This is the accepted peer-reviewed author manuscript prior to publisher proof reading and type-setting.

The final published version is available online:

Biomarkers of tolerance in immune-mediated inflammatory diseases: a new era in clinical management?

Baker KF, Sim JPX, Isaacs JD

Lancet Rheumatology, Volume 3, Issue 5, e371 - e382

[https://doi.org/10.1016/S2665-9913\(21\)00069-2](https://doi.org/10.1016/S2665-9913(21)00069-2)

Biomarkers of tolerance in immune-mediated inflammatory diseases: a new era in clinical management?

Dr Kenneth F Baker^{1,2}, Jasmine PX Sim¹, Prof John D Isaacs^{1,2}

1 Musculoskeletal Research Group, Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, UK.

2 Musculoskeletal Unit, The Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK.

Correspondence to:

Professor John Isaacs,

4th Floor William Leech Building,

Medical School, Framlington Place,

Newcastle University,

Newcastle upon Tyne,

United Kingdom,

NE2 4HH.

Word Count: 5,341 words

Abstract

Modern therapeutic agents and treatment regimens have made sustained remission an achievable target for many patients across a spectrum of immune-mediated inflammatory diseases, albeit at the risk of adverse events and the expense of drug prescription and safety monitoring. Clinicians and patients are thus increasingly faced with a novel treatment dilemma – whether and how best to stop immunomodulatory treatment in patients who achieve remission. In this final paper in a Series on therapeutic tolerance induction, we summarise our current knowledge of biomarkers of immune homeostasis in human immune-mediated inflammatory diseases and their application to the prediction and achievement of sustained drug-free remission. We summarise evidence from prospective studies of immunomodulatory drug cessation across a range of immune-mediated inflammatory diseases, including rheumatoid arthritis, juvenile idiopathic arthritis, and inflammatory bowel disease, and we consider current evidence for clinical, serologic, proteomic, metabolomic, cellular, and microbiome biomarkers of immune homeostasis. The steps necessary for clinical translation, and the potential transformative effect of these biomarkers on management of patients with immune-mediated inflammatory diseases if successfully achieved, are discussed.

Introduction

The past two decades have witnessed a remarkable revolution in the management of immune mediated inflammatory diseases. Whereas progressive end organ damage, disability, and mortality were previously viewed as inevitable consequences of unrelenting chronic inflammation, sustained remission is now achievable in substantial proportions of patients across a range of different diseases. These impressive advances have been achieved largely through a combination of early diagnosis combined with treat-to-target strategies utilising an increasingly broad armamentarium of immunomodulatory agents—a therapeutic blueprint that has been successfully replicated across a wide spectrum of immune mediated inflammatory diseases.¹⁻⁵ A prime example is in rheumatoid arthritis (RA), where management has shifted from a historical approach of symptom palliation with non-steroidal anti-inflammatory drugs and glucocorticoids to rapid and tight control of disease activity, with 50-60% of patients now achieving clinical remission (as defined by DAS28) in modern cohort studies.^{6,7}

Nevertheless, the increasing use of potent immunomodulatory agents in the treatment of immune mediated inflammatory diseases poses challenges in terms of risk of medication-related adverse events,⁸ the resources required for regular safety monitoring,⁹ and the substantial prescription costs of newer biologic and targeted therapies. These drawbacks provide impetus for the development of novel strategies of treatment de-escalation and cessation, with consequent benefits for both patients and healthcare systems. Central to this step-down therapeutic approach is an ability to define and quantify the mechanisms underlying the restoration of immune homeostasis in order to identify those patients most likely to benefit.

In this Series paper, we summarise recent developments in the search for biomarkers of immune homeostasis in human immune-mediated inflammatory diseases, from early work exploring immune tolerance biomarkers in solid organ transplantation to recent clinical trials of drug cessation in the setting of remission in patients with immune-mediated inflammatory diseases. Finally, we discuss

the key steps required to translate such biomarkers to the clinic, and the potential transformative impact on disease management pathways if this is successfully achieved.

Operational tolerance - early lessons from solid organ transplantation

Early insights into the nature of human immune tolerance biomarkers were provided by pioneering studies in solid organ transplantation. Combinations of potent immunosuppressive drugs are prescribed to recipients of solid organ allografts to prevent immune-mediated rejection, and these drugs are then usually continued for the duration of graft survival, often life-long. Occasionally immunosuppression is stopped, sometimes because of life-threatening toxicity but more commonly because of patient non-adherence. In most cases this results in rejection of the allograft, but in rare cases the graft is not rejected and the immune responses against infections and other foreign antigens is preserved—a state known as operational tolerance.¹⁰

Several international consortia have explored potential biomarkers of operational tolerance, providing unique insights into this dramatic manifestation of human immune homeostasis. Cohort studies have revealed distinct whole-blood gene expression signatures uniquely associated with operational tolerance. Intriguingly, these transcriptomic signatures differ between different organ grafts: operational tolerance in renal transplant recipients is characterised by expression of genes involved in B cell function,¹¹ whereas in liver transplantation a signature enriched in NK-cell genes is observed.¹² The relative ease of liver tissue biopsy combined with a greater propensity for operational tolerance has facilitated the prospective study of immunosuppressive drug withdrawal in patients undergoing liver transplantation. In a trial of 75 patients with stable liver graft function on minimal immunosuppression,¹³ 33 (44%) patients successfully achieved operational tolerance at 12 months after drug cessation. Operational tolerance was associated with hepatocyte expression of iron homeostasis genes at the start of tapering, and this association was validated in a further cohort of 55 patients.¹³

Although these studies included small numbers of participants, they provide important proof-of-concept evidence to support the feasibility of identifying biomarkers of tolerance in humans. While such biomarkers have potential to help identify those patients likely to benefit from withdrawal of immunosuppression, they may not necessarily help to define the underlying immune processes involved, and they might not relate to antigen-specific mechanisms. Nevertheless, the distinct transcriptomic signatures observed in renal and liver transplantation raise the possibility of distinct pathways to immune tolerance, specific to the anatomical and pathophysiological context.

Drug-free remission as a model of tolerance

With increasing numbers of patients with immune-mediated inflammatory diseases achieving sustained disease remission, clinicians and patients are faced with the dilemma of how best to manage long-term immunomodulatory treatments in this setting. Minimisation of drug treatment carries advantages of reduced medication-related adverse events and reduced prescription costs, and drug tapering (though not necessarily complete cessation) is now endorsed by international consensus management guidelines for several immune-mediated inflammatory diseases including

RA,¹ psoriatic arthritis,⁴ and inflammatory bowel disease (IBD).² Indeed, in some cases it is possible to completely stop all immunomodulatory therapy and maintain a state of drug-free remission—a potential cure akin to operational tolerance in organ transplantation. For example, interventional trials of drug cessation in patients with rheumatoid arthritis have consistently demonstrated that around half of patients who achieve remission with conventional synthetic disease-modifying anti-rheumatic drugs (DMARDs) can maintain remission for sustained periods after complete DMARD withdrawal.¹⁴⁻¹⁷

At present, whether the immune system of such individuals has returned to health, and the patients are permanently cured, can only be determined by long-term observation. However, attainment of drug-free remission at least means that immune homeostasis has been reinstated in the medium term. Prospective studies of complete drug cessation provide an ideal experimental model by which to explore possible biomarkers of tolerance or reinstated immune homeostasis in patients with immune mediated inflammatory diseases. Such studies have adopted one of two similar, but conceptually distinct, approaches: the identification of biomarkers that predict sustained drug-free remission, or identification of biomarkers that predict relapse of disease (i.e. flare). Although in practice, both approaches define outcomes based on disease activity below or above a predetermined remission threshold, the conceptual implications from an immunopathological viewpoint are worthy of consideration. Drug-free remission could be defined by the absence of immune mediators of flare, comparable to immune tolerance in the healthy state; alternatively, drug-free remission might require the presence of homeostatic mediators that actively maintain a state of remission in the context of disease-associated immune dysregulation, which may or may not reflect the healthy state. The two concepts are not mutually exclusive and, in keeping with known mechanisms of immune tolerance (such as deletional, anergic, and regulatory tolerance), it is probable that drug-free remission in patients with immune-mediated inflammatory diseases is achieved through a balance between reduced flare-promoting and increased remission-permissive factors (**figure 1**). Thus whereas biomarkers of disease flare are conceptually distinct from biomarkers of sustained drug-free remission, they can both be utilised to quantify and predict immune homeostasis (**table**).

