuals with no OA[101,102]. Overall, plasma miRNAs might correlate with levels found in synovial fluid either positively or negatively yet still show potential as systemic biomarkers of KOA.

Exploration of circulatory EV contents has also helped to identify putative biomarkers of KOA progression and associated pathologies. For example, in a longitudinal model of equine OA, the first principal component of a collection of plasma EV proteins was associated with time after OA induction[103], suggesting that plasma EV proteins might help to decipher OA progression. In another study comparing horses with or without post-traumatic OA, 658 miRNAs were differentially expressed within plasma EVs, with links to fibrosis and inflammation, among other mechanisms[44]. In a human clinical study, 199 EV peptides in synovial fluid and plasma were associated with inflammatory processes, whereas an increased frequency of plasma EVs expressing the surface markers fibrinogen alpha chain (FGA), fibrinogen beta chain (FGB), fibrinogen gamma chain (FGG), talin-1 (TLN1), and α-1-microglobulin/bikunin precursor (AMBP) was predictive of KOA progression[104]. Together, these studies suggest that analyses of EV cargo might provide useful circulatory biomarker signatures associated with KOA.

Similar to synovial fluid biomarkers, the aims of using circulatory proteins or peptides as biomarkers for KOA include both early diagnosis and personalized treatment. For instance, a profile of 82 differentially expressed proteins was identified in plasma from individuals with KOA compared with healthy individuals, with up to 25 of these proteins also correlating with worse performance-based joint function or questionnaire scores quantifying pain, functional disability, and reduced quality of life[105]. However, the origin of these proteins was not explicitly investigated, and further studies should investigate whether these proteins can also be found within the synovial fluid. Several panels of circulating proteins are predictive of overall radiographic KOA progression[106–108], bone-marrow lesions[109], or increased pain[108], with some proteins of these panels also detected in synovial fluid, including hyaluronan-binding protein 2 (HABP2), histidine-rich glycoprotein (HRG), protein Z-related protease inhibitor (ZPI), platelet factor 4 (PLF4)[106], and cartilage acidic protein 1 (CRAC1)[110]. With respect to adipokines, a recent study identified higher resistin levels in the serum of individuals with KOA compared with individuals without KOA, and resistin levels correlated strongly with circulating levels of TNF[71]. Furthermore, IL-34 in plasma was associated with synovitis and increasing KOA severity, consistent with findings on synovial fluid IL-34 levels[74]. As IL-34 levels in plasma and synovial fluid[74] were found to correlate linearly, IL-34 plasma levels appear to be directly related to local joint expression and associated pathological processes. Overall, circulatory joint-tissue degradation products, adipokines, and cytokines seem to mirror protein alterations of the joint synovial fluid and could be used as proxies for what may be modified in the local KOA joint environment to help diagnose KOA and predict KOA outcomes, as well as to understand KOA pathogenesis better.

Predictive biomarkers in circulation

Circulatory biomarkers used for the detection of KOA might also function as molecular mediators involved in altering KOA pathologies and might therefore be promising for evaluating therapeutic efficacy. Collagen degradation is a key marker of extracellular matrix (ECM) and cartilage deterioration associated with KOA, wherein an increase in collagen degradation products is indicative of cartilage degeneration. Type II collagen neo-epitope (T2CM) was found in synovial fluid of dogs with induced OA[111], reflecting cartilage degeneration. The levels of T2CM were also found to be increased in the serum of individuals with late-stage KOA (KL IV), compared with individuals with KL II-graded KOA[112], thus emerging as a circulating biomarker to track disease progression and potentially therapeutic efficacy. A phase II clinical trial with 549 participants investigated the effect of intra-articular injections of recombinant fibroblast growth factor-18 (rFGF-18; also known as sprifermin) on type II collagen formation by measuring type 2 collagen propeptide (PRO-C2) in serum and synovial fluid, as increased levels of PRO-C2 are suggestive of increased collagen and therefore cartilage formation[113]. This study found that individuals receiving intra-articular injections of rFGF-18 had increased levels of PIIBNP in the synovial fluid and increased articular cartilage thickness, although this treatment was most effective for individuals who had a low baseline serum PRO-C2 level[113]. FGF-18 is of major interest moving forward as a means of disease modification, but as pain modification remains the primary motivation for OA patients, investigating combination therapies of rFGF-18 with long-term pain-mitigating therapies will be crucial to potentially limit disease progression and pain simultaneously.

The adipokine neutrophil gelatinase–associated lipocalin (NGAL), a circulating protein used as a biomarker for inflammation, has been identified within the synovial fluid of individuals with KOA where it forms a complex with MMP-9, enhancing cartilage degeneration[114]. Although obesity, as defined by a BMI of >30 kg/m^2, is a well-characterized risk factor for KOA incidence and progression[115], caloric restriction has been shown to increase serum levels of NGAL[116]. By contrast, a phase IV clinical trial of 168 participants with obesity and KOA identified that administration of the glucagon-like peptide 1 (GLP-1) receptor agonist liraglutide promoted weight loss in participants but did not significantly alter serum NGAL expression. This study suggests that GLP-1 receptor agonists might be beneficial for weight loss in individuals with obesity and KOA without posing a threat to worsening KOA pathologies, such as cartilage degeneration and inflammation. Additionally, although this study suggests that GLP-1 receptor agonists might not modify NGAL systemically, future studies should also determine whether NGAL is reduced or unaffected in synovial fluid to determine potential local effects of GLP-1 therapy. Although GLP-1 agonists have been reported to potentially modify pain and disease activity in KOA[117–121], additional preclinical and clinical studies should be conducted to identify the potential relationships of GLP-1 receptor agonists and joint pathologies associated with KOA.

Overall, circulating non-coding transcripts and proteins derived from the synovial fluid are likely to reflect the pathological changes that occur within the osteoarthritic knee joint. Further validation studies should be conducted to determine if any of the discussed molecules in circulation can be used as biomarkers of KOA. Focusing on molecules with known positive correlations between plasma and synovial fluid levels and associations with disease pathologies or symptoms is of major interest as these molecules might be important predictors of therapeutic efficacy, act as targets for therapeutic intervention,

Table 2 | Potential molecular mediators of knee osteoarthritis pathology in synovial fluid (reported between 2021 and 2024)

Molecular mediator	Associated fluid/tissue	Pathogenetic process	Refs.
Non-coding RNAs			
miRNA-34a-5p	Synovial fluid, plasma, articular cartilage	Cartilage degeneration	100
miR-126-3p	Synovial fluid and plasma	Cartilage protection from degeneration	101,102
let-7b-5p and let-7c-5p	Synovial fluid	Cartilage degeneration	145
miRNA-21	Synovial fluid and synovium	Knee joint pain	173
miR-30b-5p	Synovial fluid	Cartilage degeneration, inflammation and joint pain	174
Proteins or peptides			
IL-40	Synovial fluid	Cartilage degeneration and inflammation	65
TDO2	Synovial fluid	Inflammation	73
IGF2, AHSG, FN1, CFB, KNG and C8	Synovial fluid, cartilage, synovium and infrapatellar fat pad	Communication between joint tissues through synovial fluid	123
FN1, SDC4, ITGA5	Synovial fluid and synovium	Communication between synovium and articular cartilage	124
TNC and NT5E	Synovial fluid and articular cartilage	Communication between synovium and articular cartilage through synovial fluid	124
IL-6	Synovial fluid, articular cartilage and synovium	Cartilage degeneration, knee joint pain and inflammation	129–133
MMP-2 and RANKL	Synovial fluid and articular cartilage	Cartilage degeneration	137
SPARC	Synovial fluid, plasma and subchondral bone	Cartilage degeneration	140,141
APOE	Synovial fluid, synovium and infrapatellar fat pad	Cartilage degeneration	142
IL-1β	Synovial fluid, infrapatellar fat pad	Cartilage degeneration	143,144,172
TNF	Synovial fluid	Inflammation and cartilage degeneration	143,144,167,170
Profile of 121 upregulated proteins	Synovial fluid and secretomes of articular cartilage, infrapatellar fat pad, synovium and meniscus	Inflammation	161
MIF	Synovial fluid and articular cartilage	KOA pathogenesis and inflammation	162,163
CD74	Articular cartilage	KOA pathogenesis and inflammation	162,163
IL-17A	Synovial fluid and serum	Joint pain, angiogenesis and inflammation	166,168–170
IL-17	Synovial fluid	Inflammation	171

AHSG, α-2-HS-glycoprotein; APOE, apolipoprotein E; CFB, complement factor B; C8, complement 8; FN1, fibronectin-1; IGF2, insulin-like growth factor 2; ITGA5, integrin α5; KNG, kininogen 1; KOA, knee osteoarthritis; let-7, lethal-7 microRNA; MIF, macrophage migration inhibitory factor; MMP-2, matrix metalloproteinase 2; NT5E, 5′-nucleotidase ecto; RANKL, receptor activator of nuclear factor-κB ligand; SDC4, syndecan-4; SPARC, secreted protein acidic and rich in cysteine; TDO2, tryptophan 2,3-dioxygenase; TNC, tenascin C; TNF, tumour necrosis factor.

or help to stratify patients for precision-medicine approaches based on molecular pathophysiology, so-called 'theratypes'[122].

Synovial fluid molecular mediators of knee osteoarthritis

As synovial fluid bathes all tissues of the joint (Fig. 1), it not only promotes cross-tissue communication but also carries molecular mediators that have the potential to promote KOA pathology. Profiling of proteins secreted from cartilage, synovium, the infrapatellar fat pad, and the meniscus into the synovial fluid has identified insulin-like growth factor 2 (IGF2), α-2-HS-glycoprotein (AHSG), fibronectin-1 (FN1), complement factor B (CFB), kininogen (KNG), and complement 8 (C8) as being increased in KOA[123]. The secretion of joint-tissue-specific ligands within synovial fluid is often induced by inflammatory stimulation: for example, inflammatory signals induce the expression of FN1 by synovial cells and tenascin C (TNC) by chondrocytes, and these in turn produce signals to modify the expression of the receptors for these ligands, namely syndecan-4 (SDC4) and integrin α5 (ITGA5), on chondrocytes and fibroblasts, respectively[124]. Below, we discuss the contribution of specific synovial fluid molecules to OA pathology, specifically cartilage degeneration and inflammation (Table 2).

Cartilage degeneration

Cartilage degeneration leads to permanent loss of chondrocytes and tissue architecture; thus, tissue regeneration in KOA is difficult to achieve[125]. Cartilage fragments and other molecular mediators released into synovial fluid contribute to synovial inflammation[3], subchondral bone remodelling, and osteophyte formation[126,127].

Multiple tissues secrete molecular mediators into synovial fluid that impact cartilage catabolism. For instance, in addition to their potential roles as biomarkers in synovial fluid and plasma, miRNA-34a-5p and miRNA-126-3p have cartilage-destructive and cartilage-protective effects, respectively[100–102]. The cytokine IL-6 is also found within synovial fluid, with synovial fluid IL-6 levels being increased in individuals with KOA compared with healthy individuals[128]. IL-6 is secreted by distinct joint tissues, including

articular cartilage, synovium and IL-6-secreting T cells within the infrapatellar fat pad[129,130], with studies showing associations with cartilage degeneration and pain[131–133]. IL-40, which, as mentioned previously, is also increased in KOA synovial fluid and within KOA cartilage, increases the secretion of IL-6, as well as IL-8, MMP-1, MMP-3, and MMP-13 (ref. 65). Thus, joint-tissue-derived IL-6 or its upstream regulators emerge as potential synovial fluid targets for reducing cartilage degeneration in KOA. Biological inhibitors of IL-6, such as tocilizumab, have shown some promise in the treatment of autoimmune diseases[134] (Fig. 2). In vitro, a combination treatment of both tocilizumab and celecoxib, a cytochrome *c* oxidase subunit 2 (COX2) selective inhibitor, on a hydrogen peroxide-induced human OA-like chondrocyte-cell model promoted anti-inflammatory effects, maintained chondrocyte viability and prevented the progression of cartilage damage[135]. A phase III study of 104 study participants with refractory hand OA tested the efficacy of systemic infusions of tocilizumab on pain control and joint function but reported little difference in pain relief between tocilizumab and placebo treatments[136]. Future studies should investigate local delivery of IL-6-targeting biologics such as tocilizumab or siltuximab, or of IL-6 upstream regulators within synovial fluid, as a monotherapy or in combination with other potential therapeutics, to determine if this is more efficient in attenuating associated cartilage degeneration than systemic IL-6-targeting therapies.

Within the joint, subchondral bone provides structural and nutritional support to the neighbouring articular cartilage[22]. In KOA, subchondral bone contributes to cartilage degeneration through vascular invasion and, indirectly, through the secretion of molecules such as MMP-2 or receptor activator of nuclear factor-κB ligand (RANKL), into synovial fluid[137]. Interestingly, a preclinical trial in horses with intercarpal or intertarsal OA determined an association between intra-articular injections of an anti-TNF antibody and a reduction in MMP-2 activity or other pro-inflammatory molecules within synovial fluid that are also involved in cartilage catabolism, as well as reduced pain[138]. This study demonstrates the applicability of anti-inflammatory treatments to influence the effects of molecules secreted by subchondral bone on cartilage degeneration and pain. However, in clinical trials, although intra-articular injection of the anti-TNF antibody infliximab was found to be most associated with a reduction in KOA-related pain compared with other anti-TNF treatments and placebo, it conferred no significant improvement in joint function and stiffness[139], suggesting that in human KOA, anti-TNF therapeutics are not efficient at improving overall joint function. Further studies should investigate the development of multispecific antibodies that inhibit TNF alongside additional molecules that have a role in cartilage degeneration to potentially provide a more holistic KOA treatment. Cartilage degeneration in female rats with ovariectomy-induced menopause and KOA was promoted through

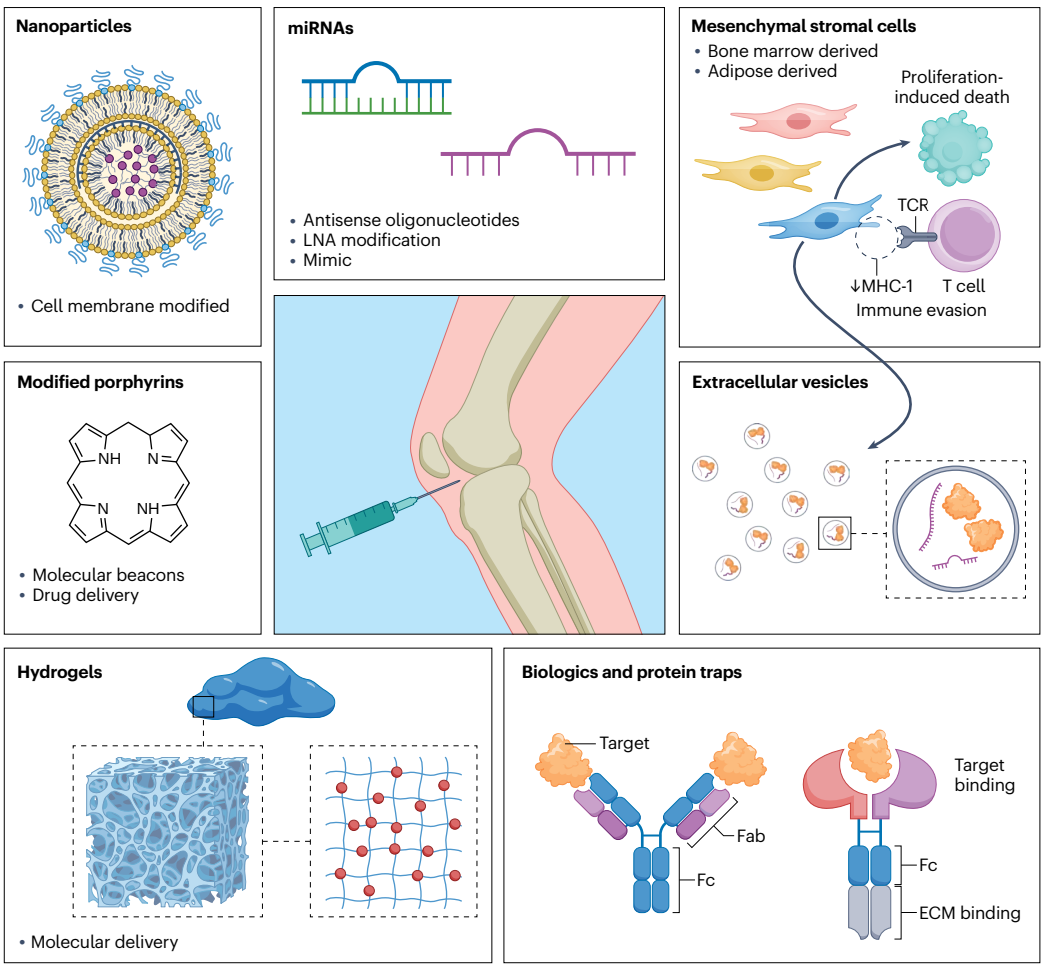

Fig. 2 | Intra-articular injection of therapeutics to mitigate knee osteoarthritis. Currently, intra-articular injection of molecular mediators is the main focus of study for treatment for knee osteoarthritis (KOA) disease attenuation. The literature highlights the use of many therapeutic approaches to intra-articular delivery, such as nanoparticles, mesenchymal stromal cells, modified porphyrins, microRNAs (miRNAs), extracellular vesicles, hydrogels, biologics and protein traps to aid in relieving disease pathologies to improve overall joint function and experienced pain. ECM, extracellular matrix; LNA, locked nucleic acid; MHC-I, major histocompatibility complex I; TCR, T cell receptor. Fc and Fab denote antibody fragments.

secreted protein acidic and rich in Cysteine (SPARC, also known as osteonectin), which was secreted by subchondral osteoblasts and downregulated AMPK–FOXO3a signalling in chondrocytes[140]. As SPARC has been identified in the synovial fluid from individuals with KOA[141], it might be a promising candidate for targeting in combination with anti-TNF treatments. With the paucity of research on subchondral bone contributions to the molecular constituents of KOA-synovial fluid, additional studies will be vital to uncover how subchondral bone influences the repertoire of synovial fluid factors that contribute to KOA.

During KOA, both the synovium and the infrapatellar fat pad become inflamed and fibrotic, and secrete molecular mediators into synovial fluid that might contribute to cartilage degeneration or pain. Single-nucleus RNA sequencing of synovium and infrapatellar fat pad tissues from individuals with or without KOA demonstrated that apolipoprotein E (APOE) was expressed and secreted into KOA synovial fluid by fibroblasts and macrophages located in these structures[142]. Synovial fluid APOE derived from synovium and infrapatellar fat pad tissue was suggested to contribute to accelerated cartilage degeneration, thus emerging as another relevant target for reducing articular cartilage degradation. IL-1β and TNF are additional molecules that are secreted into the synovial fluid from the infrapatellar fat pad, and their levels are increased in the synovial fluid from individuals with KOA compared with that from healthy individuals[143]. IL-1β and TNF secretion from KOA infrapatellar fat pad might promote cartilage degeneration by modifying p38 MAPK signalling[144]. EVs secreted specifically from KOA infrapatellar fat pad that contain miRNAs let-7b-5p and let-7c-5p were also identified in KOA synovial fluid and found to contribute to cartilage degeneration[145]. Additional studies are needed focussing on cross-communication between the synovium, the infrapatellar fat pad and articular cartilage via the synovial fluid to further parse out how the synovium and the infrapatellar fat pad influence cartilage degeneration, potentially determining therapeutic targets originating from both tissues to reduce this pathology.

Currently, there are no effective therapeutic strategies to 'cure' KOA pathological features, and most treatments focus primarily on alleviating pain. However, intra-articular injections of therapeutics have recently been explored to attenuate disease pathology, providing a promising future for KOA treatments (Fig. 2). For example, aggrecanases (ADAMTS) can be found within synovial fluid and have been previously shown to play a role in proteoglycan aggrecan degradation within articular cartilage[146]. Approaches to reduce the expression of ADAMTS include CYT-108, a recombinant protease inhibitor constructed from an α-2-macroglobulin (A2M) variant with affinity for ADAMTS proteins. Intra-articular injections of recombinant A2M in rats with anterior cruciate ligament transection-induced KOA showed greater attenuation of cartilage degeneration than wild type A2M[147]. As a result, intra-articular injections of CYT-108 are currently being investigated in a phase I clinical study (NCT06263270) to evaluate its preliminary efficacy in slowing the progression of KOA, inhibiting cartilage degeneration, reducing pain, and improving mobility.

Manipulating synovial fluid expression of miRNAs using intra-articular injection therapies might also be a promising therapeutic strategy to attenuate cartilage degeneration associated with KOA. Previously, it was found that KOA cartilage had reduced miR-1 expression, whereas overexpression of miR-1 in mouse joints was protective against cartilage degeneration[148]. Expanding upon these results, an injectable miR-1 agomir mimicking endogenous miR-1 reduced osteophyte formation and cartilage degeneration in rats with induced KOA[149]. Furthermore, miRNA modification by locked nucleic acid (LNA)-modified antisense oligonucleotide (ASO) technology can enhance the stability of miRNA blockers, and, in the case of miR-181a-5p LNA-ASO, reduced cartilage degeneration and the expression of markers reflecting cartilage catabolism, inflammation, hypertrophy, apoptotic/cell death, and type II collagen breakdown in animal models of OA[150,151]. Intra-articular injection of miR-34a-5p LNA-ASO in mice with KOA protected cartilage from degradation. Previously, miR-140-5p was shown to alter ECM homeostasis and cell senescence, and attenuate KOA pathogenesis in animal models[152,153]. Subsequently, isolated EVs from human urine-derived stem cells overexpressing miR-140-5p that were intra-articularly injected into preclinical KOA models attenuated cartilage degeneration and subchondral bone remodelling[154]. In rats, intra-articular injection of exosomes from synovial fibroblasts overexpressing miR-126-3p also attenuated surgically induced KOA[102]. Together, these results indicate that intra-articular injection of miRNAs or miRNA-overexpressing EVs are promising therapeutic modalities for attenuating KOA. Going forward, studies should apply the technologies discussed above to manipulate the expression of other miRNAs to alleviate other pathological features of KOA, including fibrosis and inflammation in addition to cartilage degeneration. As there is a lack of clinical translatability in current preclinical studies targeting miRNAs to reduce cartilage degeneration, further studies should identify the applicability and efficacy of altering miRNA expression within human KOA synovial fluid to reduce cartilage degeneration through injection of either miRNA mimics and antagomirs, or encapsulated vesicles, such as EVs.

In summary, the above-discussed studies indicate that distinct tissues within the knee joint secrete molecular mediators into synovial fluid that affect cartilage degeneration. Further development of strategies to target molecular mediators within synovial fluid contributed by distinct joint tissues should be conducted to help to attenuate disease progression.

Inflammation

Chronic joint inflammation impacts multiple distinct joint tissues in KOA, both increasing joint swelling and stiffness and contributing to cartilage degradation and fibrosis[155,156]. Joint inflammation might also contribute to KOA pain, although there is some controversy surrounding this associaton[157]. The levels of pro-inflammatory cytokines within synovial fluid have been associated with clinical and radiographic severity of KOA[158,159]. Although the synovium has historically been considered the primary source of these cytokines[3,160], inflammatory mediators are secreted into synovial fluid by many joint tissues. For example, IL-40, whose levels are increased in synovial fluid during KOA, as mentioned previously, promotes the expression of inflammatory molecules and degradative enzymes in chondrocytes[65]. Synovial fluid or cartilage, infrapatellar fat pad, synovium, and meniscus secretomes associated with KOA, as characterized by protein analysis in samples from individuals with KOA, were found to enhance pro-inflammatory signalling in a chondrocyte-like cell line[161], indicating that cartilage, while responding to inflammatory signals, also contributes pro-inflammatory molecules to synovial fluid. Interestingly, a pro-inflammatory chondrocyte population defined by snRNA-seq and spatial sequencing was found to activate macrophage migration inhibitory factor (MIF)–CD74 signalling in the joint[158]. This finding is consistent with previously reported increases in levels of MIF in KOA-associated synovial fluid and chondrocytes, as well as with the protective effects of MIF deletion in KOA pathogenesis[162,163]. Thus, cartilage-derived pro-inflammatory mediators of the synovial fluid might, in part, contribute to positive feedback reinforcing joint pathologies.

Although many KOA joint tissues support inflammation, the synovium and the infrapatellar fat pad are considered hubs for the contribution of inflammatory factors to synovial fluid[3,164,165]. For instance,

the synovium contributes to increased TDO2 levels in synovial fluid in KOA[73]. The pro-inflammatory cytokines IL-17A and TNF found in the synovial fluid contribute to inflammation and disease severity[166,167], and seem to be derived, at least in part, from the synovium. Increased IL-17A levels in both the synovial fluid and serum in KOA were associated with structural damage, decreased quality of life and increased pain[168], although the relative contributions of local and systemic IL-17A signalling to increased inflammation and pain in individuals with KOA remain to be fully elucidated. Notably, increased IL-17A has also been associated with increased expression of genes in chondrocytes and the synovium linked to angiogenesis, immune, and complement pathways[169]. IL-17A and TNF induce expression of E74-like ETS transcription factor 3 (ELF3) in synovial fibroblasts, and the downstream upregulation of pro-inflammatory genes[170]. Genes associated with IL-17 signalling were found to be among the most significantly upregulated genes related to inflammatory processes within a transcriptomic signature of the synovium in KOA[171]. The synovium might also contribute molecules with an anti-inflammatory activity, such as clusterin, which is detected in synovium, plasma, and synovial fluid of individuals with KOA, and decreases IL-1β-induced gene expression[172].

Molecules present within synovial fluid that are associated with KOA inflammation, including miRNAs, might also modify pain intensity related to KOA. For example, miR-21, the most upregulated miRNA in the synovium and detected in synovial fluid in a mouse model of anterior cruciate ligament transection-induced KOA, seems to regulate pain intensity through interacting with Toll-like receptor 7 (TLR7), a key player in the innate immune system[173]. Injection of an miR-21 inhibitor in rats with OA was associated with reduced pain, whereas injecting naive rats with miR-21 was associated with increased joint pain[173]. In addition, miR-30b-5p expression was increased in KOA synovial fluid and associated with enhanced joint pain, while also promoting chondrocyte apoptosis and inflammation[174]. These data further demonstrate that miRNAs found within KOA synovial fluid might contribute to joint pain directly. Additional research should parse out the mechanism of action of specific miRNAs within synovial fluid that function to modify tissue-innervating DRG neurons, influencing the painful sensory information transmitted to the brain.

