











2022 EULAR points to consider for the measurement, reporting and application of IFN-I pathway activation assays in clinical research and practice

Javier Rodríguez-Carrio ¹, Agata Burska,² Philip G Conaghan ², Willem A Dik,³ Robert Biesen ⁴, Maija-Leena Eloranta,⁵ Giulio Cavalli,⁶ Marianne Visser,⁷ Dimitrios T Boumpas ⁸, George Bertias,⁹ Marie Wahren-Herlenius ^{10,11}, Jan Rehwinkel,¹² Marie-Louise Frémond ¹³, Mary K Crow ¹⁴, Lars Rönnblom ⁵, Marjan A Versnel ¹⁵, Edward M Vital ²

Handling editor Josef S Smolen

For numbered affiliations see end of article.

Correspondence to

Dr Edward M Vital, NIHR Leeds Biomedical Research Centre, Leeds, UK; e.m.j.vital@leeds.ac.uk

JR-C and AB are joint first authors.
MAV and EMV are joint senior authors.

Received 15 November 2022
Accepted 4 February 2023
Published Online First
1 March 2023

ABSTRACT

Background Type I interferons (IFN-I) play a role in a broad range of rheumatic and musculoskeletal diseases (RMDs), and compelling evidence suggests that their measurement could have clinical value, although testing has not progressed into clinical settings.

Objective To develop evidence-based points to consider (PtC) for the measurement and reporting of IFN-I assays in clinical research and to determine their potential clinical utility.

Methods EULAR standardised operating procedures were followed. A task force including rheumatologists, immunologists, translational scientists and a patient partner was formed. Two systematic reviews were conducted to address methodological and clinical questions. PtC were formulated based on the retrieved evidence and expert opinion. Level of evidence and agreement was determined.

Results Two overarching principles and 11 PtC were defined. The first set (PtC 1–4) concerned terminology, assay characteristics and reporting practices to enable more consistent reporting and facilitate translation and collaborations. The second set (PtC 5–11) addressed clinical applications for diagnosis and outcome assessments, including disease activity, prognosis and prediction of treatment response. The mean level of agreement was generally high, mainly in the first PtC set and for clinical applications in systemic lupus erythematosus. Harmonisation of assay methodology and clinical validation were key points for the research agenda.

Conclusions IFN-I assays have a high potential for implementation in the clinical management of RMDs. Uptake of these PtC will facilitate the progress of IFN-I assays into clinical practice and may be also of interest beyond rheumatology.

INTRODUCTION

Effects of type I interferons (IFN-I) range from antiviral defence to the crosstalk between innate and adaptive immune responses.¹ Due to their immune stimulatory effects, IFN-I and their signalling pathway have gained attention in the breakdown of tolerance and the development and perpetuation of autoimmune and autoinflammatory phenomena.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ Type I interferons (IFN-I) play a role in a number of rheumatic and musculoskeletal conditions.
- ⇒ The IFN-I pathway activation can be measured at different levels and using different readouts.
- ⇒ Assays measuring IFN-I pathway activation have not progressed into clinical practice and uncertainty exists pertaining clinical applications.

WHAT THIS STUDY ADDS

- ⇒ These are the first EULAR endorsed points to consider (PtC) for the measurement and reporting of IFN-I assays in clinical research and practice.
- ⇒ PtC concerned terminology and reporting practices to promote consistency and harmonisation, as well as delineate clinical applications in specific settings.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ Implementation of IFN-I pathway assays show a strong potential to improve clinical management in rheumatology and other specialties.
- ⇒ This consensus document creates a framework for the future implementation of other biomarkers.

Thus, there is an extensive body of evidence supporting the participation of IFN-I in the pathogenesis of rheumatic and musculoskeletal diseases (RMDs). Compared with other cytokines, IFN-I have been implicated in a wide range of different RMDs.² Moreover, this involvement covers the whole disease process, from disease development (and diagnosis) to exacerbation (prognosis) and prediction of therapeutic responses.² At the mechanistic level, the IFN pathway activation has been reported to participate from genetic susceptibility to disease perpetuation and progression.² Finally, consistent evidence supports the IFN-I pathway as a therapeutic target.^{3–5} Taken together, all this



© Author(s) (or their employer(s)) 2023. No commercial re-use. See rights and permissions. Published by BMJ.

To cite: Rodríguez-Carrio J, Burska A, Conaghan PG, et al. *Ann Rheum Dis* 2023;**82**:754–762.

Table 1 Overarching principles and points to consider for the measurement and reporting of IFN-I pathway assays in clinical research and practice

	Level of evidence	Level of agreement (mean±SD), n (%) scorings ≥8/10
Overarching principles		
A. The IFN pathway is a complex system with multiple subtypes of IFNs and diverse downstream effects on gene and protein expression.	N/A	9.76±0.66 17 (100)
B. IFN-I pathway activation is a common hallmark in many RMDs. Although IFN-I pathway activation is associated with some clinical manifestations, the utility of IFN-I pathway assays in clinical practice requires further validation for most contexts.	N/A	9.29±0.98 16 (94.1)
Points to consider		
1. Task force consensus terminology should be considered for reporting IFN assays measurement.	5	9.58±0.79 17 (100)
2. Existing assays measure different aspects of the IFN-I pathway; they do not reflect the entirety of the pathway and some are not specific for IFN-I. The most appropriate assay will depend on the research or clinical question and should be justified.	4	9.76±0.56 17 (100)
3. Publications on novel IFN-I pathway assays should report whether they specifically reflect IFN-I, and to the extent possible, which IFN-I is measured.	5	9.58±0.61 17 (100)
4. For assays that evaluate pathways downstream of the IFN-I receptor (eg, IFN-stimulated gene expression or protein scores) the choice of components needs to be justified. For gene expression scores, the known subsets of IFN-stimulated genes should be described separately.	5	9.41±0.87 16 (94.1)
5. IFN-I pathway is consistently activated in several RMDs, but assays measuring IFN-I pathway activation cannot be currently recommended for diagnostic purposes.	2b/3b	8.58±1.83 12 (70.5)
6. IFN-I pathway assays define more severe subgroups within many RMDs, so they should be considered in stratification studies.	2b/3b	8.70±1.31 12 (70.5)
7. IFN-I pathway activation is associated with disease activity in some RMDs, especially SLE and myositis, but its added value in clinical decision making is uncertain.	2b/3b	8.82±1.18 14 (82.3)
8. IFN-I pathway assays can predict disease exacerbations, in particular flare occurrence in patients with SLE, but further work should be performed to determine to what extent they outperform current instruments.	2b	9.00±1.00 16 (94.1)
9. IFN-I pathway assays might predict progression from preclinical autoimmunity to clinical disease.	2b	8.00±1.69 11 (64.7)
10. In SLE, IFN-I pathway assays may be useful in predicting response to IFN-I targeting therapies.	2b	8.76±1.20 14 (82.3)
11. IFN-I pathway assay results may be affected by some treatments (eg, IFN-targeted therapies and high-dose glucocorticoids), and timing of sample collection should be taken into account and reported.	2b/3b	9.70±0.46 17 (100)
IFN-I, type I interferon; RMD, rheumatic and musculoskeletal disease; SLE, systematic literature review.		

evidence asserts a particularly promising role of IFN-I as (multi-faceted and multipurpose) biomarkers in rheumatology.