Here, we present data from selected studies of drug-free remission as a paradigm for immune homeostasis in immune-mediated inflammatory diseases. We do not discuss data from dose optimisation (i.e. partial tapering) studies, which is outside the scope of this review, unless such studies illustrate potential biomarkers in contexts where complete drug cessation studies do not exist. Although the majority of published data on biomarkers of immune homeostasis pertains to inflammatory arthritis (namely RA and juvenile idiopathic arthritis [JIA]), we also include the limited available data in IBD. Despite the differences in underlying disease pathogenesis, it is possible that immune homeostatic mechanisms overlap and, furthermore, in many instances the same immunomodulatory drugs are used to treat these diseases.

Biomarker studies in immune mediated inflammatory diseases

Disease activity biomarkers

Studies of drug withdrawal in immune mediated inflammatory diseases have identified common clinical characteristics of patients who can successfully achieve sustained drug-free remission. For example, cohort studies of patients with RA (and also IBD) have consistently demonstrated that elevated disease activity scores prior to DMARD cessation are associated with reduced likelihood of subsequent drug-free remission.^{16,18-21} These observations support the clinically intuitive notion of deep clinical remission, whereby robust suppression of disease activity is required before drug withdrawal may be considered.²² Nevertheless, the precise definition of remission in this context remains controversial, and a lack of standardised definitions for remission and flare in drug withdrawal studies, combined with different baseline clinical characteristics, makes it difficult to compare across different cohorts.

To address the lack of standardisation, several studies have explored the utility of more objective measures of disease remission, such as imaging or histological criteria. In various (mainly biologic agent) tapering studies of patients with RA, the presence of ultrasound measures of synovitis (power Doppler signal, alone or combined with greyscale) has been shown to predict future arthritis flare.²³ However, many of these studies included patients with low disease activity, and very few studies have examined the role of musculoskeletal ultrasound in predicting drug-free remission. In a study of 157 patients with RA in sustained clinical remission (DAS28-ESR < 2.6 for > 6 months), no significant association was observed between the presence of greyscale or power Doppler synovitis (or both) at baseline and occurrence of arthritis flare following DMARD tapering or cessation.²⁴ In the Biomarkers of Remission in Rheumatoid Arthritis (BioRRA) study,¹⁴ 44 patients with RA in remission (DAS28-CRP < 2.4 and absence of power Doppler synovitis on a 7 joint ultrasound scan) stopped conventional synthetic DMARDs and were followed for 6 months. 23 (52%) experienced an arthritis flare, with no association observed between baseline ultrasound measures of synovial or tenosynovial greyscale change, or erosions, with subsequent attainment of drug-free remission – however, the predictive utility of baseline power Doppler signal was not assessed.¹⁴ The limitations of musculoskeletal ultrasound in this setting include the potential for substantial inter-observer variability and non-specific low-level ultrasound abnormalities.²⁵ Furthermore, the negative findings of recent imaging-based treat-to-target trials in RA have shed doubt on the role of imaging modalities (both ultrasound and magnetic resonance imaging) in defining remission.²⁶⁻²⁸ Given these limitations, there is currently insufficient evidence to conclude whether imaging adds further useful information beyond clinical measures of disease activity in the prediction of future disease-free remission in patients with RA.

In summary, clinical data from studies of drug-free remission—at least in RA—point to the importance of robust and sustained remission as a clinical state favouring successful drug withdrawal and subsequent drug-free remission. While not measures of tolerogenic immune mechanisms *per se*, clinical measures of disease activity, combined with measures of subclinical inflammation, have an important role in the selection of patients who are more likely to benefit from drug withdrawal strategies. However, precise definitions of such deep remission states in immune-mediated inflammatory diseases remain debateable, as is the relative contribution of clinical versus subclinical disease activity assessments when considering strategies of immunomodulatory drug withdrawal.

Serologic biomarkers

A hallmark of many immune-mediated inflammatory diseases is the presence of autoantibodies, useful as an aid to diagnosis and, in some cases, as a biomarker of disease activity (e.g. double-stranded DNA antibodies in systemic lupus erythematosus).²⁹ The presence of autoantibodies is clear evidence of breach of self-tolerance, and their utility as biomarkers to predict attainment of drug-free remission has been explored in the context of immune-mediated inflammatory diseases, notably RA.

Approximately 80% of patients with RA are seropositive for rheumatoid factor (RF), anti-citrullinated peptide autoantibodies (ACPA), or both.³⁰ Patients with seropositive RA are more likely to develop joint erosions and disability compared with their seronegative counterparts,³¹ and the presence of ACPA and RF are negatively associated with subsequent attainment of drug-free remission.^{15-17,20,24} Indeed, the replication of this finding across multiple studies underscores the importance of seropositivity as a negative predictor of drug-free remission in RA. Other anti-posttranslationally modified protein antibodies (AMPAs), not currently in widespread clinical use, have also been negatively associated with drug-free remission in RA. In the RETRO study, 101 patients with RA in clinical remission were randomised to one of three arms: DMARD continuation, DMARD tapering to half dose, or DMARD tapering followed by complete cessation.¹⁵ In an exploratory analysis of 94 patients, increasing numbers of AMPAs at baseline was associated with increasing risk of disease relapse (18% of patients with 0-1 AMPAs relapsed vs 55% of patients with more than 5 AMPAs; $p = 0.011$).³² Similar results were observed in an exploratory analysis of the IMPROVED study, a therapeutic strategy trial of methotrexate plus sulfasalazine plus hydroxychloroquine versus methotrexate plus adalimumab, in which drug tapering to complete cessation was permitted when sustained clinical remission was achieved.³³ In an exploratory analysis of 399 seropositive patients, an increasing number of AMPAs at baseline was associated with improved response to treatment but also with lower rates of early drug-free remission (37% of patients with 1-2 AMPAs achieved drug-free remission for 4 months vs 11% in patients with 3 or more AMPAs; $p = 0.005$).³⁴ However, when using a more stringent definition of long-term drug-free remission of 12 months or longer, maintained to last study visit, no significant association was observed.

These observations have led to the notion of different levels of remission in RA, with so-called immunological remission (i.e. seroreversion from a seropositive to seronegative state) representing true restoration of immune tolerance.³⁵ Although conceptually appealing, this suggestion has limited clinical utility in view of the relative permanence of autoantibodies in RA and the rarity at which autoantibodies (particularly ACPA) are lost.³⁶ The specificity of autoantibodies as a biomarker of disease-free remission is also rather limited, as 30-45% of ACPA-positive individuals can still achieve drug-free remission,¹⁶ and in patients who achieve this state, persistence of ACPA or RF (or both) is common. In an observational study of 95 seropositive patients with RA who achieved sustained drug-free remission (median 4.2 years duration), only 12.8% became ACPA seronegative, and 19.7% became RF seronegative; seroreversion did not differ between those who maintained remission and those who relapsed.³⁷ Similarly, no significant association was seen between relative changes in autoantibody titres (including RF, ACPA and AMPA) following DMARD cessation and the subsequent achievement of drug-free remission by seropositive patients in the IMPROVED study.³⁸

Given these limitations, it is unlikely that autoantibody status alone will be sufficient for clinical use as a robust biomarker of drug-free remission; however, study of autoreactive B cells may hold promise. Using a tetramer-based approach, Kristyanto *et al.*³⁹ identified a distinct phenotype of ACPA+ memory B cells in patients with established RA that were not present in those with pre-RA. These cells—expressing high levels of the costimulatory molecules CD80 and CD86, low levels of the inhibitory receptor CD32, and having a propensity for secretion of the neutrophil chemoattractant interleukin (IL)-8—persisted in patients with established RA after treatment, even in those with low clinical disease activity scores. Such studies of antigen-specific B cell function may thus yield novel biomarkers of immune tolerance mechanisms, although these have yet to be applied to the setting of drug-free remission in immune-mediated inflammatory diseases.