To attenuate inflammation associated with KOA, molecular mediators and cell types that promote disease pathology can be targeted (Fig. 2). One strategy to modify pathological constituents of the synovial fluid locally without unwanted systemic effects is to intra-articularly inject protein traps. As an example, 'sticky traps' function like traditional biologics, but limit molecular mediator function through both binding ligands and trapping them in the local ECM. This method has previously been shown to be effective in trapping VEGF to reduce aberrant angiogenesis within the eye[175], a molecular pathology also associated with KOA[17]. In the context of KOA, intra-articular delivery of mesenchymal stromal cells expressing an IL-1β sticky-trap into knee joints of mice with surgically induced KOA reduced cartilage degradation[176]. In addition, opsonized nanoparticles loaded with anti-inflammatory agents can 'trap' pro-inflammatory M1 macrophages to promote an M1 to M2 macrophage polarization, reducing inflammation and promoting cartilage repair[177]. Overall, therapeutic traps should be further explored to modify the activity of KOA mediators within synovial fluid for disease attenuation through validation in additional preclinical models for eventual translation to human clinical trials.

In summary, joint tissues contribute distinct molecular mediators to synovial fluid that promote KOA-associated inflammation. Additional studies should focus on molecular mediators secreted into synovial fluid that are associated with other pathological features such as tissue fibrosis and subchondral bone remodelling, as well as pro-inflammatory mediators of OA in synovial fluid of the shoulder, hip, and temporomandibular joints (Box 2). Further investigations should also focus on molecular mediators with shared expression across all joint tissues and in synovial fluid to support therapeutic strategies around a 'whole-of-joint' target.

Additional strategies targeting synovial fluid

Harnessing molecules with dual disease detection and therapeutic potential is an additional avenue for pursuit when developing therapeutics (Fig. 2). For example, porphyrin molecules can be used to both track disease activity and deliver disease-modifying drugs. A porphyrin-based molecular sensor in which fluorescence is activated by MMP-13, the primary type II collagen catabolic protease upregulated in KOA, was shown to track disease activity in vitro using cultures of MMP-13-expressing synoviocytes and in a mouse model of KOA[178]. Porphyrin-based molecules should be further explored for targeted or photodynamic therapy in KOA .

One challenge when delivering therapeutics intra-articularly is their rapid clearance rate from synovial fluid. For instance, the neuropeptide substance P is associated with joint pain[179], but has been shown to reduce chronic pain when injected into the spine; however, intrathecal catheter-administered substance P is cleared from the cerebrospinal fluid relatively quickly (within 4 h)[180]. Self-assembled peptide (SAP) hydrogels containing substance P were shown to have improved knee joint retention time (6 weeks)[181]. SAP substance P hydrogels have been tested in rabbits and guinea pigs, as well as in human synoviocyte–synovial fluid co-cultures, and have been reported to reduce cartilage degeneration and inflammation-related markers, respectively[182]. Nanoparticles might also help to increase retention time and modify synovial fluid constituents. Chondrocyte membrane-coated nanoparticles had a lower clearance rate than those without the membrane using in vitro synovial fluid clearance simulation assays[183]. Additionally, drug-filled coated nanoparticles intra-articularly injected into rat and canine models of KOA attenuated periarticular bone remodelling, protected against cartilage degeneration, and restored gait[183]. Thus, hydrogels and nanoparticles might be useful as therapeutic carriers to increase drug retention time and enhance long-term efficacy. Both therapeutic technologies should now be investigated in larger animal models to determine their efficacy in relieving pain, inflammation, and cartilage degeneration for further clinical translation.

Above, we have highlighted how some molecules and EVs released from joint tissues into synovial fluid are detected in the circulation. Although not within the focus of the current review, these joint-derived factors might act beyond being passive biomarkers of joint disease, and mediate organism-wide communication or modification of distant tissues by the OA joint. There has been increasing interest in crosstalk between musculoskeletal tissues and other organs such as the brain and heart[184,185]. Large longitudinal human cohort studies have identified the prospective risk of OA and comorbid conditions such as cardiovascular disease, neurodegenerative diseases (Parkinson's and dementia), and chronic kidney disease, among others, which suggests potential bi-directional interaction or shared mechanisms of disease[186]. A single-cell transcriptomic study demonstrated that distal bone marrow immune cells are substantially modified in individuals with OA, implicating a remote organ impact of OA[187]. Furthermore, a recent preclinical study demonstrated renal pathology in mice following DMM-induced KOA but not sham surgery, directly implicating OA in

Box 2 | Synovial fluid beyond knee osteoarthritis

Hip osteoarthritis

- The hip can be impacted by osteoarthritis (OA), and several molecules in the hip synovial fluid are potential biomarkers and molecular mediators of OA pathology.
- Increased concentrations of the chemokine C-X-C motif chemokine ligand 8 (CXCL8), matrix metalloproteinase 9 (MMP-9), and vascular endothelial growth factor (VEGF) in synovial fluid of individuals with hip OA were indicative of hypertrophic bone morphology associated with hip OA, indicating a potential contribution to osteophyte formation[213].
- The metabolite profile of hip OA synovial fluid was altered based on body mass index (BMI), and levels of 1,3-dimethylurate, *N*-nitrosodimethylamine, succinate, tyrosine, pyruvate, glucose, glycine, and lactate were increased in the hip synovial fluid of individuals with hip OA and obesity compared with individuals with hip OA and a normal BMI[214].

Hand osteoarthritis

- Owing to the low volume of synovial fluid within hand synovial joints, most biomarkers used in the detection of hand OA are systemic.
- Longitudinally, higher concentrations of serum hyaluronic acid were associated with an increased incidence of hand OA and with multiple joint involvement[215].
- The levels of circulating interleukin-7 (IL-7) correlate with the severity of hand OA[216]. IL-7 has also been associated with the incidence of erosive hand OA[217].

Shoulder osteoarthritis

- Individuals with shoulder OA have altered fatty acid profiles in the shoulder synovial fluid, with increased proportions of 18:1n-7 compared with non-OA trauma synovial fluid[218].
- In a rat model of OA, IL-21 levels were increased in the shoulder synovial fluid compared with synovial fluid from healthy rats, and correlated with decreased levels of miR-361-5p in synovial fluid and shoulder OA pathogenesis[219].

Temporomandibular joint osteoarthritis

- The damage-associated molecular pattern molecule high mobility group (HMGB1) is increased in temporomandibular joint (TMJ) OA synovial fluid compared with synovial fluid from individuals with TMJ internal derangement, disc displacement without reduction, or disc displacement with reduction. Toll-like receptor 4 (TLR4), IL-1β, IL-18, prostaglandin E_2 (PGE_2), inducible nitric oxide synthase (iNOS), MMP-1, MMP-8, MMP-13, IL-6, and IL-23 are also increased in the synovial fluid of individuals with TMJ OA[220,221].
- Increased levels of tumour necrosis factor (TNF) and an increased ratio of receptor activator of nuclear factor-κB ligand (RANKL) to its receptor OPG in synovial fluid from individuals with TMJ-OA were associated with increased TMJ pain and subchondral bone degeneration[221].

causing distal organ disease[188]. Future studies should further explore the systemic impact of KOA and identify the joint-derived factors responsible that are also found within synovial fluid, which can thus act as both biomarkers of comorbid disease risk and potential therapeutic targets.

Future directions

Ongoing research into the synovial fluid of individuals with KOA has uncovered new biomarkers and molecular mediators of pathology, both within synovial fluid and in the circulation. However, there is a lack of research focussing on non-coding RNAs, including lncRNAs and circRNAs, that might contribute to KOA biomarker profiles. Expanding our understanding of the roles of growth factors within synovial fluid will be important to fully establish the contributions of proteins within synovial fluid to KOA. Comprehensive investigations directed at uncovering the molecular mechanisms by which biomarkers and molecular mediators impact KOA pathogenesis must be completed to properly understand how these molecules contribute to KOA and, in turn, allow for more targeted therapeutics to be developed.

Omics analyses, such as next-generation sequencing, enable high-resolution analysis of tissues within the joint space, identifying specific cell types, subsets and transcriptomic profiles that influence KOA pathologies[56,142,189–194]. However, most sequencing studies focus on characterizing joint tissues rather than connecting specific cell populations to molecular mediators of the synovial fluid. Sequencing several joint tissues in parallel while evaluating components of patient-matched synovial fluid will be required to identify associations between tissue cells and synovial fluid constituents. Exploring both joint tissues and synovial fluid might provide an insight into potential therapeutic targets that might aid in attenuating disease pathologies across the entire knee joint, without focussing only on a single region or structure. This approach might also reveal additional systemically detected molecules associated with KOA that better represent local joint pathological changes. The benefits of high-resolution omics technologies and bioinformatics must be taken advantage of to identify specific molecular mediators and associated pathways that can be therapeutically targeted in synovial fluid to attenuate KOA. In addition, the field is also expected to benefit from the development of methods that improve retention of the therapeutics delivered while also providing delivery of therapeutics that may include cells, biologics and complex molecules.

We recommend that studies investigating therapeutic strategies for KOA view joint disease holistically and consider diverse pathological features including pain, synovitis, cartilage degeneration, subchondral bone sclerosis, and inflammation, among others. As synovial fluid bathes all joint tissues, a specific focus should be placed on identifying therapeutic methods targeting synovial fluid molecules that interact with a variety of tissues to relieve multiple KOA pathologies.

Conclusions

The rapid expansion of studies focused on knee joint synovial fluid has provided profiles of molecules that can be harnessed as biomarkers for KOA disease and potential therapeutic targets to attenuate KOA symptoms and pathologies. Although these discoveries are promising, further innovation is necessary to effectively modify the synovial fluid landscape by creating therapeutic modalities that alleviate KOA pathogenesis.

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Review article

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Insights into chondrocyte populations in cartilaginous tissues at the single-cell level

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Abstract

Chondrocyte biology is being revolutionized by single-cell multi-omics technologies, revealing cellular heterogeneity within cartilaginous tissues. Although past research has implicated cellular heterogeneity in chondrocyte populations, advances over the past decade in single-cell transcriptomics now enable a more granular, functionally annotated classification of chondrocyte subtypes. These analyses provide crucial insights into the role of these subtypes in cartilage formation, maintenance and disease progression. Chondrocyte populations are implicated in tissue homeostasis, pathogenesis and responses to external stimuli, including pro-inflammatory mediators and novel therapeutic agents. This knowledge opens pathways for developing targeted treatments for diseases such as osteoarthritis and intervertebral disc disease. Insights into the molecular signatures of disease-critical chondrocyte populations provide a foundation for biomarker discovery and therapeutic targeting, and there are exciting opportunities for leveraging these findings to progress regenerative therapies. Spatial and temporal profiling of cellular markers, behaviour and metabolic activity will enhance understanding of disease pathogenesis and chondrosenescence and could possibly enable early intervention for osteoarthritis, thereby preventing irreversible joint damage. Future research must integrate advanced single-cell techniques with computational modelling to unravel the dynamic interplay of chondrocyte populations. These efforts could transform precision medicine in rheumatology, addressing the unmet clinical needs in cartilage-related diseases.

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Sections

Key points

- Musculoskeletal disorders, particularly osteoarthritis and intervertebral disc disease, remain therapeutic challenges owing to a focus on symptom management rather than mechanistic targeting.
- Single-cell RNA sequencing analysis has identified distinct chondrocyte subpopulations in healthy and diseased tissues, overturning the paradigm of chondrocyte homogeneity.
- Cellular diversity mapping through single-cell transcriptomics enables molecular stratification of cartilage degeneration, which forms the basis for disease-modifying therapies.
- Integrating spatial transcriptomics and subcellular proteomics will reveal microenvironment-specific chondrocyte behaviours that are critical for the maintenance of tissue homeostasis, which could better explain how chondrocyte subtypes contribute to tissue homeostasis.
- Machine learning-driven analysis of multi-omics data accelerates the discovery of network-level therapeutic targets for personalized treatment strategies.

Introduction

The increasing prevalence of musculoskeletal disorders, particularly osteoarthritis (OA) and intervertebral disc (IVD) degeneration (IVDD), represents a considerable public health challenge globally[1]. These conditions not only lead to chronic pain and disability but also impose a substantial economic burden on healthcare systems[2]. Current treatment options focus on symptomatic relief rather than addressing the underlying pathophysiological mechanisms. As the population ages, the incidence of these musculoskeletal disorders is expected to rise, necessitating a deeper understanding of their underlying mechanisms to develop effective therapeutic strategies[3].

Chondrocytes, the most abundant and functionally important cell type in cartilage, are essential for skeletal development and musculoskeletal function[4–6]. Chondrocyte phenotypes in developmental cartilage disorders (such as chondrodysplasia) and cartilage following traumatic joint injuries have been reviewed elsewhere[7,8]; however, their specific roles in developmental cartilage disorders remain poorly understood. This Review focuses on how single-cell technologies are unravelling chondrocyte heterogeneity in prevalent degenerative disorders such as OA and IVDD, with implications for biomarker discovery and targeted therapies. Chondrocyte phenotype is determined and maintained by the local physio-chemical microenvironment provided by the cartilage-specific extracellular matrix (ECM)[9]. Previous conceptions of cartilage structure portrayed chondrocytes as nearly uniformly distributed within the ECM, with limited appreciation for their spatial organization or functional diversity. Over the past two decades, imaging-based studies have revealed heterogeneity in cell morphology and distribution, including fibroblast-like chondrocytes with cytoplasmic processes, particularly in non-degenerate cartilage[10–12]. However, advances over the past 5–10 years in multi-omics approaches, which integrate single-cell RNA sequencing (scRNA-seq) and proteomics, have transformed the understanding of the complexity of cellular heterogeneity in cartilage and IVD[13]. Studies using these approaches have uncovered previously unrecognized chondrocyte populations that are associated with both healthy and diseased tissue, providing insights into their specific roles in cartilage formation and maintenance[14]. Defining the cell populations present in different types of cartilage, which we summarize in this Review article, is indispensable for future cartilage tissue engineering strategies, and provides important insights related to pathogenesis. Such insights are critical for identifying potential biomarkers and therapeutic targets for OA, IVDD and other cartilage-related disorders[15]. Moreover, integrating spatial transcriptomics and subcellular proteomics could provide a more comprehensive view of chondrocyte behaviour within their native microenvironment. Mapping the spatial distribution of chondrocyte subtypes and their associated signalling pathways in developing (Box 1 and Fig. 1) and mature cartilage will improve understanding of how these cells contribute to cartilage homeostasis and the progression of degenerative diseases and could also facilitate development of targeted therapies for these conditions.

In this Review we provide a comprehensive update on chondrocyte populations in cartilaginous tissues at the single-cell level in health, disease and senescence, and highlight the applications for these technologies for deciphering the phenotypic cues that could be developed into sensitive, specific biomarkers and therapeutic targets for cartilage disorders in synovial joints or in the IVD.

Cell phenotype and phenotypic markers at the single-cell level

Understanding the diversity of cell phenotypes in joint and spinal tissues is crucial for interpreting their roles in development, homeostasis and disease. Notably, in addition to chondrocytes, synoviocytes and synovial fibroblasts have emerged as central regulators of synovitis in OA, interacting dynamically with chondrocytes to propagate inflammatory mediators and cartilage-degrading pathways under mechanical or metabolic stress[16]. This crosstalk exacerbates disease progression, positioning synoviocytes and synovial fibroblasts as important therapeutic targets alongside chondrocytes. Over the past decade, the integration of high-resolution techniques such as scRNA-seq, spatial transcriptomics and proteomics has enabled unprecedented insight into cell populations across cartilaginous tissues. This section explores how these tools have revealed tissue-specific heterogeneity and phenotypic markers in hyaline cartilage, the meniscus and the IVD, with emphasis on both healthy and pathological contexts.

Cellular complexity in hyaline cartilage

The morphology of chondrocytes within hyaline cartilage varies depending on their function and location within the tissue. In articular cartilage, the characteristic roundish cell morphology is predominantly observed in the chondrocytes of the middle layer, where cells are sparse. These cells are embedded in an ECM that is rich in proteoglycans and collagen type II, which aids in the absorption and distribution of mechanical compressions applied to the joint. By contrast, chondrocytes in the deep zone exhibit an enlarged pre-hypertrophic or hypertrophic appearance and are often arranged in columns oriented perpendicular to the surface. In the superficial layer, which faces the synovial fluid of the joint space and shields the deeper layers from shear stress, chondrocytes are abundant, have a flattened morphology and are tangentially oriented relative to the cartilage surface, as demonstrated by 3D synchrotron imaging of the intact tissue[11,17] (Fig. 2).

In contrast to other cell types, such as mesenchymal stem cells (MSCs), which are recognized by the presence or absence of a defined set of surface markers[18], there is no widely acknowledged set of surface

markers for identifying chondrocytes. Some of the proposed markers (such as CD44, CD73, CD90 and CD105) are non-specific and overlap with MSCs and fibroblasts, and donor variability and methodological challenges hinder consensus[19–21]. This lack of distinct markers might, in part, reflect the unique niche in which chondrocytes reside (embedded within a dense ECM and largely isolated from direct cell–cell contact), which might result in limited biological pressure to maintain a robust repertoire of cell-surface proteins for intercellular communication. Instead, depending on their location, chondrocytes from various zones exhibit differences in the expression of specific markers associated with their unique ECM. Mature chondrocytes in the middle layer express characteristic cartilage components such as collagen type II, IX and XI, aggrecan and link protein[22]. By contrast, pre-hypertrophic and hypertrophic chondrocytes in the deep zone and calcified zone are marked by the presence of collagen type X[23]. Cells in the superficial zone are exclusive producers of lubricin (also known as proteoglycan-4 (PRG4)), a surface protein crucial for joint lubrication. A small proportion of superficial zone chondrocytes in non-degenerate cartilage express collagen type I and largely lack expression of the collagen types typical of deeper layers of articular cartilage, such as collagen type II[24], which challenges earlier assumptions about the absence of collagen type I in healthy hyaline cartilage[10]. In addition to zonal variation, transcriptomic evidence from OA cartilage reveals the presence of *METRNL*$^+$ and *PRG4*$^+$ chondrocyte subtypes, which seem to reflect early and intermediate states of dedifferentiation and are regulated by Hippo signalling via Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), a key pathway in cartilage remodelling[25].

Although a small number of studies previously described cells in cartilage with progenitor-like features[26,27], the dominant paradigm for many years viewed cartilage as being composed of a nearly uniform cell population[28]. Therefore, the recognition that chondrocytes are in fact not a uniform population predates single-cell sequencing. Previous morphological and immunohistochemical studies using confocal microscopy, cytoskeletal staining and protein-level markers have highlighted heterogeneity in chondrocyte morphology and phenotype in situ[10–12,29,30]. These investigations revealed differences in cell volume, cytoplasmic projections and differential expression of collagen types I and VI, IL-1β and ECM-degrading enzymes, such as ADAMTS4, within macroscopically healthy cartilage.

Although these foundational studies underscored the functional diversity of chondrocytes, the advent of single-cell and spatial multi-omics has dramatically enhanced the resolution of this heterogeneity, revealing transcriptionally distinct subtypes and their roles in homeostasis, inflammation and degeneration. Only advancements in scRNA-seq technologies over the past decade have revealed the full extent of heterogeneity within cartilaginous tissues. Further supporting this heterogeneity, seven transcriptionally distinct chondrocyte subpopulations were identified in OA cartilage, including stress-metabolizing and ECM-synthesis-related subtypes that dominate at early and late stages of damage, respectively[31]; these shifts highlight functional transitions during OA progression. Cellular subpopulations with distinct phenotypes have been identified in the different layers of articular cartilage[32,33] but also from weight and non-weight-bearing areas of articular cartilage tissues[34]. In rheumatoid arthritis (RA), studies using single-cell transcriptomics have also revealed immune-associated chondrocyte populations with distinct spatial distributions depending on mechanical load, which emphasizes the relevance of tissue location even under inflammatory conditions[35].

Box 1 | Signalling pathways involved in chondrogenesis

Cartilage development starts with cartilage progenitor cells differentiating from mesenchymal stem cells. Limb cartilage originates from the sclerotome, whereas head cartilage derives from the cranial neural crest. Articular cartilage progenitors arise from the interzone at future joint sites. Limb development begins with the condensation of cartilage progenitor cells into chondrogenic nodules (a process mediated by cell junctions), which enhances local gradients of chondrogenic growth factors (Fig. 1). This condensation commits mesenchymal cells to the chondrogenic lineage, a process that requires the activation of numerous signalling pathways.

Meniscus fibrocartilage development starts with interzone cells derived from embryonic mesenchyme. The gene signature associated with meniscus development is unique and differs from that of cartilage and ligament development, with the *IGF1*, *GDF5*, *LGR5*, *SCX* and *GLI1* pathways having prominent roles.

Intervertebral disc formation shares regulatory factors with chondrogenesis, but shows key differences in cell types and tissue composition. The annulus fibrosus and cartilaginous endplates are mesenchymal in origin, whereas the nucleus pulposus develops from the notochord, initially containing notochordal cells replaced by chondrocyte-like cells (Fig. 1). SOX9 is essential for nucleus pulposus and annulus fibrosus development, with annulus fibrosus and cartilaginous endplate cells derived from SCX and SOX9 double-positive progenitors.

A separate scRNA-seq study that focused on healthy and OA human articular cartilage also identified seven distinct chondrocyte subpopulations, providing a high-resolution transcriptional map of cell types within macroscopically healthy tissue[36]. In addition, single-cell transcriptomic advances enable a more granular and functionally annotated classification of chondrocyte subtypes than previous in situ immunolabelling-based studies that suggested phenotypic heterogeneity among chondrocytes[12]. These seven clusters were classified as fibrocartilage chondrocytes-1 and fibrocartilage chondrocytes-2 (expressing *SH3BGRL3*, *S100A6*, *MYL9* and *IGFBP5*, *LMCD1*, respectively), cartilage progenitor cells-1 and cartilage progenitor cells-2 (which express *KIAA0101*, *BIRC5* and *CDC20*, *UBE2C*, *CENPF*, *KIAA0101*, *BIRC5*, respectively), regulatory chondrocytes (expressing *EIF5A*, *PGK1*, *ANXA1*, *TUBA1A*), pre-hypertrophic chondrocytes (expressing *SOX9*, *COL9A3*, *COL11A1*) and homeostatic chondrocytes (expressing *TXNIP*, *IFITM3*, *GDF15* and *TIMP1*). The most apparent differences between healthy and OA cartilage were an enrichment of regulatory and pre-hypertrophic chondrocytes in OA and an abundance of cartilage progenitor cells in healthy cartilage. A subpopulation of hypertrophic chondrocytes (expressing *SLC39A14* and *COL10A1*) and distinct from hypertrophic chondrocytes in healthy cartilage was further identified in the superficial region of damaged cartilage in human OA tissue[36] (Supplementary Table 1).