The IFN pathway activation can be measured at different levels, including several targets (IFN proteins, transcripts, etc) and methods (immunoassays, qPCR, etc) reported in the literature. A number of studies have revealed associations between assays measuring IFN-I pathway activation (or IFN-I assays) and clinical features in different RMDs, thereby suggesting potential roles in several clinical applications such as diagnosis, prognosis, prediction of response to therapy and patient stratification. However, results have been heterogeneous and IFN-I assays have largely not progressed into routine clinical practice, with few exceptions mostly in infectious diseases.⁶ A key impediment has been the enormous diversity of approaches used for measuring IFN-I pathway activation, which ranged from IFN-I proteins, IFN-stimulated protein scores, the assessment of IFN-stimulated gene expression scores and signatures, to cell-based functional assays. In addition to the intrinsic differences across assay methods, the use of different biological samples, the lack of standardisation within each approach as well as the lack of a reference standard for all IFN-I assays have challenged the comparison and synthesis of the results. Under these circumstances, the exact added value of IFN-I measurements and the need of such assays for the clinical setting remains to be established.

For these reasons, a EULAR task force was convened to elaborate points to consider (PtC) to cover this gap, in order to enable more consistent reporting and facilitate uptake into

clinical practice as well as to appraise the current evidence on the clinical value of IFN-I measurements in RMDs to determine potential clinical utility.

METHODS

The EULAR Standardised Operating Procedures (SOPs) were followed to produce these PtC.⁷ After approval from the EULAR Executive Committee, the convenors (MAV and EMV) together with the methodologist (PGC) formed a multidisciplinary task force of 17 members (from 8 EULAR countries and the USA), including rheumatologists, immunologists, virologists, translational researchers and experts in interferonopathies. Two EMEUNET members and one patient representative (member of PARE) were also involved. A first meeting was held in July 2019 to introduce the project agenda and define the research questions (PICO structure). Systematic literature reviews (SLR) were performed with all the literature published until September 2019.^{8,9}

A second meeting (held remotely on two consecutive days in January 2021) was organised to present the evidence collected and after an iterative process, the overarching principles (OPs) and PtC were derived.

The level of evidence for each point was scored according to the Oxford Centre for Evidence-Based Medicine. Furthermore, scorings on the level of agreement (LoA) for each OP/PtC were retrieved by an online survey using a numeric scale (ranging from

Table 2 Consensus terminology

Term (abbreviation)	Definition
Interferon (IFN)	Proteins (cytokines) with anti-viral activity; IFNs are mediators of an anti-viral response. They belong to the type I, type II and type III IFN families.
Type I interferon (IFN-I)	The IFNs alpha, beta, omega, kappa, epsilon, secreted by any nucleated cell and binding to the IFNAR, which is expressed on any nucleated cell.
Type II interferon (IFN-II)	IFN gamma, mostly secreted by T cells, binding to the IFNGR, which is expressed on most leucocytes.
Type III interferon (IFN-III)	IFN lambda, which are structurally more similar to IL-10 but share downstream signalling and gene expression with IFN-I.
Interferon-stimulated genes (ISG)	Genes whose expression is known to be upregulated by any kind of IFN. Individual ISGs may not exclusively represent Type I IFN pathway activation.
Type I Interferon pathway	Type I IFN pathway is a dynamic, biological system that includes the secretion of type I IFN protein, binding to the IFNAR, initiation of Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways, expression of IFN-stimulated genes and the expression of IFN-stimulated proteins.
Type I Interferon pathway activation	Any evidence for changes in function or levels of the components of the type I IFN pathway.
Type I interferon pathway assay	An assay measuring one or more components of the Type I IFN pathway at a molecular or functional level.
Interferon stimulated gene expression signature	A qualitative description of coordinated expression of a set of ISGs that is indicative of type I IFN pathway activation.
Interferon stimulated gene expression score	A quantitative variable derived from expression of a defined set of ISGs that is indicative of type I IFN pathway activation.
Interferon stimulated protein score	A variable derived from expression of a defined set of soluble biomarkers known to be upregulated by IFN, although not specific for type I IFN.
Interferonopathy	Mendelian diseases in which there is constitutive type I IFN pathway activation with a causal role in pathology. The clinical picture may resemble RMDs. However, most diseases with IFN pathway activation are polygenic disorders and not mendelian interferonopathies.
RMDs, rheumatic and musculoskeletal diseases.	

0 = 'completely disagree' to 10 = 'fully agree'). The final manuscript was reviewed and approved by all task force participants.

RESULTS

Two OP and 11 PtC pertaining the IFN-I measuring and reporting in RMDs were produced (table 1).

The IFN pathway is a complex system with multiple subtypes of IFNs and diverse downstream effects on gene and protein expression

The IFN pathway comprises multiple types of IFNs (IFN-I, IFN-II and IFN-III) and receptors. A total of 16 subtypes can be distinguished within IFN-I proteins: 12 for IFN α , IFN β , IFN κ , IFN ω and IFN ϵ . IFN-II (IFN γ) and IFN-III (IFN λ -1, IFN λ -2, IFN λ -3 and IFN λ -4) have different proteins and receptors. On ligation with their shared surface receptor (IFNAR), IFN-Is regulate the expression of hundreds of IFN-stimulated genes (including signalling proteins, transcription factors, cytokines, etc), which have diverse functional effects on multiple cell types.¹⁰ However, there is a large overlap between the signalling pathways and IFN-stimulated genes induced by ligation of IFNAR with the receptors for IFN-II and IFN-III. The composition and intensity of the IFN-stimulated response are

dynamic, variable, context-dependent, influenced by multiple other stimuli, degree of activation, duration of the stimuli and negative regulation, and other factors, including the distribution of the receptors. Because of this complexity, care must be taken when planning and describing studies of this pathway.

IFN-I pathway activation is a common hallmark in many RMDs. Although IFN-I pathway activation is associated with some clinical manifestations, the utility of IFN-I pathway assays in clinical practice requires further validation for most contexts

Sustained IFN-I pathway activation has been demonstrated in a wide range of RMDs, with stronger evidence in systemic lupus erythematosus (SLE) studies, followed by polymyositis/dermatomyositis (PM/DM), rheumatoid arthritis (RA), primary Sjögren's syndrome (pSS), systemic sclerosis (SSc) and anti-phospholipid syndrome (APS). This activation has been demonstrated using different approaches and biological samples in most RMDs.⁹ The level of activation differs across conditions. IFN-I pathway activation has been related to several clinical features, but laboratory and clinical methodological issues preclude translation to clinical practice for the most contexts. The use of a whole blood four-gene IFN-I gene signature to predict response to anifrolumab is a more strongly validated application. Standardisation and clinical validation for other applications are critical clinical unmet needs for future biomarker research. Moreover, it must be noted that IFN-I pathway activation also occurs in immune responses apart from RMDs, so measurements of IFN-I pathway activation should be interpreted with caution and attention must be paid to clinical and biological contexts.

Task force consensus terminology should be considered for reporting IFN assays measurement

An important source of heterogeneity in reporting IFN research is the lack of a uniform terminology.^{11–13} The current task force has developed a consensus-based list of terms to cover key aspects related to IFN measurement and reporting, to ensure comparability in future research efforts (table 2).⁸ It includes a clear definition of all the elements under the umbrella term of 'IFN-I pathway' that we found to be relevant from the biomarker literature (from IFN proteins to IFN-stimulated mediators and effects), whose changes reflect IFN-I pathway activation and thus represent targets of the different assays. This terminology can be applied beyond the field of rheumatology.