Proteomic biomarkers

The measurement of circulating inflammatory mediators provides a convenient approach to biomarker discovery that is compatible with a wide range of commercially available clinical-grade assays. Individual cytokines and chemokines have demonstrated predictive value in drug-free remission in some settings; for example, circulating IL-6 levels are negatively associated with drug-free remission in patients with RA following withdrawal of the anti-IL-6 agent tocilizumab.⁴⁰ Alternatively, several cytokines and chemokines can be combined to give a composite measure of immune activity, such as the so-called multibiomarker disease activity score (MBDA),⁴¹ which combines the measurement of 12 pro-inflammatory cytokines and chemokines in a single score that has been shown to correlate with clinical measures of disease activity in RA⁴² and to predict future radiographic progression.⁴³⁻⁴⁵ In the RETRO study, a high MBDA score prior to DMARD withdrawal was associated with a greater risk of flare in a multivariate model (OR 8.5, 95% CI 2.0-36.4, $p = 0.004$).⁴⁶ Furthermore, the combination of baseline MBDA with ACPA status yielded an even greater discrimination for future flare (76% of patients positive for both MBDA and ACPA experienced flare compared with 32% of patients positive for MBDA alone, and 13% of patients negative for both).⁴⁶ These results demonstrate that the presence of subclinical inflammation is sufficient, at least in susceptible individuals, to manifest in overt clinical disease relapse upon DMARD withdrawal. In contrast, high MBDA score at the point of diagnosis has been shown to predict higher rates of future drug-free remission in seronegative (but not seropositive) RA, suggesting distinct subgroups of disease distinguished by differing propensities for drug-free remission.⁴⁷

Circulating inflammatory biomarkers have also shown promise in the prediction of drug-free remission in patients with juvenile idiopathic arthritis. In a randomised trial,⁴⁸ elevated baseline concentrations of serum calprotectin (a phagocyte activation marker) and S100A12 (a neutrophil activation marker) predicted subsequent disease flare within the first 3 months after methotrexate discontinuation with an area under the receiver operating characteristic curve (ROC_{AUC}) of 0.75 (0.60 - 0.90) for calprotectin and 0.70 (0.53 - 0.87) for S100A12. Serum calprotectin was a strong predictor of arthritis flare at 12 months after methotrexate discontinuation in another study of 22 patients with JIA in remission (ROC_{AUC} 0.95, 95% CI 0.85 - 1.00)⁴⁹ and also predicted flare in some studies of patients who did not discontinue treatment,^{50,51} but not in others.^{52,53} Thus serum calprotectin appears to be a sensitive biomarker of subclinical disease activity in JIA rather than a measure of underlying immune homeostasis.

In a multi-parameter exploratory analysis of the U-ACT-Early study, Teitsma *et al*⁵⁴ showed that among 85 inflammatory proteins measured, higher levels of C-C motif chemokine ligand 18 (CCL18), CCL20, and soluble IL2 receptor alpha (sIL2-R α) were predictive of future drug-free remission when measured prior to initiation of methotrexate, tocilizumab, or both.. However, it is likely that these cytokines and chemokines are predictors of response to treatment rather than biomarkers of immune tolerance mechanisms.

Metabolomic biomarkers

Recent years have witnessed an increasing interest in the application of high-throughput mass spectrometry and nuclear magnetic resonance spectroscopy technologies to measure small molecule metabolites as an indicator of underlying immune processes.⁵⁵ If immune homeostasis in immune-mediated inflammatory disease is restored through distinct metabolic processes, it is conceivable that the metabolites produced or consumed by such processes could be utilised as clinical biomarkers.

In an exploratory analysis of the U-Act-Early study, mass spectrometry was used to quantify 263 metabolites in baseline serum samples from 60 patients with early RA prior to treatment with methotrexate, tocilizumab, or both.⁵⁶ Network analysis revealed distinct metabolic pathways associated with drug-free remission depending on the treatment arm: arachidonic acid metabolism was associated with tocilizumab monotherapy, arginine and proline metabolism with methotrexate monotherapy, and histidine metabolism with dual therapy. As only pre-treatment samples were obtained, the metabolic pathways identified likely reflect responsiveness to therapy (i.e. theragnostic biomarkers) rather than biomarkers of immune homeostatic processes. Nevertheless, the distinct pathways observed for different treatment arms raises the possibility of medication-specific routes to drug-free remission, even within a common disease.

Cellular biomarkers

All of the methodologies discussed thus far aim to quantify biomarkers in the circulation (whole blood, serum, or plasma). Whereas these approaches are inexpensive and allow for rapid laboratory processing, they may not provide sufficient resolution to identify faint biomarkers signals or biomarkers that are specific to a rare immune cellular compartment. This is further compounded by wide variations in the cellular composition of peripheral blood between different individuals. To address these problems, several research groups have explored cell-type specific biomarkers of disease-free remission (phenotypic or transcriptional) in immune-mediated inflammatory diseases, an approach that offers the potential for richer phenotyping and better resolution of candidate biomarkers at the cellular level.

In the aforementioned BioRRA study,¹⁴ disease flare following cessation of conventional synthetic DMARDs could be predicted by the baseline expression of three genes within circulating CD4+ T cells: *FAM102B*, *AC073343.2*, and *EPS15-AS1*. Although the functional relevance is unclear, a composite score combining expression of these three genes together with baseline concentrations of circulating IL-27 and a clinical measure of disease remission (ACR/EULAR Boolean remission) could

predict future arthritis flare versus remission, with an AUC_{ROC} of 0.96 (95% CI 0.91–1.00); work is ongoing to validate these findings in an independent cohort.

In another exploratory analysis of the U-Act-Early study, distinct CD4⁺ T cell transcriptomic modules associated with subsequent disease-free remission were observed in the different treatment arms: leukocyte migration and G-protein signalling pathway modules were associated with tocilizumab monotherapy, response to bacteria or biotic stimuli modules with methotrexate monotherapy, and transcription and translation modules with dual therapy.⁵⁷

Subsets of circulating lymphocytes associated with flares have been defined in immune-mediated inflammatory diseases, and several groups have explored whether they are predictive of sustained remission following withdrawal of treatment. In a study of 36 patients with JIA who stopped anti-TNF therapy, an increased abundance of CD45RA⁺–TNF⁺ memory CD4⁺ T cells deficient in immune checkpoint molecules (PD1[–]CD152[–]) prior to anti-TNF withdrawal was seen in patients who experienced flare compared with those who achieved sustained remission.⁵⁸ In this small study, the baseline ratio of CD45RA⁺–TNF α ⁺ to CD45RA⁺–TNF α [–] CD4⁺ T cells predicted flare with an AUC_{ROC} of 0.94. Whether this biomarker can predict drug-free remission remains to be established, as several patients in this study continued treatment with conventional synthetic DMARDs.

In another study of 47 patients with RA who stopped anti-TNF therapy (but continued methotrexate), lower frequencies of so-called inflammation-related CD4⁺ T cells (CD4⁺CD45RB^{high}CD45RA⁺CD45RO^{low}CD62L[–])⁵⁹ were associated with sustained remission (OR_{remission} 16.20, p=0.041).⁶⁰ However this and other flare-associated cellular subsets, such as the recently-discovered pre-inflammatory mesenchymal (PRIME) cells (CD45[–]CD31[–]PDPN⁺),⁶¹ remain to be studied in the context of drug-free remission.

