RMD Open
Rheumatic & Musculoskeletal Diseases

ORIGINAL RESEARCH

Type I interferon pathway assays in studies of rheumatic and musculoskeletal diseases: a systematic literature review informing EULAR points to consider

Agata Burska,[1] Javier Rodríguez-Carrio ,[2] Robert Biesen ,[3] Willem A Dik,[4] Maija-Leena Eloranta,[5] Giulio Cavalli,[6,7] Marianne Visser,[8] Dimitrios T Boumpas ,[9] George Bertsias,[9] Marie Wahren-Herlenius ,[10,11] Jan Rehwinkel,[12] Marie-Louise Frémond,[13] Mary K Crow,[14] Lars Ronnblom,[5] PG Conaghan ,[1] Marjan Versnel,[15] Ed Vital[1]

To cite: Burska A, 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;**9**:e002876. doi:10.1136/rmdopen-2022-002876

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/rmdopen-2022-002876).

AB and JR-C contributed equally.
MV and EV contributed equally.

AB and JR-C are joint first authors.
MV and EV are joint senior authors.

Received 18 November 2022
Accepted 8 February 2023

For numbered affiliations see end of article.

Correspondence to
Dr Ed Vital;
E.M.J.Vital@leeds.ac.uk

ABSTRACT

Objectives To systematically review the literature for assay methods that aim to evaluate type I interferon (IFN-I) pathway activation and to harmonise-related terminology.

Methods Three databases were searched for reports of IFN-I and rheumatic musculoskeletal diseases. Information about the performance metrics of assays measuring IFN-I and measures of truth were extracted and summarised. A EULAR task force panel assessed feasibility and developed consensus terminology.

Results Of 10 037 abstracts, 276 fulfilled eligibility criteria for data extraction. Some reported more than one technique to measure IFN-I pathway activation. Hence, 276 papers generated data on 412 methods. IFN-I pathway activation was measured using: qPCR (n=121), immunoassays (n=101), microarray (n=69), reporter cell assay (n=38), DNA methylation (n=14), flow cytometry (n=14), cytopathic effect assay (n=11), RNA sequencing (n=9), plaque reduction assay (n=8), Nanostring (n=5), bisulphite sequencing (n=3). Principles of each assay are summarised for content validity. Concurrent validity (correlation with other IFN assays) was presented for n=150/412 assays. Reliability data were variable and provided for 13 assays. Gene expression and immunoassays were considered most feasible. Consensus terminology to define different aspects of IFN-I research and practice was produced.

Conclusions Diverse methods have been reported as IFN-I assays and these differ in what elements or aspects of IFN-I pathway activation they measure and how. No 'gold standard' represents the entirety of the IFN pathway, some may not be specific for IFN-I. Data on reliability or comparing assays were limited, and feasibility is a challenge for many assays. Consensus terminology should improve consistency of reporting.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Type I and type II interferons play a role in a broad spectrum of rheumatic musculoskeletal diseases (RMDs).

⇒ There is a large body of literature indicating that many different assay methodologies evaluating distinct steps of type I interferon (IFN-I) pathway activity may have roles in the diagnosis, prognosis, therapy selection and stratification for therapy in RMD patients. However, no consensus on the best method has been proposed.

WHAT THIS STUDY ADDS

⇒ This study provides a systemic literature review and synthesis of all published data on IFN-I assays reported in basic and clinical research in RMDs, especially the most substantial literature on gene expression and protein assays in systemic lupus erythematosus.

⇒ We provide an appraisal and commentary of an expert group on content and criterion validity, reliability and feasibility of these assays.

⇒ We also propose consensus terminology for future IFN assay reporting in basic and clinical research.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This work will assist physicians and researchers to select the most appropriate assays for the analysis of IFN-I pathway activity and facilitate the translation of IFN-I pathway activation assays into clinical practice.

INTRODUCTION

Type I interferons (IFN-Is) are a group of cytokines with antiviral and immune-modulatory function. IFN-Is have roles in

several rheumatic musculoskeletal diseases (RMDs), Many IFN-I assays have been proposed to aid in clinical management of RMDs but, despite a large body of literature, not yet adopted into routine clinical practice.[1]

The IFN-I family comprises 13 functional IFN-α genes and one IFN-β gene on human chromosome 9, as well as IFN-ε, IFN-κ, IFN-τ and IFN-ω. IFN-I subtypes are produced by all nucleated cells. In acute viral infection circulating haematopoietic immune cells, especially plasmacytoid dendritic cells, are the most important producers. In other contexts production by stromal and parenchymal cells in most tissues may be more important.[2 3] These proteins bind to a shared receptor (IFNAR), initiating a cascade of downstream molecular and cellular effects. Signalling via the JAK-STAT pathway leads to expression of IFN-stimulated genes (ISG) that contain the IFN-sensitive response element. These ISGs encode intracellular, surface and soluble proteins that have diverse effects on immune regulation and on antiviral response, with significant remodelling of mRNA processing, post-translational modifications, metabolism, cellular trafficking, chromatin organisation and the cytoskeleton, among others.[4 5] Although there are distinctions in the signalling pathway and response elements between type I, type II (IFN-γ) and type III IFNs (IFN-λs), there is also considerable overlap between these systems which may make interpretation of these downstream pathways difficult.[6] ISG expression on similar stimuli may differ between cell types and tissues.[1]

IFN-I have pathogenic roles in a broad range of human diseases including autoimmunity, infection, cancer and cardiovascular disease.[7 8] In RMDs, IFN-I acts as a mediator linking innate and adaptive immunity, with special significance in diseases in which self-nucleic acids are sensed by IFN-producing innate immune cells, in which they probably promotes the production of antinuclear antibodies.[9 10] Therapeutic monoclonal antibody blockade of IFNAR was effective in phase III clinical trials in systemic lupus erythematosus (SLE), and is being investigated in other RMDs, and IFN-I pathway blockade may be involved in the mechanism of action of other therapies such as anti-malarials and JAK inhibitors.[11 12]