Single-cell and spatial transcriptomic analysis of healthy and OA human knee articular cartilage identified 33 cell population-specific marker genes that define 11 chondrocyte populations, including 9 known populations and 2 newly defined populations: pre-inflammatory and inflammatory chondrocytes[37]. This study established that the

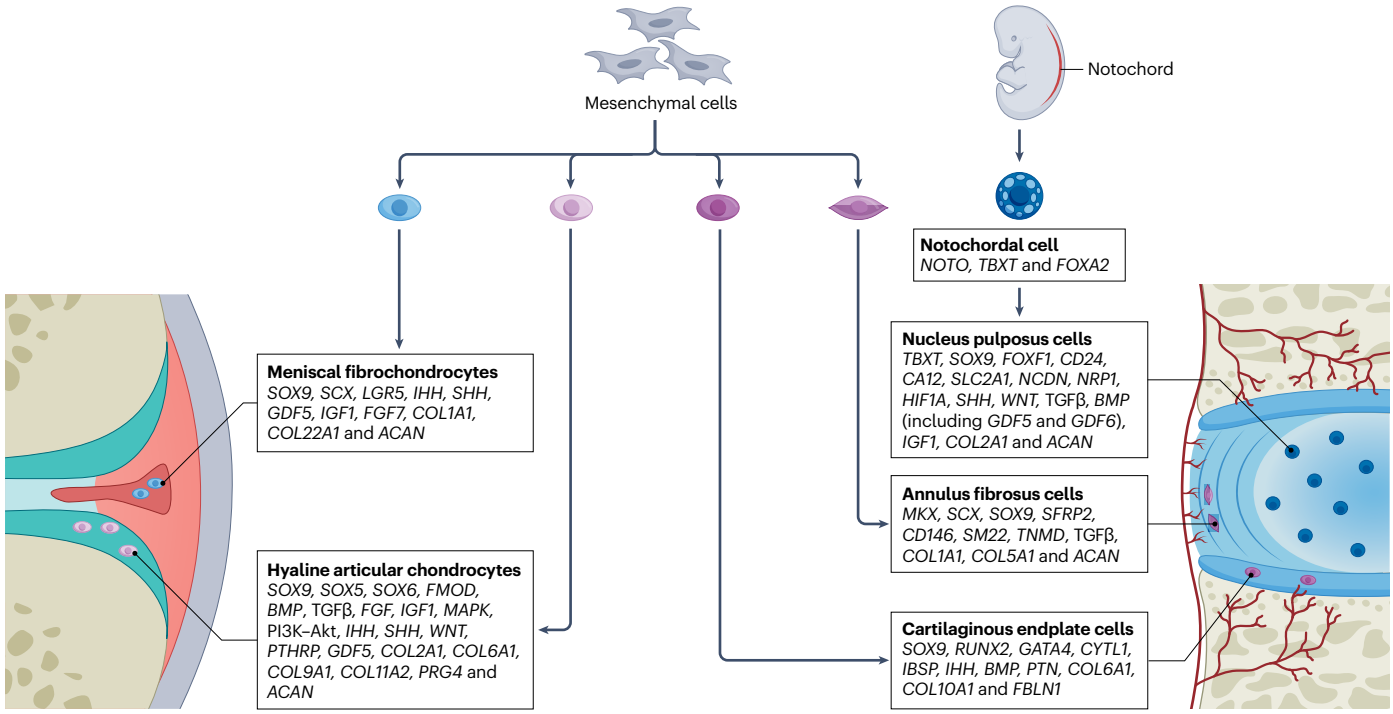

Fig. 1 | Main signalling pathways and markers that regulate the development of cartilaginous tissues. Mesenchymal cells differentiate into progenitor cells giving rise to various chondrogenic lineages, characterized by partially overlapping signalling pathways, and the expression of transcription factors and extracellular matrix (ECM) components. Nucleus pulposus cells are derived from the notochord. Hyaline articular chondrocytes: *SOX9*, *SOX5* and *SOX6* (chondrogenesis); *FMOD* (collagen organization); *BMP*, TGFβ, *FGF*, *IGF1*, *MAPK*, PI3K–Akt and *WNT* (proliferation and differentiation); *IHH*, *SHH* and *PTHRP* (also known as *PTHLH*) (homeostasis and differentiation); *GDF5* (joint development); *COL2A1*, *COL6A1*, *COL9A1*, *COL11A2*, *PRG4* and *ACAN* (ECM components). Meniscal fibrochondrocytes: *SOX9* and *SCX* (differentiation); *LGR5* (progenitor marker); *IHH* and *SHH* (homeostasis and differentiation); *GDF5* (joint development); *IGF1* (proliferation and differentiation); *FGF7* (differentiation); *COL1A1*, *COL22A1* and *ACAN* (ECM components). Annulus fibrosus cells: *MKX*, *SCX* and *SOX9* (differentiation); *SFRP2* (ECM remodelling); *CD146* (also known as *MCAM*; progenitor marker); *SM22* (also known as *TAGLN*; contractile phenotype); *TNMD* (tenomodulin, tendon-like identity); TGFβ (developmental signalling); *COL1A1* and *COL5A1* (tensile strength); *ACAN* (ECM components). Cartilaginous endplate cells: *SOX9* and *RUNX2* (differentiation); *GATA4* and *CYTL1* (boundary formation); *IBSP* (mineralization inhibition); *IHH*, *BMP* and *PTN* (ossification signals, hypertrophy and ECM remodelling); *COL6A1* and *COL10A1* (ECM components); *FBLN1* (ECM organization and mechanical stability). Nucleus pulposus cells: *TBXT*, *SOX9* and *FOXF1* (differentiation); *CD24*, *CA12* and *SLC2A1* (involved in hypoxic and microenvironmental adaptation); *NCDN* (lineage maintenance and ECM stability); *NRP1* (developmental patterning); *HIF1A* (hypoxia response); *SHH*, *WNT*, TGFβ and *BMP* (including *GDF5* and *GDF6*), *IGF1* (developmental signalling); *COL2A1* and *ACAN* (ECM components).

pre-hypertrophic chondrocyte and hypertrophic chondrocyte populations are potentially essential for disease progression in OA, and that the pre-fibrocartilage chondrocyte population, a distinct entity from the previously described fibrocartilage chondrocytes, is a major contributor to the stratification of patients with OA[37]. Another study investigated OA human knee articular chondrocyte populations under different mechanical loading conditions via scRNA-seq[38]. In line with previous studies, 12 chondrocyte subtypes were identified, and their functions, development and interactions with other cells were described. The study also identified a new chondrocyte subset, termed hypertrophic chondrocytes-C. These findings underscore the importance of delineating major cell populations within healthy cartilage and comparing them with pathological cells; such comparisons are key for comprehending the distinct roles of various chondrocyte populations and their respective pathogenic mechanisms, which contribute to the development of diseases such as OA.

In a 2023 study, a subset of chondrocytes with high expression of *SPP1* (also known as osteopontin) was identified in human OA cartilage using scRNA-seq[39]. These *SPP1*$^{+}$ chondrocytes exhibited the highest SenMayo score, a transcriptomic index used to quantify cellular senescence, among all chondrocyte subgroups and demonstrated strong angiogenic potential. Furthermore, the SPP1 signalling network was more abundant in OA cartilage than in healthy cartilage, and the receptor–ligand binding pattern of SPP1–CD44 appeared to have an important role in this network.

A 2024 single-cell study further refined the understanding of chondrocyte populations that are critical for the progression of OA. In a post-traumatic model of OA, pre-inflammatory and inflammatory chondrocyte subtypes emerge early and contribute to disease progression through cytokine-mediated crosstalk[37]. In parallel, angiogenic (*Smoc2*$^{+}$*Angptl7*$^{+}$) and osteogenic (*Col1a1*$^{+}$) chondrocytes have been identified as drivers of pathological vascularization and subchondral bone remodelling in later stages of disease[40]. These findings align with trajectory analyses that reveal time-dependent shifts in chondrocyte states following joint injury, with inflammatory and ECM-degrading signatures progressively dominating the transcriptomic landscape[41].

Similar to single-cell transcriptomics data, cytometry by time of flight (CyTOF) single-cell proteomics using a panel of 33 markers (which included cell-surface receptors, adhesion molecules, signalling mediators and transcription factors) revealed three cartilage progenitor cell (CPC) variants (CPC I–III) in healthy and OA human cartilage[42], which also included the previously identified migratory CPCs. CPC I was characterized by low CD105 and high CD54 (also known as ICAM-1) expression, and very active ERK1–2 signalling; CPC II had high levels of CD73 expression and the CPC III population was enriched for pro-inflammatory pathways, including nuclear factor-κB (NF-κB), signal transducer and activator of transcription 3 (STAT3), β-catenin and hypoxia-inducible factor 2α (HIF2α). Furthermore, a rare chondrocyte population, termed inflammation-amplifying (Inf-A) chondrocytes, was identified in patients with OA using CyTOF-based single-cell proteomics. Despite their atypical signalling profile, these cells were confirmed to express classical chondrogenic markers such as CD44 and SOX9, affirming their chondrocyte identity. They exhibited high levels of IL1R1 (also known as CD121a) and TNFRII (also known as CD120b), as well as exclusive activation of JNK and SMAD1–5 signalling pathways, and accounted for ~2% of the chondrocyte population based on single-cell proteomic and transcriptomic analyses[33]. Owing to the established role of CD24 in mitigating inflammation, CD24-enriched chondrocytes were termed inflammation-dampening chondrocytes and displayed enrichment of inflammation and immune cell trafficking-related pathways. Thus, a combination strategy of enhancing these rare inflammation-dampening chondrocytes and inhibiting the inflammation-amplifying chondrocyte populations could be effective in mitigating inflammation in OA cartilage[42]. In a follow-up study, four senescent CPC populations were identified in human OA cartilage based on $p16^{INK4a}$ expression[43]. These senescent subsets, which included and expanded upon the previously defined CPC I–III populations, exhibited distinct inflammatory and catabolic signalling profiles.

Cellular complexity in the meniscus

The meniscus comprises three zones, the avascular (white) inner zone, the outer vascular (red) zone, and a transitional red–white zone. The avascular inner zone is subject to compressive loading, whereas the outer vascular zone is under tensile and torsional loading[44]. Cells within the meniscus have historically been described as fibrochondrocytes, a mixed phenotype reflecting both fibroblastic and chondrogenic features, although microarray and scRNA-seq analyses have since uncovered specific cell types and gene signatures, both within healthy and OA meniscus and across its distinct inner and outer zones.

In a study in which microarray analysis was used to investigate the differences in transcriptomes between OA and non-OA human meniscal tissues, bone-related genes such as *SPARCL1*, *COL10A1* and *WIF1* were upregulated, whereas *VEGFA* and *POSTN* were downregulated within OA meniscal tissues. Cluster analysis of the array data showed that pro-inflammatory genes were highly expressed in the

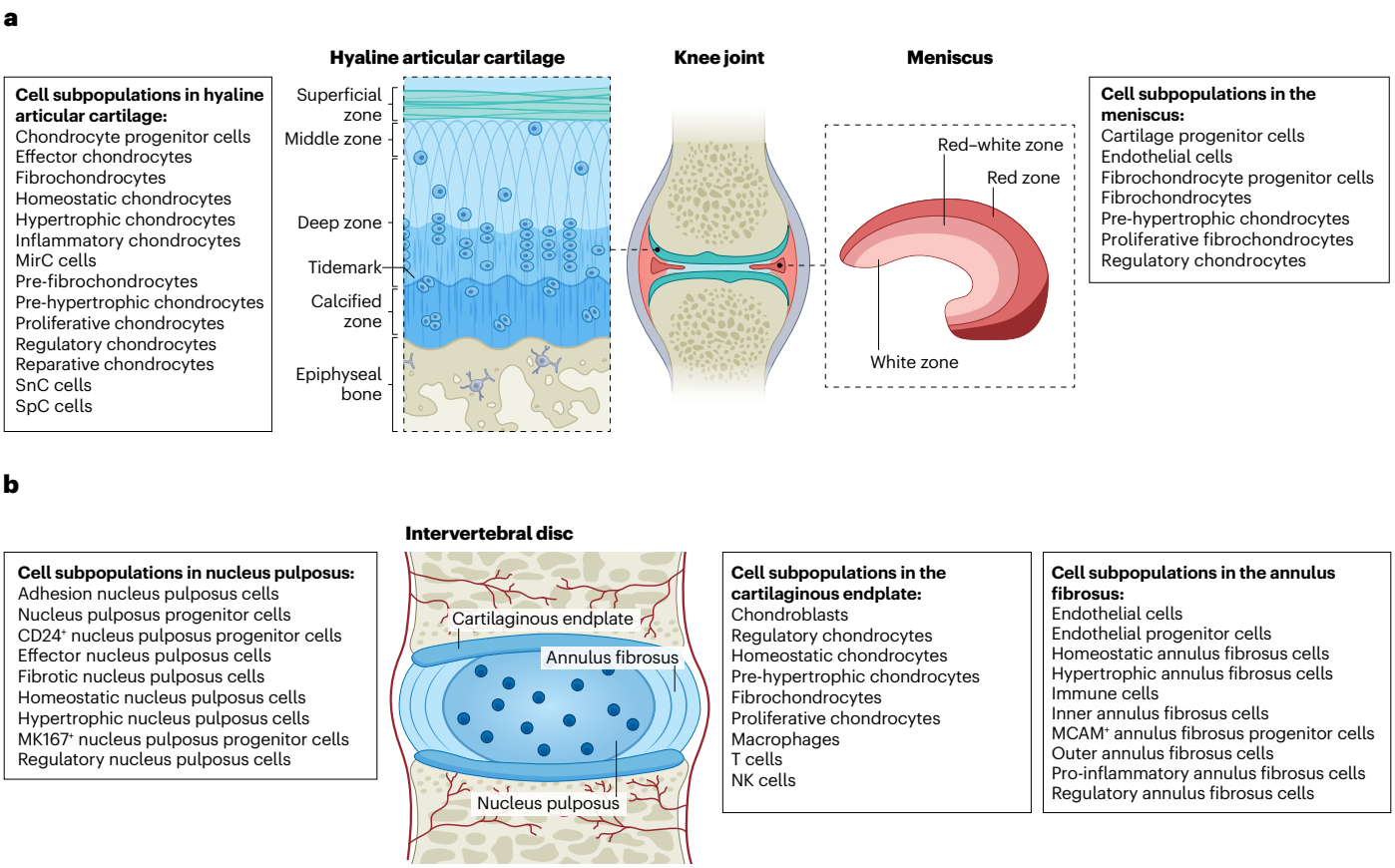

Fig. 2 | Cell populations in cartilaginous tissues. Single-cell RNA sequencing has identified numerous cell populations (only the main populations are shown in this figure) in hyaline articular cartilage and the meniscus (**a**) and the intervertebral disc (**b**). MirC, metal ion-related chondrocyte; NK cell, natural killer cell; SnC, senescent cluster; SpC, splicing chondrocyte.

OA meniscus, whereas genes associated with tissue regeneration were more prominently expressed in the non-OA meniscus[45]. Healthy (non-OA) meniscus samples were taken from patients with partial meniscus tears that showed no macroscopic evidence for OA or other joint diseases; however, even a partial tear can influence the expression of specific genes, as there could be upregulation or downregulation in post-traumatic inflammation-associated genes upon injury. In one study, scRNA-seq analysis of healthy human meniscus from patients undergoing amputation (mean Kellgren–Lawrence grade 0) compared with OA meniscus (mean Kellgren–Lawrence grade 3) was used to identify specific markers for OA meniscus. In contrast to the microarray analysis discussed earlier in this article, *LCN2*, *RAB27B*, *PRDM1* and *SERPINB2* were upregulated in human OA meniscal tissues compared with healthy tissues, with *LCN2* and *RAB27B* emerging from gene ontology as potential early-stage OA meniscus-specific markers[46]. The expression of both *Lcn2* and *Rab27b* was increased for up to 6 months in spontaneously aged mice with OA[46]. However, the authors did not evaluate human meniscus at different disease stages of OA to observe whether these genes can be used as specific meniscus markers for early OA. Owing to meniscus tears being a potential start point for OA, finding an early-stage marker within the meniscus is vital to prevent the onset of disease[47].

scRNA-seq analysis has also provided a greater understanding of the cell types within the meniscus. Specifically, studies have identified seven cellular populations within the human meniscus: endothelial cells, cartilage progenitor cells, regulatory chondrocytes, fibrochondrocytes, pre-hypertrophic chondrocytes, fibrochondrocyte progenitors (also described as CD146$^+$ pericyte-like cells) and proliferative fibrochondrocytes[48,49] (Supplementary Table 2). The avascular zone of the tissue also contains lymphocytes and myeloid cells, whereas the vascular zone has a greater proportion of endothelial cells and also Schwann cells that correlate with the presence of nerves within this region[48]. In both of the aforementioned studies, the presence of fibrochondrocyte progenitors within the tissue indicates the presence of regenerative populations within both the healthy and degenerative meniscus. A *CD146*$^+$ (a typical pericyte marker, also known as *MCAM*) population that was isolated from healthy human meniscus had a multilineage differentiation capacity and expressed stem cell markers; however, within the degenerative meniscus, a loss of *CD146*$^+$ cells led to an increase in a *CD318*$^+$ (also known as *CDCP1*) cell population that displayed progenitor-like characteristics. The latter population could have a crucial role in meniscal degeneration and has been proven to be a marker for meniscus progenitor populations isolated from degenerative meniscus[50]. *CD318* expression in injured meniscus tissue was reduced upon treatment with TGFβ; thus, *CD138* could be a potential marker for meniscal degeneration[49]. The study supports the presence of progenitor populations within the meniscus described in previous human and bovine in vitro studies[50–53].

At the tissue level, in vivo post-traumatic destabilized medial meniscus (DMM) mouse models of OA have demonstrated pathological mineralization in the lateral joint compartment, a process known as lateral chondrocalcinosis, which can drive medial articular cartilage damage via LEF1 signalling[54]. These findings highlight the relevance of Wnt signalling, as LEF1 acts as a key downstream effector in the canonical Wnt beta-catenin pathway, in regulating meniscal stiffness and pathological mineralization. The data also suggest that alterations originating in the lateral compartment, such as chondrocalcinosis, might contribute to degenerative changes in adjacent joint structures, including the medial articular cartilage.

Cellular complexity in the intervertebral disc

Phenotyping studies using omics technologies at the transcriptome and proteome level have identified a wide range of markers of human notochordal cells[55–58], healthy nucleus pulposus, annulus fibrosus, and cartilaginous endplate (CEP) cells and tissues[59,60], as well as markers of degeneration[59,61–64]. These efforts to understand the nucleus pulposus cell phenotype resulted in the publication of an international consensus statement in 2015 on markers to distinguish nucleus pulposus cells from annulus fibrosus and CEP cells[65]. Nucleus pulposus cells express markers found in human and bovine notochordal cells[66,67], suggesting that at least a proportion of human nucleus pulposus cells are notochord-derived. However, additional progenitor cell populations have been identified within the human and mouse IVD (most notably Tie2$^+$ GD2$^+$ nucleus pulposus progenitor cells[68]), including cells from the nucleus pulposus, annulus fibrosus and CEP, which possess MSC-like properties such as multipotency[69]. Although some of these populations have been proposed to have regenerative potential, they highlight the complexity of IVD formation and the diversity of cells that exist within the disc during development, ageing and degeneration.

scRNA-seq is beginning to provide a more detailed understanding of the cell subpopulations within the human IVD. Comparisons of the cells within the healthy human IVD have revealed differences in transcriptional profiles between nucleus pulposus cells and annulus fibrosus cells[70], and comparisons of non-degenerate and degenerate IVD cells from the same donor have revealed a panel of potential biomarkers of disease[71]. Additionally, multiple distinct cell sub-types within both the human nucleus pulposus and annulus fibrosus have been identified[72–77], with studies showing a shift in IVDD tissues towards populations with a more fibrotic phenotype, populations that might drive angiogenesis and an increased presence of immune cell-like populations, most notably macrophages, when compared with non-degenerate discs. Although the function of these subpopulations requires further investigation and functional validation, the alterations in cell populations might underpin the tissue-level changes observed during degeneration, and these studies highlight the diversity of cell phenotypes present within the human IVD throughout ageing and degeneration (Supplementary Table 3).

Alongside studies investigating cell populations associated with degeneration, scRNA-seq has also enabled identification of a putative *PROCR*$^+$ progenitor cell population within the human nucleus pulposus[78]. Additionally, transcriptomic and protein-level analyses of human and mouse IVD during early embryonic development have identified populations during early (*SOX10*$^+$) and late (cathepsin K$^+$ (encoded by *CTSK*)) IVD formation as well as populations that are responsible for ECM homeostasis (*CTSK*$^+$ and brachyury$^+$ (encoded by *TBXT*))[79]. An integrated analysis of proteome sequencing, bulk RNA sequencing and scRNA-seq data identified *SERPINA1* as a biomarker to regulate or predict the progress of IVDD[80]. Identification and functional characterization of these subpopulations within the adult human IVD could further elucidate their roles in tissue homeostasis and identify progenitor cell populations with potential for therapeutic application.

Ageing, inflammation and chondrosenescence

With age, cartilaginous tissues might gradually become damaged, which can lead to prevalent joint diseases such as OA and IVDD[81]. Notably, these degenerate tissues do not present a widespread apoptotic phenotype[82], leading researchers to investigate causal drivers of structural damage. In the past decade, research has focused on elucidating the role of senescence in OA and IVDD pathophysiology[83].

Cell senescence (also termed chondrosenescence in articular cartilage) is characterized by an irreversible halt in cell division[84,85]. Cell senescence increases with age and correlates with progressive tissue degeneration and functional loss[86–89].

Senescence in hyaline cartilage

Senescent cells often display dramatic changes in structure, metabolism and secretory profile, indicating that senescent cells have a pleotropic phenotype. These cells often display an increase in cell volume, senescence-associated β-galactosidase activity, senescence-associated heterochromatic foci and the expression of cell-cycle-related proteins, such as $p16^{INK4a}$, $p19^{ARF}$, $p14^{ARF}$ and $p21^{CIP1}$ (refs. 85,89,90). Moreover, senescent cells contribute to a systemic increase in pro-inflammatory mediators[88], as they secrete exosomes (known as senescence-associated secretory phenotype (SASP)) that contain pro-inflammatory mediators, chemokines (IL-1β, IL6 and CXCL8) and ECM-degrading enzymes, such as matrix metalloproteinases (MMPs) and cathepsins[88,91]. In addition to the systemic effect of senescent cells, it has been suggested that SASP-secreting senescent cells confer a 'bystander effect' that affects neighbouring cells, resulting in further induction or reinforcement of tissue senescence[92,93] (Fig. 3). This process seems to be mediated by SASP-related factors and cytokines, which also contribute to age-related chronic inflammation[90,94]. Notably, mechanical insults to human hyaline articular cartilage contribute to senescence in the superficial zones, displaying telomere erosion and reduced cell doubling[95]. In line with these observations, data from animal models of ageing and post-traumatic OA (such as those that use the anterior cruciate ligament transection procedure) show that the number of senescent cells is highest in the superficial zone (which is directly exposed to mechanical loading)[96]. Given that the superficial zone is enriched in stem-cell populations, the accumulation of senescent chondrocytes might interfere with the regenerative potential of the tissue after loading[97]. Cumulatively, these data suggest that mechanical loading entices the initial emergence of superficial senescent chondrocytes, which could be further increased with time via the SASP-mediated 'bystander effect'. To this end, ageing and the inflammaging process can only contribute to the proportion of chondrosenescence in articular cartilage.

Humans and rodents exhibit a chronological increase in the senescence biomarkers $p14^{ARF}$ and $p16^{INK4a}$, respectively; but these changes are not associated with increased levels of SASP, nor does loss of murine $p16^{INK4a}$ result in a mitigated OA phenotype[98]. Notably, the gradual acquisition of the chondrosenescent phenotype is suggested to be accompanied by chondrocyte hypertrophy and mineralization[99], posing a specialized profile of senescent chondrocytes. Mechanistically, this hypertrophy-related chondrosenescent feature is not fully understood, but evidence shows that cartilage-specific ablation of SIRT1 (which is known to repress senescence[100]) resulted in severe post-traumatically induced ectopic osteophyte formation, meniscal mineralization and cartilage damage[54], accompanied by increased chondrosenescent $p16^{INK4a}$ staining[101]. In a recent study, age-associated transcriptional changes, such as *GATA4* upregulation, impaired chondrocyte ECM synthesis and amplified pro-inflammatory responses, providing a mechanistic link between cellular ageing and OA susceptibility[102].

The relationship between inflammation and cellular senescence in the context of musculoskeletal disorders remains unclear, with both chronic and acute inflammation potentially contributing to the accumulation of senescent cells in ageing tissues or after injury[96,101]. Although chronic inflammation can induce senescence, and anti-inflammatory treatments might clear senescent cells (Fig. 3), the effects of acute injury-related inflammation on senescence are not fully understood, suggesting a complex interplay between these processes.

Most in vivo studies, including studies describing cartilage senescence, are traditionally performed in male mice given that they are reported to harbour a more severe OA phenotype than female mice[103], and therefore the male models better support this chronological accumulation in SASP and senescent cell phenotype[96,104]. In a 2024 study that examined a targeted treatment to enhance SIRT1 activity, aged female mice did not display a senescent phenotype, whereas aged male mice did have the senescent hallmark of H2Aγ[105]. These data insinuate that, at least in preclinical models, different phenotypes of senescence can occur owing to sex-related differences, which should be addressed in future research.

Senescence in the meniscus

The specific association of senescence in meniscal tissues has predominantly focussed on its association with articular cartilage. Thus, studies specifically examining senescence in meniscus are rare and more commonly related to studies investigating the aged meniscus[106]. A study that used gene databases from previous microarray analyses identified four genes (*RRM2*, *AURKB*, *CDK1* and *TIMP1*) and microRNAs associated with these genes in senescent human meniscal tissues[107], whereas another study showed that downregulation of *FOXO1* and *FOXO3* transcription factors in aged meniscal tissues increased susceptibility to OA[108].

A study that aimed to identify specific OA markers using scRNA-seq analysis of healthy and OA human meniscal tissues, found a subset of cells with upregulated expression of fibroblast activating protein and the transcription factor *ZEB1*, and promoted ECM degradation and senescence[109]. Senotherapeutic drugs (therapies that target senescent cells) have yet to be directly applied to meniscal tissues, although the specific mechanisms that induce senescence remain to be elucidated.

Senescence in the intervertebral disc

Senescence often correlates with skeletal ageing, a major risk factor for IVDD and OA[90]. Other pathogenic factors, including oxidative, genotoxic and inflammatory stress, along with nutritional constraints that contribute to IVDD, all correlate with cell senescence. Consequently, senescence has an important role in the pathophysiology of IVDD[110].

Early work showed a positive correlation between $p16^{INK4a}$ expression levels and disc degeneration in patients[87]. Later studies, using $p16^{tdTOM}$ reporter mice, showed increased levels of $p16^{INK4A}$, p21 and senescence burden in aged mouse IVD[111]. These authors, using a model of conditional deletion of $p16^{INK4A}$ ($Acan^{creERT2}$;$p16^{INK4a}$), showed a compensatory role of $p19^{ARF}$ in the senescence process. Although $p16^{INK4A}$ was dispensable for the induction and maintenance of senescence, this study established a causal relationship between $p16^{INK4A}$ with SASP and altered ECM homeostasis. These findings aligned with studies of a mouse model of cyclin dependent kinase inhibitor 2A (*Cdkn2a*; encoding $p16^{INK4A}$) germline deletion, which showed a reduction in oxidative stress and disc degeneration following tail suspension injury[112]. Furthermore, a study using a genetically engineered $p16^{INK4A}$-3MR transgenic mouse model showed that systemic clearance of $p16^{INK4a}$-positive cells ameliorated age-related disc degeneration[113]; mice that lacked $p16^{INK4A}$-positive senescent cells had decreased ECM catabolism and reduced inflammation. These findings support the causal relationship between senescent cells and IVD degeneration[114,115].