Biomarkers of autoreactivity

As mentioned above, assessing the presence and diversity of autoantibodies provides an opportunity to define underlying antigen-specific mechanisms that can perturb immune homeostasis in immune-mediated inflammatory disease. Extending this concept, an increasing number of research groups are using modern and emerging laboratory techniques to quantify and monitor rare autoreactive cells in response to antigen-specific tolerogenic therapeutic strategies (discussed further in a previous review in this Series⁶²). It is possible that cell-specific characteristics (e.g. surface markers, gene expression, cytokine production etc.) could be used as biomarkers of immune homeostasis. Such autoreactive cellular subsets could have either pro-inflammatory or pro-tolerogenic effects, and knowledge of their function could help illuminate underlying disease mechanisms. However, such biomarkers of autoreactivity are yet to be studied in the context of drug-free remission in immune-mediated inflammatory disease, and their suitability for translation to a commercially viable assay remains to be established.

Tissue biomarkers

Although convenient to acquire, peripheral blood-borne biomarkers can only infer the underlying disease processes occurring at the site of disease. Tissue biopsies, although usually more difficult to obtain, arguably provide the most comprehensive insight into immunopathology. This approach has already been used to study operational tolerance in liver transplantation, and more recently it has been applied to studies of drug-free remission in immune-mediated inflammatory diseases.

Using single cell RNAseq and multiparameter flow cytometry to study synovial biopsies from patients with RA, Alivernini *et al*⁶³ identified a higher abundance of MerTK⁺CD206⁺ synovial tissue macrophages in patients in remission versus those with active disease. Furthermore, in patients in remission who tapered biologic therapy (but continued methotrexate), a low baseline proportion of MerTK⁺CD206⁺ synovial tissue macrophages was predictive of subsequent disease flare (OR 13.5, 95% CI 2.3 - 80.8). Intriguingly, *in vitro* studies have shown that MerTK⁺ synovial tissue macrophages produce significantly lower concentrations of pro-inflammatory cytokines and more IL-10 in response to LPS stimulation compared with their MerTK-negative counterparts. This, coupled with MerTK-dependent upregulation of a distinct transcription factor signature in MerTK⁺ synovial tissue macrophages from patients in remission (but not those with active RA or healthy controls), suggests a potential pro-tolerogenic role for this cellular subset in RA remission. Whether these cells are predictive of drug-free remission in RA remains to be confirmed.

In a study of Crohn's disease, 45 patients in clinical and endoscopic remission discontinued infliximab (but continued maintenance conventional synthetic DMARDs) and were followed for up to 104 months.⁶⁴ Normalised mucosal expression of *TNF* in baseline colonic biopsies was predictive of sustained remission, with a median relapse free survival of 20 in those with normal *TNF* expression compared with 5 months in those with high expression. Again, whether these results are applicable to the context of drug-free remission has not been examined.

Microbiome

Recent years have witnessed a surge of interest in commensal human microbiota and their modulation of host immunity. The intimate relationship between microbiome and host suggests a wide range of potential mechanisms for the promotion of immune-mediated inflammatory diseases, including formation of neoautoantigens by post-translational modification of host antigens, molecular mimicry, mucosal permeabilisation, and polarisation of immune responses.⁶⁵ Strong associations have been observed between the presence of specific species of commensal bacteria and both the onset and severity of a range of immune-mediated inflammatory diseases. For example, an increased abundance of the intestinal commensal bacterium *Prevotella copri* is observed in individuals with early RA versus healthy controls,⁶⁶ with anti-*P. copri* antibody titres correlating with serum T helper-17 cytokine profiles;⁶⁷ furthermore, transfer of *P. copri* derived from RA patients can induce arthritis in experimental murine models.⁶⁸

In contrast, it is likely that specific commensal bacteria may instead exert anti-inflammatory effects to maintain immune tolerance. For example, several studies in patients with IBD have observed lower levels of commensal organisms that produce and convert butyrate in patients versus healthy controls;⁶⁹ butyrate has been shown to exert anti-inflammatory and intestinal homeostatic roles in both animal and human studies.⁷⁰

Therapeutic manipulation of the microbiome in order to create a more favourable balance of anti-inflammatory versus pro-inflammatory microbes is being actively investigated across a range of immune-mediated inflammatory diseases. Microbiome manipulation may be achieved through the use of dietary modification, probiotics, prebiotics (i.e. agents that promote the expansion of pro-tolerogenic microbes), and most strikingly through the use of faecal transplantation from healthy donors.⁷¹ In a recent meta-analysis of four studies including 277 patients with ulcerative colitis, faecal transplant recipients were twice as likely to achieve remission after 8 weeks compared to placebo recipients (RR 2.03, 95 % CI, 1.07 to 3.86).⁷² With these emerging results, it appears feasible that microbiome-related biomarkers could provide a possible measure of immune homeostasis. At present no published studies have explored the potential association between microbiome biomarkers with drug-free remission in immune-mediated inflammatory disease, although we expect this to be an area of development in the near future.

Limitations of current studies

Recent studies have offered tantalising glimpses of potential biomarkers of immune homeostasis in immune-mediated inflammatory diseases, which have shown impressive prognostic performance in small clinical cohorts. Nevertheless, there are several limitations common to these studies. First and foremost is a lack of validation of biomarkers in large independent cohorts; to date, no biomarker of immune homeostasis in these diseases has been externally validated. However, this remains an active area of research with several tolerance biomarker validation studies currently in recruitment: for example, the ongoing BIOlogical Factors that Limit sustAined Remission in rhEumatoid arthritis (BIO-FLARE) study [ISRCTN16371380]).

Furthermore, there remains a lack of standardisation across drug cessation studies, including the definition of remission used and differences between study populations in terms of demographics (e.g. age), disease stage (i.e. early vs. established disease), and previous drug treatment (e.g. previous biologics vs. biologic-naïve). Study quality also varies, particularly in terms of power, with overfitting of complex datasets a common limitation. The net effect of these non-standardised protocols is to make it very difficult, if not impossible, to compare and analyse results across different studies. In addition, the laboratory biomarkers explored by different studies are largely non-overlapping, with the exception of routine clinical serology such as ACPA and RF in RA.

Finally, there is a lack of consensus on the optimal approach to drug cessation, including the timing (e.g. abrupt vs. tapering) and the order of drug withdrawal in those treated with combinations of therapies. Indeed, very few studies have explored the comparative effectiveness of different strategies for drug cessation. In the TApering strategies in Rheumatoid Arthritis (TARA) study, 189 patients in remission were randomised to taper and then stop conventional synthetic DMARDs prior to anti-TNF, or vice versa, with a non-significant trend of increased drug-free remission in those who tapered conventional synthetic DMARDs first (20% versus 11%; $p = 0.07$).⁷³ Whether such an effect would reach statistical significance in a larger study remains to be seen and, furthermore, would need to be balanced against the relative prescription costs and side effect profiles of each individual therapy.

Translation to clinical practice

Several hurdles remain in translating the use of biomarkers of immune homeostasis to routine clinical practice (**Figure 2**). Assuming that immune homeostasis biomarkers are successfully validated (an essential first step), it will then be necessary to demonstrate their clinical efficacy in biomarker-driven protocols of drug withdrawal. Trials comparing no stratification (i.e. drug cessation in all) versus biomarker stratification (i.e. drug cessation only in biomarker positive group) are required to demonstrate superior ratios of remission to flare for biomarker-driven strategies, and thus provide evidence of clinical efficacy and, ultimately, cost-effectiveness of this approach. For example, the ongoing Liver Immunosuppression Free Trial (LIFT - NCT02498977) uses such a design to assess the efficacy of a biomarker-driven strategy of immunosuppression withdrawal in the setting of liver transplantation.