Existing assays measure different aspects of the IFN pathway; they do not reflect the entirety of the pathway and some are not specific for IFN-I. The most appropriate assay will depend on the research or clinical question and should be justified

The IFN-I pathway (table 2) is a complex, dynamic biological entity encompassing a large number of upstream and downstream processes.^{12–15} Whether it is important to measure the direct production of IFN-I or its downstream effects (and which ones) should be taken into consideration, depending on the clinical or research question. For example, assays measuring IFN-I proteins directly may not assess all relevant IFN subtypes, and cellular sources, and tissues, nor the strength of downstream effect induced. Whereas on the other hand, assays measuring downstream effects (certain chemokines, sets of IFN-stimulated genes, etc) may not be specific for IFN-I pathway activation¹ or may differ in their degree of specificity^{11–15} and responsiveness to change (see PtC11).

Hence, existing assays each only capture a limited aspect of the whole pathway.⁸ As such, their readouts and their added value may differ, should not be considered as interchangeable, and must be interpreted in the context of the clinical application. In fact, different assays differ in their associations with clinical outcomes even in the same cohorts.^{8 16} Even though technical advances have allowed the development of highly sensitive and specific assays for some IFN proteins, such as Simoa, such assays still only evaluate part of the pathway and depend on specific antibodies, and their (clinical) superiority cannot currently be established. Therefore, there is not a single gold standard for IFN-I assays, and the most appropriate assay (or combination of assays) must be chosen (and justified) by a combination of theoretical, experimental, feasibility and clinical evidence requirements. The same applies to sample choice.^{111 11 15}

Publications on novel IFN-I pathway assays should report whether they specifically reflect IFN-I, and to the extent possible, which IFN-I is measured

Assays that evaluate downstream effects of IFN-I may be influenced by multiple IFNs, or other inflammatory mediators.^{3 8 11 12} This is not consistently tested in the literature. For reporting novel assays measuring IFN-I pathway activation, experimental demonstration to what degree they specifically measure IFN-I pathway activation is recommended. An analysis of the comparative effect of other IFN proteins (eg, IFN-II or γ and/or IFN-III or λ) as well as non-IFN controls on assays results should be included.

For assays that evaluate pathways downstream of the IFN-I receptor (eg, IFN stimulated gene expression or protein scores) the choice of components needs to be justified. For gene expression scores, the known subsets of IFN-stimulated genes should be described separately

Despite the broad use of assays measuring the indirect effects of IFN-I through downstream mediators (IFN-stimulated genes or proteins), a lack of consistency (and thus, replication and validation of clinical associations) was observed for both the choice of gene or protein components analysed as well as for their combinations.⁸ Reasons underlying these choices were not frequently disclosed. Considering that not all downstream mediators are specific for IFN-I, they may differ in their degree of specificity and responsiveness to change, results from different IFN-I scores may yield to different results, which has been shown to influence clinical associations.^{17–20}

Therefore, for assays measuring pathway changes downstream IFN-I receptor, the specificity for IFN-I must be proven to the extent possible, and the choice of the actual components (including number of components and their analyses) needs to be justified based on experimental evidence of existing literature demonstrating their specificity and clinical associations.^{17–20}

IFN-I pathway is consistently activated in several RMDs, but assays measuring IFN-I pathway activation cannot be currently recommended for diagnostic purposes

There is compelling evidence of IFN-I pathway activation in several RMDs compared with healthy controls.^{3 14 15 21} The strongest evidence in terms of numbers of studies and assays came from SLE.^{19 22–25} SSc^{26–29} and pSS^{30–33} were also evaluated by different assays, followed by RA^{34–37} and PM/DM,^{38–40} where

more consistent evidence was observed for DM compared with PM. However, despite the considerable number of studies, these generally test association in preselected groups. We found few well-designed diagnostic studies with appropriate diagnostic statistics, pretest/post-test probability assessment, the inclusion of disease controls and replication cohorts. Consequently, most of this evidence was overall judged as having high risk of bias for this application. Further limitations include: (1) IFN-I assays are not specific for RMDs, since IFN-I pathway activation is also observed in viral infections, monogenic interferonopathies and even cardiovascular disease; (2) IFN-I pathway activation seems to be present in several RMDs with different clinical presentation, so they may differentiate RMDs from normal, but not between specific RMDs; (3) IFN-I assays only capture a certain aspect of the IFN-I pathway, so a negative IFN-I assay cannot fully rule out the possibility that a patient had an IFN-I pathway activation, perhaps in non-circulating tissues, and variation among assays make difficult the comparison among studies and (4) IFN-I activation seems to be present in some patients but not always in a disease population as a whole (see PtC 6). These observations suggest that IFN-I pathway activation assays may be used in combination with other features (clinical signs or auto-antibodies) to improve patient diagnosis, but this has received reduced attention in the literature and studies suffered from the same methodological limitations as above. Furthermore, this application may be of limited impact beyond SLE and PM/DM populations, since the level of IFN-I pathway activation is much lower (see PtC6) and thus less likely to aid in diagnosis. Taken together, the use of IFN-I pathway assays for RMDs diagnosis cannot currently be recommended.

IFN-I pathway assays define more severe subgroups within many RMDs, so they should be considered for stratification studies

Although several RMDs are hallmarked by a sustained IFN-I pathway activation,^{3 14 15 21} evidence suggests that the level of activation differs across the RMD spectrum.^{41 42} A higher activation in blood has been observed in SLE, followed in order by PM/DM (especially in DM compared with PM), RA, pSS, SSc and APS,⁴¹ although methodological differences do not allow firm group comparisons. Overall, patients with IFN-I pathway activation are often associated with more severe clinical features, such as disease activity,^{11 22 26 31 32 41 43 44} organ involvement,^{20 23 25 26 45 46} damage^{25 47} or glucocorticoid use,^{48–50} across several RMDs. IFN-I pathway activation was found to have a greater effect than other clinical features in subanalyses and multivariate analyses, hence confirming an incremental value.^{20 22 25 49} Further evidence published after the accompanying SLR reconfirmed these findings in observational longitudinal studies¹⁶ as well as in clinical trials.^{13 51} Taken together, IFN-I pathway activation is indicated for patient stratification in RMDs.

IFN-I pathway activation is associated with disease activity in some RMDs, especially SLE and myositis, but its added value in clinical decision making is uncertain

There is substantial evidence that activation of the IFN-I pathway is associated with disease activity in some RMDs, especially in SLE^{20 23 24 41 43 47 52 53} and PM/DM.^{54 55} The association in other diseases such as RA^{34 44} or SSc^{26 27} depends on clinical subsets or disease duration. It is less clear whether knowledge of IFN pathway activation status would change a decision compared with the existing standard of using symptoms, signs and existing biomarkers such as acute phase markers. There

were no studies that evaluated the clinical impact of including IFN-I biomarkers in assessment of disease activity. Therefore, although the associations with disease activity are solid and consistent, the actual added value for clinical management is unknown.

In appraising the literature and in planning future research it must be noted that some disease activity instruments include laboratory biomarkers (eg, C-reactive protein, erythrocyte sedimentation rate, complement and anti-dsDNA levels) that may be directly influenced by IFN-I. Indices that only assess symptoms and signs are recommended for studies analysing IFN-I pathway activation. In addition, disease activity instruments such as the SLEDAI weigh organ-related activity differently, which makes testing association of assays with specific organ manifestations more complex.

Further, it must be considered that some IFN-I assays, and certain interferon stimulated genes (ISG), are more variable over time than others or present differential associations with some clinical aspects than others, which can affect conclusions about correlations with disease activity in cross-sectional or longitudinal analyses.

IFN-I pathway assays can predict disease exacerbations, in particular flare occurrence in patients with SLE, but further work should be performed to determine to what extent they outperform current instruments

There is evidence from many longitudinal studies reporting that IFN-I pathway activation can predict flare occurrence in patients with SLE.^{20 52 53 56–59} However, similar limitations as described in point 7 apply; despite evidence being consistent among studies using different IFN-I assays, the added value of such measurements over conventional clinical features and existing laboratory markers has to be established,^{52 53 56 58 60} and therefore, also whether an IFN-I assay would affect decision making.