Assays for IFN-I have been proposed to have roles in the diagnosis, prognosis, therapy selection and stratification for therapy in RMD patients, to reclassify RMDs, as well as predict disease onset.[13] A limitation in the progression of these assays into routine practice has been the number and heterogeneity of methods and clinical studies published.[1] Assays include methods to measure IFN-I proteins,[14] gene expression assays for ISGs, assays for proteins encoded by ISGs, DNA methylation and functional assays. These are paralleled by heterogeneity in the diseases studied, clinical questions addressed and design of clinical studies. As a result, there has been no consensus on the type of IFN-I assay that should be used, nor in what clinical indications. An additional issue is the use of varying, and sometimes contradictory, terminology to refer to aspects of the biological pathway and systems for evaluating it.

To address these issues, a EULAR task force was convened. We aimed to conduct a systematic literature review (SLR) on the principles and performance metrics of the assays described in the field of RMDs and to develop consensus terminology for use in future studies.

METHODS

EULAR standardised operating procedures for EULAR-endorsed recommendations were followed.[15] A multidisciplinary task force of 17 members (from 8 EULAR countries and the USA) was convened including experts in all techniques used for IFN-I pathway activation assays, as well as autoimmune rheumatic disease, viral immunology and monogenic interferonopathies. The task force included an expert in EULAR methodology, two members of the EULAR emerging network (EMEUNET) and a patient representative. Six Population Intervention Comparator and Outcome questions (PICOs) were formulated (online supplemental text 1). PICO 1, 'What methods have been employed to assess type I IFN pathway activation in people with RMDs? What are their performance metrics (including aspects of content, criterion, construct validity as well as reliability and feasibility) of these methods?' is the basis of this SLR. PICOs 2–6 refer to clinical applications and will be reported separately (reference to SLR2) (online supplemental text 1).

Search strategy and eligibility criteria

A protocol for the SLR was developed and approved by the task force. Ovid Medline, Embase and Web of Science were searched for reports of IFN and RMDs up to October 2019. Search strategy is provided in online supplemental text 2–4. In addition to the RMD terms, papers were eligible for inclusion if they fulfilled the following criteria: (1) presented data on human patients with RMDs (with or without healthy controls); (2) design as cross-sectional; randomised control trials, case–control studies, non-controlled trials, diagnostic accuracy studies, cohort studies, intervention studies; (3) studies that described results on biological material derived from peripheral blood (ie, serum, whole blood, cell subsets); (4) written in English. Exclusion criteria were: (1) non-human studies; (2) conference abstracts, case studies, non-original articles such as editorials, review, opinion pieces, (3) articles that did not specify the type of IFN that the assay measures; (4) papers that did not describe their results as an assay, biomarker, test, score or similar in the abstract, (5) studies purely on IFN-I pathway genetics (see online supplemental text 5 for details). A minimum sample size was not considered within eligibility criteria as it could hid studies with more complex methods.

Titles and abstracts, followed by full-text screening was performed by two reviewers (AB and JR-C). The agreement between reviewers was high (>95%) and

discrepancies were resolved by discussion or consultation with the convenor (EV).

Data extraction

An extraction template addressing all PICOs was developed. For the present report, the following fields were collected: method name, population studied, type of assay, material analysed, pathway element, detailed description of method and calculation of reported result, validity (including face validity, criterion validity, concurrent validity, discussed further below), reliability and feasibility. Association with clinical endpoints (ie, diagnostic accuracy) as well as assay responsiveness were analysed in a separate SLR (reference to SLR2). Due to the heterogeneity in methods and analyses reported, comparative statistical analysis or meta-analysis was not performed, and the results are presented in narrative form.

Interpretation of IFN assay validity

All assays included in SLR met face validity since the literature review filter implied a plausible role in the IFN pathway. For content validity, we described what aspects of the IFN pathway are measured in a description of principle of the assay. For criterion validity, no objective gold standard type I IFN pathway activation assay exists. We therefore described criterion validity as 'evidence that assay measures IFN-I' and four options were considered: (1) experimental stimulation of cells with IFN-I in vitro and demonstration of assay induction, (2) assays in patients receiving IFN-I therapy, (3) standard curves where appropriate, for example for an IFN-alpha ELISA but not IFN-inducible chemokines, (4) blocking with anti-IFN antibodies in vitro or as a therapeutic agent. For concurrent validity, we evaluated whether putative IFN-I assays were shown to correlate with other putative assay(s). For reliability, we sought evidence of reanalysis of samples in independent laboratories by the same method or repeat analysis of a sample at different times. Information on feasibility was provided based on review of the paper methodologies by the TaskForce panel as well as review of data in the paper.

Development of consensus terminology

After review of extracted data, terminology to describe aspects of type I IFN pathway, and assays designed to measure it, was developed by the task force. First, common themes with ambiguous concepts or that require harmonisation were identified. Next, definitions were produced by an iterative process until consensus was achieved.

RESULTS

Summary of RMDs and assays reported in the literature

Study selection is summarised in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses diagram (figure 1). A total of 10 037 abstracts were identified. A total of 276 of these reports fulfilled eligibility criteria and were used for data extraction. Some used more than one technique to measure IFN-I pathway activation. Hence, these 276 papers generated data on 412 methods.

Assays measured diverse aspects of the IFN-I pathway (figure 2). A summary of the assays used and their classification is in table 1. The most frequently studied RMD was SLE (n=204 reports), followed by RA (n=43), SS/pSS (n=41), SSc (n=32), myositis (n=29), antiphospholipid syndrome (APS) (n=6), multiple disease groups (n=44), other single RMDs such as AS, PsA, AAV, Behçet's disease, IgG4-RD (n=13). Sample sizes ranged from n=6 to n=1760.[16 17] Types of assays also varied across RMDs (figure 3).