Following proof of efficacy, it would then be necessary to develop robust assays suitable for clinical use. Such assays would need to be compliant with necessary good clinical practice standards, yield accurate and reproducible results, and ideally be compatible with existing equipment and staff skillsets within healthcare clinical laboratory facilities. Furthermore, these assays would have to be acceptable to patients, and ideally be minimally invasive to avoid risk of significant adverse effects (especially when applied to clinically well sustained remission populations). Finally, the assays would need to be commercially viable by identifying a sufficient proportion of patients who are able to achieve drug-free remission, such that the treatment cost savings to healthcare providers outweigh the manufacturing, marketing, and sample processing costs of the assays.

Immune tolerance - a future target of disease control?

Assuming biomarkers are successfully validated and provided their efficacy is confirmed and commercially viable clinical assays are developed, what impact can we expect to see on clinical practice?

One major impact would be to facilitate the widespread adoption of personalised drug tapering and cessation in the routine clinical management of patients with immune-mediated inflammatory diseases. Current techniques to measure disease activity, while successfully used to identify those in need of escalation of therapy in treat-to-target approaches, are typically poor at discriminating those at the opposite end of the spectrum who can achieve drug-free remission. In this context, effective biomarkers of immune homeostasis would enable a personalised prediction of drug-free remission, identifying those most likely to benefit from drug withdrawal.³⁵ This would minimise the risk of disease flare, thus reducing potential flare-related complications, limiting use of resources needed to regain disease remission, and increasing patient acceptability. Furthermore, longitudinal monitoring of such biomarkers following drug cessation might identify those in whom immune homeostasis is subsequently perturbed, allowing for drug holidays followed by the pre-emptive re-initiation of therapy before disease flare occurs (**Figure 3**).

One further likely impact would be to provide insights into mechanisms that maintain immune homeostasis, or tolerance, in immune-mediated inflammatory disease. A greater knowledge of such mechanisms may identify potential therapeutic targets by which immune tolerance can be restored,

catalysing a novel class of tolerising therapies in immune-mediated inflammatory disease. Tolerance biomarkers should also provide the tools to identify those patients most likely to benefit from such therapies, and to quantify their clinical response.

Conclusion

A growing body of research has provided evidence for a range of potential biomarkers of drug-free remission across different immune-mediated inflammatory diseases. Such biomarkers of immune homeostasis have the potential to usher in a new era of disease management, in which the success of current treat-to-target approaches in the early stages of disease control are later augmented by personalised tapering and cessation of immunomodulatory treatment. Combined with the potential for tolerising therapies, it is possible that the current gold-standard treatment target of remission could be replaced in the future by drug-free remission, at least in some patients.⁷⁴ Considerable research, innovation, and commercial development will be required to achieve this. However, reflecting on the remarkable progress in immune-mediated inflammatory disease management over the past three decades, and recent progress in identifying potential biomarkers of immune homeostasis, another therapeutic revolution in immune-mediated inflammatory disease management may be closer than we think.

Search strategy and selection criteria

We identified references by searching PubMed with combinations of the terms "biomarker", "drug free remission", "tapering", "withdrawal", "cessation", "autoimmune", "operational tolerance" and various IMIDs including "rheumatoid arthritis", "psoriatic arthritis", "ankylosing spondylitis", "systemic lupus erythematosus", "inflammatory bowel disease", "psoriasis", "uveitis", "multiple sclerosis", "nephrotic syndrome", "vasculitis", and "juvenile idiopathic arthritis". We only reviewed English-language articles published in peer-reviewed journals, and deliberately focussed on reports of human disease rather than animal studies. The final reference list is not an exhaustive systematic literature review, but rather a selection of highlighted articles applicable to our aim of presenting a broad overview of current research in biomarkers of immune homeostasis in human IMID.

Competing interests

KFB reports grants to explore drug-free remission in rheumatoid arthritis from the British Society for Rheumatology, Academy of Medical Sciences, JGW Patterson Foundation, and Wellcome Trust. KFB and JDI are named inventors on a patent application by Newcastle University ("Prediction of Drug-Free Remission in Rheumatoid Arthritis"; International Patent Application Number PCT/GB2019/050902), and have an ongoing collaboration with Genentech to measure cytokines/chemokines in rheumatoid arthritis. JPXS declares no competing interests.

Acknowledgements

KFB is supported by a Clinical Lectureship from the National Institute for Health Research (NIHR) (grant number CL-2017-01-004). JPXS is supported by a PhD Studentship from the JGW Patterson Foundation (grant number 30015.088/27of18/PA/IXS). Work in JDI's laboratory is supported by the NIHR Newcastle Biomedical Research Centre, the Research Into Inflammatory Arthritis Centre Versus Arthritis, and Rheuma Tolerance for Cure (European Union Innovative Medicines Initiative 2, grant number 777357). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care.

References

1. Smolen JS, Landewé RBM, Bijlsma JWJ, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. *Ann Rheum Dis* 2020; **79**: 685–99.
2. Lamb CA, Kennedy NA, Raine T, et al. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut* 2019; **68**(Suppl 3): s1.
3. Smith CH, Yiu ZZN, Bale T, et al. British Association of Dermatologists guidelines for biologic therapy for psoriasis 2020: a rapid update. *British Journal of Dermatology* 2020; **183**(4): 628–37.
4. Gossec L, Baraliakos X, Kerschbaumer A, et al. EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2019 update. *Annals of the Rheumatic Diseases* 2020; **79**(6): 700–12.
5. Fanouriakis A, Kostopoulou M, Alunno A, et al. 2019 update of the EULAR recommendations for the management of systemic lupus erythematosus. *Annals of the Rheumatic Diseases* 2019; **78**(6): 736–45.
6. Matthijssen XME, Niemantsverdriet E, Huizinga TWJ, van der Helm–van Mil AHM. Enhanced treatment strategies and distinct disease outcomes among autoantibody-positive and -negative rheumatoid arthritis patients over 25 years: A longitudinal cohort study in the Netherlands. *PLOS Medicine* 2020; **17**(9): e1003296.
7. Ramiro S, Landewé RB, van der Heijde D, et al. Is treat-to-target really working in rheumatoid arthritis? a longitudinal analysis of a cohort of patients treated in daily practice (RA BIODAM). *Ann Rheum Dis* 2020; **79**(4): 453–9.
8. Sepriano A, Kerschbaumer A, Smolen JS, et al. Safety of synthetic and biological DMARDs: a systematic literature review informing the 2019 update of the EULAR recommendations for the management of rheumatoid arthritis. *Annals of the Rheumatic Diseases* 2020; **79**(6): 760–70.
9. Ledingham J, Gullick N, Irving K, et al. BSR and BHPR guideline for the prescription and monitoring of non-biologic disease-modifying anti-rheumatic drugs. *Rheumatology (Oxford)* 2017; **56**(6): 865–8.
10. Rickert CG, Markmann JF. Current state of organ transplant tolerance. *Current Opinion in Organ Transplantation* 2019; **24**(4): 441–50.