IFN-I pathway assays might predict progression from preclinical autoimmunity to clinical disease

There is good quality and consistent evidence, although from a smaller number of longitudinal studies, associating IFN-I pathway activation in ‘at risk’ preclinical autoimmunity individuals with progression to SLE/CTD or RA. In RA, two studies (microarray and qPCR) both supported association between an IFN gene expression signature and progression from arthralgia to RA.^{61 62} IFN-I pathway activation showed a predictive value equivalent to that of autoantibodies (RF/ACPA) and improved the predictive power of the latter when combined.⁵³ Other classical risk factors such as age, shared epitope or acute-phase reactants did not exhibit predictive power. In antinuclear antibody (ANA)-positive individuals, a predefined set of ISGs predicted progression to SLE or pSS in a prospective study.⁵⁹ This effect was independent of other clinical characteristics and routine immunology features as demonstrated in a multivariate analysis.⁵⁹

Taken together, IFN-I pathway activation has been demonstrated to have an independent and incremental value in predicting progression to RMD. The field of preclinical disease is still emerging, and therefore, so is the role of novel biomarkers, but existing evidence suggests an equivalent effect than some autoantibodies, a greater effect than other conventional risk factors and a promising potential to improve prediction over traditional features.

In SLE, IFN-I pathway assays may be useful in predicting response to IFN-I targeting therapies

A qPCR IFN signature may be useful to predict treatment outcomes in patients with SLE undergoing IFN-I-targeting treatments, as differences in clinical response were observed depending on the level of IFN-I pathway activation.^{49 50 63 64} At the time of this SLR, the evidence is limited to phase II trials. Since that time, an analysis of pooled phase III data has been published validating the greater efficacy of anifrolumab in patients with high interferon gene signature, so this clinical application is the most strongly supported by the literature.⁵ The use of IFN-I assays to predict treatment outcomes in other conditions (RA, PM/DM) and non-IFN targeted therapies was inconclusive. In patients with RA, a higher IFN pathway activation was associated with worse outcomes on some treatments (conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs),^{34 44} tumour necrosis factor inhibitors (TNFi),^{18 34 65–67} tocilizumab⁶⁸ and rituximab^{69–72}), using different approaches, but heterogeneity and lack of replication prevented firm conclusions to be drawn.

IFN-I pathway assay results may be affected by some treatments (eg, IFN-targeted therapies and high-dose glucocorticoids), and timing of sample collection should be taken into account and reported

IFN-I pathway activation may be suppressed by some treatments such as IFN-targeted therapies^{47 73–77} and high-dose glucocorticoids,^{78 79} whereas the effect of other drugs (TNFi, hydroxychloroquine or rituximab) may be weaker or absent. However, treatment duration, dosages, existing RMD and the assay used (and the choice of ISG, if applicable) should be taken into account. Overall, most of the studies with no group-level changes in treatments or disease exacerbation reported little or no change over time across different RMD and techniques.

DISCUSSION

This is the first systematic approach to evaluate the use of IFN-I assays in clinical research and practice in rheumatology. The task force agreed on the formulation of 2 OP and 11 PtC, which represent the consensus of a multidisciplinary, international group covering all the range of professionals and stakeholders in this field. The level of agreement was overall high, thus supporting the broad acceptability of the statements produced. These PtC are expected to facilitate the validation and use of IFN-I assays in routine practice and clinical trials, to guide future steps in IFN-I research (box 1) where the evidence was lower, and to facilitate international collaborations.

Current literature on IFN-I pathway activation in RMDs is characterised by a great heterogeneity, which represents major pitfall to obtain clinical validation and establish clinical utility. Heterogeneity on IFN-I research is a multilevel issue, related to the complexity of the pathway biology itself, but also to the assay choice, clinical applications, clinical context, terminology, study designs and diversity in analysis and reporting practices. Assay-specific issues, such as the low reliability of direct IFN protein measurements due to sensitivity, the presence of multiple subtypes of IFN-I, cross-reactivity and potential interferences, also add to this complexity.^{8 80 81} This heterogeneity may account for the lack of transition of IFN-I assays into clinical practice and represents a major limitation that may preclude IFN-I potential to be realised. Under these circumstances, the task force aimed at providing uniform guidelines for terminology, assay choice, analysis and reporting. Of note, this set of statements (PtC 1–4)

Box 1 Research agenda

Fundamental/basic unmet needs

- ⇒ A better understanding of whether different type I interferons (IFN-I), in particular IFN α s, have unique and/or redundant functions may help in the development of more precise tools for clinical use.
- ⇒ For IFN-stimulated genes:
 - ⇒ Identify the sets of ISGs induced by different IFNs in relevant primary cell types.
 - ⇒ Characterise differences in cell sensitivity to IFN-I and tissue and cell-specific ISGs profiles.
 - ⇒ Characterise molecular, cellular and biochemical functions of ISGs.
 - ⇒ Identify which of the hundreds of ISGs typically induced actually mediate pathology in rheumatic and musculoskeletal diseases (RMDs).
 - ⇒ Investigate IFN-repressed factors.
- ⇒ Development of assays that directly, sensitively and specifically measure subtypes of IFN-I.

Methodological unmet needs

- ⇒ For downstream assays (IFN stimulated gene expression, IFN stimulated protein assays) the sensitivity and specificity for subtypes of IFNs, including appropriate positive and negative controls needs to be tested.
- ⇒ For interferon-stimulated gene expression assays:
 - ⇒ Confirmation of the most appropriate reference genes (across RMD spectrum).
 - ⇒ Investigation of the mechanistic explanation for the subgroupings of ISGs to decide which should be included in assays.
 - ⇒ Minimum number of genes needed to capture the information in existing scores.
 - ⇒ To confirm whether whole blood assays represent associations reported in peripheral blood mononuclear cell (PBMC) or cell subset literature.
- ⇒ For soluble interferon-stimulated protein assays:
 - ⇒ Most appropriate sample type (eg, serum or plasma).
 - ⇒ Appropriate selection of proteins to be analysed, how many to include and how to summarise results.
 - ⇒ To evaluate potential confounding factors such as neutralising antibodies and rheumatoid factors.
- ⇒ For high sensitivity interferon protein assays (eg, SiMoA)
 - ⇒ Investigation of the effects of non-circulating interferons and other interferon subtypes that may not be captured by a serum IFN- α SiMoA.
 - ⇒ Evaluation of the potential confounding effect of other pathogenic factors, such as neutralising antibodies and rheumatoid factors.
 - ⇒ Comparison of the results using a pan-IFN- α or an IFN- α subtype (eg, IFN- α) antibody.
- ⇒ For cellular interferon-stimulated protein assays (ie, flow cytometry).
 - ⇒ Confirmation of sample stability and transportation when used in routine clinical laboratories.

Clinical unmet needs in RMDs

- All of the following clinical studies must account for above technical validation
- ⇒ Diagnosis.