Description of assays reported in the literature

A detailed description of the principles of each assay is given in online supplemental text 6.

Immunoassays: IFN-I proteins (IFN-α and IFN-β)

Fifty-eight studies were identified for IFN-α and nine for IFN-β.

Content: Antibody-based methods to quantify IFN-I proteins in biosamples including ELISA, dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA), multiplex assays and radioimmunoassays (RIA).

Criterion validity: These assays use specific antibodies, mostly commercial and validated by manufacturers, and most include controls and calibration curves. It is less clear whether a single sample and IFN subtype, such as serum IFN-alpha, is the most clinically relevant to a patient.

Concurrent validity: Concurrent validity was provided in 12/57 reports IFN-α and 4/9 for IFN-β against other assays such as expression of individual ISG,[18 19] ISG expression scores,[20] soluble IP-10 levels,[21] flow cytometric measurement of SIGLEC1,[22] bioassays,[23] virus inhibition assays[24] or reporter cell assays. Outcomes of these comparisons were highly variable. Some showed no clear association with IFN-α levels (ie, for IP-10, expression of individual genes),[20 21 25 26] weak associations (IFN inducible gene expression, bioassay),[20 21] or strong association (ie, between RIA and virus inhibition assays or SIGLEC-1 by flow cytometry or IFN-α and expression of individual ISGs).[19 22]

SiMoA (single molecule assay)

Eight studies were identified.

Content: A proprietary technology for ultrasensitive digital protein detection with a commercial assay for IFN-α (and some IFN-induced proteins).

Criterion validity: This was shown in six of eight studies. The study by Rodero *et al* showed that SiMoA could detect all subtypes of IFN-α, and excluded crossreactivity testing antibodies against recombinant IFN-β, IFNλ−1, IFNλ−2, IFN-ω and IFN-γ. This paper also described criterion validity showing that addition of anti-IFN-α antibody

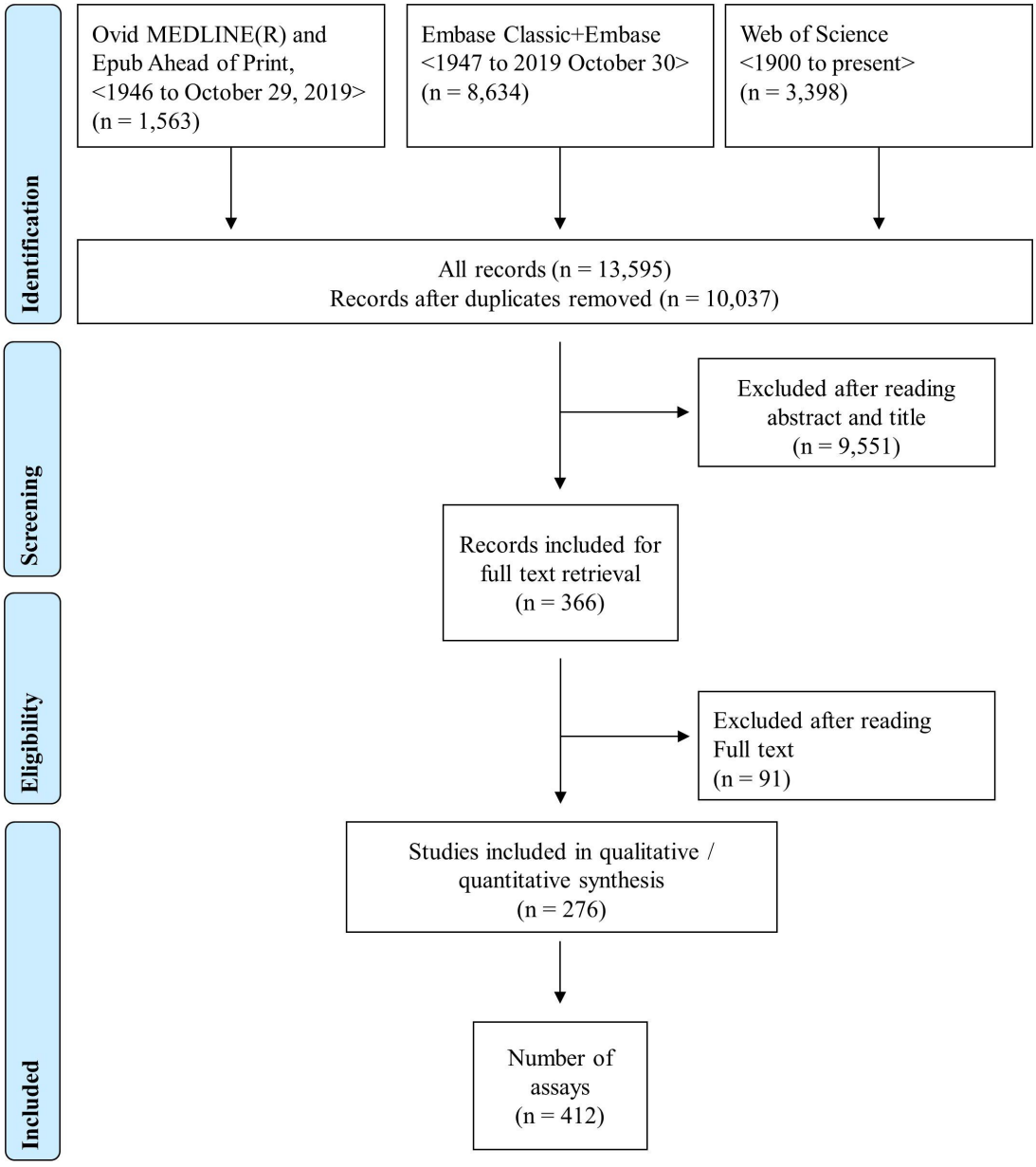

Figure 1 PRISMA chart. Search and selection strategy of publications. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

depleted the signal. Reproducibility was also confirmed. Matched plasma and serum samples tested for IFN-α showed strong correlation indicating a negligible influence of blood processing on IFN-α concentration and the ability to use either sample for retrospective patient screening. The potential implication of missing non-circulating sources was emphasised since intracellular IFN-I was detected in samples from patients with gain of function mutations in Stimulator of IFN Genes (STING) but not SLE despite high expression of ISGs in both. The IFN-α concentrations that are detected by SiMoA are often below the levels required to induce a cellular response in vitro.