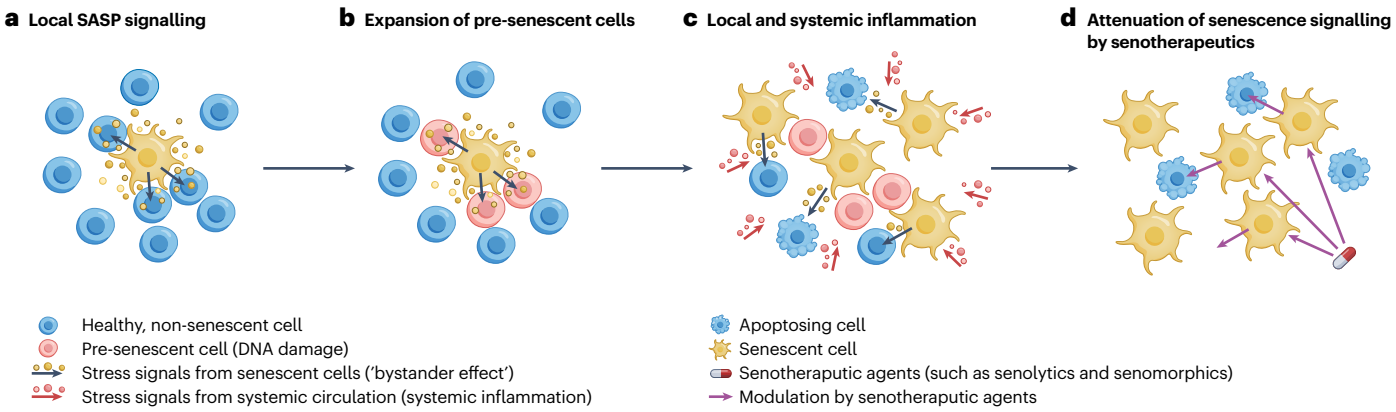

Fig. 3 | Senescence in cartilaginous tissues. A proposed trajectory wherein the frequency of cellular senescence in cartilage increases in proportion to both chronic and acute inflammation. Although the frequency of senescent cells in the tissue is initially low (**a**), healthy cells accumulate DNA damage over time with increasing levels of inflammation and oxidative stress (**b**). Moreover, senescent cells negatively affect neighbouring healthy cells through the production and secretion of senescence-associated secretory phenotype (SASP) factors (known as the 'bystander effect'), which predisposes them to senescence. Various external stimuli, such as trauma, injury and infection, promote further inflammation and amplify the bystander effect, causing healthy, non-senescent cells to undergo apoptosis, whereas senescent cells are more resistant to inflammatory conditions and instead undergo cellular dysfunction. Local (that is, intra-articular) and systemic inflammatory mediators arising from chronic co-morbidities (such as obesity, diabetes and cardiovascular disease) further exacerbate the process (**c**). Senotherapeutic agents selectively induce apoptosis in senescent cells or modulate their secretory phenotype, thereby reducing their accumulation and mitigating the harmful effects of their pro-inflammatory secretome, especially the senescence-inducing effects of SASP factors (**d**).

Another study used scRNA-seq to identify compartment-specific changes in human IVDD and observed gene signatures of cell senescence, as well as a notable reduction in cells, particular stem cells and fibroblast progenitors, expressing markers of immature cell types in both the annulus fibrosus and the nucleus pulposus[116]. Other studies that investigated the cellular heterogeneity during human IVDD have observed changes in processes such as ferroptosis, which, similar to senescence, are linked to oxidative stress and inflammation[117]. A 2024 study using scRNA-seq, identified autophagy-related protein 9 A (*ATG9A*) as a key marker associated with IVDD, whereby ATG9A expression is diminished during degeneration, which suggests reduced autophagic flux[118]. Notably, basal autophagy has a protective function during ageing-related pathologies, and dysregulated autophagy contributes to many pathologies that affect the spinal column[119,120]. However, a 2021 study also showed the importance of autophagy in establishing full senescence through regulated protein stability and the importance of this process during human OA[121]. Further investigations are needed to understand this relationship in the context of IVDD. Although similarities have been noted between other skeletal tissues and the IVD, pathways (such as cGAS–STING) that are linked to cell senescence did not contribute to senescence burden in the ageing mouse with IVD, highlighting the cell and tissue type specificity of the mechanisms driving cell senescence[122].

Senotherapeutic agents are therapies that target cellular senescence and include both senolytics (which reduce inflammation and improve tissue function by removing senescent cells) and senomorphics (which help maintain tissue function by reducing the negative impact of senescent cells, such as chronic inflammation, without eliminating these cells). These therapies show great potential for treating OA and IVDD by targeting senescent cells in affected tissues. However, further research is needed to elucidate the broader effects of senescence on joint health, to develop reliable biomarkers for patient selection and to optimize treatment protocols for disease models and cell-based therapies. For developing novel senotherapeutic strategies, single-cell analysis could be instrumental in identifying specific cellular subpopulations and their roles in senescence, thereby allowing for targeted interventions to mitigate age-related tissue dysfunction. This approach could help to elucidate the complex interactions between senescent cells and their native niche, potentially leading to novel senotherapeutic agents that could improve tissue regeneration and function (Box 2).

Future prospects

Although genomic and transcriptomic analyses, including single-cell transcriptomic analyses, have transformed the understanding of chondrocyte heterogeneity, their use in predicting functional ECM outcomes remains limited. For instance, transcript levels of *ACAN* or *COL2A1* alone do not reflect the sulfation patterns of glycosaminoglycans or the cross-linking density of collagen fibrils, both of which are critical for load-bearing capacity[123–125]. This example highlights the need to better integrate single-cell multi-omics approaches with tissue-level multi-omics analyses, as well as to complement transcriptomics data with direct assessments of ECM biomechanics and post-translational modifications, as gene expression alone might not faithfully predict tissue-level functionality[126].

Notably, differences in study outcomes often arise from methodological and biological variables; for instance, time points can critically influence results: early-stage OA tends to involve transient inflammatory or proliferative chondrocyte states, whereas late-stage disease predominantly exhibits catabolic or senescent populations[127]. Species-specific differences (such as rodent versus human cartilage)[128] and OA induction methods (such as surgical destabilization versus chemical injury)[129] yield distinct pathophysiology, which influences the observed transcriptional profiles. For example,

mechanical injury models predominantly activate mechanosensitive pathways (such as YAP and TAZ)[130], whereas inflammatory models (such as collagenase-induced OA) amplify cytokine-driven responses[131]. Sex-specific differences in hormone signalling and immune regulation might underlie divergent cellular subpopulations in men and women[132]. Furthermore, technical variables such as cell isolation protocols (for example, the effects of enzymatic digestion on stress-response genes) and sequencing depth can skew population distributions[133]. Acknowledging these factors is essential for understanding differences across studies and advancing translational insights into chondrocyte heterogeneity.

Despite advances in the field, several critical questions remain unanswered. The precise molecular mechanisms that regulate the transition of chondrocytes from healthy to pathological states are still poorly understood. Additionally, the role of systemic factors, such as age, sex, metabolic health and mechanical loading, in shaping chondrocyte subtype distributions necessitates further investigation[134]. Addressing these gaps could substantially refine the understanding of cartilage degeneration and inform the development of targeted therapeutic strategies. Longitudinal single-cell analyses of post-traumatic OA models are needed to resolve temporal shifts in chondrocyte subtypes (such as fibrocartilage and pre-inflammatory chondrocytes) and their causal roles in fibrosis and inflammation[37,135].

Critically evaluating the translatability of animal models to human OA, particularly given the anatomical disparities in cartilage thickness and biomechanical loading patterns between quadrupedal rodents and bipedal humans[136], is crucial. Although animal models such as anterior cruciate ligament (ACL) rupture or DMM provide controlled systems for studying OA progression, they often fail to replicate the chronic, multifactorial nature of human disease, which involves ageing, systemic inflammation and cumulative mechanical stress[136,137]. Notably, cartilage that is classified as 'non-degenerate' in OA joints might still exhibit molecular alterations owing to prolonged exposure to pro-inflammatory mediators and abnormal mechanical stresses, as evidenced by proteomic and transcriptomic profiling[34,138]. Even in macroscopically intact regions, osteoarthritic chondrocytes can display upregulated catabolic pathways (such as MMPs, ADAMTS4 and ADAMTS5) and reduced anabolic activity[138], highlighting the need for cautious interpretation of 'healthy' cartilage.

Looking to the future, integrating spatially resolved multi-omics technologies, such as spatial transcriptomics and proteomics, will enable chondrocyte subsets to be mapped within their native niches. Such approaches could reveal dynamic changes in cellular behaviour during disease progression and provide insights into the molecular drivers of cartilage disorders. Moreover, longitudinal studies using these technologies might help to identify biomarkers for early detection of joint diseases, offering opportunities for timely and more effective interventions.

We propose that future applications of machine learning and artificial intelligence for the analysis of complex multi-omics datasets will uncover previously unrecognized patterns in chondrocyte gene expression and interactions, potentially leading to the discovery of novel therapeutic networks and safer druggable targets. Furthermore, developing predictive models on the basis of patient-specific data could facilitate personalized medicine approaches, tailoring treatments to individual disease trajectories. From a translational perspective, these findings have the potential to substantially improve clinical outcomes for patients affected by conditions such as OA and IVDD. Distinct chondrocyte phenotypes could offer new opportunities to refine current therapeutic strategies. In OA, inflammatory chondrocyte subsets represent potential targets for biologic therapies aimed at suppressing catabolic signalling, whereas progenitor-like populations could be harnessed for regeneration. Therapies that target these inflammatory chondrocyte subtypes could mitigate cartilage degradation in OA and enhancing the regenerative potential of homeostatic or reparative subpopulations could improve cartilage repair. Conversely, failed OA trials targeting broad-spectrum MMPs highlight the need for subtype-specific approaches to avoid disrupting homeostatic ECM maintenance. For meniscus-tissue engineering, hypertrophic chondrocyte subsets, which drive calcification in degenerated menisci, could be selectively inhibited, whereas ECM-producing phenotypes might be expanded to enhance graft integration. Similarly, in IVDD, nucleus pulposus cells with notochord-like signatures show enhanced proteoglycan synthesis[139], suggesting their potential in cell-based IVD regeneration. Therefore, future efforts should explore combined approaches that simultaneously suppress pro-inflammatory pathways and activate regenerative ones, optimizing therapeutic efficacy (Box 3).

Conclusions

Single-cell technologies are transforming the understanding of chondrocyte heterogeneity and functionality across cartilage types and disease states. Distinct chondrocyte subtypes have been identified in

Box 2 | Senotherapeutic agents for joint diseases

Senotherapeutic agents, which aim to modulate or eliminate senescent cells, are emerging as potential treatments for osteoarthritis (OA) and intervertebral disc (IVD) degeneration (IVDD). Intra-articular administration of senotherapeutic modulators has shown promise in reducing OA severity by modulating senescent chondrocytes in preclinical rodent models. Additionally, senolytics can also induce apoptosis in senescent IVD cells, thereby mitigating IVDD.

Most research focuses on the effects of senescence on chondrocytes during OA and IVDD but less is known about its role in other joint tissues or pain transmission. Senotherapeutic drugs have yet to show notable progression in clinical trials, which suggests that improved patient selection using senescence-related biomarkers is needed for more effective and quantifiable clinical outcomes.

Acute post-traumatic OA models might require different senotherapeutic drug dosages or administration methods compared with age-induced OA models. However, it should be noted that intra-articular administration of senotherapeutic agents is likely to target the senescent chondrocyte population that is located in the superficial zone, eliminating their detrimental effects on the tissue and promoting a pro-regeneration milieu. Similarly, the efficacy of cell transplantation approaches could be affected by the chondrosenescent environment, highlighting the need to consider the 'seno-severity' of the host. Future research should focus on understanding the broader effects of senescence on the entire joint and use biomarkers to identify suitable candidates for senotherapeutic therapies. Pretreatment with senotherapeutic agents could potentially enhance the outcomes of cell-based therapies by creating a more favourable environment for transplanted cells.

Box 3 | Exploiting chondrocyte heterogeneity for cartilaginous tissue regeneration

Current cartilage repair strategies, such as microfracture, autologous chondrocyte implantation and matrix-assisted chondrocyte implantation, face issues with tissue quality and durability. Future tissue engineering approaches that leverage the functional diversity of chondrocyte subpopulations that are emerging could be promising targeted therapies. Using mesenchymal stem cells or engineered cells that have been differentiated into specific chondrocyte subtypes could also enhance cartilage regeneration.

Meniscal regeneration focuses on stem cells and meniscal progenitor cells, potentially involving culturing these cells in 3D bioprinted scaffolds. Discovering specific progenitor populations within the meniscus, particularly from healthy and osteoarthritis tissues, could lead to better translational therapies for treating small defects or even whole meniscal tissues.

Approaches to intervertebral disc regeneration include intradiscal injections, gene therapies and cell implantation. Autologous and allogeneic cells, including nucleus pulposus and annulus fibrosus cells, chondrocytes and stem cells, are currently being tested; however, the harsh microenvironment of the degenerate disc poses challenges, necessitating pre-conditioning to improve cell survival. Future strategies might involve regenerative therapies without cell implantation and building on the secretome of notochordal cells.

hyaline articular cartilage, meniscal cartilage and the IVD, which exhibit unique gene expression profiles that correlate with their functional roles in health and disease. Understanding how these cells interact within their native niches and with cells in other joint compartments is crucial for developing more effective regenerative therapies. A deeper understanding of the cellular and molecular diversity of these cell populations, their crosstalk and relative influence can help to develop therapeutic candidates that can tilt the inflammatory and catabolic balance towards restoration of homeostasis and tissue regeneration. This approach will be particularly beneficial in the early stages of disease pathogenesis and progression. An enhanced knowledge of cartilage biology and its molecular regulation is invaluable, not only for understanding joint disorders but also for bone trauma repair. This paradigm shift will open up new avenues for targeted therapeutic strategies in diseases such as OA and IVDD. The identification of key molecular markers associated with specific chondrocyte states could lead to novel biomarkers for early diagnosis and therapeutic targets for these disorders.

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Review article

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Platelets as drivers of immunothrombosis in rheumatic diseases

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Abstract

Platelets are central players in inflammatory and thrombotic responses that drive the onset and progression of rheumatic diseases. In particular, they regulate immunothrombosis, a defence mechanism in which the immune and blood-clotting systems cooperate to contain infections or vascular damage. Although immunothrombosis can help to preserve blood-vessel integrity and promote healing, it becomes harmful when exaggerated or chronic. In rheumatic diseases, such as systemic lupus erythematosus, systemic sclerosis and antiphospholipid syndrome, immunothrombosis contributes to persistent inflammation, abnormal blood-clot formation and long-term damage to the small blood vessels. It has also been implicated in maintaining autoimmune responses to autoantigens released by neutrophils. Platelets are among the first responders to vascular injury and influence the activity of immune cells, particularly neutrophils, by promoting the formation of neutrophil extracellular traps. Platelets express proteins such as P-selectin and the damage-associated molecule high-mobility group box 1 (HMGB1), which have distinct and non-redundant roles, both via direct interactions locally at sites of vascular damage and systemically via the release of extracellular vesicles. Understanding how platelets contribute to vascular inflammation and clotting in autoimmune settings elucidates disease mechanisms and might lead to the identification of new therapeutic targets.

Sections

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Key points

- Immunothrombosis integrates innate immune defence with coagulation, when dysregulated, it sustains maladaptive immune responses, driving inflammation, thrombotic complications and pathological tissue remodelling — highlighting its relevance as a key therapeutic target.
- Platelets are central orchestrators of immunothrombosis, bridging vascular injury and immune activation in rheumatic diseases.
- Although the hallmarks of immunothrombosis are shared across rheumatic diseases, the cellular mediators and initiating pathways vary according to disease-specific inflammatory contexts.
- Pharmacological targeting of immunothrombosis holds promise not only for reducing autoimmune-driven cardiovascular risk but also for controlling chronic inflammation and limiting tissue damage.

Introduction

Platelets are traditionally recognized for their role in haemostasis, but their functions extend well beyond clot formation. They are increasingly understood as pivotal regulators of inflammation, particularly through their involvement in immunothrombosis — a coordinated process that bridges innate immune activation with coagulation[1]. During this process, platelet activation is triggered by pathogen-associated molecular patterns and damage-associated molecular patterns, which are recognized by pattern recognition receptors on platelets. This activation leads to the formation of a clot and the recruitment of immune cells, thereby linking innate immune responses to the coagulation cascade aimed at containing intravascular infections, clearing immune complexes and sustaining vessel integrity. In systemic autoimmune diseases, such as systemic sclerosis (SSc), systemic lupus erythematosus (SLE), antiphospholipid syndrome (APS) and rheumatoid arthritis (RA), platelets become hyperactivated, triggering immunothrombosis in the absence of a pathogen or infection[2–4]. This inappropriate activation leads to a state of chronic inflammation, as the immune system mistakenly responds as if a pathogenic threat were present.

Widespread immune dysregulation, which leads to microvascular injury and tissue remodelling, is a feature of many rheumatic diseases. Platelets have an underappreciated role as drivers and amplifiers of immune responses[4]. Beyond their haemostatic function, platelets interact with immune cells through surface receptors, extracellular vesicles and the release of bioactive molecules[5]. These interactions underlie neutrophil activation and the formation of neutrophil extracellular traps (NETs), exacerbate endothelial dysfunction and enhance the production of autoantibodies, which establishes a self-perpetuating cycle of inflammation and vascular damage.

Advances over the past decade have reshaped understanding of platelet heterogeneity, revealing that distinct subsets of platelets contribute to immune responses[4,6]. Although some platelets primarily contribute to haemostasis, others have immunomodulatory roles[4]. This diversity is relevant in rheumatic diseases, whereby platelet activation profiles influence disease severity and progression. In SSc, for example, platelet-derived extracellular vesicles carrying high-mobility group box 1 (HMGB1) exacerbate fibrosis and microvascular involvement[7,8], whereas in SLE, platelet interactions with immune cells fuel immunothrombosis and autoimmunity[9,10]. APS represents a paradigm of pathological immunothrombosis, in which autoantibody-driven platelet activation leads to complement activation and thrombosis[11,12]; however, direct evidence of distinct platelet subsets in these diseases is still lacking.

This Review highlights the evolving role of platelets in rheumatology, focusing on their contributions to immunothrombosis and their potential as therapeutic targets. By integrating insights from studies published in the past decade, we aim to provide a perspective on how platelet activation shapes disease outcomes in SSc, SLE, APS, RA and other rheumatic conditions. Recognizing platelets as key orchestrators of immune responses might pave the way for innovative treatment strategies in rheumatic patients that extend beyond conventional anti-thrombotic approaches.

The activation and function of platelets

Platelet release occurs through iterative fission events, during which megakaryocyte-derived proplatelets extend into blood vessels and fragment into platelets[13]. Under a steady state, megakaryocytes produce platelets mostly within the bone marrow[14,15]. A stress-responsive population of megakaryocytes emerges in aged mice, which seems to promote the production of a distinct subset of platelets that exhibit heightened sensitivity to stimuli and contribute to age-related thrombocytosis and thrombotic responses triggered by vascular injury[16,17]. During inflammation the demand for platelets increases, also triggering adaptations in megakaryocytes. Stress conditions, such as sepsis, activate alternative sites of thrombocytopoiesis, such as the spleen and lung[18–20], alongside diverse mechanisms that regulate platelet production in the bone marrow and lung[20]. Human lungs contain haematopoietic progenitor cells that exhibit a bias towards erythroid and megakaryocyte lineages within the haematopoietic progenitor tree[21]. Although only a small fraction (approximately 10%) of platelets originate from lung megakaryocytes under steady-state conditions, this proportion during thrombocytopenia, which is induced by sterile or infectious triggers, nearly doubles[21]. Although similar reprogramming of platelet production probably occurs in rheumatic diseases, driven by antibody-mediated platelet clearance and potentially by inflammatory alterations in the pulmonary microenvironment[22,23], the understanding of these processes in humans remains limited, underscoring a critical gap in current research.

Lung megakaryocytes possess immune functions, including antigen processing and presentation to $CD4^+$ T cells[23]. Distinct stem cells have been identified that use separate pathways for the generation of distinct megakaryocyte-restricted progenitors, relying on a slower, steady-state multipotent pathway alongside a fast-track, emergency-activated, platelet-restricted pathway[24]. These findings align well with the notion that stress-responsive thrombocytopoiesis programs platelets for enhanced immune interactions, for example, through adhesion mechanisms in lung-derived platelets[25] and CD40 ligand (CD40L) expression in spleen-derived platelets, rather than exclusively prioritizing haemostasis (Table 1).

The lung is a frequent and particularly vulnerable target in systemic autoimmune diseases, including SLE, SSc, RA, antineutrophil cytoplasmic antibody (ANCA)-associated small-vessel vasculitis, idiopathic inflammatory myopathies and Sjögren syndrome.

Interstitial lung disease (ILD), a frequent manifestation in systemic autoimmune diseases, is marked by inflammation and/or fibrosis of the pulmonary interstitium and can progress to irreversible fibrosis

Table 1 | Comparison of megakaryocytes from the bone marrow, lung and spleen

Feature	Bone marrow megakaryocytes	Lung megakaryocytes	Spleen megakaryocytes	Refs.
Ploidy	2N to 32N	2N to 16N	8N to 64N	19,163
Platelet production	Yes	Produce fewer platelets under steady-state conditions, but increase output in response to thrombocytopenia	Not known	14–16,20, 21,164,165
Haemostasis	Yes	Not known	Not known	14,16,19
Role in thrombosis	Yes	Yes	Not known	14,16, 19,66
Role in inflammation	Yes	Yes	Linked to acute inflammatory responses (such as sepsis)	14–19,24, 25,28,165
Role in extracellular vesicle production	Yes	Not known	Not known	166

'N' represents the number of chromosome sets.

owing to abnormal wound healing and excessive fibroblast activation[26]. Clinically apparent lung fibrosis is particularly common in patients with SSc, affecting up to 60% of individuals[27]. Although various immune mechanisms contribute to fibrosis, early fibrotic events remain poorly defined. Notably, in the bleomycin-induced model of lung fibrosis, megakaryocytes accumulate within the lung, where they promote fibroblast proliferation and trans-differentiation into myofibroblasts[28]. Whether a similar contribution of lung megakaryocytes occurs in patients with autoimmune diseases remains to be determined. Further research is warranted to clarify whether platelets derived from these lung-resident megakaryocytes[18] contribute to local fibrotic processes or systemic inflammatory responses.

Mature platelets, which have an average lifespan of 7–10 days, circulate in the bloodstream, patrolling the vasculature; platelets circulate near the vascular wall, without substantial detectable interaction with the endothelium[29]. A bundle of peripherally oriented microtubules that interact with the cytoplasmic spectrin skeleton maintain the lens-like shape of platelets[30,31]. Despite their seemingly inert appearance, non-activated platelets are integral to the maintenance of a stable intravascular environment. They preserve microvascular barrier integrity, preventing the spontaneous leakage of fluids and solutes across the endothelium, even in the absence of detectable vascular injury or inflammation[32,33]. Moreover, resting platelets generate extracellular adenosine, which is known for its anti-inflammatory properties and regulatory effects, via the action of membrane ectonucleotidase such as CD73 (refs. 34,35). Fascinatingly, platelets from long-term immobilized, hibernating brown bears, which are protected from venous thromboembolism, resist activation and display an antithrombotic signature, which includes a substantial reduction in heat shock protein 47 (ref. 36); this decrease in heat shock protein 47 can attenuate immune cell activation across various mammalian species[36].

Thus, healthy platelets dynamically adapt to organismic needs based on environmental conditions by regulating their activation threshold. Accordingly, platelets readily respond to changes in their microenvironment, maintaining lymphatic structure and supporting functional contraction capacity[37], and also seal gaps in the vascular endothelial lining during leukocyte extravasation. A failure in these actions can exacerbate microvascular damage, particularly in rheumatic diseases.

The role of platelets in vascular injury

In response to vascular injury, platelets are activated by changes in rheological forces — such as altered shear stress and disturbed blood flow — and subsequently collide with the exposed subendothelial matrix, which is primarily composed of fibronectin, laminin and fibrillar collagen[38]. von Willebrand factor (vWF), which is immobilized onto the exposed matrix, interacts with the platelet mechanoreceptor, GPIb. Under shear stress, vWF binds to the platelet GPIb–IX–V complex and to αIIbβ3 integrin (also known as GPIIb/GPIIIa). Additionally, platelets release stored moieties, including fibrinogen, and undergo a rapid metamorphosis, altering their morphology, function and lifespan to meet the physiological requirements of the vasculature[3].

Upon tethering to the subendothelial matrix, platelets undergo morphological and functional changes, including firm adhesion and spreading across the damaged vascular wall. These changes facilitate interactions with other platelets and their subsequent aggregation, which is mediated by the αIIbβ3 integrin–fibrinogen interactions, culminating in the formation of the primary haemostatic plug that prevents bleeding. Exposed anionic phospholipids provide a negatively charged scaffold for the assembly of coagulation factors. Thrombin converts fibrinogen to fibrin, reinforcing and stabilizing the developing haemostatic plug[39,40]. Activation of protease-activated receptors 1 and 4 further enhances platelet activation, spreading and contracting[41]. Integral to this process are molecules that interact with subendothelial components, including integrins and tyrosine kinase-linked receptors, such as the GPIb–IX–V complex and GPVI glycoproteins. Platelet aggregation also reflects their ability to sense sudden accelerations and decelerations in shear stress within the vessel wall. The relevance of mechanotransduction in platelet activation has been reviewed elsewhere[42].

α-granules are specialized storage organelles within platelets that undergo fusion with the plasma membrane, enabling the release of preformed bioactive mediators. These granules, which constitute 10% of platelet volume, are mobilized after the fusion of the granule membrane with the platelet open canalicular system and plasma membrane[43]. They contain molecules that are crucial for platelet haemostatic actions (such as vWF, fibrinogen and factor V), angiogenic and anti-angiogenic signals (such as vascular endothelial growth factor (VEGF), angiostatin and CXCL4 (also known as platelet factor 4)), growth factors (such as platelet-derived growth factor (PDGF)), CXCL7 (also known as β-thromboglobulin), thrombospondin and matrix metalloproteases[44]. Most molecules stored in α-granules are released into the bloodstream. Other signals that are crucial for cell-to-cell interactions, including P-selectin and CD40L, are upregulated on the surface of platelets, which ensures that activated platelets exclusively interact with and are recognized by leukocytes, enabling them to guide inflammatory responses[44].

Dense granules mostly store small molecules, including ADP, ATP and Ca^{2+}. The interaction of ADP with purinergic receptors at injury sites contributes to further activation of αIIbβ3 integrin. This paracrine

activation, in conjunction with thromboxane A2 production, cytoskeletal contraction and the recruitment of additional platelets, works synergistically to sustain and amplify platelet aggregation[43,44].

The role of platelets beyond haemostasis

Platelets serve as early responders, alongside neutrophils, at sites of inflammation[45–47]. Leukocyte extravasation damages the integrity of endothelial barriers owing to the release of proteolytic enzymes and other mechanisms of damage that remain poorly understood. This phenomenon has been consistently demonstrated across diverse models, including middle cerebral artery occlusion, cremaster muscle inflammation, experimental colitis, peritonitis, acute lung injury and various malignancies[48]. Electron microscopy studies from the 1960s showed that individual platelets localize to sites of leukocyte transmigration[49] where they can accumulate in response to vascular cues such as sensing density gradients of deposited fibrinogen[50,51]. This haptotactic process enables platelets to plug holes made at endothelial cell junctions by extravasating neutrophils, thus preventing bleeding. Moreover, platelets accumulate at sites where they can surround invading bacteria and alert the immune system[29,48,50,52].