Continued

Box 1 Continued

- ⇒ Well-designed and powered formal diagnostic studies, controlling for existing clinical and routine laboratory tests, and in patient populations that are representative of the intended clinical context.
- ⇒ Evaluation of the added value of interferon assays in combination with other parameters (eg, autoantibodies or clinical features) for each specific RMD.
- ⇒ Patient stratification
 - ⇒ Establish the role of patient stratification within each RMD context according to management unmet needs.
- ⇒ Disease activity
 - ⇒ Confirmation of the added value of an interferon assay in determining disease activity as compared with an endpoint of an objective gold standard (eg, imaging or biopsy) or a subsequent clinical outcome.
- ⇒ Prediction of flare
 - ⇒ Well-designed and powered formal prognostic studies, controlling for existing clinical and routine laboratory tests, and in patient populations that are representative of the intended clinical context.
- ⇒ Progression in at-risk cohorts
 - ⇒ Validation studies for existing results in cohorts at risk of RA or CTD, including evaluation of appropriate clinical covariates.
 - ⇒ Confirmation of the added value of an interferon assay compared with an established, validated clinical instrument.
 - ⇒ Assessment of the added value of interferon over conventional risk factors for progression (eg, autoantibody profiling) once established.
- ⇒ Response to treatment
 - ⇒ Validation of data for prediction of response to anifrolumab in phase III trials.
 - ⇒ Replication of similar studies for other conventional and targeted therapies.
- ⇒ Responsiveness
 - ⇒ For specific therapies: evaluation of IFN-I assays at multiple time points from baseline in a population receiving similar therapy.
 - ⇒ For change in disease activity: evaluation of IFN-I assays at multiple time points in patients who are experiencing a change in clinical status (eg, flare or improvement), which may not depend on a specific therapy.

RA, rheumatoid arthritis.

showed the highest agreement, thus reinforcing their urge/priority and appropriateness for the experts. The use of these PtC will also enable international collaborations to solve clinical unmet needs. Moreover, these PtC create a framework for the implementation of biomarkers in the long-term, especially for complex pathways.

A greater understanding is imperative to maximise the clinical applications of the IFN-I pathway activation, especially with the advent of IFN-I-targeted therapies. Despite decades of research, the complexity of the IFN-I pathway remains only partially understood. In fact, specific and redundant functions of IFN-I subtypes are not firmly established, the sets of genes induced by different IFN-I subtypes in different types of cells or tissues are often partially known and many known ISGs remain functionally uncharacterised. The harmonising procedures here

developed are expected to foster the advancement towards the proposed research agenda (box 1).

Based on the existing literature, the task force strengthens that currently there is not a single, unique, universal assay for IFN-I pathway activation in RMDs. Consequently, none of the assays can be currently considered as a gold standard, and thus, assay decisions must be made considering both assay technical properties and the clinical question. The lack of harmonisation and the absence of universal gold standard(s) as well as comparative studies challenged the comparisons among the multiplicity of assays described in the literature. Moreover, as different assays measure distinct biological entities of the IFN-I pathway activation, they may likely capture distinct layers of information which differ in terms of their clinical correlate(s). This may account, at least in part, for the discrepancy among assay results within the same clinical purpose in a given disease, as observed in the SLR. The fact that evidence across RMDs was skewed represents an additional limitation in defining considerations across the whole spectrum of RMDs. Therefore, the potential integration of these PtC into clinical management needs to be evaluated within each RMD according to the detected clinical unmet needs and potential of IFN-I assays.

Evidence was however higher in SLE, not only in number of studies, but also in terms of quality and coverage of clinical applications. Therefore, SLE-specific PtC were formulated, which also received a high agreement. These clinical applications were mostly derived from qPCR, immunoassays and flow cytometry methods, which the task force considered as the most informative for the setting of SLE. More recent evidence on these assays is reassuring,^{82–84} including phase III trials.¹³ Of note, these methods differ in terms of assay methodology and biosamples, which provides a reassuring message on the clinical value of the IFN-I pathway activation itself, regardless of the method performed. Nevertheless, although certain parallelism may exist with other RMDs, whether this inference could be generalisable cannot be established at this point.

Clinical heterogeneity in some RMDs, especially SLE and RA, may also represent a substantial obstacle for the development and validation of IFN-I assays for clinical management. However, IFN-I pathway activation may be a powerful instrument to decipher the biological complexity of these heterogeneous conditions. As distinct from application in disease diagnosis, evidence was stronger and more consistent for a role in patient stratification, which may guide differences in management and perhaps resolve the apparent heterogeneity. Hence, assays measuring IFN-I pathway activation have high likelihood of instructing the molecular taxonomy of RMDs, enabling patient stratification and allowing reclassification into ‘molecular hubs’ or mechanistically distinct subsets.⁸⁵

Apart from RMDs, IFN-I has numerous roles in other autoimmune, infectious, cardiovascular and oncological contexts. These guidelines may, therefore, also be of interest for other specialties. The observation of these statements beyond rheumatology will help to gain understanding towards the IFN-I pathway activation in other clinical scenarios compared with RMDs. The task force felt that one of these areas are monogenic interferonopathies, where clinical heterogeneity may be linked to differential tissue expression of the constitutive IFN-I production and/or signalling, which is characteristic of these rare disorders.⁸⁶ Assessment of IFN-I pathway activation may be of help in the screening of interferonopathies in some subsets of RMDs and may represent a strong tool for diagnosis assessment in this scenario.

This study has some limitations that should be noted. These PtC were built on SLRs covering all IFN research until 2019,

and further evidence has been published subsequently. However, recent evidence by no means changes the current PtC but confirm the value of IFN-I pathway activation to predict therapeutic responses in SLE (PtC10),¹³ to measure disease activity in SLE and DM (PtC7),^{16 87} and to demonstrate stability in the absence of treatment changes/disease exacerbations.⁸⁸ Additional evidence has demonstrated that IFN-I pathway activation can be useful to segregate patients (PtC6) but different assays measure different pathway aspects and thus are not fully interchangeable (PtC2).^{89 90} Of note, the latest evidence consistently exhibits the same weaknesses raised in these PtC, such as heterogeneous nomenclature, lack of clinical validation for some applications and assessment of added value, hence reinforcing the need for uniform practices and a consistent research agenda. Moreover, the lack of clinical instruments in certain areas, such as progression from at-risk phases, may represent an additional limitation to realise the potential of IFN-I assays.

In conclusion, the assessment of the IFN-I pathway activation has a high potential for implementation in the clinical management of several RMDs, although further research is needed. We have developed a set of PtC that creates a framework for harmonisation, validation and application of IFN-I assays in clinical research and practice with the ultimate goal of translating these assays into clinical care. Uptake of these considerations along with gains in understanding from the proposed research agenda will facilitate updating of these statements that may eventually be considered in the category of recommendations. Finally, this work represents a model for the translation of other biomarkers, beyond the field of IFNs and rheumatology.

Author affiliations

¹Department of Functional Biology, University of Oviedo, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Asturias, Spain

²Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds & NIHR Leeds Biomedical Research Centre, Leeds, UK

³Erasmus MC, University Medical Center Rotterdam, Laboratory Medical Immunology, Department of Immunology, Rotterdam, The Netherlands

⁴Charité University Medicine Berlin, Department of Rheumatology, Berlin, Germany

⁵Uppsala University, Department of Medical Sciences, Rheumatology, Uppsala, Sweden

⁶Vita-Salute San Raffaele University, Unit of Immunology, Rheumatology, Allergy and Rare Diseases, Milan, Italy

⁷EULAR PARE Patient Research Partner, Amsterdam, The Netherlands

⁸Medicine, University of Crete, Medical School, Department of Internal Medicine, Heraklion, Greece

⁹University of Crete, Medical School, Department of Rheumatology-Clinical Immunology, Heraklion, Greece

¹⁰Karolinska Institutet, Division of Rheumatology, Stockholm, Sweden

¹¹Broegelmann Research Laboratory, Department of Clinical Science, University of Bergen, Bergen, Norway

¹²Medical Research Council Human Immunology Unit, Medical Research Council Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

¹³Université de Paris Cité, Hôpital Necker-Enfants Malades, Immuno-Hématologie et Rhumatologie pédiatriques, Paris, France

¹⁴Hospital for Special Surgery, Weill Cornell Medical College, Mary Kirkland Center for Lupus Research, New York, New York, USA

¹⁵Erasmus MC, University Medical Center Rotterdam, Department of Immunology, Rotterdam, The Netherlands

Twitter Javier Rodríguez-Carrio @javierrcarrio, Dimitrios T Boumpas @none and Edward M Vital @edvital

Contributors JR-C, AB, PGC, EMV and MAV led the literature search, data extraction and formulated the draft PtC versions. All authors participated in the definition of the final versions of the PtC and provide feedback for their interpretation and discussion. JR-C and AB drafted the manuscript. PGC, EMV and MAV edited the manuscript draft. All authors contributed and approved the final version of the manuscript. EV is guarantor for the manuscript.