11. Baron D, Ramstein G, Chesneau M, et al. A common gene signature across multiple studies relate biomarkers and functional regulation in tolerance to renal allograft. *Kidney Int* 2015; **87**(5): 984–95.
12. Martínez-Llordella M, Lozano JJ, Puig-Pey I, et al. Using transcriptional profiling to develop a diagnostic test of operational tolerance in liver transplant recipients. *J Clin Invest* 2008; **118**(8): 2845–57.
13. Bohne F, Martínez-Llordella M, Lozano J-J, et al. Intra-graft expression of genes involved in iron homeostasis predicts the development of operational tolerance in human liver transplantation. *J Clin Invest* 2012; **122**(1): 368–82.
14. Baker KF, Skelton AJ, Lendrem DW, et al. Predicting drug-free remission in rheumatoid arthritis: A prospective interventional cohort study. *J Autoimmun* 2019; **105**: 102298.
15. Haschka J, Englbrecht M, Hueber AJ, et al. Relapse rates in patients with rheumatoid arthritis in stable remission tapering or stopping antirheumatic therapy: interim results from the prospective randomised controlled RETRO study. *Ann Rheum Dis* 2016; **75**(1): 45–51.
16. Klarenbeek NB, van der Kooij SM, Guler-Yuksel M, et al. Discontinuing treatment in patients with rheumatoid arthritis in sustained clinical remission: exploratory analyses from the BeSt study. *Ann Rheum Dis* 2011; **70**(2): 315–9.
17. ten Wolde S, Breedveld FC, Hermans J, et al. Randomised placebo-controlled study of stopping second-line drugs in rheumatoid arthritis. *Lancet* 1996; **347**(8998): 347–52.
18. Moghadam M, Vonkeman HE, Ten Klooster PM, et al. Stopping Tumor Necrosis Factor Inhibitor Treatment in Patients With Established Rheumatoid Arthritis in Remission or With Stable Low Disease Activity: A Pragmatic Multicenter, Open-Label Randomized Controlled Trial. *Arthritis Rheumatol* 2016; **68**(8): 1810–7.
19. Kuijper TM, Luime JJ, de Jong PHP, et al. Tapering conventional synthetic DMARDs in patients with early arthritis in sustained remission: 2-year follow-up of the tREACH trial. *Annals of the Rheumatic Diseases* 2016; **75**(12): 2119.
20. van der Woude D, Young A, Jayakumar K, et al. Prevalence of and predictive factors for sustained disease-modifying antirheumatic drug-free remission in rheumatoid arthritis: results from two large early arthritis cohorts. *Arthritis Rheum* 2009; **60**(8): 2262–71.
21. Little DHW, Tabatabavakili S, Shaffer SR, Nguyen GC, Weizman AV, Targownik LE. Effectiveness of Dose De-escalation of Biologic Therapy in Inflammatory Bowel Disease: A Systematic Review. *The American journal of gastroenterology* 2020; **115**(11): 1768-74.
22. Tanaka Y, Smolen JS, Jones H, Szumski A, Marshall L, Emery P. The effect of deep or sustained remission on maintenance of remission after dose reduction or withdrawal of etanercept in patients with rheumatoid arthritis. *Arthritis Research & Therapy* 2019; **21**(1): 164.
23. Simpson E, Hock E, Stevenson M, et al. What is the added value of ultrasound joint examination for monitoring synovitis in rheumatoid arthritis and can it be used to guide treatment decisions? A systematic review and cost-effectiveness analysis. *Health Technol Assess* 2018; **22**(20): 1–258.

24. El Miedany Y, El Gaafary M, Youssef S, et al. Optimizing therapy in inflammatory arthritis: prediction of relapse after tapering or stopping treatment for rheumatoid arthritis patients achieving clinical and radiological remission. *Clin Rheumatol* 2016; **35**(12): 2915-23.
25. Padovano I, Costantino F, Breban M, D'Agostino MA. Prevalence of ultrasound synovial inflammatory findings in healthy subjects. *Ann Rheum Dis* 2016; **75**(10): 1819-23.
26. Haavardsholm EA, Aga A-B, Olsen IC, et al. Ultrasound in management of rheumatoid arthritis: ARCTIC randomised controlled strategy trial. *BMJ* 2016; **354**: i4205.
27. Dale J, Stirling A, Zhang R, et al. Targeting ultrasound remission in early rheumatoid arthritis: the results of the TaSER study, a randomised clinical trial. *Ann Rheum Dis* 2016; **75**(6): 1043-50.
28. Møller-Bisgaard S, Hørslev-Petersen K, Ejbjerg B, et al. Effect of Magnetic Resonance Imaging vs Conventional Treat-to-Target Strategies on Disease Activity Remission and Radiographic Progression in Rheumatoid Arthritis: The IMAGINE-RA Randomized Clinical Trial. *Jama* 2019; **321**(5): 461-72.
29. Pisetsky DS, Lipsky PE. New insights into the role of antinuclear antibodies in systemic lupus erythematosus. *Nature Reviews Rheumatology* 2020; **16**(10): 565-79.
30. Sun J, Zhang Y, Liu L, Liu G. Diagnostic accuracy of combined tests of anti cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis: a meta-analysis. *Clin Exp Rheumatol* 2014; **32**(1): 11-21.
31. Farragher TM, Lunt M, Plant D, Bunn DK, Barton A, Symmons DPM. Benefit of early treatment in inflammatory polyarthritis patients with anti-cyclic citrullinated peptide antibodies versus those without antibodies. *Arthritis Care Res (Hoboken)* 2010; **62**(5): 664-75.
32. Figueiredo CP, Bang H, Cobra JF, et al. Antimodified protein antibody response pattern influences the risk for disease relapse in patients with rheumatoid arthritis tapering disease modifying antirheumatic drugs. *Ann Rheum Dis* 2017; **76**(2): 399-407.
33. Heimans L, Akdemir G, Boer KVCW-d, et al. Two-year results of disease activity score (DAS)-remission-steered treatment strategies aiming at drug-free remission in early arthritis patients (the IMPROVED-study). *Arthritis research & therapy* 2016; **18**: 23-.
34. de Moel EC, Derksen VFAM, Stoeken G, et al. Baseline autoantibody profile in rheumatoid arthritis is associated with early treatment response but not long-term outcomes. *Arthritis research & therapy* 2018; **20**(1): 33-.
35. Schett G, Emery P, Tanaka Y, et al. Tapering biologic and conventional DMARD therapy in rheumatoid arthritis: current evidence and future directions. *Ann Rheum Dis* 2016; **75**(8): 1428-37.
36. Jansen DTSL, Emery P, Smolen JS, et al. Conversion to seronegative status after abatacept treatment in patients with early and poor prognostic rheumatoid arthritis is associated with better radiographic outcomes and sustained remission: post hoc analysis of the AGREE study. *RMD Open* 2018; **4**(1): e000564.
37. Boeters DM, Burgers LE, Toes RE, van der Helm-van Mil A. Does immunological remission, defined as disappearance of autoantibodies, occur with current treatment strategies? A long-term follow-up study in rheumatoid arthritis patients who achieved sustained DMARD-free status. *Ann Rheum Dis* 2019; **78**(11): 1497-504.