Platelets can also prevent microvascular haemorrhage in the absence of detectable plug formation; however, their pro-coagulant properties are crucial. Fibrinogen and thrombin are recruited to the membrane of phosphatidylserine-expressing platelets, contributing to fibrin deposition and the sealing of disruptions to microvascular integrity. This process involves the release of vasoactive signals, such as angiopoietin 1, from dense granules and α-granules, which stabilizes endothelial junctions, hindering further leukocyte transmigration, and ultimately terminating the inflammatory response[53].

Leukocytes that interact with endothelial and platelet selectins traverse the vessel wall and migrate via the perivascular matrix. Interactions between P-selectin and P-selectin granulocyte ligand 1 (PSGL-1, expressed on neutrophils and monocytes) activates outside-in signalling events that lead to the transactivation of β2 integrins on leukocytes[54], which slows neutrophil rolling and facilitates adhesion to endothelial cells. Conversely, interactions between E-selectin and E-selectin ligand-1 result in the activation of the αMβ2 integrin (also referred to as Mac-1) on the leading edge of the migrating leukocyte[55,56].

These pathways work cooperatively. Neutrophils that fail to roll and adhere properly will struggle to interact properly with E-selectin and will be unable to crawl. Platelet P-selectin is necessary for neutrophil transmigration through vessel walls at sites of injury[57]. The extent, kinetics and sites of leukocyte and platelet interactions change depending on the vessel conditions and can determine the onset and termination of the vascular response. Moreover, extracellular vesicles released by activated platelets accumulate in the lymph and interact with lymphatic endothelial cells contributing to lymphatic contractility and function[37]. This observation supports the idea that platelet-derived extracellular vesicles exert protective effects at sites distant from their original locations of activation, aligning with the concept that activated platelets are integral to an 'immune continuum' that is involved in tissue repair and the maintenance of homeostasis.

Platelets as sensors of inflammation

Alongside receptors for identifying vascular defects, platelets express an array of receptors for microbial (pathogen-associated molecular patterns) or sterile (damage-associated molecular patterns) inflammatory signals. These receptors include Toll-like receptors (TLRs), Nod-like receptors and C-type lectin receptors, which can be found at various cellular locations, including the plasma membrane, endosomes and the cytosol[58]. Platelets also recognize immune complexes through Fcγ receptors, and respond to inflammatory cytokines such as IL-1β and TNF via dedicated receptors[58,59].

Thus, platelets, even in the absence of direct vascular threats, perceive and react to environmental inflammatory signals. The success of biologic agents that neutralize TNF in patients with immune-mediated diseases emphasizes the role of pro-inflammatory cytokines in chronic human diseases[59,60]. These agents have off-target effects, such as conferring protection from cardiovascular events, notable in patients with RA[61] and spondyloarthropathies[62]. TNF activates platelets independently of agonists such as collagen, ADP or thrombin receptor agonists. Conversely, TNF inhibitors used to manage synovial manifestations and prevent articular damage inhibit platelet activation, suggesting that TNF-induced platelet activation contributes to the heightened cardiovascular risk observed in people with rheumatic disease[59].

Activated platelets, if not promptly cleared from the bloodstream, can bind to fibrin and chondroitin sulfate A[63]. As a result, the platelet surface serves as a template for the deposition of immunoglobulins, irrespective of their antigen specificity, in a configuration capable of activating complement. This cascade promotes interactions with myeloid cells, ultimately propagating thrombosis[63]. Given the critical roles of antibody production and complement activation in systemic autoimmune diseases, these findings further link platelet activation, thrombosis and subsequent downstream events to the pathogenesis of rheumatic diseases.

Platelet–neutrophil interactions

Although platelets can interact with numerous different cells[44], their interactions with neutrophils are particularly frequent and biologically important, and have an important role in both physiological responses and pathological processes[64]. Platelet–neutrophil interactions occur at the earliest stages of platelet formation. Within the bone marrow, megakaryocytes reside in the perisinusoidal space, which serves as both the exit and the entry point for neutrophils entering or returning from the bloodstream[65]. Neutrophils returning from the blood to the bone marrow will migrate towards bone marrow megakaryocytes in a CXCR4–CXCL12-dependent manner and can 'pluck' proplatelets, which accelerates their shedding, a physiological process that, under conditions such as myocardial infarction, might exacerbate the production of pro-thrombotic immature platelets[66].

Moreover, live neutrophils can enter the cytoplasm of bone marrow megakaryocytes via a process called emperipolesis, which has emerged as a key contributor to platelet heterogeneity. This interaction activates megakaryocytes, prompting increased platelet production. Emperipolesis enables neutrophils to enter megakaryocytes in membrane-bound vesicles before forming membrane continuity with the demarcation membrane system, which facilitates the transfer of neutrophil-derived entire membrane domains and proteins to nascent platelets[67]. These so-called 'hybrid platelets' exhibit increased phosphatidylserine exposure, a feature linked to procoagulant and inflammatory platelet phenotypes. Emperipolesis occurs under basal conditions but is amplified considerably during haematopoietic stress and inflammatory diseases, such as myeloproliferative neoplasms and grey platelet syndrome[67–69]. Notably, inflammatory neutrophils that evade clearance by phagocytes can invade megakaryocytes via emperipolesis, promoting both platelet production and bone marrow fibrosis – an event implicated in the pathogenesis of myeloproliferative

neoplasms, including myelofibrosis[70]. The potential for megakaryocytes to selectively engage activated neutrophils based on integrin signalling raises the possibility that inflammatory conditions could favour the generation of hyperreactive platelets, which supports the concept that 'angry neutrophils make angry platelets'[67]. Further investigation into the molecular cargo exchanged during emperipolesis, and the subsequent effects on platelet function and immune signalling, might reveal novel pathways linking bone marrow inflammation to peripheral vascular pathology.

Outside the confines of the bone marrow, mature activated platelets and neutrophils often converge at sites of haemorrhage, vessel wall injuries, thrombotic occurrences and instances in which intact vessel walls undergo leukocyte trans-endothelial cell migration and extravasation, as previously discussed. The co-localization of neutrophils and platelets is not haphazard but rather orchestrated in response to 'find-me' signals. One potential source of these signals is platelet serotonin, which is released from dense granules. The serotonin metabolite, 5-hydroxyindoleacetic acid, engages the neutrophil G protein-coupled receptor (GPCR), GPR35, promoting adhesion to activated lining endothelial cells and facilitating neutrophil recruitment to inflamed tissues[71]. GPR35 expression has a pivotal role in mediating the interactions between transmigrating neutrophils and platelets[71].

Multiple signals within inflamed vessels culminate in platelet–leukocyte adhesion that sustains neutrophil activation and extravasation. The best characterized molecular interaction involves P-selectin on the platelet surface binding to the constitutively expressed PSGL-1 receptor on neutrophils and monocytes (Fig. 1). This initial tethering primes neutrophils, triggering intracellular reorganization and the redistribution of their granular contents. As a result, neutrophils begin to express bioactive molecules, such as myeloperoxidase and tissue factor, on their surface[59,72–74], a process that facilitates their extravasation. Activated leukocytes also upregulate β2 integrins, which undergo conformational changes that increase their affinity for fibrinogen. Fibrinogen is frequently presented by activated platelet αIIbβ3 integrins, which facilitates firm adhesion and promotes leukocyte–platelet interactions. These interactions lead to the formation of heterotypic platelet–leukocyte aggregates, which can be readily detected in circulating blood[75,76].

These aggregates are characteristic of various inflammatory conditions, such as SLE, APS, RA and other autoimmune diseases[64,76–79]. Besides serving as biomarkers of platelet activation[80], heterotypic aggregates propagate inflammatory vascular damage. Their recruitment to inflamed or damaged endothelium is guided by interactions with neutrophils at lower shear rates, and platelet binding to vWF or exposed extracellular matrix constituents at higher shear rates[81]. Efforts to limit neutrophil–platelet interactions and aggregate formation have yielded promising results[82].

The formation of heterotypic aggregates is just one of the possible outcomes of the interaction between neutrophils and activated platelets. Following adhesion, neutrophils that interact with platelets can either phagocytose them, sequestering them from the microenvironment and quenching their thrombogenic and inflammatory potential[8,83–85]. Alternatively, platelets can promote the production of NETs by neutrophils[45] (NETs are three-dimensional structures composed of decondensed chromatin and microbicidal moieties such as histones and proteolytic enzymes). The factors that influence neutrophils to undergo NET generation over phagocytosis are only partially understood, although a neutrophil metabolic state is probably involved[86,87].

In a seminal study, platelet activation via TLR4 was shown to drive their adhesion to neutrophils, triggering subsequent neutrophil activation and the formation of NETs[45]. TLR4-dependent platelet–neutrophil

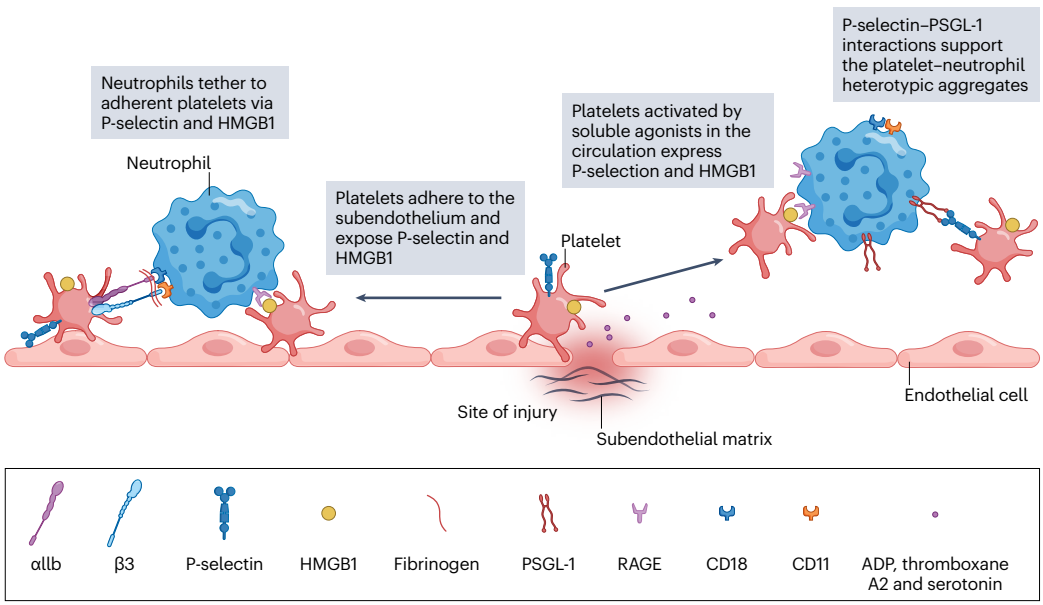

Fig. 1 | Acute microvascular injury drives the early interaction between activated platelets and neutrophils. Activated platelets respond to subendothelial components or soluble signals (such as ADP, thromboxane A2 and serotonin) by expressing P-selectin and high-mobility group box 1 (HMGB1) on their outer membrane. Extravasating neutrophils tether to platelets that adhere to the subendothelial matrix via the P-selectin–PSGL-1 axis, initiating firm adhesion through fibrinogen-mediated interactions between platelet αIIbβ3 integrin and the neutrophil CD11–CD18 receptor. Platelet HMGB1 triggers the activation of receptor for advanced glycation end products (RAGE) on neutrophils. These events promote neutrophil activation and migration into perivascular tissues. Additionally, activated circulating platelets can adhere to neutrophils, forming platelet–neutrophil aggregates.

interactions were induced by signals present in the blood of patients with sepsis, resulting in the entrapment of bacteria by NETs within the vasculature, notably in the liver sinusoids and pulmonary capillaries, through mechanisms independent of P-selectin[45].

NETs released in a regulated manner are probably protective and non-pathogenic. However, components of NETs can activate the coagulation system, which contributes to intravascular coagulation in patients with sepsis regardless of the inciting microorganism involved[88]. Interactions between H4 histone in NETs, platelets and inorganic polyphosphates have an essential role in promoting coagulation and immunothrombosis[89,90]. Strategies to target NETs in sepsis face inherent difficulties, as digesting NETs can release entrapped, potentially dangerous microbes into the systemic circulation. Interestingly, factors released from platelet α-granules, such as CXCL4, have a protective role by stabilizing NETs, which enhances microbial entrapment and limits the release of prothrombotic and cytotoxic degradation products — such as extracellular histones and DNA fragments — that can cause oxidative vascular damage[91].

Platelet signals and immunothrombosis

Immunothrombosis refers to a physiological defence mechanism whereby platelets, neutrophils and the coagulation cascade coordinate to form microthrombi that help to contain pathogens and limit systemic spread[1]. This process is tightly regulated and typically beneficial, especially in the setting of infection or acute tissue damage; however, when persistent or dysregulated, immunothrombosis gives rise to thromboinflammation, a maladaptive state in which thrombosis and inflammation reinforce one another, resulting in vascular occlusion, tissue injury and organ dysfunction[92]. Although this conceptual distinction is widely adopted, the boundary between protective and pathological outcomes is often difficult to define, particularly in rheumatic diseases, in which the same cellular and molecular mediators contribute to vascular repair and to maladaptive remodelling. For instance, thromboinflammatory responses might initially aid in restoring vascular integrity but later promote fibrosis, vasculopathy or chronic immune activation[46]. As such, both immunothrombosis and thromboinflammation should be viewed as dynamic processes whose roles, protective or pathogenic, depend heavily on context, duration and regulation. This complexity is particularly relevant in systemic autoimmune diseases, in which chronic inflammation continuously reshapes the vascular environment.

Mechanistically, the sequence of events underlying immunothrombosis can be summarized as follows: initially, platelets are activated upon receiving microbial signals, such as those encountered during sepsis, or sterile signals, both of which act through pattern recognition receptors on platelets. Platelet activation can then be further amplified by fibrin-dependent assembly of autoantibodies and complement activation, which is often observed in autoimmune diseases[63]. Activated platelets undergo degranulation, releasing the contents of their α-granules, including P-selectin, which translocates to the plasma membrane. This event is crucial for the subsequent interaction between platelets and neutrophils, setting the stage for further immune cell recruitment and establishing the foundation for thrombus formation driven by immune cell interactions[44,46,63] (Fig. 2).

In parallel, activated platelets release HMGB1, a key inflammatory mediator and a prototypic damage-associated molecular pattern. HMGB1 can be released in several forms: as a soluble entity, as a membrane-associated moiety or packed into extracellular vesicles[7,93,94]. HMGB1 amplifies the inflammatory response by promoting further platelet activation and enhancing the thrombotic response in the vasculature. It interacts with neutrophils, particularly those in direct contact with platelets, by binding to receptors such as receptor for advanced glycation end products (RAGE), triggering their activation and enhancing their inflammatory response[93–96]. Additionally, $HMGB1^+$ platelet-derived extracellular vesicles can influence distant neutrophils, conveying signals locally and propagating the thrombotic cascade.

RAGE activation upon recognition of platelet HMGB1 initiates a cascade of intracellular signalling events leading to autophagy within neutrophils, which is essential for generating the ATP required to form NETs, which in turn contribute to the development of a thrombus[89–91,95,97,98].

Platelet-derived extracellular vesicles are released during diverse forms of platelet activation and death[99]. Procoagulant platelets, marked by calcium-dependent phospholipid exposure, heightened P-selectin expression and robust extracellular vesicle shedding, are key contributors to immunothrombosis[100]. Extracellular vesicles generated under these conditions can carry distinct immunomodulatory cargo, but their functional diversity remains poorly defined. Moreover, platelet-derived extracellular vesicles include distinct subtypes such as microparticles (also known as microvesicles), which are generated by membrane budding, and exosomes that differ in size, biogenesis and cargo composition (including cytokines, nucleic acids, transcription factors, organelles and inflammatory mediators such as HMGB1). Their heterogeneity probably reflects the nature and strength of platelet activation signals. Notably, platelet-derived extracellular vesicles can traffic beyond the vascular compartment and deliver bioactive cargo to target tissues[101].

Platelets express components of the inflammasome machinery, including NLRP3 and caspase-1, which can be activated by sterile inflammatory stimuli[102,103]. Once activated, caspase-1 cleaves pro-IL-1β, which enables platelets to release IL-1β and contribute to vascular inflammation. Elucidating how platelet-induced inflammasome activation intersects with extracellular vesicle release, HMGB1 signalling and neutrophil priming might reveal novel therapeutic targets for modulating inflammation in autoimmune diseases.

Much of the current knowledge regarding platelet–neutrophil interactions stems from models that are unrelated to autoimmunity, in which immune complex formation and Fc receptor engagement are not predominant drivers of inflammation. Consequently, the extent to which autoantibodies modulate the requirement for specific adhesion molecules, signalling pathways or the temporal orchestration of cellular crosstalk remains poorly defined. To advance mechanistic insights relevant to systemic autoimmune diseases, further investigation into how immunothrombosis is regulated within disease-specific microenvironments is warranted.

Platelets in rheumatic diseases

Across rheumatic diseases, platelets function as key modulators of immune and vascular dysfunction, linking localized injury to systemic pathology. This section explores how platelet activation and crosstalk with neutrophils, endothelial cells and the innate immune system drive tissue damage, fibrosis and thromboinflammation in disease-specific contexts such as SSc, SLE, APS, vasculitis and RA. Although immunothrombosis serves as the unifying framework, each disease presents a distinct pathological scenario that is paradigmatic for understanding how platelets contribute to immune-mediated vascular injury.

Systemic sclerosis

Early and extensive microvascular activation is a hallmark of SSc, a prototypic autoimmune and fibrotic disease, with continuous platelet activation accompanying microvascular injury[104]. Transcriptome profiling has confirmed the association between platelet degranulation and activation with microvascular damage and neutrophil dysfunction[105,106].

As a result of continuous and unrestricted platelet activation, extracellular vesicles accumulate in the bloodstream, which is further amplified by the defective clearance of activated platelets[88,107]. Clearance of activated platelets by phagocytes involves an initial tethering phase, which is dependent on the P-selectin–PSGL-1 axis, followed by an internalization event that requires exposure of anionic phospholipids on the platelet plasma membrane[83]. Dysregulated expression and function of PSGL-1 in SSc has been described, and *Psgl1*$^{-/-}$ mice spontaneously develop key features of the human disease[107,108].

Circulating platelets in patients with SSc express HMGB1 on their outer membranes, alongside P-selectin[109,110]. This activation profile contrasts with those observed under conditions in which platelet activation occurs acutely, such as in response to ongoing coronary syndromes or infectious agents[111]. Despite the prominent expression of activation markers, defects in PSGL-1 seem to compromise the recognition of activated platelets, thereby limiting their clearance through interaction with professional phagocytes[8,107].

Another consequence of defective early interactions between activated platelets and phagocytes is the underrepresentation of heterotypic platelet–leukocyte aggregates in patients with SSc. This deficiency reflects the limited recognition of P-selectin on platelet membranes by phagocytes, possibly owing to intense proteolysis within plasma membrane domains. Redistribution on the neutrophil membrane of bioactive proteolytic granular enzymes, as previously identified[7,110], might reduce the expression of specific receptors.

Moreover, the unrestricted activity of transmigrating leukocytes in bystander perivascular tissues probably exacerbates vascular activation, perpetuating and amplifying the systemic vasculopathy characteristic of SSc. Extracellular vesicles released by activated platelets and other vascular cells serve as a source of bioactive HMGB1, further accelerating and amplifying widespread microvascular damage and obliteration[7,8,93]. Persistent oxidative stress, a hallmark of SSc, might further enhance the inflammatory effects of HMGB1 on extracellular vesicles, which perpetuates microvascular injury[110]. Injecting extracellular vesicles from patients with SSc into immunodeficient mice that are receptive to human cells and tissues mimics the neutrophil activation, NET formation, microvascular inflammation and lung fibrosis observed in patients with SSc. This evidence highlights platelet activation as a potential driver of disease progression in predisposed individuals, which suggests a mechanism whereby an initially localized microvascular injury propagates to systemic involvement of the microcirculation. Importantly, extracellular vesicles derived from the lungs of patients with SSc can induce experimental fibrosis when injected into the lungs of mice[112], which highlights the considerable role of tissue-generated signals that are packaged in locally produced extracellular vesicles in shaping disease outcomes.

HMGB1, carried by platelet-derived extracellular vesicles, seems to mediate their pathogenic actions, as genetic and pharmacological approaches targeting vesicle-associated HMGB1 have specifically been shown to effectively abrogate the extracellular vesicle-induced microvascular inflammation and lung fibrosis in experimental models[7,8]. These findings highlight the crucial role of platelet-derived HMGB1-expressing extracellular vesicles in amplifying and perpetuating systemic vascular damage in SSc (Fig. 3) and suggest that targeting HMGB1 could be a promising therapeutic strategy for preventing the systemic spread of microvascular injury in this disease.

Although findings from the past decade suggest that platelets generated by megakaryocytes in different tissues, such as the bone marrow versus the lung, might have distinct functional properties[101], this concept has not yet been extended to the extracellular vesicles that they release. Such an extension could be particularly relevant in SSc, in which inflammatory involvement of the lung is prominent[27]. Targeting the production, uptake or inflammatory cargo of specific extracellular vesicle subtypes, such as HMGB1 or mitochondrial components, might, therefore, represent a promising therapeutic strategy

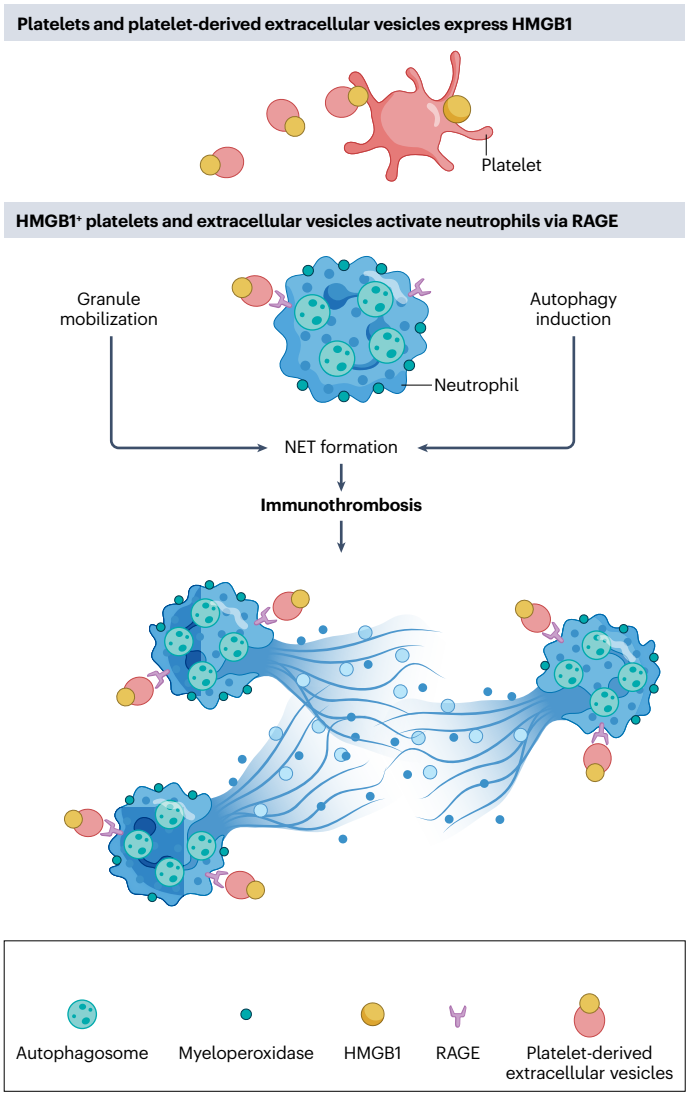

Fig. 2 | Platelet-derived HMGB1 promotes neutrophil NET formation. Recognition of high-mobility group box 1 (HMGB1), expressed by activated platelets or platelet-derived extracellular vesicles, via the receptor for advanced glycation end products (RAGE) triggers neutrophil metabolic reprogramming, autophagy and granule mobilization with the transfer of enzymes, such as myeloperoxidase, to the plasma membrane. This process ultimately promotes the formation of neutrophil extracellular traps (NETs), contributing to immunothrombosis.

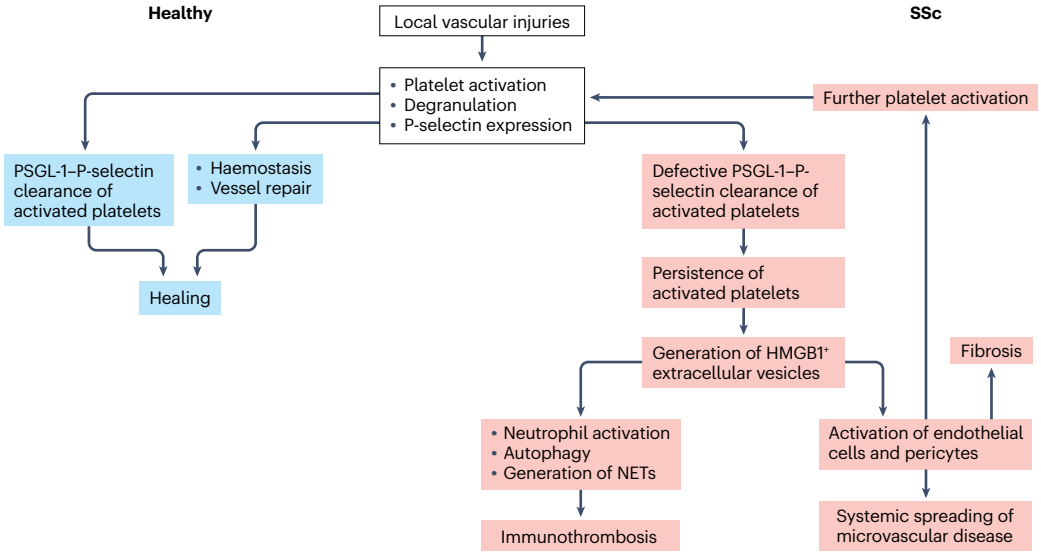

Fig. 3 | Responses to vascular injury in healthy people and patients with systemic sclerosis. In healthy people (left, blue), platelet activation after vascular injury promotes haemostasis, vessel repair and resolution through P-selectin–PSGL-1-mediated clearance of activated platelets by phagocytes. In systemic sclerosis (SSc, right, red), impaired platelet clearance leads to the persistence of activated platelets and the continuous release of HMGB1$^+$ extracellular vesicles. This process perpetuates neutrophil activation, neutrophil extracellular trap (NET) formation, endothelial damage and immunothrombosis, which contributes to vasculopathy and fibrosis. The resulting feedback loop sustains microvascular disease and platelet activation.

for limiting the progression of microvascular damage and systemic fibrosis in SSc.