Funding This work was funded by the European Alliance of Associations for Rheumatology (EULAR) (grant number SCI019). PGC and EMV are supported in part

by the UK National Institute for Health and Care Research (NIHR) Leeds Biomedical Research Centre.

Competing interests MKC has received consulting fees from AstraZeneca, Bristol Myers Squibb, Lilly, and Shannon Pharmaceuticals, as well as grant/research support from Gilead. LR has received consulting fees from AstraZeneca. EMV served in the speakers' bureau of GSK, received consulting fees from AURINIA, SANDOZ, GSK, AstraZeneca, Roche, and Modus, as well as grant/research support from AstraZeneca. PGC has received consultancies or speaker fees from AbbVie, Amgen, AstraZeneca, BMS, Eli Lilly, Galapagos, GSK, Merck, Pfizer, Novartis and UCB.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement No data are available. The data used to formulate these guidelines were the results of two systematic literature reviews, will be published as separate manuscripts 10.1136/rmdopen-2022-002876 and 10.1136/rmdopen-2022-002864.

ORCID iDs

Javier Rodríguez-Carrio <http://orcid.org/0000-0002-0011-5102>
 Philip G Conaghan <http://orcid.org/0000-0002-3478-5665>
 Robert Biesen <http://orcid.org/0000-0002-0434-7832>
 Dimitrios T Boumpas <http://orcid.org/0000-0002-9812-4671>
 Marie Wahren-Herlenius <http://orcid.org/0000-0002-0915-7245>
 Marie-Louise Frémond <http://orcid.org/0000-0002-2798-9141>
 Mary K Crow <http://orcid.org/0000-0002-7881-2020>
 Lars Rönnblom <http://orcid.org/0000-0001-9403-6503>
 Marjan A Versnel <http://orcid.org/0000-0003-0245-5386>
 Edward M Vital <http://orcid.org/0000-0003-1637-4755>

REFERENCES

- Ivashkiv LB, Donlin LT. Regulation of type I interferon responses. *Nat Rev Immunol* 2014;14:36–49.
- Muskardin TLW, Niewold TB. Type I interferon in rheumatic diseases. *Nat Rev Rheumatol* 2018;14:214–28.
- Psarras A, Emery P, Vital EM. Type I interferon-mediated autoimmune diseases: pathogenesis, diagnosis and targeted therapy. *Rheumatology (Oxford)* 2017;56:1662–75.
- Crow MK. Type I interferon in organ-targeted autoimmune and inflammatory diseases. *Arthritis Res Ther* 2010;12 Suppl 1:S5.
- Morand EF, Furie R, Tanaka Y, et al. Trial of anifrolumab in active systemic lupus erythematosus. *N Engl J Med* 2020;382:211–21.
- Shirley M. FebrDiX[®]: A rapid diagnostic test for differentiating bacterial and viral aetiologies in acute respiratory infections. *Mol Diagn Ther* 2019;23:803–9.
- van der Heijde D, Aletaha D, Carmona L, et al. 2014 update of the EULAR standardised operating procedures for EULAR-endorsed recommendations. *Ann Rheum Dis* 2015;74:8–13.
- Burska AN, Rodríguez-Carrio J, Biesen R, et al. Type I interferon pathway assays in studies of rheumatic and musculoskeletal diseases: a systematic literature review informing EULAR points to consider. *RMD Open* 2023.
- Rodríguez-Carrio J, Burska A, Conaghan PG, et al. Association between type I interferon pathway activation and clinical outcomes in rheumatic and musculoskeletal diseases: a systematic literature review informing EULAR points to consider. *RMD Open* 2023.
- Schoggins JW. Interferon-stimulated genes: what do they all do? *Annu Rev Virol* 2019;6:567–84.
- Rodero MP, Decalf J, Bondet V, et al. Detection of interferon alpha protein reveals differential levels and cellular sources in disease. *J Exp Med* 2017;214:1547–55.
- Lamot L, Niemietz I, Brown KL. Methods for type I interferon detection and their relevance for clinical utility and improved understanding of rheumatic diseases. *Clin Exp Rheumatol* 2019;37:1077–83.
- Vital EM, Merrill JT, Morand EF, et al. Anifrolumab efficacy and safety by type I interferon gene signature and clinical subgroups in patients with SLE: post hoc analysis of pooled data from two phase III trials. *Ann Rheum Dis* 2022;81:951–61.
- Rönnblom L, Eloranta M-L. The interferon signature in autoimmune diseases. *Curr Opin Rheumatol* 2013;25:248–53.
- Crow MK, Olfertiev M, Kirou KA. Type I interferons in autoimmune disease. *Annu Rev Pathol* 2019;14:369–93.
- Wahadat MJ, Schonenberg-Meinema D, van Helden-Meeuwse CG, et al. Gene signature fingerprints stratify SLE patients in groups with similar biological disease profiles: a multicentre longitudinal study. *Rheumatology (Oxford)* 2022;61:4344–54.
- Somers EC, Zhao W, Lewis EE, et al. Type I interferons are associated with subclinical markers of cardiovascular disease in a cohort of systemic lupus erythematosus patients. *PLoS One* 2012;7:e37000.
- Reynier F, Petit F, Paye M, et al. Importance of correlation between gene expression levels: application to the type I interferon signature in rheumatoid arthritis. *PLoS One* 2011;6:e24828.
- Psarras A, Md Yusof MY, El-Sherbiny YM, et al. A9.05 distinct subsets of interferon-stimulated genes are associated with incomplete and established systemic lupus erythematosus. *Ann Rheum Dis* 2016;75:A72.
- El-Sherbiny YM, Psarras A, Md Yusof MY, et al. A novel two-score system for interferon status segregates autoimmune diseases and correlates with clinical features. *Sci Rep* 2018;8:5793.
- Barrat FJ, Crow MK, Ivashkiv LB. Interferon target-gene expression and epigenomic signatures in health and disease. *Nat Immunol* 2019;20:1574–83.
- Bauer JW, Petri M, Batliwalla FM, et al. Interferon-Regulated chemokines as biomarkers of systemic lupus erythematosus disease activity: a validation study. *Arthritis Rheum* 2009;60:3098–107.
- Baechler EC, Batliwalla FM, Karypis G, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci U S A* 2003;100:2610–5.
- Bauer JW, Baechler EC, Petri M, et al. Elevated serum levels of interferon-regulated chemokines are biomarkers for active human systemic lupus erythematosus. *PLoS Med* 2006;3:e491.
- Kirou KA, Lee C, George S, et al. Activation of the interferon-alpha pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease. *Arthritis Rheum* 2005;52:1491–503.
- Eloranta M-L, Franck-Larsson K, Lövgren T, et al. Type I interferon system activation and association with disease manifestations in systemic sclerosis. *Ann Rheum Dis* 2010;69:1396–402.
- Liu X, Mayes MD, Tan FK, et al. Correlation of interferon-inducible chemokine plasma levels with disease severity in systemic sclerosis. *Arthritis Rheum* 2013;65:226–35.
- Tan FK, Zhou X, Mayes MD, et al. Signatures of differentially regulated interferon gene expression and vasculotrophism in the peripheral blood cells of systemic sclerosis patients. *Rheumatology (Oxford)* 2006;45:694–702.
- Brkic Z, van Bon L, Cossu M, et al. The interferon type I signature is present in systemic sclerosis before overt fibrosis and might contribute to its pathogenesis through high BAFF gene expression and high collagen synthesis. *Ann Rheum Dis* 2016;75:1567–73.
- Wildenberg ME, van Helden-Meeuwse CG, van de Merwe JP, et al. Systemic increase in type I interferon activity in Sjögren's syndrome: a putative role for plasmacytoid dendritic cells. *Eur J Immunol* 2008;38:2024–33.
- Brkic Z, Maria NI, van Helden-Meeuwse CG, et al. Prevalence of interferon type I signature in CD14 monocytes of patients with Sjögren's syndrome and association with disease activity and BAFF gene expression. *Ann Rheum Dis* 2013;72:728–35.
- Maria NI, Brkic Z, Waris M, et al. Mxa as a clinically applicable biomarker for identifying systemic interferon type I in primary Sjögren's syndrome. *Ann Rheum Dis* 2014;73:1052–9.
- Bodewes ILA, Al-Ali S, van Helden-Meeuwse CG, et al. Systemic interferon type I and type II signatures in primary Sjögren's syndrome reveal differences in biological disease activity. *Rheumatology (Oxford)* 2018;57:921–30.
- Rodríguez-Carrio J, Alperi-López M, López P, et al. Heterogeneity of the type I interferon signature in rheumatoid arthritis: a potential limitation for its use as a clinical biomarker. *Front Immunol* 2017;8:2007.
- Rodríguez-Carrio J, de Paz B, López P, et al. IFN α serum levels are associated with endothelial progenitor cells imbalance and disease features in rheumatoid arthritis patients. *PLoS One* 2014;9:e86069.
- van der Pouw Kraan TCTM, Wijbrandts CA, van Baarsen LGM, et al. Rheumatoid arthritis subtypes identified by genomic profiling of peripheral blood cells: assignment of a type I interferon signature in a subpopulation of patients. *Ann Rheum Dis* 2007;66:1008–14.
- Cooles FAH, Anderson AE, Hilken CMU, et al. A1.13 the prevalence of a raised interferon gene signature is increased in early RA and is associated with worse disease activity. *Ann Rheum Dis* 2016;75:A6.
- Liao AP, Salajegheh M, Nazareno R, et al. Interferon β is associated with type 1 interferon-inducible gene expression in dermatomyositis. *Ann Rheum Dis* 2011;70:831–6.
- Walsh RJ, Kong SW, Yao Y, et al. Type I interferon-inducible gene expression in blood is present and reflects disease activity in dermatomyositis and polymyositis. *Arthritis Rheum* 2007;56:3784–92.
- Greenberg SA, Higgs BW, Morehouse C, et al. Relationship between disease activity and type 1 interferon- and other cytokine-inducible gene expression in blood in dermatomyositis and polymyositis. *Genes Immun* 2012;13:207–13.
- Higgs BW, Liu Z, White B, et al. Patients with systemic lupus erythematosus, myositis, rheumatoid arthritis and scleroderma share activation of a common type I interferon pathway. *Ann Rheum Dis* 2011;70:2029–36.
- Assassi S, Mayes MD, Arnett FC, et al. Systemic sclerosis and lupus: points in an interferon-mediated continuum. *Arthritis Rheum* 2010;62:589–98.
- Biesen R, Demir C, Barkhadarova F, et al. Sialic acid-binding Ig-like lectin 1 expression in inflammatory and resident monocytes is a potential biomarker for monitoring disease activity and success of therapy in systemic lupus erythematosus. *Arthritis Rheum* 2008;58:1136–45.