38. de Moel EC, Derksen VFAM, Trouw LA, et al. In rheumatoid arthritis, changes in autoantibody levels reflect intensity of immunosuppression, not subsequent treatment response. *Arthritis research & therapy* 2019; **21**(1): 28-.
39. Kristyanto H, Blomberg NJ, Slot LM, et al. Persistently activated, proliferative memory autoreactive B cells promote inflammation in rheumatoid arthritis. *Sci Transl Med* 2020; **in press**.
40. Nishimoto N, Amano K, Hirabayashi Y, et al. Drug free REmission/low disease activity after cessation of tocilizumab (Actemra) Monotherapy (DREAM) study. *Mod Rheumatol* 2014; **24**(1): 17–25.
41. Centola M, Cavet G, Shen Y, et al. Development of a multi-biomarker disease activity test for rheumatoid arthritis. *PLoS One* 2013; **8**(4): e60635.
42. Hirata S, Dirven L, Shen Y, et al. A multi-biomarker score measures rheumatoid arthritis disease activity in the BeSt study. *Rheumatology (Oxford)* 2013; **52**(7): 1202–7.
43. Markusse IM, Dirven L, van den Broek M, et al. A multibiomarker disease activity score for rheumatoid arthritis predicts radiographic joint damage in the BeSt study. *J Rheumatol* 2014; **41**(11): 2114–9.
44. Hirata S, Li W, Kubo S, et al. Association of the multi-biomarker disease activity score with joint destruction in patients with rheumatoid arthritis receiving tumor necrosis factor-alpha inhibitor treatment in clinical practice. *Mod Rheumatol* 2016; **26**(6): 850–6.
45. Li W, Sasso EH, van der Helm-van Mil AHM, Huizinga TWJ. Relationship of multi-biomarker disease activity score and other risk factors with radiographic progression in an observational study of patients with rheumatoid arthritis. *Rheumatology* 2016; **55**(2): 357–66.
46. Rech J, Hueber AJ, Finzel S, et al. Prediction of disease relapses by multibiomarker disease activity and autoantibody status in patients with rheumatoid arthritis on tapering DMARD treatment. *Ann Rheum Dis* 2016; **75**(9): 1637–44.
47. Boeters DM, Burgers LE, Sasso EH, Huizinga TWJ, van der Helm – van Mil AHM. ACPA-negative RA consists of subgroups: patients with high likelihood of achieving sustained DMARD-free remission can be identified by serological markers at disease presentation. *Arthritis Research & Therapy* 2019; **21**(1): 121.
48. Gerss J, Roth J, Holzinger D, et al. Phagocyte-specific S100 proteins and high-sensitivity C reactive protein as biomarkers for a risk-adapted treatment to maintain remission in juvenile idiopathic arthritis: a comparative study. *Annals of the Rheumatic Diseases* 2012; **71**(12): 1991.
49. Foell D, Frosch M, Schulze zur Wiesch A, Vogl T, Sorg C, Roth J. Methotrexate treatment in juvenile idiopathic arthritis: when is the right time to stop? *Ann Rheum Dis* 2004; **63**(2): 206-8.
50. Schulze zur Wiesch A, Foell D, Frosch M, Vogl T, Sorg C, Roth J. Myeloid related proteins MRP8/MRP14 may predict disease flares in juvenile idiopathic arthritis. *Clin Exp Rheumatol* 2004; **22**(3): 368-73.
51. Holzinger D, Frosch M, Kastrup A, et al. The Toll-like receptor 4 agonist MRP8/14 protein complex is a sensitive indicator for disease activity and predicts relapses in systemic-onset juvenile idiopathic arthritis. *Ann Rheum Dis* 2012; **71**(6): 974-80.

52. Barendregt AM, Veldkamp SR, Hissink Muller PCE, et al. MRP8/14 and neutrophil elastase for predicting treatment response and occurrence of flare in patients with juvenile idiopathic arthritis. *Rheumatology (Oxford)* 2020; **59**(9): 2392-401.
53. Hinze CH, Foell D, Johnson AL, et al. Serum S100A8/A9 and S100A12 Levels in Children With Polyarticular Forms of Juvenile Idiopathic Arthritis: Relationship to Maintenance of Clinically Inactive Disease During Anti-Tumor Necrosis Factor Therapy and Occurrence of Disease Flare After Discontinuation of Therapy. *Arthritis Rheumatol* 2019; **71**(3): 451-9.
54. Teitsma XM, Jacobs JWG, Concepcion AN, et al. Explorative analyses of protein biomarkers in patients with early rheumatoid arthritis achieving sustained drug-free remission after treatment with tocilizumab- or methotrexate-based strategies: from transcriptomics to proteomics. *Clin Exp Rheumatol* 2018; **36**(6): 976-83.
55. Bantug GR, Galluzzi L, Kroemer G, Hess C. The spectrum of T cell metabolism in health and disease. *Nature Reviews Immunology* 2018; **18**(1): 19-34.
56. Teitsma XM, Yang W, Jacobs JWG, et al. Baseline metabolic profiles of early rheumatoid arthritis patients achieving sustained drug-free remission after initiating treat-to-target tocilizumab, methotrexate, or the combination: insights from systems biology. *Arthritis Res Ther* 2018; **20**(1): 230.
57. Teitsma XM, Jacobs JWG, Mokry M, et al. Identification of differential co-expressed gene networks in early rheumatoid arthritis achieving sustained drug-free remission after treatment with a tocilizumab-based or methotrexate-based strategy. *Arthritis Res Ther* 2017; **19**(1): 170.
58. Leong JY, Chen P, Yeo JG, et al. Immunome perturbation is present in patients with juvenile idiopathic arthritis who are in remission and will relapse upon anti-TNF α withdrawal. *Annals of the Rheumatic Diseases* 2019; **78**: 1712-21.
59. Burgoyne CH, Field S, Brown AK, et al. Abnormal T cell differentiation persists in patients with rheumatoid arthritis in clinical remission and predicts relapse. *Ann Rheum Dis* 2008; **67**: 750-7.
60. Saleem B, Keen H, Goeb V, et al. Patients with RA in remission on TNF blockers: when and in whom can TNF blocker therapy be stopped? *Annals of the Rheumatic Diseases* 2010; **69**: 1636-42.
61. Orange DE, Yao V, Sawicka K, et al. RNA Identification of PRIME Cells Predicting Rheumatoid Arthritis Flares. *New England Journal of Medicine* 2020; **383**: 218-28.
62. Stanway JA, Isaacs JD. Tolerance-inducing medicines in autoimmunity: rheumatology and beyond. *The Lancet Rheumatology* 2020; **2**(9): e565-e75.
63. Alivernini S, MacDonald L, Elmesmari A, et al. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. *Nat Med* 2020; **26**(8): 1295-306.
64. Olsen T, Rismo R, Gundersen MD, et al. Normalization of mucosal tumor necrosis factor- α : A new criterion for discontinuing infliximab therapy in ulcerative colitis. *Cytokine* 2016; **79**: 90-5.
65. Reyes-Castillo Z, Valdés-Miramontes E, Llamas-Covarrubias M, Muñoz-Valle JF. Troublesome friends within us: the role of gut microbiota on rheumatoid arthritis etiopathogenesis and its clinical and therapeutic relevance. *Clinical and Experimental Medicine* 2020.
66. Scher JU, Sczesnak A, Longman RS, et al. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *Elife* 2013; **2**: e01202-e.

67. Pianta A, Arvikar S, Strle K, et al. Evidence of the Immune Relevance of *Prevotella copri*, a Gut Microbe, in Patients With Rheumatoid Arthritis. *Arthritis Rheumatol* 2017; **69**(5): 964–75.
68. Maeda Y, Kurakawa T, Umemoto E, et al. Dysbiosis Contributes to Arthritis Development via Activation of Autoreactive T Cells in the Intestine. *Arthritis Rheumatol* 2016; **68**(11): 2646–61.
69. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A* 2007; **104**(34): 13780–5.
70. Couto MR, Gonçalves P, Magro F, Martel F. Microbiota-derived butyrate regulates intestinal inflammation: Focus on inflammatory bowel disease. *Pharmacological Research* 2020; **159**: 104947.
71. Manasson J, Blank RB, Scher JU. The microbiome in rheumatology: Where are we and where should we go? *Annals of the Rheumatic Diseases* 2020; **79**(6): 727–33.
72. Imdad A, Nicholson MR, Tanner-Smith EE, et al. Fecal transplantation for treatment of inflammatory bowel disease. *Cochrane Database of Systematic Reviews* 2018; **11**(11).
73. van Mulligen E, Weel AE, Hazes JM, van der Helm-van Mil A, de Jong PHP. Tapering towards DMARD-free remission in established rheumatoid arthritis: 2-year results of the TARA trial. *Annals of the Rheumatic Diseases* 2020; **79**(9): 1174.
74. Verstappen M, van Mulligen E, de Jong PHP, van der Helm-Van Mil AHM. DMARD-free remission as novel treatment target in rheumatoid arthritis: A systematic literature review of achievability and sustainability. *RMD open* 2020; **6**(1): e001220.

Table - Summary of interventional studies of drug withdrawal that have identified biomarkers of drug-free remission in IMiD.