Concurrent validity: This was reported in five of eight studies. SiMoA IFN-α measurements were highly correlated with a 6 ISG qPCR score as well as Nanostring and soluble Siglec-1.[3 27 28] IFN-α protein by SiMoA correlated with cytopathic effect using Madin–Darby bovine kidney cells challenged with vesicular stomatitis virus.[3] In some SLE cases, SiMoA detected IFN-α while a bioassay was negative, so the biological significance of the low IFN-α concentrations detected by SiMoA may require further confirmation.

Immunoassays: IFN-inducible proteins

Forty-two studies were identified.

Content: Immunoassays for soluble proteins encoded by ISGs in biosamples including ELISA, DELFIA, multiplex assays and radioimmunoassays.

Criterion validity: These assays do not evaluate IFN-I directly and production of some of the IFN-inducible proteins reported is known to be also induced by

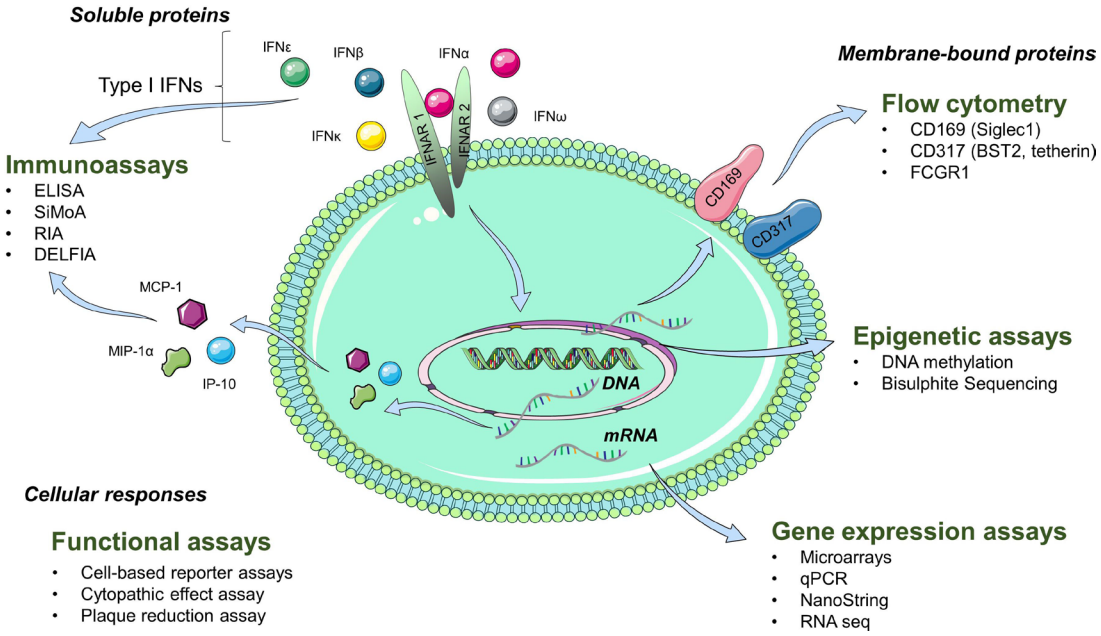

Figure 2 Aspects of the IFN-I pathway evaluated by each assay. The IFN pathway is a complex system with multiple subtypes of IFNs and diverse downstream effects on gene and protein expression. Existing assays measure different aspects of the IFN pathway; they do not reflect the entirety of the pathway and some are not specific for IFN-I. See text for full description of these assays.

IFN-II or other inflammatory mediators. This is likely a particular problem if a single 'IFN-inducible' protein is reported. Of 42, 21 studies measuring chemokines cited papers showing they are IFN-I-induced. Only 3/42 studies provided experimental evidence. Bauer *et al*[29] selected IFN chemokines whose transcripts were induced by IFN-α in a microarray study but did not check whether other IFNs or inflammatory mediators also induced these proteins. Thanarajasingam *et al*[30] evaluated stimulation of whole blood including IFN-α, oligonucleotides with cytosine-guanine repeats, Resiquimod (R848), LPS, IFN-α+LPS and null (no stimulant) then measured cytokines/chemokines and IFN-α. Most of the other studies cite the evidence from Bauer *et al*.[29] The IFN-inducible proteins included in assays are summarised in figure 4.

Concurrent validity: Of 42, 16 studies demonstrated concurrent validity against another test for IFN-I. 11/16 used an ISG expression assay.[29–38] In two studies concurrent validity was tested between the IFN-inducible protein and two other techniques (flow cytometry for SIGLEC-1[22 39] as well as IFN-α protein[21 26]; and gene expression as well as a reporter cell assay).[30 40]

Flow cytometry

Twelve studies reported using flow cytometry to analyse IFN-I-inducible markers.

Content: Evaluation of IFN-inducible proteins, mostly on the cell surface, using flow cytometry. It allows cell-specific measurement that avoids artefacts due to changes in the cellular composition of the sample. The most common target was SIGLEC-1. Other markers reported were CD64 (FCGR1), MxA, IFITM1, PRKRA. Only monocytes can be analysed using SIGLEC-1 (CD169), so monocyte data represent the bulk of this literature.