Systemic lupus erythematosus

SLE is characterized by enhanced cardiovascular and atherothrombotic risks[113]. Platelets are constitutively activated in patients with SLE[9,114,115] and platelet transcriptome alterations correlate with clinical status[116], which is consistent with the observation that platelets interact with neutrophils, T cells and endothelial cells in SLE[115,117–120]. Platelet-induced neutrophil activation is particularly important because the NETs that are released during this process serve as scaffolds for immune thrombi. NETs are enriched in bona fide nuclear antigens, which are essential for the activation of autoreactive B cells and T cells, fuelling the autoimmune process central to SLE pathogenesis[117,121,122] (Table 2).

Unlike in SSc, platelet activation in SLE is associated with an increased presence of leukocytes adhering to platelets, which form circulating aggregates. Low-density neutrophils, a cell population responsible for high levels of NET generation, preferentially adhere to activated platelets[117,118]. This interaction seems to depend on platelet TLR7 and leads to enhanced generation of NETs, particularly in patients with active nephritis[118].

In patients with active SLE, neutrophils exhibit reduced expression of PSGL-1 (ref. 119). Although PSGL-1 might have a less crucial role in SLE than in SSc, impaired interactions between PSGL-1-expressing phagocytes and P-selectin-expressing activated platelets could contribute to defective platelet clearance. Notably, defective clearance mechanisms are a well-known feature of SLE, particularly in the removal of apoptotic material and cellular debris. This impaired clearance might promote the persistence of activated platelets and an increased generation of platelet-derived extracellular vesicles, as observed in SSc[8]. Indeed, reduced PSGL-1 expression is associated with enhanced concentrations of both platelet extracellular vesicles and NET fragments in people with SLE[119], and soluble PSGL-1 might represent an interesting biomarker in the clinic[123].

T cells specific for bona fide cell-associated autoantigens have dual roles in SLE, both promoting and restraining immune-mediated tissue damage. The mechanisms that regulate their clonal expansion remain poorly understood[124]. Notably, activated P-selectin-positive platelets from patients with SLE preferentially interact with regulatory T cells, and P-selectin expression also seems to be crucial for the generation of anti-double-stranded DNA antibodies and the development of lupus nephritis[120].

Platelets also interact with and activate endothelial cells in SLE, a phenomenon in which IL-1β released from platelets has a major role[115]. Other platelet-derived factors link platelet activation to core features of SLE pathogenesis, including the secretion of IFNα by plasmacytoid dendritic cells through CD40–CD40L interactions[125]. Among these factors, platelet LGALS3BP (galactoside-binding soluble 3-binding protein) has emerged as a marker of disease severity and lupus nephritis activity[126]. Importantly, type I interferons, key cytokines in SLE natural history, directly induce LGALS3BP transcription and translation in megakaryocytes[126], further implicating the platelet lineage in the dysregulated immune response that drives SLE.

Dysregulation of type I interferons and aberrant neutrophil activation are central to the vascular damage observed in SLE. Type I interferons directly damage endothelial cells and prime leukocytes, which enhances their capacity to injure the vessel lining[122]. SLE neutrophils are highly sensitive to interferon signalling and are key drivers of NET formation, which further amplifies type I interferon production by activating plasmacytoid dendritic cells[127,128].

NETs serve as DNA scaffolds for AIM2-like receptors, a family of receptors that recognize double-stranded DNA and sustain interferon signalling[129]. This interaction creates DNase-resistant nucleoprotein structures that become immunogenic and promote sustained

interferon signalling[130,131]. HMGB1 also has roles both as an amplifier of neutrophil activation and as a facilitator of neutrophil survival through autophagy[7], and also acts as a natural adjuvant when associated with DNA fragments or neutrophil enzymes such as elastase[132]. HMGB1-containing complexes can predict renal outcomes in patients with SLE, further emphasizing their importance in disease pathogenesis[132].

Antiphospholipid syndrome

APS is characterized by thrombotic, non-thrombotic and obstetric complications driven by autoantibodies that recognize complexes of anionic phospholipids and protein cofactors, such as β2-glycoprotein I (β2-GPI), which become exposed on activated endothelial cells and platelets, particularly in settings of vascular injury or cell activation. These interactions lead to immune complex formation and complement activation[11] (Fig. 4). Importantly, in APS, immune complex formation typically occurs on the cell surface, where the binding of β2-GPI to anionic phospholipids induces conformational changes that expose neoepitopes for immune recognition[11]. This localized, surface-bound mechanism contrasts with SLE, in which immune complexes more commonly form freely in the circulation.

Neutrophils rapidly detect these events and respond by generating NETs, which further amplify platelet activation and contribute to ongoing vascular damage. NETs serve as structural components in arterial coronary thrombi and are key constituents of the large thrombi formed following anti-phospholipid antibodies administration in experimental models of disease[133]. β2-GPI directly binds NETs[134], potentially enhancing autoantibody binding and further propagating inflammation and thrombosis. Anti-NET antibodies, which are observed in patients with APS, impair NET degradation, prolonging their persistence and biological actions, and correlate with recurrent thrombosis[78,135].

Genes related to complement activation, interferon pathways and NET generation are highly expressed in the kidneys of people with APS (who undergo chronic tissue remodelling), which suggests the persistent involvement of these pathways even outside acute thrombotic events, as observed in APS nephropathy[12]. Additionally, decreased ectonucleotidase activity on neutrophils and platelets in APS has been linked to enhanced extracellular nucleotide activity, which facilitates interactions between platelets and neutrophils, further promoting immunothrombosis[78].

Neutrophils in APS exhibit persistent activation and functional changes even outside critical disease phases, when thrombotic events are absent. This phenotype, associated with reduced ectonucleotidase activity, includes a metabolic shift towards glycolysis (especially in patients with a history of microvascular disease), integrin transactivation and a lowered threshold for NET generation[136,137].

Platelets exhibit persistent activation, with increased P-selectin expression, thromboxane A2 production, granule content release, extracellular vesicle generation and acquisition of a procoagulant phenotype[77,78,138]. Mechanistic studies in mouse models of APS underscore the critical role of platelet–neutrophil interactions in APS-associated thrombosis[78,135].

Although APS is characterized by recurrent thrombosis, patients experience prolonged asymptomatic periods, suggesting the presence of mechanisms that terminate pathological immunothrombosis and restore vascular homeostasis. NET degradation, complement downregulation and other resolution processes might fail in catastrophic APS, leading to thrombotic microangiopathy, the primary pathological finding of this condition, which reflects multi-organ activation of the endothelium, platelets and neutrophils[11].

Further investigation into the pathways that halt immunothrombosis in APS might provide important insights into why some patients experience extended asymptomatic periods despite ongoing serological markers of disease. Identifying these regulatory mechanisms could help to prolong asymptomatic phases and reduce clinical complications.

Table 2 | Key platelet interactions in systemic lupus erythematosus

Cells that interact with platelets	Effects in SLE	Refs.
Neutrophil	Neutrophil–platelet interactions induce immunothrombosis via NET formation, which forms a scaffold for immune thrombi and is enriched in nuclear antigens that activate autoreactive B cells and T cells	116–119, 127,128
Low-density neutrophils	Preferentially adhere to activated platelets via TLR7 on platelets, which enhances NET generation, especially in active nephritis	117,122
Regulatory T cells	Interact with platelets via P-selectin, which weakens suppression of acquired immunity (such as T cells and B cells), favouring autoimmunity and promoting anti-dsDNA antibody production and nephritis	120,124
Endothelial cells	Platelets activate endothelial cells and promote vascular damage in SLE, partly via IL-1β release by activated platelets	115
Plasmacytoid dendritic cells	Platelets interact with pDCs via CD40–CD40L, which stimulates IFNα production and amplifies immune dysregulation. Platelet-derived LGALS3BP, which is upregulated in SLE, is linked to increased interferon activity and disease severity	125,126
Monocytes and macrophages	Defective clearance of activated platelets by monocytes and macrophages leads to sustained platelet activation and accumulation of platelet-derived extracellular vesicles	8,119

dsDNA, double-stranded DNA; NET, neutrophil extracellular trap; pDCs, plasmacytoid dendritic cells; SLE, systemic lupus erythematosus; TLR7, Toll-like receptor 7.

Vasculitis

In addition to systemic autoimmune diseases characterized by microvascular involvement, the interaction between platelets and neutrophils, which culminates in immunothrombosis, has a crucial role in vasculitis. Byproducts of NETs accumulate in the plasma of patients with ANCA-associated small-vessel vasculitis[139], and this accumulation correlates with clinical severity. Additionally, the degradation of NETs – a mechanism that restricts their bioactivity – is impaired, and anti-NET antibodies have been described in people with ANCA-associated small-vessel vasculitis. The biological effects of NETs seems to be twofold: they might modulate the immunogenicity of specific neutrophil antigens in the context of vascular inflammation and necrotic damage, but they might amplify and sustain this process, which drives vascular injury[140].

Platelets are activated in patients with ANCA-associated small-vessel vasculitis and can directly trigger the generation of NETs through a mechanism involving the release of CXCL4 (ref. 141). The clinical relevance of this interaction, particularly in relation to small-vessel and organ involvement, such as the lungs and kidneys, warrants further

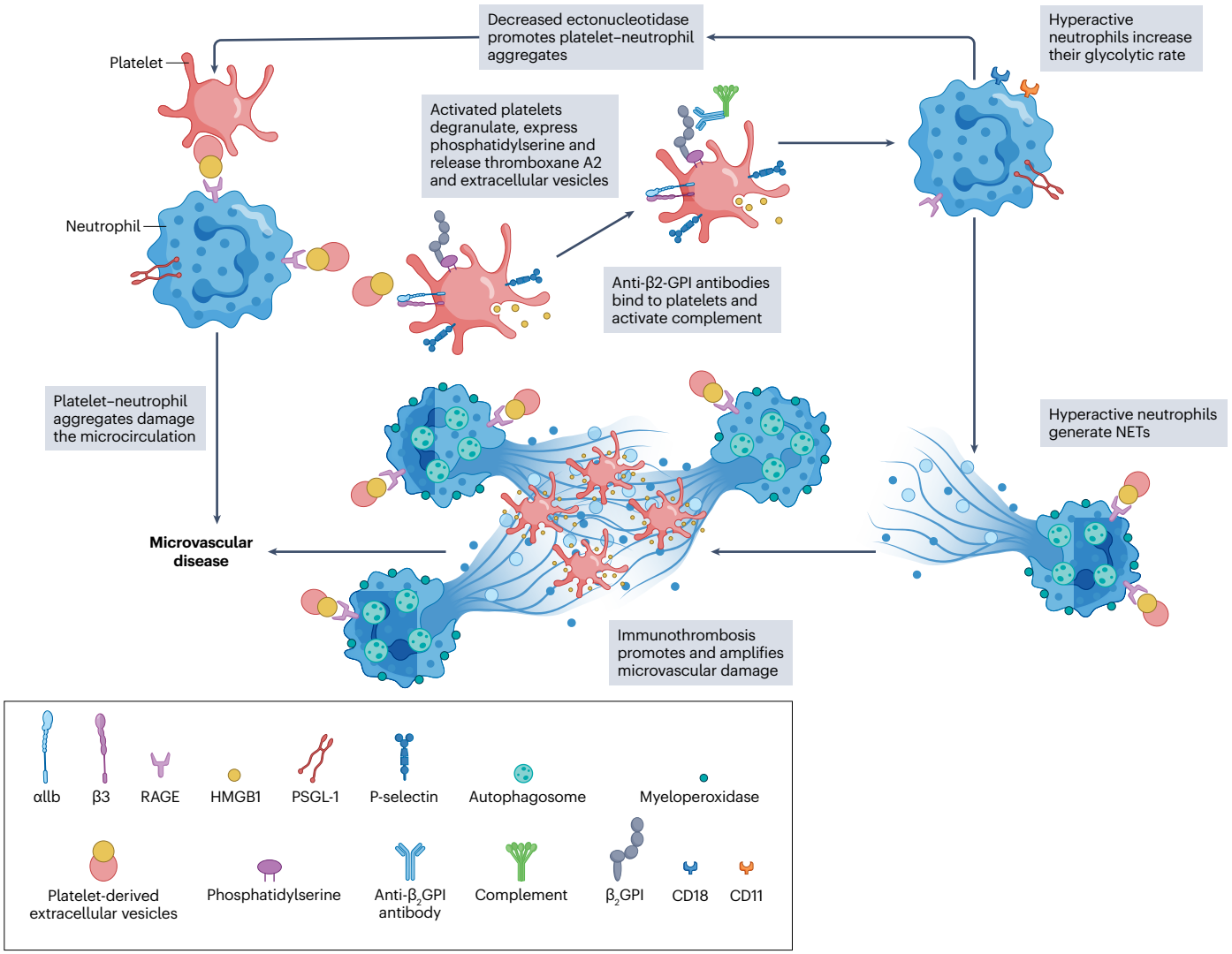

Fig. 4 | Mechanisms that link platelet activation and immunothrombosis in antiphospholipid syndrome. In antiphospholipid syndrome (APS), platelet activation induces degranulation, thromboxane A2 release and extracellular vesicle generation. Phosphatidylserine on platelet surfaces serves as a scaffold for β2-glycoprotein I (β2-GPI) binding, enabling recognition by anti-β2-GPI autoantibodies and subsequent complement activation. Neutrophils, which are hyperactivated and metabolically reliant on glycolysis, form aggregates with platelets and generate neutrophil extracellular traps (NETs). These interactions drive acute immunothrombosis and chronically sustain microvascular injury. HMGB1, high-mobility group box 1; RAGE, receptor for advanced glycation end products.

investigation. Notably, pharmacological inhibition of neutrophil cathepsin C, a key signal responsible for activating neutrophil serine proteases, has proven effective in experimental models of ANCA-associated vasculitis[142]. This intervention not only suppresses NET production but also limits the clinical severity of the disease[142]. Intriguingly, similar strong evidence that links NET generation to neutrophil inflammatory priming, potentially triggered by activated platelets, has been observed in patients with large-vessel vasculitis, such as giant cell arteritis and Takayasu arteritis[143,144]. This is particularly notable given that these diseases lack the widespread microvascular inflammation or systemic endothelial activation typically required to support neutrophil priming and NET formation. Notably, the physical disruption of the vasa vasorum by infiltrating leukocytes, including neutrophils, has an important role in these diseases and might serve as a portal of entry for autoreactive lymphocytes that infiltrate the vessel wall and reach the adventitia[145]. Indeed, neutrophils are consistently present in the temporal arteries of patients with giant cell arteritis, comprising a considerable proportion of total infiltrating cells throughout the arterial wall. However, NETs are only observed in the adventitial regions adjacent to the vasa vasorum, sites where neutrophils might still interact productively with other blood constituents, particularly platelets[146].

Rheumatoid arthritis

Other biological fluids apart from blood can provide an environment that sustains the productive interaction between platelets and neutrophils. A notable example is in RA, in which synovial remodelling

drives the disease process. Neutrophil accumulation within the synovial fluid generates reactive oxygen species, the release of proteases and NET production, all of which contribute to the establishment and maintenance of the synovial pannus[147].

Importantly, the stimuli that promote the initial activation of neutrophils in the synovium are still poorly understood. The early role of platelets in the RA synovium, which is reviewed elsewhere[148], has been known for several years. In a seminal study, the authors traced bioactive platelet-derived extracellular vesicles in RA joints and found that platelets can recognize collagen moieties via glycoprotein VI, which subsequently triggers platelet activation and extracellular vesicle generation[149]. Intriguingly, platelets seem to be directly responsible for the permeability of the synovial vessels, which might facilitate the establishment of the synovial pannus[150] and the selective access of platelet-derived extracellular vesicles, but not extracellular vesicles from activated leukocytes, to the lymphatic circulation that drains the synovial tissue[151].

Box 1 | Targeting immunothrombosis in rheumatic diseases

Antiplatelet strategies beyond cardiovascular risk management

- P2Y12 inhibitors (such as clopidogrel and ticagrelor) reduce platelet–leukocyte interactions and IFNα signalling, highlighting platelet activation as a driver of systemic lupus erythematosus progression.
- Ongoing clinical trials are investigating the potential of clopidogrel in systemic sclerosis (NCT05098704).
- The addition of ticagrelor to methotrexate is effective in severe rheumatoid arthritis.

Inhibition of platelet-driven neutrophil activation

- Targeting P-selectin and its ligand (using agents such as crizanlizumab) and high-mobility group box 1 (HMGB1) blockers to prevent neutrophil extracellular trap (NET) formation.

Modulation of neutrophil function and NET formation

- Low-molecular-weight heparin reduces NET formation by interfering with neutrophil autophagy.
- Modulating neutrophil reactive oxygen species production and PAD4 activity might further reduce NET generation.

NET dismantling

- Heparin and DNases actively degrade existing NETs.

Coagulation modulation and factor X inhibition

- Factor Xa inhibitors show coagulation-independent benefits, attenuating immunothrombosis and protecting tissues via regulation of platelet granule release and modulation of neutrophil reactivity during maturation.

Targeting downstream NET pathways

- Strategies focusing on complement activation and cytokine modulation to mitigate immunothrombosis.
- IL-6 receptor inhibition (such as tocilizumab) has shown promise in reducing NET-mediated damage.

Given the evidence that platelet–neutrophil interactions promote NET formation, it is tempting to speculate that platelet activation, elicited in response to the recognition of collagen moieties, causes localized immunothrombosis within the rheumatoid joint, eventually leading to NET generation.

In addition to contributing to important features of RA, such as joint damage, osteoclast activation and synovial angiogenesis[152], NETs provide an important source of post-translationally modified chromatin, including both citrullinated and carbamylated histones. These modifications render NETs a preferential substrate for the generation of autoantibodies. Anti-citrullinated protein antibodies (ACPAs) are a hallmark of RA and are associated with disease severity, whereas aberrant immune responses to carbamylated antigens in RA have been linked to osteoclast activation and erosive bone damage[153].

Platelets express the molecular machinery required for protein translational and post-translational modifications, including the key enzyme, PAD4 (ref. 154). Thus, platelet proteins undergo citrullination, and can thus be recognized by ACPAs from RA sera and synovial fluids, leading to further platelet activation[154]. Conversely, the expansion and accumulation of subsets of neutrophils in the peripheral blood that are prone to generating NETs seem to be a consistent feature of RA flares. These 'rogue' neutrophils might be recruited in response to endogenous alarmins, and potentially release NETs that promote immunothrombosis through interactions with platelets via TLR4-mediated signalling[155].

Further investigation is needed to determine whether platelet-induced neutrophil activation that leads to immunothrombosis specifically interferes with physiological inhibitory mechanisms — such as neutrophil clearance, DNase-mediated NET degradation, complement regulation or anti-thrombotic signalling — and to explore whether overlapping regulatory pathways help to limit the potential threats to organismal homeostasis associated with this process.

Immunothrombosis as a therapeutic target in rheumatic diseases

Immunothrombosis is a consistent feature in persistent, self-sustaining rheumatic diseases and has a crucial role not only in the heightened cardiovascular risk associated with these conditions but also in tissue remodelling and organ damage. Targeting immunothrombosis might therefore improve therapeutic strategies for many rheumatic diseases.

Numerous efforts aimed at targeting immunothrombosis in rheumatic diseases are ongoing (Box 1), including the assessment of antiplatelet strategies beyond cardiovascular risk management. For example, platelet–leukocyte interactions and IFNα-induced gene expression are elevated in megakaryocytes and platelets in SLE, and P2Y12 inhibition (a platelet-inhibiting therapy) alone is sufficient to normalize these alterations[156]. The central role of IFNα in SLE pathogenesis highlights the therapeutic potential of targeting the megakaryocyte–platelet axis in systemic autoimmune diseases such as SLE[156].

A clinical trial evaluating the potential efficacy of clopidogrel, a P2Y12 inhibitor commonly used as an antiplatelet agent to prevent arterial thrombosis, in modifying the natural history of SSc is currently ongoing (NCT05098704). Furthermore, the addition of ticagrelor, another P2Y12 inhibitor that also inhibits adenosine reuptake, to methotrexate therapy has shown increased benefits in patients with severe RA compared with methotrexate alone[157], an effect that could be caused by the dual action of this inhibitor. These mechanisms can

Glossary

α-granules
Platelet granules that contain haemostatic proteins (such as fibrinogen and von Willebrand Factor), growth factors, angiogenic signals and adhesion molecules (such as P-selectin).

αIIbβ3 integrin
Also known as GPIIb/GPIIIa. Mediates platelet aggregation by binding fibrinogen and contributing to clot formation.

Dense granules
Store small molecules such as ADP, ATP, serotonin and calcium ions, which contribute to platelet aggregation and activation.

Emperipolesis
A cellular process in which one living cell (such as a neutrophil) actively enters and resides within another cell (such as a megakaryocyte) without being destroyed. In the context of haematopoiesis, emperipolesis contributes to platelet heterogeneity by enabling the transfer of membrane components from neutrophils to developing platelets.

GPIb–IX–V complex
A mechanoreceptor that interacts with von Willebrand Factor on the subendothelial matrix during platelet adhesion.

High-mobility group box 1
(HMGB1). A damage-associated molecular pattern released from platelets that amplifies inflammation and neutrophil activation in immunothrombosis.

Immunothrombosis
A physiological process in which components of the innate immune system and coagulation system, including platelets and neutrophils, cooperate to form intravascular microthrombi, which help to contain pathogens and limit their systemic spread.

Iterative fission events
A stepwise process by which megakaryocytes produce platelets, involving the repeated extension and fragmentation of proplatelet projections into the bloodstream to generate mature platelets.

ST-segment elevation myocardial infarction
(STEMI). A type of acute myocardial infarction characterized by persistent ST-segment elevation on electrocardiography, indicating complete or prolonged occlusion of a coronary artery.

Thrombocytopoiesis
The biological process by which platelets are produced from megakaryocytes, primarily in the bone marrow but also in other tissues such as the spleen and lungs during stress or inflammation.

Thromboinflammation
A pathological state that results from dysregulated or excessive immunothrombosis, in which inflammation and thrombosis amplify one another, leading to tissue damage, vascular dysfunction and organ injury.

Thromboxane A2
Promotes platelet activation, aggregation and vasoconstriction.

Von Willebrand factor
(vWF). Binds GPIb to mediate platelet adhesion under shear stress.

be difficult to disentangle, as adenosine also acts as a platelet agonist under certain conditions.

Agents that selectively inhibit platelet-driven neutrophil activation, including those that target P-selectin (such as crizanlizumab) or block HMGB1, might prevent NET generation. Moreover, therapies that modulate neutrophil metabolism, such as low-molecular weight heparin, can reduce NET formation[158]. Modulating the generation of neutrophil reactive oxygen species and key enzymes involved in NET formation, such as PAD4, might also interfere with NET production. Notably, PAD4 inhibitors show promise for reducing the generation of extracellular traps from other cells, such as eosinophils[158], whereas both heparin and DNases can actively dismantle existing NETs[159]. Additional approaches include factor X inhibition and strategies targeting downstream pathways activated by NETs, such as complement activation and cytokine release[12,160–162]. Evidence from a 2025 study indicates a coagulation-independent role of chronic factor Xa inhibition[160], which attenuates immunothrombosis and protects tissues by influencing platelet expression of molecules that control α-granule and dense granule release and by modulating the reactivity of maturing neutrophils[160,161].

Results from the ASSAIL-MI study emphasize the potential of reducing neutrophil activation and NET formation as a key mechanism underlying the improvement of myocardial salvage in patients with ST-segment elevation myocardial infarction (STEMI) through IL-6 receptor inhibition with tocilizumab[162]. In this condition, NET generation has a crucial role in determining the clinical outcome of the vascular event[93].

Although promising results have been obtained thus far — both preclinically and clinically — using these strategies as single interventions, combining therapeutic approaches in diseases in which immunothrombosis has a role might lead to better outcomes. Such combinations could not only reduce thromboembolic risks but also exert broader beneficial effects, such as limiting tissue remodelling and potentially preventing organ damage in patients with rheumatic diseases; however, careful consideration is needed when using combination treatments, as they might synergistically increase the likelihood of adverse effects. These risks could range from an elevated risk of bleeding, particularly when anticoagulants and antiplatelet agents are used together, to increased susceptibility to infections when immunosuppressive agents are involved. Striking the right balance between efficacy and safety is crucial for the optimization of combination therapies for immunothrombosis, ensuring maximal therapeutic benefit while minimizing complications.

Future directions

In this Review, we have explored the relationship between platelet activation and systemic rheumatic diseases. Immunothrombosis, a specialized form of thrombosis, is an essential physiological response to infections and tissue injury; however, when dysregulated, this process can contribute to microvascular injury and maladaptive tissue remodelling. Platelets, as guardians of vascular integrity, interact with immune cells, notably neutrophils, amplifying inflammatory cascades that promote thrombosis and tissue damage.

In systemic autoimmune diseases, persistent platelet activation caused by different events amplifies vascular injury and sustains autoimmunity. Platelet-derived extracellular vesicles might have an essential role in transforming local injuries in self-maintaining conditions to

systemically activate the microcirculation and circulating leukocytes. One of the most striking advances in the past 10 years has been the recognition of heterogeneity within megakaryocytes and their derived platelets, suggesting that platelet subpopulations might differentially contribute to disease pathogenesis; however, direct evidence linking this heterogeneity to specific disease mechanisms in rheumatic conditions is lacking. Understanding how platelets contribute to both the initiation and resolution of immunothrombosis could lead to novel therapeutic approaches that balance immune protection with the prevention of pathological thrombosis. At the same time, it is important to consider that platelets also contribute to tissue repair and vascular homeostasis, and their complete inhibition might inadvertently impair these protective functions.