- 44 Cooles FAH, Anderson AE, Lendrem DW, *et al.* The interferon gene signature is increased in patients with early treatment-naïve rheumatoid arthritis and predicts a poorer response to initial therapy. *J Allergy Clin Immunol* 2018;141:445–8.
- 45 Bos CL, van Baarsen LGM, Timmer TCG, *et al.* Molecular subtypes of systemic sclerosis in association with anti-centromere antibodies and digital ulcers. *Genes Immun* 2009;10:210–8.
- 46 Airò P, Ghidini C, Zanotti C, *et al.* Upregulation of myxovirus-resistance protein A: a possible marker of type I interferon induction in systemic sclerosis. *J Rheumatol* 2008;35:2192–200.
- 47 Kennedy WP, Maciuga R, Wolslegel K, *et al.* Association of the interferon signature metric with serological disease manifestations but not global activity scores in multiple cohorts of patients with SLE. *Lupus Sci Med* 2015;2:e000080.
- 48 Dutton K, Psarras A, El-Sherbiny Y, *et al.* OP0124 In sle patients in sustained low disease activity, novel interferon assays predict flares and glucocorticoid requirements. Annual European Congress of Rheumatology, EULAR 2018, Amsterdam, 13–16 June 2018; June 2018
- 49 Furie R, Khamashta M, Merrill JT, *et al.* Anifrolumab, an anti-interferon- α receptor monoclonal antibody, in moderate-to-severe systemic lupus erythematosus. *Arthritis Rheumatol* 2017;69:376–86.
- 50 Kalunian KC, Merrill JT, Maciuga R, *et al.* A phase II study of the efficacy and safety of roplaximab (rhumb interferon- α) in patients with systemic lupus erythematosus (ROSE). *Ann Rheum Dis* 2016;75:196–202.
- 51 Merrill JT, Werth VP, Furie R, *et al.* Phase 2 trial of iberdomide in systemic lupus erythematosus. *N Engl J Med* 2022;386:1034–45.
- 52 Mathian A, Mouries-Martin S, Dorgham K, *et al.* Ultrasensitive serum interferon- α quantification during SLE remission identifies patients at risk for relapse. *Ann Rheum Dis* 2019;78:1669–76.
- 53 Rose T, Grützkau A, Hirsland H, *et al.* Irfn α and its response proteins, IP-10 and SIGLEC-1, are biomarkers of disease activity in systemic lupus erythematosus. *Ann Rheum Dis* 2013;72:1639–50.
- 54 Baechler EC, Bauer JW, Slatery CA, *et al.* An interferon signature in the peripheral blood of dermatomyositis patients is associated with disease activity. *Mol Med* 2007;13:59–68.
- 55 Huard C, Gullà SV, Bennett DV, *et al.* Correlation of cutaneous disease activity with type 1 interferon gene signature and interferon β in dermatomyositis. *Br J Dermatol* 2017;176:1224–30.
- 56 Mathian A, Mouries-Martin S, Dorgham K, *et al.* Monitoring disease activity in systemic lupus erythematosus with single-molecule array digital enzyme-linked immunosorbent assay quantification of serum interferon- α . *Arthritis Rheumatol* 2019;71:756–65.
- 57 Rose T, Grützkau A, Klotsche J, *et al.* Are interferon-related biomarkers advantageous for monitoring disease activity in systemic lupus erythematosus? A longitudinal benchmark study. *Rheumatology (Oxford)* 2017;56:1618–26.
- 58 Munroe ME, Vista ES, Merrill JT, *et al.* Pathways of impending disease flare in african-american systemic lupus erythematosus patients. *J Autoimmun* 2017;78:70–8.
- 59 Md Yusof MY, Psarras A, El-Sherbiny YM, *et al.* Prediction of autoimmune connective tissue disease in an at-risk cohort: prognostic value of a novel two-score system for interferon status. *Ann Rheum Dis* 2018;77:1432–9.
- 60 Mackay M, Oswald M, Sanchez-Guerrero J, *et al.* Molecular signatures in systemic lupus erythematosus: distinction between disease flare and infection. *Lupus Sci Med* 2016;3:e000159.
- 61 van Baarsen LG, Wijbrandts CA, Rustenburg F, *et al.* Regulation of IFN response gene activity during infliximab treatment in rheumatoid arthritis is associated with clinical response to treatment. *Arthritis Res Ther* 2010;12:R11.
- 62 Lübbers J, Brink M, van de Stadt LA, *et al.* The type I IFN signature as a biomarker of preclinical rheumatoid arthritis. *Ann Rheum Dis* 2013;72:776–80.
- 63 Petri M, Wallace DJ, Spindler A, *et al.* Sifalimumab, a human anti-interferon- α monoclonal antibody, in systemic lupus erythematosus: a phase I randomized, controlled, dose-escalation study. *Arthritis Rheum* 2013;65:1011–21.
- 64 Merrill JT, Furie R, Werth VP, *et al.* Anifrolumab effects on rash and arthritis: impact of the type I interferon gene signature in the phase IIb MUSE study in patients with systemic lupus erythematosus. *Lupus Sci Med* 2018;5:e000284.
- 65 Wright HL, Thomas HB, Moots RJ, *et al.* Interferon gene expression signature in rheumatoid arthritis neutrophils correlates with a good response to tnfi therapy. *Rheumatology (Oxford)* 2015;54:188–93.
- 66 Mavragani CP, La DT, Stohl W, *et al.* Association of the response to tumor necrosis factor antagonists with plasma type I interferon activity and interferon-beta/alpha ratios in rheumatoid arthritis patients: a post hoc analysis of a predominantly Hispanic cohort. *Arthritis Rheum* 2010;62:392–401.