Criterion validity: Of 12, 4 studies reported in vitro experiments showing stimulation of cells (PBMCs cells subsets or cell lines) with IFN-I and induction of the flow cytometry markers. Of 12, 9 studies cited evidence from the literature that the genes encoding the flow cytometry marker are IFN-inducible, or prior papers that report the marker as an IFN assay. These studies did not report whether other subtypes of IFN or other inflammatory mediators induced the flow cytometry markers.

Concurrent validity: Of 12, 5 studies demonstrated correlation with another IFN assay such as the same protein in serum by ELISA, the expression of the protein's transcript by qPCR, against IFN-α protein measured by ELISA, or against IFN scores derived from qPCR assays.[22 35 39 41–43] Some comparator assays compared ELISA or IFN Score results from unsorted blood with a specific cell population analysed by flow cytometry.

RNA microarrays

Microarrays were reported in 70 studies in total, with several differences in the methods section.

Content: A gene expression assay using probes that provide broad coverage of the transcriptome.

Of the 70 studies, 3 main methods of data analysis were used: (1) reporting an 'IFN signature', referring to the presence or absence of a cluster of ISGs, usually readily visible on a heat map; (2) an 'IFN score', usually referring to a continuous variable calculated from a predefined set of ISGs or (3) modules of ISGs, which allows the clustering of ISGs into two or more groups, and then a

Table 1 Summary of IFN-I assays reported in the literature

	Category	Name	What it measures	No of studies	Validation that assay measures IFN-I (n/N)	Evidence of concurrent validity (n/N)	Quantitative or categorical	Ref
1.	Immunoassay	Immunoassays for IFN -α protein	IFN-α protein	60	58/58	12/57	Quantitative	18–26 46 47 57–103
		SiMoA	IFNs or chemokines	8	6/8	5/8	Quantitative	3 27 28 38 39 104 105
		Immunoassays for IFN -β protein	IFN -β protein	9	9/9	4/9	Quantitative	16 18 20 66 73 82 95 105 106
		Immunoassays for IFN-inducible proteins	IFN-inducible soluble proteins	42	3/42	16/42	Quantitative	21 22 26 29–40 61 62 74 77 78 83 103 106–125
2	Flow cytometry	Flow cytometry	IFN inducible surface markers	12	4/12	5/12	Quantitative	22 35 39 41 43 70 77 120 121 126–128
3	Microarrays	Microarray modules	Subsets of ISGs (modules)	10	1/10	4/10	Either	38 113 118 129–135
		Microarray scores	Selected gene analysis	25	8/25	14/25	Quantitative	20 25 29 37 40 112 125 133 135–150
		Microarray signatures	Whole transcriptome unsupervised analysis	35	7/35	14/35	Categorical	17 34 43 62 70 82 127 151–178
4.1	qPCR	pPCR for IFNs	IFN-α or β gene expression	4	1/4	1/4	Quantitative	57 89 98
		qPCR: individual ISGs	Individual ISG expression	30	4/30	15/30	Quantitative	57 65 129 137 139 144 179–187
		qPCR IFN scores	Expression of a set of ISGs	82	8/82	25/82	Quantitative or categorical	3 13 27 28 32–35 41 48–54 105 109 121 125 136 137 145 157 161 166 168 171 173 188–237
		qPCR Chemokines	IFN induced chemokines	4	1/4	2/4	Quantitative	201–203 238
		qPCR ISG clusters	ISG signature	2	0/2	0/2	Categorical	153 170
5	RNASeq	RNASeq	Whole transcriptome analysis	9	3/9	3/9	Categorical	28 44–46 105 116 211 239 240
6	Nanostring	Nanostring	Expression of a set of ISGs	5	1/5	4/5	Quantitative	17 28 39 46 47
7	DNA methylation	DNA methylation	ISGs in whole genome DNA methylation arrays	14	0/14	10/14	Categorical	45 125 181 241–251
8	Bisulphite sequencing	Bisulphite sequencing	Validation for genes from DNA methylation studies	3	1/3	0/3	Categorical	244 246 252

Continued

Table 1 Continued

	Category	Name	What it measures	No of studies	Validation that assay measures IFN-I (n/N)	Evidence of concurrent validity (n/N)	Quantitative or categorical	Ref
9.1	Reporter cell assay	Reporter cell qPCR	Cell line or PBMCs) stimulated with patient sample then ISG qPCR	31	14/31	8/31	Quantitative	30 32 40 65 82 182 204 212 229 253–273
9.2	Reporter cell assay	Reporter cell other	Cell line or PBMCs stimulated with patient sample then luminometric or colorimetric methods	9	2/9	5/9	Quantitative	20 24 137 149 163 274–276
10	Cytopathic effect assay	Cytopathic effect assay	Tests whether the functional effect of IFNs in protecting cells from viral infection can be observed by cytopathic features in cultured cells	11	9/11	3/11	Semiquantitative	3 23 27 277–284
11	Plaque reduction assay	Plaque reduction assay	Tests whether the functional effect of IFNs in protecting cells from viral infection can be observed by size of plaque of cultured cells	7	7/7	0/7	Semiquantitative	42 285–290
12	IHC	IHC in whole blood	Whole blood stained for MxA	1	0/1	0/1	Semiquantitative	122

IHQ, Immunohistochemistry; ISG, IFN-stimulated genes; PBMCs, Peripheral Blood Mononuclear Cells.