The latest research underscores the importance of platelet–leukocyte interactions, particularly through the P-selectin–PSGL-1 axis, and the role of platelet HMGB1 and NET constituents in driving the immune-thrombotic response. Notably, targeted therapies have shown promise in modulating these pathways, offering potential strategies to mitigate platelet-mediated inflammation. However, much remains to be explored in terms of the molecular mechanisms underlying these processes, especially in the context of chronic human inflammation and immune dysregulation. The interplay between platelet activation and the immune system in the context of immunothrombosis raises several questions regarding the bidirectional influences that exacerbate rheumatic diseases. How platelet-mediated signalling cascades modulate immune cell recruitment and activation — and how chronic inflammation alters platelet function — remains incompletely understood. Further studies are needed to elucidate these dynamics, particularly in the context of disease-specific microenvironments such as the inflamed synovium in RA or damaged microvasculature in SSc.

Although much of the existing literature on platelet–neutrophil interactions in inflammation stems from research outside the specific context of autoantibody-driven diseases, the role of Fc receptors in neutrophil and platelet activation is well documented, particularly in autoimmunity[121]. Fc receptors, especially in autoantibody-driven diseases, contribute considerably to platelet activation and inflammatory responses[44]; however, platelets from mice lack Fc receptors, which limits the translatability of many findings from mouse models to human autoimmunity. Future research should address this gap and focus on identifying biomarkers for early detection and monitoring of platelet activation in autoimmune diseases, with an emphasis on extracellular vesicles, HMGB1 and other molecules implicated in the regulation of the immune response. Understanding how platelets contribute to both the initiation and resolution of immunothrombosis could lead to novel therapeutic approaches that balance immune protection with the prevention of pathological thrombosis. Furthermore, investigating the potential for personalized treatment strategies based on platelet activation profiles could enhance the management of systemic rheumatic diseases and related thrombotic complications.

Conclusions

Platelet activation is a double-edged sword in immunothrombosis, fuelling thrombosis and inflammation at the core of autoimmune diseases. Beyond driving cardiovascular risk, dysregulated immunothrombosis sustains chronic inflammation, vascular damage and fibrosis, revealing an intricate interplay between platelet hyperactivity, neutrophil reprogramming and NET generation. These mechanisms offer a unique lens for dissecting how immune dysfunction translates into progressive tissue injury.

Therapeutic intervention is no longer a question of if but how to best disrupt this pathogenic loop. Numerous promising therapeutic strategies are under investigation, including P2Y12 inhibitors, P-selectin inhibitors, HMGB1 blockers, low-molecular-weight heparin, PAD4 inhibitors and cytokine and complement inhibition; however, the challenge of targeting immunothrombosis without tipping the balance towards excessive bleeding or infection remains.

A paradigm shift is underway; targeting immunothrombosis is no longer just about reducing thromboembolic risk, it is about halting autoimmune-driven organ damage. The future of rheumatic disease management lies in striking this delicate equilibrium, unlocking novel strategies for disease modification and prevention.

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Perspective

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CAR T cell therapy for children with rheumatic disease: the time is now

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Abstract

Initial success with B cell-targeted chimeric antigen receptor (CAR) T cells for the treatment of systemic lupus erythematosus and other rheumatic diseases has generated enthusiasm for the broad application of this technology outside of the field of oncology. Paediatric patients with severe rheumatic diseases require lifelong therapy with a substantial toxicity burden and a high cost of care. Paradigm-shifting treatments, including CAR T cells, are desperately needed. Although CAR T cell therapy shows promise for paediatric rheumatic diseases, there are unique aspects of care compared with adults, which require careful consideration and expertise. In response, we established the Integrated Multidisciplinary Paediatric Autoimmunity and Cell Therapy (IMPACT) working group, comprising international experts in the fields of paediatric rheumatology, oncology and cellular therapy, immunology and nephrology, to address the challenges of introducing cell therapies to patients with paediatric-onset autoimmune diseases. Given the possible benefits, we advocate for the study of CAR T cells in paediatric patients with rheumatic diseases who carry a lifelong risk of morbidity and mortality from chronic illness and medication toxicity. As this patient population is relatively small, consensus around definitions of success, robust study of predictors of response and uniform assessment and reporting of toxicities are critical to advancing the field.

Sections

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Introduction

Chimeric antigen receptor (CAR) T cells are immune effector cells engineered to express a synthetic receptor combining an antigen-binding extracellular domain with an intracellular T cell stimulatory domain. CAR T cell potency is best demonstrated by the success of CD19-targeting CAR T cells (anti-CD19 CAR T cells) for adult B cell non-Hodgkin lymphoma and paediatric and adult B cell acute lymphoblastic leukaemia (ALL), whereby a single dose can achieve a complete response in up to 85% of patients[1–4]. The use of CAR T cells for adult and paediatric oncological indications thus far has led to a growing understanding of CAR T cell biology, efficacy and toxicities (such as cytokine release syndrome (CRS) and immune cell-associated neurotoxicity syndrome (ICANS)). B cell aplasia (also referred to as on-target, off-tumour B cell ablation) that results from anti-CD19 CAR T cells led to the hypothesis that this therapy could be leveraged to treat B cell-mediated rheumatic diseases, including systemic lupus erythematosus (SLE), idiopathic inflammatory myopathies, systemic sclerosis, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and other B cell-mediated autoimmune diseases[5–9].

With this rationale, the potential of this approach was first demonstrated in 2019 with the effective treatment of a mouse model of lupus[10,11]. In 2024, the largest case series to date of anti-CD19 CAR T cell therapy for patients with rheumatic diseases was published. Müller et al.[12] described the feasibility, safety and efficacy of anti-CD19 CAR T cells in patients with SLE ($n = 8$, five of whom had paediatric-onset disease), idiopathic inflammatory myopathies ($n = 3$) and systemic sclerosis ($n = 4$), all of whom were refractory to multiple lines of therapy, including 8 out of 15 individuals previously treated with rituximab[12]. All patients achieved disease remission or major clinical response per validated disease criteria, accompanied by B cell aplasia lasting an average of 4 months (median follow-up period 15 months, ranging from 4 to 29 months). Importantly, all patients were able to completely discontinue immunosuppressive therapy at the time of the final follow-up.

Similar to their adult counterparts, the pathogenesis of paediatric-onset SLE (pSLE), juvenile dermatomyositis (JDM), juvenile systemic sclerosis (jSSc) and paediatric AAV (pAAV) are characterized by B cell dysfunction, suggesting that B cell-targeting CAR T cells might be an effective treatment strategy[13–16]. Although B cell depletion with the anti-CD20 monoclonal antibody, rituximab, can be effective in subsets of patients with paediatric rheumatic diseases, drug-free remission remains rare. In pSLE, various case series suggest that rituximab is primarily used for refractory lupus nephritis, with potential benefit for some patients that partially reflects the outcomes described in adults with lupus nephritis[17,18]. The Rituximab in Myositis trial, which included patients with JDM, did not meet its primary end point; however, post hoc analyses suggested that paediatric-onset disease and other patient factors (such as antibody subsets) might predict efficacy, and paediatric rheumatologists frequently report the use of rituximab for refractory JDM[19–21]. The efficacy of rituximab in pAAV seems to be similar to that of cyclophosphamide, which was expected based on studies of adult AAV[22,23]. As the depth and duration of B cell and plasmablast depletion have been shown to predict response to rituximab[24], it is reasonable to hypothesize that a more potent 'reset' of the B cell compartment with anti-CD19 CAR T cells might be more effective[25,26].

The first paediatric patients (12–15 years old) with rheumatic disease treated with anti-CD19 CAR T cells have now been reported with a similar safety profile and encouraging outcomes[27–29]. Consistent with the Müller study, all three paediatric patients with lupus achieved drug-free remission after CAR T cell therapy, including a patient with rapidly progressing lupus nephritis requiring haemodialysis who achieved dialysis-free remission[27,28]. Similarly, a 12-year-old patient with JDM treated with CAR T cells showed major improvement in skin disease, normalization of muscle strength, resolution of MRI-detected myositis and improvement in calcinosis and was able to discontinue immunosuppressive medications[29]. Adverse effects in paediatric patients have mirrored adult patients, with only grade one CRS and ICANS reported[27–29].

Although the use of CAR T cells in adults with rheumatic diseases has been reviewed in the literature, there is a paucity of focus on paediatric patients[25,30–32]. We formed the Integrated Multidisciplinary Paediatric Autoimmunity and Cell Therapy working group, bringing together international experts in paediatric rheumatology, oncology and cell therapy, immunology and nephrology to address the challenges of applying cell therapies in paediatric autoimmunity (Fig. 1). Here we review the rationale supporting CAR T cell therapy for B cell-mediated paediatric rheumatic diseases, focusing on pSLE, JDM, jSSc and pAAV. We describe the unique aspects of CAR T cell therapy implementation and clinical trial considerations in these paediatric populations (Box 1).

Unmet needs in paediatric rheumatic diseases

Paediatric patients with rheumatic diseases endure a lifelong burden of disease that is associated with progressive accrual of organ damage, both from their disease and from long-term immunosuppressive treatments and substantial associated toxicities. Compared with patients who experience disease onset during adulthood, children with rheumatic diseases often exhibit more severe disease presentations with high disease and treatment-related morbidity and mortality. For example, patients with pSLE have higher rates of nephritis, neuropsychiatric disease and a higher standardized mortality ratio (relative to healthy individuals) than their adult-onset SLE counterparts, and patients with JDM suffer more frequent vasculopathic complications (such as intestinal perforation)[33–39].

Frequent flares and chronic, refractory disease activity are well described in many patients with pSLE, JDM and pAAV, whereas jSSc often follows a progressive course despite intensive treatment[40–44]. Moreover, disease often persists into adulthood, with an ongoing risk

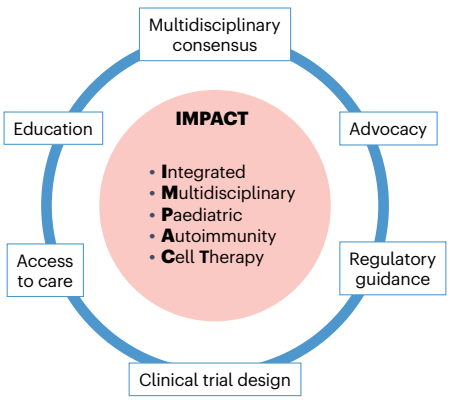

Fig. 1 | The Integrated Multidisciplinary Paediatric Autoimmunity and Cell Therapy (IMPACT) working group. The IMPACT working group currently comprises paediatric specialists in the fields of rheumatology, oncology and cell therapy, nephrology and immunology. In the future we plan to expand our group to include additional disease-related subspecialties (such as pulmonology) and patient and caregiver partners.

of disease flare, progressive organ damage and medication-related adverse effects[36,45–50]. Onset of rheumatic diseases in childhood also increases the risk of irreversible organ damage compared with adult-onset disease; for example, patients with pSLE and pAAV are at a higher risk of end-stage renal disease, which is particularly relevant given the 5-year mortality rate of 22% in lupus nephritis with end-stage renal disease[51]. Similarly, patients with JDM develop calcinosis more frequently than their adult counterparts, and patients with jSSc develop relentless skin and/or lung fibrosis, severe gastrointestinal smooth muscle dysfunction and digital pulp ischaemia[47,52–65].

Damage accrual also occurs from toxicity that is associated with aggressive immunosuppressive therapy, such as high-dose corticosteroids and cyclophosphamide, which have deleterious effects on growth, bone health, cardiometabolic health, fertility and protective immunity[47,52]. As patients accumulate increasingly severe medication toxicity, treatment intensity might need to be decreased, and long-term disease control becomes more difficult to maintain (Fig. 2). Therefore, early interventions that achieve drug-free remission in paediatric patients with rheumatic diseases would improve outcomes considerably and reduce associated morbidity and mortality.

These lifelong challenges have serious implications for quality of life and psychosocial well-being. Multiple studies have demonstrated a high burden of mental health disorders in pSLE and JDM, with persistence of depression, anxiety and stress even during disease quiescence[66–70]. Compared with their unaffected counterparts, children with rheumatic diseases consistently exhibit diminished health-related

Box 1 | Key considerations for CAR T cell therapy in paediatric rheumatic diseases

Unmet needs in paediatric rheumatic diseases

- Paediatric-onset rheumatic diseases present with a more severe phenotype than adult-onset diseases.
- Paediatric patients with rheumatic diseases experience high cumulative medication toxicity and drug-free remission is rare.
- Psychosocial burdens of paediatric-onset rheumatic disease include mental health comorbidities and diminished health-related quality of life, which often persist even when disease is well controlled.
- B cell-directed chimeric antigen receptor (CAR) T cell therapy has shown promise in adults with rheumatic diseases and is expected to provide similar efficacy in paediatric-onset rheumatic diseases including paediatric-onset systemic lupus erythematosus, juvenile systemic sclerosis and paediatric anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis.

Unique considerations for paediatric patients with rheumatic diseases

- Delivery of CAR T cell therapy requires collaborative, multidisciplinary teams, including paediatric rheumatic disease-specific experts and immune effector cell therapy specialists.
- Access to CAR T cell therapy might be challenging owing to the distance from experienced paediatric tertiary care centres.
- As paediatric patients with rheumatic diseases might have a high genetic load and/or monogenic driver mutations that are associated with early-onset, severe phenotypes, the role of genetic moderators in CAR T cell therapeutic outcomes requires special attention.
- Compared with adults, differences in T cell repertoire and humoral immunity in paediatric patients with rheumatic diseases could affect CAR T cell efficacy and short-term and long-term risk of infection related to B cell aplasia and hypogammaglobulinaemia.
- Children and adolescents with rheumatic diseases receiving CAR T cells require tailored vaccination strategies that account for pretreatment immunization status as well as post-treatment seroprotective status and immune reconstitution.
- Safety assessment of CAR T cell therapy in paediatric patients with rheumatic disease should consider fertility preservation and related concerns, secondary malignancy risk, infectious risk and disease-specific organ toxicity.

Clinical trial considerations

- International consensus on paediatric rheumatic diseases response criteria, clinical trial endpoints and desired time to achievement of response are needed to ensure the comparability of CAR T cell studies.
- Engaging paediatric patients with rheumatic diseases and their caregivers to define and select clinical endpoints and patient-reported outcomes will enhance feasibility, meaningfulness and the effect of CAR T cell therapy clinical trials.
- Paediatric-specific studies of immunobiology correlatives (such as final CAR T cell product characteristics, CAR T cell dose and cellular kinetics) are needed to identify predictors of safety and efficacy.
- Incidence and predictors of paediatric rheumatic disease flares after CAR T cell therapy, and the role of CAR T cell redosing warrant further study.
- Uniform assessment, grading and reporting of CAR T cell toxicities such as cytokine release syndrome, immune effector cell-associated neurotoxicity syndrome, immune effector cell-associated haematotoxicity and immune effector cell-associated haemophagocytic lymphohistiocytosis-like syndrome should be adopted, based on findings from paediatric oncology, adult oncology and rheumatology populations.

Regulatory considerations

- The risk–benefit assessment of CAR T cell therapy in paediatric patients with rheumatic diseases should include the long-term morbidity and mortality associated with chronic organ damage and cumulative toxicity from standard-of-care immunosuppressive regimens.
- Given the high unmet needs of paediatric patients with rheumatic diseases, early inclusion of this population in CAR T cell therapy trials is warranted (such as adaptive designs with sequential enrolment of adolescents followed by younger patients).
- Extrapolation of efficacy and safety data from adult rheumatic disease CAR T cell trials warrants consideration given known similarities of adult reference and paediatric target populations.
- Ongoing pharmacosurveillance and patient safety programmes will be needed to ensure the identification of emergent or late toxicities in real-world settings.

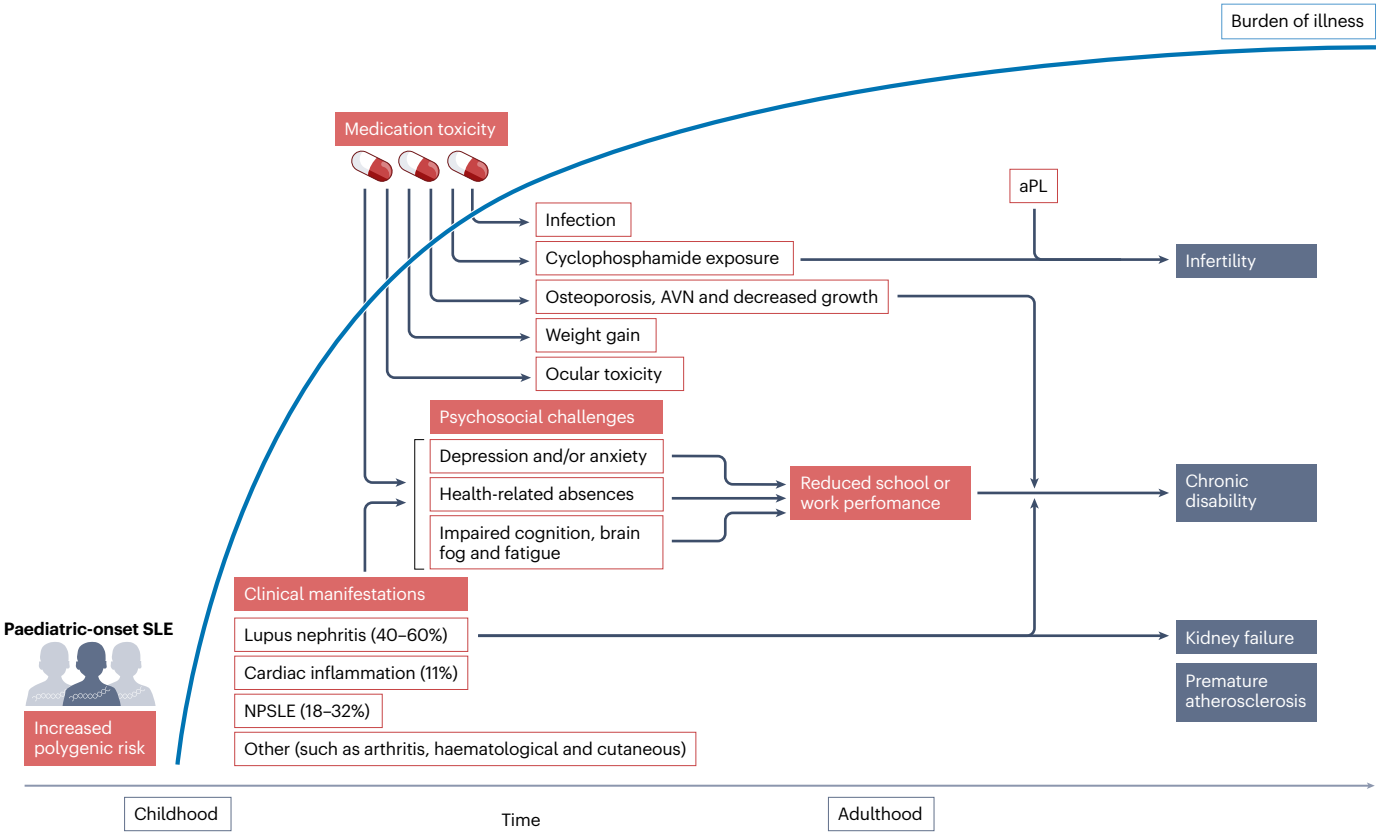

Fig. 2 | Paediatric-onset systemic lupus erythematosus as an example of the burden of paediatric-onset rheumatic disease. Paediatric-onset rheumatic disease might be associated with earlier and more severe presentation in the setting of increased polygenic risk (that is, genetic load). During critical periods of growth, development and education, paediatric patients with rheumatic diseases experience more severe clinical features relative to adult-onset disease, shared and unique medication toxicities and associated psychosocial burdens that cause substantially worse health outcomes over the life course. Early intervention with chimeric antigen receptor T cell therapy could dramatically improve disease outcomes for a select population of paediatric patients with rheumatic diseases, but clinical trials are needed to assess efficacy and safety. aPL, antiphospholipid antibodies; AVN, avascular necrosis; NPSLE, neuropsychiatric systemic lupus erythematosus.

quality of life and global health status, attributable to the combined effects of active disease, organ damage and medication toxicities[44,71–76]. These factors are associated with worse academic and adult social role outcomes as determined by rates of employment, higher education, partnering and childbearing, which affect both the individual patient and broader society[40,77–80]. Additionally, paediatric-onset rheumatic diseases are costly to treat, with intensive, complex care regimens unfolding over a lifetime that lead to a high economic and financial burden for both the individual patient and the health system as a whole[81,82].

Given the chronicity and severity of paediatric rheumatic diseases, early intervention with paradigm-shifting treatments such as CAR T cell therapy might offer substantial benefits and prevent irreversible organ damage before it occurs. These therapies could be particularly beneficial for paediatric patients as achieving durable, drug-free remission might translate into a substantial increase in the number of quality-adjusted life years compared with adults. Emerging case reports describe patients with pSLE and JDM receiving anti-CD19 CAR T cell therapy with promising efficacy and tolerable safety, even in the setting of severe disease[12,27,29]. Although these early reports are encouraging, rigorously designed clinical trials are required to address the unique features of paediatric rheumatic disease.

Unique considerations of CAR T cell therapy in paediatric patients with rheumatic diseases

Although many guiding principles in CAR T cell therapy apply across different diseases and age groups, there are unique aspects of treating paediatric patients with rheumatic diseases that need to be considered for success. Implementation of CAR T cell programmes outside of oncology and understanding disease-specific factors that could affect CAR T cell safety and efficacy require close collaboration between disease experts.

CAR T cell therapy programme implementation and patient access

Institutional infrastructure and multidisciplinary collaboration are essential to ensure the safe delivery of CAR T cell products to patients with paediatric-onset rheumatic diseases. Delivery of cellular therapies has historically fallen under the umbrella of haematology, oncology and haematopoietic stem cell transplant programmes. The Foundation for the Accreditation of Cellular Therapy (FACT) established standards for the use of cellular therapy over 25 years ago and subsequently added standards to encompass immune effector cell products, including CAR T cells[83]. FACT-accredited programmes have integrated complex

team dynamics and care management coordination for interface with apheresis, cell therapy laboratories, manufacturing, clinical research and clinical groups with expertise in cell therapy. Regulatory reporting requirements for cell therapy products are extensive, with FACT providing oversight from the time of cell collection through delivery and long-term follow-up. Patients with paediatric rheumatic diseases will require evaluation and treatment by multidisciplinary teams that include disease-specific experts alongside immune effector cell therapy specialists, with clearly defined team member responsibilities mapped to phases of care, including long term follow up. Multidisciplinary teams are needed to ensure complementary expertise in identifying and managing post-CAR T cell therapy toxicities, evaluating treatment efficacy using disease-appropriate endpoints, and assessing for and managing disease flares over time. In addition, the FDA currently recommends up to 15 years of dedicated long-term follow-up for patients receiving gene-modified cell therapy products to monitor for delayed adverse events[84]. As paediatric patients approach young adulthood, systematic approaches to ensure effective transitions to adult care providers will be needed for long-term follow-up after CAR T cell therapy.

Given the complexity of care and expertise required, CAR T cell therapy is generally administered at tertiary care centres, which might limit access to care for paediatric patients who often face difficulties participating in clinical trials owing to workforce shortages and uneven geographic distribution of paediatric subspecialists[85–87]. Many paediatric patients and their families already travel long distances for both routine care and research studies; thus, CAR T cell therapy will be difficult to access for many paediatric patients and their families. Moreover, patients might be required to reside nearby for at least several weeks following CAR T cell administration for toxicity monitoring, adding to the financial burden and psychosocial stress on patients and families. These burdens are disproportionately experienced by patients and families facing greater socioeconomic disadvantages[88–90]. Financial assistance and robust psychosocial support will be important facilitators of trial participation and post-approval access to CAR T cell therapy[88,91–93]. Ensuring robust insurance coverage of CAR T cell therapy for paediatric rheumatic disease indications will be essential, given published findings that differences in insurance type among patients with cancer are associated with the likelihood of receiving CAR T cell therapy and with subsequent clinical outcomes[94–96]. Given that non-biological factors (such as income) are already associated with differential outcomes in pSLE and JDM, efforts must be made towards ensuring broad access to CAR T cell therapy trials for all eligible patients[82,97–100].

Potential genetic influences on CAR T cell efficacy in patients with paediatric rheumatic diseases

Although we expect considerable benefits of CAR T cell therapy in children with rheumatic disease, we predict that genetic factors might distinctly influence CAR T cell efficacy in certain paediatric populations. Adult-onset SLE is typically polygenic[101], whereas >30 genes have been identified in monogenic pSLE via family studies, whole-exome sequencing and panel sequencing[102–104]. Monogenic pSLE manifests frequently in early childhood, with increased severity and treatment resistance compared with adult-onset SLE and paediatric patients with polygenic disease[105]. The prevalence of monogenic driver mutations in pSLE varies across studies, ranging from 7 to 30%[106–108]. In addition, across the SLE age spectrum, increasing genetic load correlates with earlier disease onset[109]. For this reason, although anti-CD19 CAR T cells might induce durable disease remission in patients with pSLE[12,110,111], we hypothesize that genetic influences could affect the efficacy and durability of CAR T cell response in a small subset of patients. Beyond SLE, the paediatric-age onset of other autoimmune conditions, such as juvenile idiopathic arthritis, inflammatory bowel disease, autoimmune cytopenias and vasculitis, can present with signs of primary immune regulatory disorders (such as STING-associated vasculopathy with onset in infancy (SAVI), Aicardi–Goutières syndrome and SOCS1 haploinsufficiency)[112,113]. Suggestive features of primary immunoregulatory disorders include very early onset disease, atypical patterns of multisystem autoimmunity and a strong family history. Depending on disease severity and whether the genetic drive for immune dysregulation is confined to the haematopoietic compartment, haematopoietic stem cell transplantation could be considered an alternative therapy[114]. In the absence of a high suspicion for a primary immunoregulatory disorder, excluding patients from CAR T cell clinical trials owing to genetic testing results is not recommended, as patients with pathogenic variants in immune-related genes could still benefit. However, to better understand the potential modifying role of paediatric-specific genetic factors on CAR T cell efficacy and durability, it might be prudent to perform genetic sequencing as a correlative assessment. Evaluating the effect of genetic variants could ultimately facilitate refinement of CAR T cell therapy design and implementation and inform strategies to optimize long-term disease control.