- 67 Wampler Muskardin T, Vashist P, Dorschner JM, *et al.* Increased pretreatment serum IFN- β / α ratio predicts non-response to tumour necrosis factor α inhibition in rheumatoid arthritis. *Ann Rheum Dis* 2016;75:1757–62.
- 68 Sanayama Y, Ikeda K, Saito Y, *et al.* Prediction of therapeutic responses to tocilizumab in patients with rheumatoid arthritis: biomarkers identified by analysis of gene expression in peripheral blood mononuclear cells using genome-wide DNA microarray. *Arthritis Rheumatol* 2014;66:1421–31.
- 69 Thurlings RM, Boumans M, Tekstra J, *et al.* Relationship between the type I interferon signature and the response to rituximab in rheumatoid arthritis patients. *Arthritis Rheum* 2010;62:3607–14.
- 70 Vosslander S, Raterman HG, van der Pouw Kraan TCTM, *et al.* Pharmacological induction of interferon type I activity following treatment with rituximab determines clinical response in rheumatoid arthritis. *Ann Rheum Dis* 2011;70:1153–9.
- 71 Raterman HG, Vosslander S, de Ridder S, *et al.* The interferon type I signature towards prediction of non-response to rituximab in rheumatoid arthritis patients. *Arthritis Res Ther* 2012;14:R95.
- 72 de Jong TD, Vosslander S, Blits M, *et al.* Effect of prednisone on type I interferon signature in rheumatoid arthritis: consequences for response prediction to rituximab. *Arthritis Res Ther* 2015;17:78.
- 73 Lauwerys BR, Hachulla E, Spertini F, *et al.* Down-regulation of interferon signature in systemic lupus erythematosus patients by active immunization with interferon α -kinoid. *Arthritis Rheum* 2013;65:447–56.
- 74 Casey KA, Guo X, Smith MA, *et al.* Type I interferon receptor blockade with anifrolumab corrects innate and adaptive immune perturbations of SLE. *Lupus Sci Med* 2018;5:e000286.
- 75 Lee PY, Li Y, Richards HB, *et al.* Type I interferon as a novel risk factor for endothelial progenitor cell depletion and endothelial dysfunction in systemic lupus erythematosus. *Arthritis Rheum* 2007;56:3759–69.
- 76 Bennett L, Palucka AK, Arce E, *et al.* Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J Exp Med* 2003;197:711–23.
- 77 Merrill JT, Wallace DJ, Petri M, *et al.* Safety profile and clinical activity of sifalimumab, a fully human anti-interferon α monoclonal antibody, in systemic lupus erythematosus: a phase I, multicentre, double-blind randomised study. *Ann Rheum Dis* 2011;70:1905–13.
- 78 Li Y, Lee PY, Kellner ES, *et al.* Monocyte surface expression of fcgama receptor RI (CD64), a biomarker reflecting type-I interferon levels in systemic lupus erythematosus. *Arthritis Res Ther* 2010;12:R90.
- 79 Kawasaki M, Fujishiro M, Yamaguchi A, *et al.* Possible role of the JAK/STAT pathways in the regulation of T cell-interferon related genes in systemic lupus erythematosus. *Lupus* 2011;20:1231–9.
- 80 Jabs WJ, Hennig C, Zawatzky R, *et al.* Failure to detect antiviral activity in serum and plasma of healthy individuals displaying high activity in ELISA for IFN-alpha and IFN-beta. *J Interferon Cytokine Res* 1999;19:463–9.
- 81 Niewold TB, Hua J, Lehman TJA, *et al.* High serum IFN-alpha activity is a heritable risk factor for systemic lupus erythematosus. *Genes Immun* 2007;8:492–502.
- 82 Zorn-Pauly L, von Stuckrad ASL, Klotsche J, *et al.* Evaluation of SIGLEC1 in the diagnosis of suspected systemic lupus erythematosus. *Rheumatology (Oxford)* 2022;61:3396–400.
- 83 Chasset F, Mathian A, Dorgham K, *et al.* Serum interferon- α levels and IFN type I-stimulated gene score perform equally to assess systemic lupus erythematosus disease activity. *Ann Rheum Dis* 2022;81:901–3.
- 84 Huijser E, Göpfert J, Brkic Z, *et al.* Serum interferon- α 2 measured by single-molecule array associates with systemic disease manifestations in Sjögren's syndrome. *Rheumatology (Oxford)* 2022;61:2156–66.
- 85 Boutrid N, Rahmoune H. Reframing immune-mediated inflammatory diseases. *N Engl J Med* 2021;385:e75.
- 86 Lodi L, Melki I, Bondet V, *et al.* Differential expression of interferon-alpha protein provides clues to tissue specificity across type I interferonopathies. *Journal of Clinical Immunology* 2021;41:603–9.
- 87 Graf M, von Stuckrad SL, Uruha A, *et al.* SIGLEC1 enables straightforward assessment of type I interferon activity in idiopathic inflammatory myopathies. *RMD Open* 2022;8:e001934.
- 88 Höppner J, Casteleyn V, Biesen R, *et al.* SIGLEC-1 in systemic sclerosis: A useful biomarker for differential diagnosis. *Pharmaceuticals (Basel, Switzerland)* 2022;15:1198.
- 89 Smith MA, Chiang C-C, Zerrouki K, *et al.* Using the circulating proteome to assess type I interferon activity in systemic lupus erythematosus. *Sci Rep* 2020;10:4462.
- 90 Trutschel D, Bost P, Mariette X, *et al.* Variability of primary sjögren's syndrome is driven by interferon- α and interferon- α blood levels are associated with the class II HLA-DQ locus. *Arthritis Rheumatol* 2022;74:1991–2002.