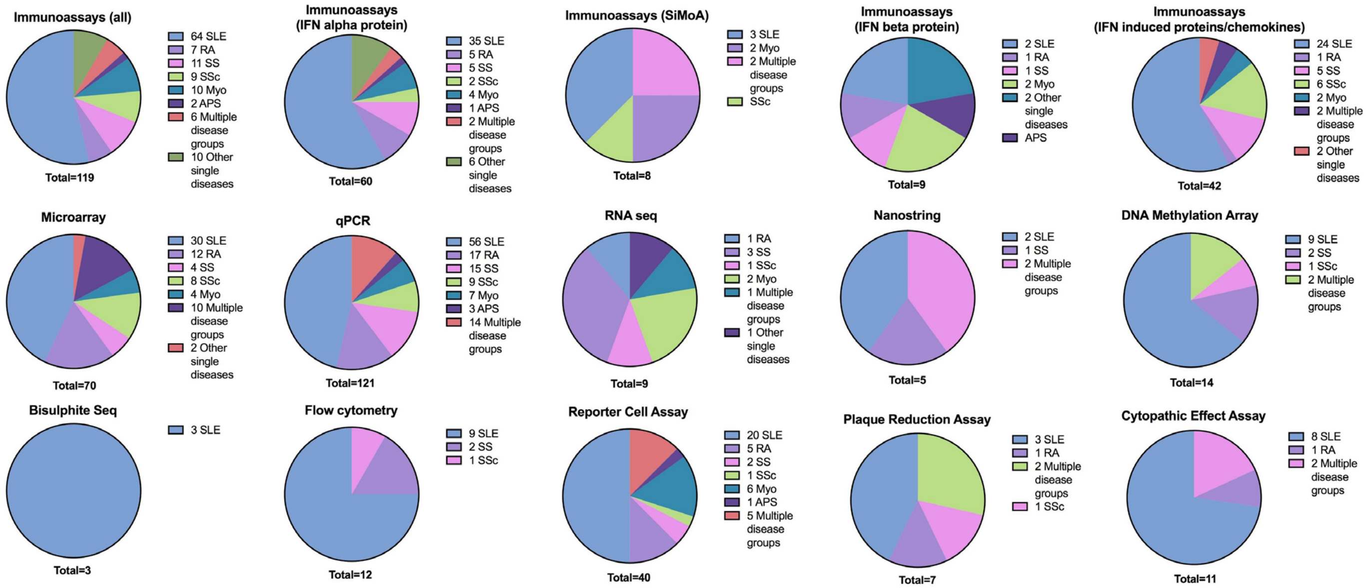

Figure 3 Summary of RMDs evaluated using each type of IFN-I assay. Pie charts indicate the number of reports of assays for each methodology and each rheumatic musculoskeletal disease. Some publications include more than one assay, so number of papers may differ from the numbers on this figure. RMDs, rheumatic musculoskeletal diseases.

signature or score to be provided for each module or differences across modules are screened. The terms for these methods have not always been consistently used.

Of 70, 36 studies reported IFN signatures, 23/70 as IFN scores and 10/70 studies presented results as IFN modules. The most popular platform used was Affymetrix (39/70), followed by Illumina (15/70), Stanford microarrays (4/70), Agilent (3/70) or custom-made arrays. Microarrays were performed mainly using RNA from whole blood (41/70). Blood was collected using PAXgene (36/70) or Tempus tubes (5/70). Others used PBMCs or various combinations of isolated cell subsets.

Criterion validity: Of 70, 16 studies presented stimulation experiments showing induction of ISGs by IFNs-I or used public datasets to derive ISG sets. A few studies also reported ISG downregulation or IFN signature neutralisation after experiments or administration of IFN-targeted medications. The modular analysis provided data suggesting that modules may represent the relative abundance of type I versus type II IFNs. As for all gene expression assays described, a potential limitation when analysing unsorted cells is artefactual change due to changes of the cellular composition of the sample. Indeed, ISG expression differs between cell subsets and

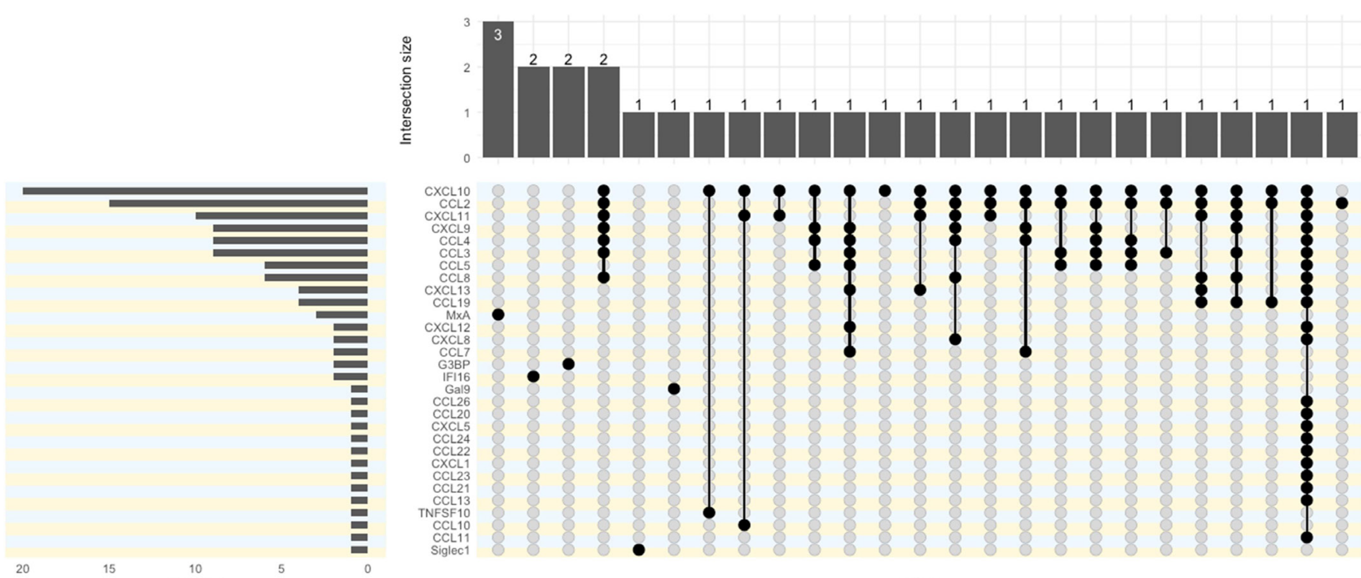

Figure 4 UpSet plot for constituents of immunoassays. Dots and bars indicate what combinations of proteins were measured in reports of IFN immunoassays. The left-hand chart shows the number of reports for each protein. The upper chart shows the number of reports for each combination.

patients with RMDs often have cytopenias or expansion of cell subsets secondary to autoimmunity or immunosuppressive therapy.