The immune landscape and the potential effects on CAR T cell fitness

In the setting of a haematological malignancy, several studies have attempted to identify variables (including age-related variables) in the composition of the initial autologous CAR T cell product that might affect clinical response. These variables include the degree of T cell exhaustion and senescence, the T cell memory composition (with higher naive and stem cell-like memory T cells correlating with better proliferative and antitumour capacity), the CD4:CD8 T cell ratio, the fraction of regulatory T cells and the T cell receptor repertoire diversity[115–117]. More generally, younger individuals have fewer senescent cells, and one hypothesis for the higher response rate in paediatric B cell ALL versus adults >60 years of age with multiple myeloma to CAR T cell therapy is that CAR T cell products from older patients are less functional[115]. The importance of these product characteristics in autoimmunity is unknown, as, for example, long-term persistence of CAR T cells might not be a pre-requisite for a durable response.

In addition to age, T cell composition in the starting material for a CAR T cell product might vary based on the underlying rheumatological disease, pre-leukapheresis immunosuppressive therapy and disease severity[118]. To circumvent concerns about product variability and immune cell fitness of products derived from patients with autoimmunity, strategies for using allogeneic products from healthy donors are being considered, including the use of CAR T cells generated from virus-specific T cells (NCT06429800) and CAR natural killer cells (NCT06557265).

Other considerations are potential differences in how anti-CD19 CAR T cell therapy affects humoral immunity in paediatric versus adult patients. Although anti-CD19 CAR T cells eliminate both naive and $CD27^+$ memory B cells, long-lived CD19-negative plasma cells remain unaffected by this treatment. These plasma cells, which can persist for decades in bone marrow niches, represent the accumulated immunological memory from previous pathogen exposures and vaccinations, a reservoir that is naturally smaller in children owing to fewer years of immune exposures. Biological differences in

humoral immunity between children and adults manifest clinically. Children experience higher rates of hypogammaglobulinaemia following anti-CD19 CAR T cell therapy compared with adults and consequently require intravenous immunoglobulin replacement more frequently[119]. As hypogammaglobulinaemia increases susceptibility to infection, the use of anti-CD19 CAR T cells in paediatric autoimmunity requires systematic monitoring of immunoglobulin levels coupled with timely and appropriate intravenous immunoglobulin replacement strategies.

Vaccination principles

Children and adolescents with rheumatic diseases who are being considered for CAR T therapy have often been treated with immunosuppressive drugs for years and, therefore, might not be up to date on age-appropriate immunizations. Moreover, depending on the age of presentation, patients might not have completed the full vaccination series, such as immunizations aimed at preventing the human papillomavirus or *Neisseria meningitidis*. Given that CAR T therapy is aimed at eliminating B cells, patients could be both more susceptible to vaccine-preventable illnesses and less likely to respond to vaccines immediately following CAR T therapy. Therefore, considering these unique factors when developing a vaccination strategy in children and adolescents with rheumatic diseases receiving CAR T cell therapy is important. Fortunately, immunogenicity data in patients treated with CAR T cell therapy for malignancy suggests that patients will probably retain vaccine seropositivity, even after achieving B cell aplasia, although these data are primarily derived from the evaluation of adult patients[120–123]. Notably, some CAR targets, such as B cell maturation antigen, might result in greater loss of seroprotection from previously administered vaccines than anti-CD19 CAR T cells[124].

In addition to existing rheumatology immunization guidelines, which focus on non-cellular immunosuppressive therapies[125], expert recommendations have been developed by the oncology community for children and adults receiving CAR T cell therapy[123,126–129]. Although these studies have not yet been replicated in paediatric rheumatology patients receiving CAR T cells, the same infection prevention principles could apply. First, updating seasonal vaccines and the pneumococcal vaccine series is recommended for immunocompromised individuals prior to leukapheresis[123,128–137]. Fortunately, the evaluation period for CAR T cell therapy can be long enough to allow for a vaccination window. Second, routine inactivated vaccines that would be due in the months following CAR T cell therapy might need to be deferred until the immune system has reconstituted enough for the patient to respond to these vaccines. Expert opinion recommends waiting at least 3 months from the start of CAR T cell therapy and ensuring that the $CD19^+$ and $CD4^+$ count are both $\geq 200 \times 10^6$/l before initiating routine immunizations[123,126–128]. Third, establishing routine monitoring of vaccine serologies after immune reconstitution could be beneficial for assessing the degree of protection a patient has and, in circumstances where titres remain low, offer revaccination[127]. Finally, live attenuated vaccines might need to be deferred for a longer period given the potential risk of infection. In the oncology field, live vaccines are deferred for at least 6–12 months after CAR T cell treatment, with evidence of immune reconstitution, response to prior inactivated vaccines and discontinuation of immunoglobulin replacement being prerequisites for delivery[123,126,127,138]. Studies should be conducted to assess vaccine immunogenicity and determine optimal vaccination timing strategies for paediatric patients with rheumatic diseases receiving CAR T cell therapies, with consideration given to duration and intensity of immune suppression prior to CAR T cell administration.

Toxicity in children, adolescents and young adults

Adverse effects of immune effector cell therapies are well described in oncological indications and include early immune mediated toxicities (such as CRS, ICANS, immune effector cell-associated haemophagocytic lymphohistiocytosis-like syndrome) and delayed toxicities (such as immune effector cell-associated haematotoxicity, impaired immunity and B cell aplasia)[139–143]. Preclinical models and clinical studies have enhanced understanding of the biological mechanisms of these inflammatory toxicities and have allowed for improved predictive models[144], early intervention strategies[145,146] and targeted treatments such as tocilizumab[141,147]. As a result, current post-marketing real-world oncology data chronicle improvement in the toxicity profile of anti-CD19 CAR T cell therapy, with only 16% of paediatric patients with ALL developing severe CRS compared with 48.1% in the first paediatric clinical trial in ALL[1]. Importantly, the CAR T cell toxicity profile in paediatric patients has generally mirrored the adult experience and is more closely correlated with the underlying disease and CAR construct than patient age[148–150]. The most important predictor of severe inflammatory toxicities in oncology patients is $CD19^+$ leukaemia cell burden at the time of infusion[148,151,152]. Therefore, inflammatory toxicities are expected to be milder in patients with rheumatic disease who have a physiological $CD19^+$ target cell burden and not an expanded malignant population[153]. CAR T cell therapy also includes a risk of infection stemming from antibody deficiency from B cell depletion, which could be accentuated in the setting of pre-existing lymphodepletion or multidrug immunosuppression[154]. The risk of CAR T cell therapy-related toxicity must be weighed against the substantial cumulative organ damage and psychosocial impact of living with serious paediatric-onset rheumatic disease[155].

Despite initial evidence supporting the safety of CAR T cells, immune-mediated toxicities might present differently in patients with rheumatic disease who have preexisting immune dysregulation and organ dysfunction. Therefore, although we hypothesize that the benefits of CAR T cell therapy for appropriately selected patients will outweigh the risks, rigorous clinical trials are needed to assess efficacy and safety. Bedside-to-bench efforts to understand the full range of CAR T cell-mediated toxicity in oncology provides insight into the importance of a high index of suspicion for emergent toxicities, rigorous reporting mechanisms and robust immunological and biological correlatives[156]. Emergent CAR T cell immune-mediated toxicities that are specific to autoimmunity are being reported. Local immune effector cell-associated toxicity syndrome (LICATS) is a term describing transient organ dysfunction in organs previously affected by autoimmunity (such as transient worsening of renal function in patients with lupus nephritis)[157]. Although it is hypothesized that this local, self-limited toxicity is related to CAR T cell destruction of tissue-resident B cells and immunological clearing, the pathophysiology is yet to be elucidated. Understanding the immunological basis for this phenomenon is important to predict individual patient risk, tailor treatment strategies and distinguish this syndrome from disease flares. The incidence and severity of LICATS might be different in paediatric patients with rheumatic disease, who have differences in disease organ involvement, severity, chronicity and histopathological features compared with adults[35,38,41,43,158].

Heightened attention to a secondary risk of malignancy is reflected in the boxed warning issued by the FDA on all currently approved

CAR T cell therapies following separate reports of CAR-positive T cell malignancies[159], indicating potential genotoxicity from the integration of the CAR transgene into proto-oncogenic sites. Follow-up reports investigating the incidence of these rare events have suggested that the rate of T cell malignancies is 0.1% of all CAR T cell adverse event reports[160]. Encouragingly, a 2023 global survey reviewing over 3,500 child, adolescent and young adult patients treated with CAR T cells did not reveal any other known cases of CAR T cell-related malignancy and cautioned against amplifying concerns of excess risk given a preponderance of data supporting the long-term safety of CAR T therapies[161]. Nevertheless, thorough education and informed consent discussions should be prioritized for paediatric patients and their caregivers to ensure tailored assessment of individual patient risks and benefits[162]. These secondary malignancy risks, although minimal, might also be influenced by an individual's prior treatments, family history, genetic and environmental risks, and particularly important, the diagnosis meriting the use of CAR T cells.

Reproductive health concerns are particularly important for adolescent and young adult patients with rheumatic diseases, as both the disease and cytotoxic and gonadotoxic treatments can impair fertility and increase the risk of adverse maternal and neonatal outcomes[163]. In oncology, long-lasting remission following CAR T cell therapy can potentially spare patients additional chemotherapy and stem cell transplant conditioning regimens that include gonadotoxic agents (such as cyclophosphamide and radiation)[164]. Similarly, drug-free remission following CAR T cell therapy in patients with paediatric rheumatic diseases might improve fertility and pregnancy outcomes by sparing exposure to gonadotoxic therapy, facilitating discontinuation of teratogenic medications and extending periods of disease quiescence. However, CAR T cells can persist for decades post-infusion in oncology patients[164,165], and their long-term effects on fertility remain unknown. Although it remains unclear if CAR T cells can cross the placental barrier and affect a developing fetus, reports of healthy live births following CAR T cell therapy describe favourable maternal and neonatal outcomes[166]. As prior cumulative exposure to gonadotoxic drugs among patients is variable, the effect of lymphodepleting chemotherapy on fertility might not be uniform. There has been considerable progress in the field of oncofertility, with established clinical practice guidelines recommending early consultation for all patients receiving anticancer treatment[167]. Fertility counselling and preservation practices in other subspecialties, including paediatric rheumatology, are not as well-defined[168,169]. Therefore, incorporating routine consultations with fertility specialists prior to lymphodepleting chemotherapy and CAR T cell infusion, and insurance coverage of such, will be critical to CAR T cell therapy programmes for paediatric patients with rheumatic diseases.

Clinical trial considerations for CAR T cell therapy in paediatric rheumatic diseases

A complete understanding of the risk–benefit profile of CAR T cells in paediatric rheumatic diseases is dependent on robust and intentional clinical trial design. In this rare disease group, uniform definitions of success and early engagement with regulatory groups will be critical to move the field forward. Using lessons learned from CAR T cell studies in oncology provides a foundation for developing correlative studies to better understand the biology and determinants of toxicity and response.

Efficacy assessment

Tisagenlecleucel was the first FDA-approved cell therapy in paediatrics, with this approval based on the dramatic complete response rate and durability of remission for patients with B cell ALL[170]. Although the definition of a complete response in ALL is universal, variability in disease characteristics, treatment protocols, study endpoints and response definitions across paediatric rheumatic diseases could make comparison across interventional studies challenging[171]. Therefore, the use of internationally accepted measures of response and remission, such as the American College of Rheumatology (ACR) criteria for clinically relevant improvement in pSLE, the ACR–European Alliance of Associations for Rheumatology myositis response criteria for JDM and the international consensus-proposed response criteria for jSSc, should be implemented to facilitate standardized assessment across clinical trials[172–174]. Consensus guidelines will need to specify the use of existing core set measurements as well as additional efficacy assessments that can characterize the effects of CAR T cells on the organs that are commonly affected in each condition. For example, skin disease is common in pSLE, JDM and jSSc, so consensus guidelines could specify the use of validated skin disease activity scoring tools, such as the Cutaneous Lupus Area and Severity Index (CLASI), Cutaneous Dermatomyositis Area and Severity Index (CDASI) and modified Rodnan Skin Score (mRSS), to provide additional detail on skin disease activity that might not be fully captured by existing core set of measurements for lupus, dermatomyositis and scleroderma. New response criteria that include 'drug-free' remission status within a prespecified time interval might also serve as a desirable end point, as this could represent the ultimate goal of CAR T cell therapy[175,176]. In addition to traditional efficacy outcome measures, which can provide an early clinical signal, the durability of drug-free remission over extended periods of time will be a novel and important aspect of efficacy assessment that is likely to be of substantial importance in regulatory decision-making by oversight agencies (such as the FDA and EMA). Collaborative efforts to align on response definitions, disease assessment time points and expected durability of drug-free remission across clinical trials are critical for ensuring the comparability of CAR T cell studies.

Invasive procedures such as tissue biopsies or radiation-exposing imaging are often considered the 'gold standard' for assessing disease activity in paediatric rheumatic diseases (such as biopsy-obtained kidney samples for the assessment of lupus nephritis disease activity). The frequency of these evaluations should be carefully considered to balance risks and benefits in younger patient populations. In some disease-specific circumstances, these invasive or high-radiation assessments might be justified to comprehensively assess response to CAR T cell therapy, such as with serial kidney biopsy for lupus nephritis or computed tomography for assessing interstitial lung disease.

Engagement of paediatric patients and caregivers as partners in defining and selecting appropriate clinical endpoints and outcome measures will be essential for ensuring that the most meaningful outcomes of CAR T cell therapy are captured in trial designs[177]. Similarly, the use of patient-reported outcome measures will provide an insight into the effects of CAR T cell therapy on health-related quality of life, psychological well-being and social function[178]. Patient preference information studies should be conducted to determine the balance of short-term and long-term risks and benefits of CAR T cell therapy that would be acceptable to paediatric patients and their caregivers, as has been done for gene therapies and other novel therapeutics that have the potential to fundamentally alter disease trajectories[179].

CAR T cell kinetics and determinants of response

Given that CAR T cells are a 'living drug' that expand in vivo after infusion, the total cell exposure and consequently, clinical efficacy, is

influenced not only by the infused cell dose but also by patient- and product-specific factors such as disease burden[4], CAR construct[180] and the immune phenotype of the final CAR T cell product[3]. Complete responses have been observed across a wide range of cell doses in B cell malignancies[181]. Although higher CAR T cell doses have generally been associated with improved outcomes in haematological malignancies, this relationship is not strictly linear and must be balanced by the risk of treatment-related toxicities[182]. Moreover, cellular kinetic (pharmacokinetics) principles gleaned from CAR T cell studies in malignant disease could differ in non-malignant settings and warrant special attention when studying the use of CAR T cell therapies in paediatric patients with rheumatic diseases.

Although some early-phase studies of CAR T cells in lupus use dose escalation to determine a recommended dose, others use a single weight-based or flat dose derived from therapeutic doses in malignant diseases. This approach might be sufficient to determine safety, but this 'one size fits all' strategy could lead to a knowledge gap in understanding optimal dosing in paediatric patients with rheumatic diseases. In contrast to malignant diseases, in which tumour burden can vary greatly, the target cell burden in paediatric rheumatic diseases might be less variable and evaluating higher dosing strategies could be necessary to optimize clinical efficacy. In addition to cell dose, variability in cell source, final product characteristics and patient factors at the time of both leukapheresis and infusion should be prospectively assessed to determine how these factors might influence or predict the initial efficacy and durability of drug-free remission. For example, as in patients with leukaemia with prior non-response to the B cell-targeting agent blinatumumab[151], rheumatology patients with prior failure of rituximab might have lower response rates than patients who are rituximab naive. Preliminary findings in autoimmunity appear to validate this principle, with lower peak CAR T cell expansion found in patients who have previously received rituximab than in those who had not[12].

Finally, future studies should evaluate the efficacy of redosing CAR T cells in paediatric patients with rheumatic diseases who flare after the initial infusion. Although attempts at CAR T cell redosing in haematological malignancies have largely been unsuccessful[183], the precise mechanisms underlying failure of a second CAR T cell infusion are unknown. Malignant cell-mediated suppression of CAR T cell function, target antigen loss or modulation and rejection of CAR T cells by the host immune system are probably all contributing factors to the limited success of secondary infusions[183–185]. Given that the underlying pathophysiology and immunological environment of malignancies and rheumatic diseases are different, determinants of CAR T cell failure are also probably unique. In addition, as the importance of CAR T cell persistence and prolonged B cell aplasia is not yet understood in autoimmunity, it is unclear if strategies to prevent immune-mediated rejection to enhance CAR T cell engraftment and persistence are necessary in this population. As the goal in this setting might be a potent short-term immunological reset, comparing the immune milieu (including tissue-resident cells) between treatment responders and non-responders will help to inform optimal dosing strategies and selection of therapeutic targets. For example, given that anti-CD19 CAR T cells might not effectively target long-lived plasma cells that contribute to autoantibody production[186], alternative targets (such as B cell maturation antigen and B cell-activating factor receptor (BAFF-R)) or dual targeting might be needed to broadly eliminate autoantibody-producing cells and optimize treatment efficacy in paediatric patients with rheumatic diseases[187,188].

Toxicity assessment

Systematic safety assessment should include known adverse events of interest in CAR T cell studies and incorporate consensus grading criteria for these toxicities, including CRS, ICANS[189], immune effector cell-associated haemophagocytic lymphohistiocytosis-like syndrome[143], immune effector cell-associated haematotoxicity[190], infectious complications and incidence of any secondary malignancies. Adverse outcomes and toxicities might require assessment via multiple measures, including the common terminology criteria for adverse events, disease-specific damage measures (such as the Systemic Lupus International Collaborating Clinics (SLICC)–ACR damage index and the myositis damage index), patient and physician global scores and patient- or parent-reported adverse event assessments[191–193]. Given that some manifestations of rheumatic disease might mimic or overlap with features of CAR T cell therapy toxicities (such as neuropsychiatric lupus versus ICANS and organ-specific disease flares versus LICATS), rigorous case definitions and adjudication processes for determining symptom attribution will be needed.

Baseline patient characteristics that predict the risk of post-CAR T cell therapy toxicity should be determined for each paediatric rheumatic disease population. Risk factors for acute toxicity already identified in oncology patients receiving CAR T therapy could be studied and validated in this population[194]. For example, one predictor of CAR T cell therapy toxicity in oncology patients is endothelial activation, which might be particularly prominent in patients with active pSLE, JDM, jSSc and pAAV, all of which prominently feature vasculopathy[195]. Patients with pre-existing antiphospholipid antibodies could be at a high risk of new thrombotic events after CAR T cell infusion, particularly those who develop severe CRS[196]. Such predictors could be used to inform and standardize preventive strategies, such as the early use of immunomodulatory agents, which could minimize rates of severe CRS or other acute post-CAR T cell therapy toxicities[145,146].

Finally, subacute and late toxicities will require continued attention in paediatric rheumatic disease populations. Given the known adverse effects of standard-of-care immunosuppressive treatments on growth, puberty, fertility and bone density, these parameters should be closely monitored. Monitoring vaccine-related antibody titres before and after infusion can guide revaccination strategies and help to elucidate strategies to reduce the risk of infection. Studies that assess the extent to which CAR T cell therapy either ameliorates or worsens neurocognitive dysfunction, which is a prevalent and debilitating feature of pSLE and potentially other paediatric rheumatic diseases, are needed[197]. Thorough documentation of additional adverse effects, including haematotoxicity and infectious complications, will enhance the understanding of the risk–benefit profile of CAR T cell therapy in paediatric patient populations with rheumatic diseases.

Regulatory considerations

Insights from the use of CAR T cell therapy in paediatric oncology provide a robust foundation of pharmacokinetic, efficacy and safety information with which to inform clinical trial and regulatory considerations pertinent to patients with paediatric rheumatic diseases. Nevertheless, the same complex regulatory challenges faced by the initial CAR T cell products will need to be addressed in paediatric rheumatic diseases. Balancing safety and efficacy considerations in patients with paediatric rheumatic diseases might differ from oncological indications, given that many patients with pSLE, JDM, jSSc and pAAV accrue organ damage and cumulative toxicity from standard-of-care immunosuppressive regimens over long periods of time, suggesting the need for

a lifelong perspective on the risk–benefit profile of CAR T cell therapy. Similarly, eligibility criteria must balance the need to enrol patients with paediatric-onset rheumatic diseases who have demonstrated some degree of refractoriness to standard-of-care therapies with the goal of administering CAR T cell therapy early enough in the disease course to mitigate disease activity, prevent damage and minimize cumulative toxicity from glucocorticoids and other traditional immunosuppressants. Given the high unmet needs in paediatric rheumatic diseases, early inclusion of adolescent patients in clinical trials with subsequent expansion into younger age groups strongly deserves consideration. As small sample sizes are inherent in rare paediatric diseases, extrapolation of efficacy and safety data from adult autoimmune disease CAR T cell trials warrants consideration given established similarities, including toxicities, in adult reference and paediatric target populations[198]. Data from large, multicentre registries and observational cohort studies can support causal inference in open-label studies of CAR T cell therapy in which assignment to placebo or standard of care is untenable but comparison with historical controls derived from such registries would be desirable. Internationally harmonized guidance on paediatric extrapolation studies could have an important role in facilitating the advancement of this therapy given that these rare disease populations could require international recruitment to meet an informative sample size[199]. Finally, ongoing pharmacosurveillance and patient safety programmes (such as risk evaluation and mitigation strategy programmes) will be needed to continue the identification of rare or late toxicities whilst maximizing safety in real-world settings[200].

Conclusions

Progress in immuno-oncology with potent and precise targeting of B cell malignancies with CAR T cells brings great hope to paediatric patients living with B cell-mediated rheumatic diseases. Thousands of paediatric patients with cancer have been treated with CAR T cells thus far, and the field is well positioned to safely and successfully bring this therapy to patients with paediatric rheumatic disease, who stand to reap major benefits from the prospect of durable drug-free remission. Given that paediatric patients arguably have the most to gain, we advocate for the inclusion of paediatric rheumatology patients in early-phase clinical trials. Although we are optimistic about the future of this field, formal evaluation is needed to determine which patients, and at what point in the disease course, will be best suited for this therapy. Differences in genetic predisposition and clinical phenotype between adults and children could influence the efficacy and durability of remission in children treated with CAR T cells, emphasizing the need for inclusion of genetic evaluations and robust correlative biology studies to understand predictors of safety and efficacy. The IMPACT working group brings together multidisciplinary experts to facilitate consensus on patient selection and trial design, education for patients and providers and advocacy for broad-based access to care. This collaborative approach will be fundamental to bringing this potentially life-changing therapy to patients with the greatest need.

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S.P., K.A., H.K., S.W.J., J.C.C., S.P.A., H.W., C.L.B, N.N.S. and M.L. researched data for the article and wrote the manuscript. All authors contributed substantially to discussion of the content and reviewed and/or edited the manuscript before submission.

Competing interests

H.W. has stock ownership in Regatta Bio and has pending patent applications related to cellular therapies. C.L.B. has awarded and pending patent applications describing the use of engineered cells as therapeutics and has received research support from Merck, Sharp and Dohme, Bristol-Myers Squibb and Kiadis Pharma. J.C.C. is a consultant at Sana Biotechnology and Synthekine and is a Paediatric SLE Advisory Board Member for Bristol-Myers Squibb. H.K. is supported by the Intramural Research Program of the National Institutes of Health (NIH), National Institute of Arthritis and Musculoskeletal and Skin diseases (NIAMS) (AR041215), is a juvenile myositis expert panel member for Cabaletta Bio and is part of NIAMS CRADA with provision of a drug (deucravacitinib) with Bristol-Myers Squibb, and previously part of NIAMS CRADA, with study support and the drug (baricitinib) with Eli Lilly and Company. L.L is supported by ZIA AR04121404. M.K. receives author royalties from Wolter-Kluwer (UpToDate) and is a consultant for Chiesi Pharmaceuticals and M3 Global Research. R.A.C. is supported by ZIAAR041184. C.E. is a site PI of the Cabaletta Bio-sponsored RESET-SLE CAR T cell trial for SLE. S.W.J. is a consultant for Merck, IgM BioSciences and Sail BioMedicines and previously served as a consultant for Bristol-Myers Squib, Variant Bio and ChemoCentryx. S.W.J. has funding provided by the National Institutes of Health (1R01DK136980 and 1K24AR085177) and Lupus Research Alliance (Lupus Mechanisms and Targets Award and Global Team Science Award). S.P. receives support for the conducting of clinical trials through Boston Children's Hospital from Atara and Jasper, is the inventor of IP related to development of third-party viral specific T cells programme with all rights assigned to Memorial Sloan Kettering Cancer Center, has received honoraria from Pierre Fabre, has engaged in consulting with Atara Biotherapeutics, Ensomo, HEOR, Pierre Fabre and VOR, is a DSMB member for Stanford University and NYBC and has equity interest in Regatta Biotherapies. N.N.S. receives research funding from Lentigen, VOR Bio and CARGO Therapeutics, has attended advisory board meetings (no honoraria) for VOR, ImmunoACT, and Sobi, receives royalties from CARGO and funding that supports this work was provided in part by the Intramural Research Program, Center of Cancer Research, National Cancer Institute and NIH Clinical Center, National Institutes of Health (ZIA BC 011823, N.N.S). K.A. has served on a scientific advisory board of Cabaletta Bio, completed paid consulting for Cabaletta Bio, serves as site PI of the Cabaletta Bio sponsored RESET-Myositis trial, serves as the Vice Chair of the Juvenile Dermatomyositis Committee for the Childhood Arthritis & Rheumatology Research Alliance (paid consultant position), has received honoraria/travel reimbursement from the Rheumatology Research Foundation and the American College of Rheumatology and receives research funding from the Rheumatology Research Foundation, Childhood Arthritis & Rheumatology Research Alliance/Arthritis Foundation, Chan Zuckerberg Initiative, Cure JM Foundation, Patient-Centered Outcomes Research Institute, and National Institutes of Health/Technical Resources International. M.L. participates in sponsored research funded by Luminary Biotherapeutics. The Integrated Multidisciplinary Paediatric Autoimmunity and Cell Therapy (IMPACT) Working Group has no competing interests to disclose. The remaining authors do not have any competing interests to disclose. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services or the National Institutes of Health, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

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