Author	Disease	Number of patients	Treatment	Duration of follow-up	Proportion maintaining remission	Biomarkers	Effect size
Baker <i>et al.</i> (2019) ¹⁴	Rheumatoid arthritis	44	csDMARDs	6 months after DMARD withdrawal	21/44 (48%)	Composite score of 3 CD4 ⁺ T cell transcripts + IL27 + Boolean remission	ROC _{AUC} flare(95% CI) = 0.96 (0.91–1.00)
El Miedany <i>et al.</i> (2016) ²⁴	Rheumatoid arthritis	157	Arm 1: Half dose bDMARDs Arm 2: half dose bDMARDs and csDMARDs Arm 3: stop bDMARDs, half dose csDMARDs Arm 4: stop all DMARDs Arm 5: continue all DMARDs	12 months after DMARD withdrawal	Arm 1: 18/31 (58%) Arm 2: 13/32 (41%) Arm 3: 10/31 (32%) Arm 4: 7/31 (23%) Arm 5: 30/32 (94%)	Positive ACPA	OR _{flare} Arm 1 = 5.35, p < 0.02 Arm 2 = 5.68, p < 0.01 Arm 3 = 5.46, p < 0.01 Arm 4 = 8.64, p < 0.003

Author	Disease	Number of patients	Treatment	Duration of follow-up	Proportion maintaining remission	Biomarkers	Effect size
Figueiredo <i>et al.</i> (2017) ³² RETRO study	Rheumatoid arthritis	94	Various biological and non-biological DMARDs, prednisolone ≤ 5mg/day	12 months after enrolment	Not stated	AMPA reactivity	Proportion flare: 0-1 AMPAs = 18% 2-5 AMPAs = 34% >5 AMPAs = 55% $\chi^2 = 6.46$, $p = 0.011$
Gerss <i>et al.</i> (2012) ⁴⁸	Juvenile idiopathic arthritis	188	MTX	At least 12 months after MTX withdrawal	120/188 (64%)	Elevated calprotectin (≥690ng/ml) Elevated S100A12 (≥175ng/ml)	HR _{flare} (95% CI) Calprotectin = 2.24 (1.39 – 3.62, $p = 0.0009$) S100A12 = 2.81 (1.70 – 4.65, $p < 0.0001$)
Haschka <i>et al.</i> (2016) ¹⁵ RETRO study	Rheumatoid arthritis	101	Various biological and non-biological DMARDs, prednisolone ≤ 5mg/day	12 months after enrolment	13/27 (48%) withdrawal arm (versus 22/36 (61%) reduction arm and 36/38 (84%) continuation arm)	Positive ACPA	OR _{flare} (95% CI) = 5.23 (1.10 – 24.87), $p = 0.038$

Author	Disease	Number of patients	Treatment	Duration of follow-up	Proportion maintaining remission	Biomarkers	Effect size
Klarenbeek <i>et al.</i> (2011) ¹⁶ BeSt study	Rheumatoid arthritis	508	Various DMARDs	5 years	115/508 (23%) achieved DMARD-free remission, of which 59/115 (51%) maintained remission at 5 year time-point	Positive ACPA Positive RF	OR _{flare} (95% CI): Positive ACPA = 5.3 (2.4–11.8), p < 0.05 Positive RF = 2.9 (1.3–6.4), p < 0.05
Nishimoto <i>et al.</i> (2014) ⁴⁰ DREAM study	Rheumatoid arthritis	187	TOC +/- glucocorticoids	52 weeks after TOC cessation	17/187 (9.1%)	Low serum IL-6 (< 35 pg/ml) Normalised MMP-3	HR _{flare} (95% CI): IL-6 = 0.41 (0.27–0.63) MMP-3 = 0.29 (0.19–0.43)
Rech <i>et al.</i> (2016) ⁴⁶ RETRO study	Rheumatoid arthritis	94	Various biological and non-biological DMARDs, prednisolone ≤ 5mg/day	12 months after enrolment	63/94 (67%)	Moderate/high MBDA score	OR _{flare} 8.5 (p = 0.004)

Author	Disease	Number of patients	Treatment	Duration of follow-up	Proportion maintaining remission	Biomarkers	Effect size
Teitsma <i>et al.</i> (2018) ⁵⁴ U-Act-Early study	Rheumatoid arthritis	60	MTX, TOC, HCQ, TNF α inhibitor	2 years after enrolment	37/60 (62%)	Elevated CCL18 Elevated CCL20 Elevated sIL2-R α (TOC + MTX group)	Negative effect estimates (β): CCL18 = -3.31, p = 0.047 CCL20 = -1.24, p = 0.035 sIL2-R α = 1.40, p = 0.039
Teitsma <i>et al.</i> (2018) ⁵⁶ U-Act-Early study	Rheumatoid arthritis	60	MTX, TOC, HCQ, TNF α inhibitor	2 years after enrolment	37/60 (62%)	Histidine metabolism (TOC + MTX) Arachidonic acid metabolism (TOC) Arginine and proline metabolism (MTX)	Histidine metabolism, p < 0.001 Arachidonic acid metabolism, p = 0.018 Arginine and proline metabolism, p = 0.022

ACPA = anti-citrullinated peptide antibodies, AMPA = anti-modified protein antibodies, bDMARDs = biological disease-modifying anti-rheumatic drugs, CCL18 = C-C chemokine ligand 18, CCL20 = C-C chemokine ligand 20, CCRP-2 = anti-cyclic citrullinated peptide 2, CDAI = clinical disease activity index, CI = confidence interval, csDMARDs = conventional synthetic disease-modifying anti-rheumatic drugs, DAS28-CRP = disease activity score 28 joint count with C-reactive protein, DAS28-ESR = disease activity score 28 joint count with erythrocyte sedimentation rate, DFR = drug-free remission, DMARDs = disease-modifying anti-rheumatic drugs, HCQ = hydroxychloroquine, HR = hazard ratio, IL-6 = interleukin 6, MBDA = multi biomarker disease activity, MMP-3 = matrix metalloproteinase 3, MTX = methotrexate, na = not applicable, OR = odds ratio, RF = rheumatoid factor, ROC_{AUC} = area under the receiver operating characteristic curve, RR = relative risk, SDAI = simplified disease activity index, sIL-R α = soluble interleukin 2 receptor alpha, SJC28 = swollen joint count in 28 joints, STM = synovial tissue macrophages, TJC28 = tender joint count in 28 joints, TNF α = tumour necrosis factor alpha, TOC = tocilizumab, ULN = upper limit of normal.

Figure 1 - Theoretical model of immune homeostasis as a balance of pro-inflammatory and pro-tolerogenic mechanisms, with clinical disease manifesting above a threshold of autoreactivity. Pro-inflammatory processes dominate in disease states and vice versa in health. The extent to which these mechanisms overlap, and whether specific compensatory pro-tolerogenic mechanisms exist exclusively in health or disease, remains uncertain.

Figure 2 - The necessary steps in translation of biomarkers of drug-free remission from discovery research through to clinical practice.

Figure 3 - Schematic representation of the potential application of future biomarkers to a personalisation of therapy in rheumatoid arthritis. Biomarkers of disease susceptibility could be used in the asymptomatic pre-RA stage, to guide use of novel strategies to prevent clinical disease. Once clinical disease is manifest, prognostic biomarkers would allow identification of those patients destined to develop severe disease, allowing early instigation of combination therapy with choice of agent guided by theragnostic biomarkers. Once disease remission is achieved, longitudinal measurement of immune homeostasis biomarkers would permit a personalised withdrawal of immunomodulation. Permissive biomarker test results (white arrows) would identify when drug tapering is appropriate, or when drug-free remission (DFR) can be extended where this has already been achieved. In contrast, non-permissive results (grey arrows) would indicate when drug regimens should be continued or reinstated, preventing relapse of clinical disease.