Concurrent validity: Provided in 33/70 reports. Microarray results were validated against qPCR scores, individual ISGs expression, serum IFN-α, -β and IFN-inducible chemokines and a methylation array.

RNA-sequencing

Nine studies reported RNA-sequencing (RNASeq).

Content: Sequencing of the whole transcriptome providing qualitative and quantitative data on any RNA type.

Studies reported differentially expressed genes including ISGs in whole blood (3/9), isolated CD19+B cells (3/9), monocytes CD14+, pDCs, neutrophils (1/9 each subset). RNASeq was performed on Illumina.

Criterion validity: As for other gene expression methods. Stimulation with IFN-I was shown in one out of nine study, in another ISGs were derived from the Interferome database. ISG were not reported in subsets as for micro-arrays and qPCR studies.

Concurrent validity: provided in three ouf of nine reports, against chemokine score in serum,[44] methylation profile of selected ISGs[45] and qPCR.[46]

Nanostring

Five studies reported gene expression using Nanostring.

Content: A proprietary probe-based gene expression usually analysing 800 transcripts in manufacturer designed or customisable panels.

Nanostring was reported in in five out of nine studies to evaluate expression of ISGs in whole blood, PBMCs and CD19+B cells.

Criterion validity: as for other gene expression assays. Commercial predefined panels were provided by the manufacturer.

Concurrent validity: Provided in four out of nine studies where results were compared with serum protein IFN-α or SIGLEC1, IFN-I scores by qPCR or RNA-seq.[28 39 46 47]

IFN-I scores by qPCR

qPCR was reported in 122 studies with differences in the methods section.

Content: All qPCR studies report a set of real-time PCR techniques based on commercial or custom probes and primers for quantification of predefined ISG transcripts. Of the 122 studies, 5 main methods of data analysis were used: (1) 4/122 measured qPCR for genes encoding IFN proteins; (2) 30/122 reported expression of individual ISGs; (3) 82/122 studies reported an IFN Score—that is, expression of a set of ISGs as a continuous value; (4) 4/122 studies reported IFN-inducible chemokines as scores or individual genes; (5) 2/122 reported clusters of ISGs as a categorical signature.

Criterion validity: as for other gene expression assays. A total of 14/122 studies contained direct evidence based on experimental stimulation with appropriate positive and negative controls. The ISGs and reference genes chosen were highly variable between studies, and the rationale for these choices was not always given. A list of transcripts in scores used in RMDs is shown in figure 5. Sources and preanalytical features also differ across studies.

Some papers derived and used more than one score, related to the modular data from microarray studies or from unsupervised approaches (factor analysis or principal component analysis). Many studies reported correlations among ISGs, providing additional criterion validity. While many papers suggest these reflect balance of IFN-I and IFN-II, this was not demonstrated in any paper.[46 48 48 49 49 50 50–54] However, when multiple scores were analysed, particular sets of ISGs, such as IFN-Score-B, show a stronger correlation with clinical endpoints than others in certain conditions. The metric properties of the scores, dynamic ranges and calculations to derive them may be other reasons that some sets of ISGs provide better clinical correlations, which also applies to other gene expression assays.

Concurrent validity: Provided for 43/122 studies, mainly by comparing new gene expression scores against previously published scores that use different ISGs. Several studies validate scores against levels of IFN-α protein measured by ELISA, SiMoA, etc); IFN-stimulated proteins (SIGLEC1, GAL9, G3BP); chemokines/chemokine scores; or expression of individual ISGs (MxA).

DNA Methylation arrays

Fourteen studies analysed an aspect of the IFN pathway using DNA methylation arrays.

Content: Analysis of methylation of genomic DNA to identify changes in actively transcribed genes

The material analysed included isolated cell subsets (most frequently CD4+T cells, but also CD8+T cells, CD19+B cells or neutrophils) as well as whole blood and PBMCs. The Illumina Human Methylation 450 BeadChip (HM450) was the most commonly method used for high-throughput human methylome analyses in 13/14 studies. Other studies used a newer Illumina assay based on the EPIC chip (Illumina Infinium MethylationEPIC BeadChip). Differential methylation was reported for ISGs as well as *IFNA* gene across all tested blood cell types and samples.

Criterion validity: These assays do not directly measure the transcription of IFNs or ISGs, nor their protein products so are only an indirect indication of activation of IFN-related pathways based on prior knowledge. The studies retrieved did not add any direct evidence to support their specificity.

Concurrent validity: Of 14, 10 reports presented concurrent validity. In three cases, results were validated against bisulphite sequencing, pyroseqencing and others looked at impact of hypomethylation on gene expression by qPCR (comparing to selected representative individual genes) or microarray results (IFN score) or RNA-seq. One study validated methylation results against IFN-α and -β

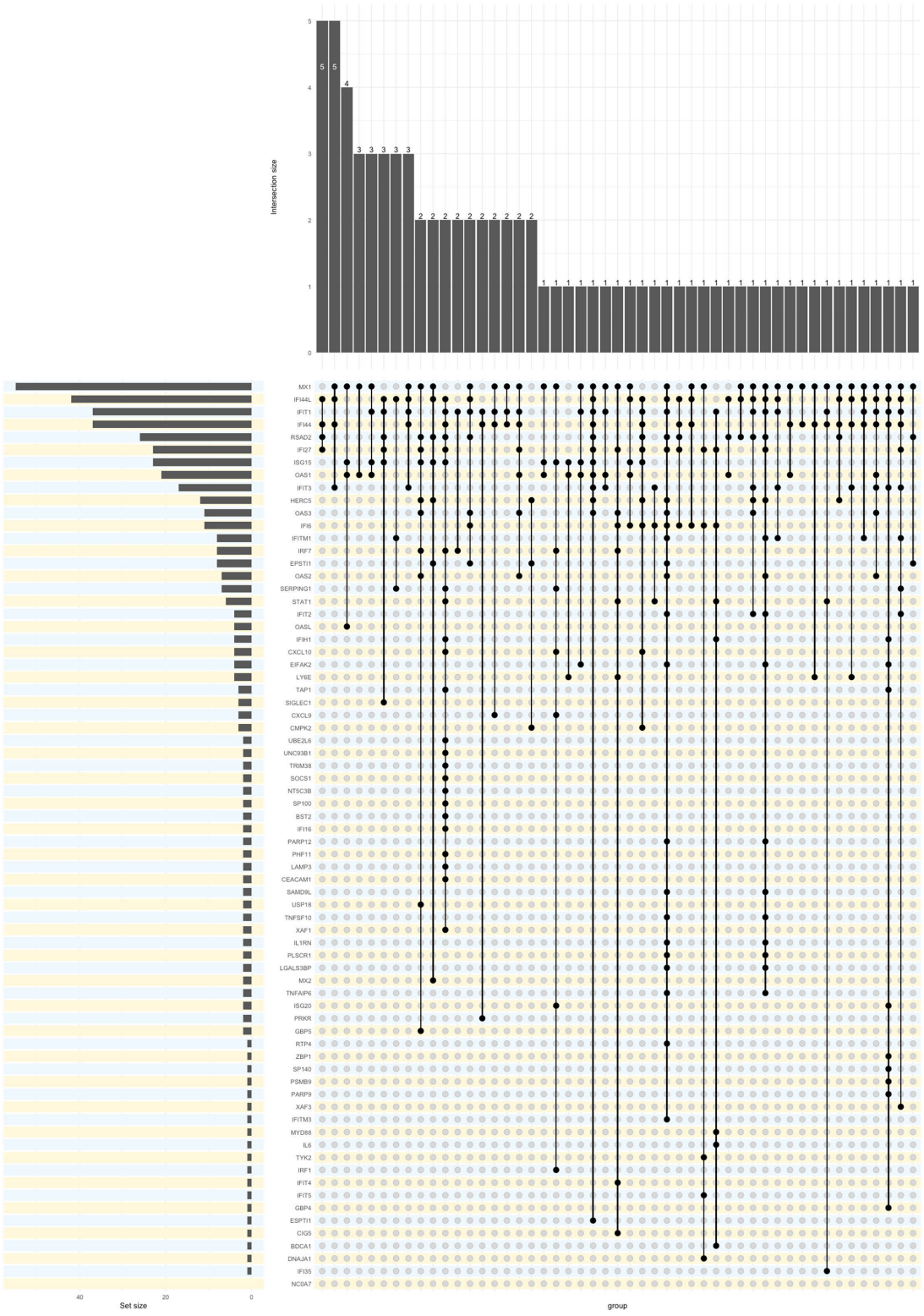

Figure 5 UpSet plot for constituents of gene expression assays. Dots and bars indicate what combinations of genes were measured in reports of IFN gene expression assays. The left-hand chart shows the number of reports for each gene. The upper chart shows the number of reports for each combination.

protein levels in serum. Of 14, 2 studies validated their results against publicly available methylation datasets.

Reporter cell assays

Forty studies reported data from reporter cell assays.

Content: Quantitative analysis of the ability of IFNs in biosamples (ie, serum) to upregulate ISGs in a reporter cell line.

Various combinations of cells, biosamples and methods of readout have been published. Most studies analysed

serum, with plasma in a smaller number. Samples were used to stimulate a reporter cell.

Two main groups of reports were identified. A first group of 31 studies used reporter cell assays with a qPCR readout. Most of these used a WISH cell line (human amnion) combined with patient serum (22 studies); 4 studies used HeLa cells; others used PBMCs from healthy controls, THP1 cells or HUVEC. The output readout was measured by qPCR using combination of 1–6 ISGs. The ISGs and reference genes chosen were highly variable between studies, and the rationale for these choices was not given. Results were usually presented as individual gene expression or IFN scores. The ISGs and reference genes chosen were highly variable between studies, and the rationale for these choices was not given. Results were usually presented as individual gene expression or IFN scores.

A second group of nine studies used measurement of output by luminescence or spectrophotometry. In this group, three studies used A549 cells (human lung carcinoma); two used novel HEK-Blue IFN-α/β and/or donor PBMCs; one used Hil3 (hepatoma cell line); one used U937 cells stably expressing an Mx1 promoter; one used U937-Mx1-Luc (containing luciferase reporter construct) and one used Fibroblast cells (FL). The results were presented in various formats such as: 'Activity' (titres, dilutions or arbitrary units) or concentration of IFN-α, etc.

Criterion validity: From both subcategories of reporter cell assays 14/29 and 2/9 gave experimental evidence on measuring IFN-I by using positive controls, inhibition with anti-IFN-I antibodies, stimulation with IFN-I or describing using a standard curve.

Concurrent validity: 8/29 and 5/9 studies, respectively, from these two groups presented concurrent validity. In most cases this was compared with IFN scores by qPCR measured in PBMCs or whole blood as well as against IFN-α protein levels in serum or plasma

Cytopathic effect assay

Eleven studies reported cytopathic effect assays.

Content: Measures the capability of IFN-I in a biosample to suppress the cytopathic effect of viral replication on a target cell in vitro. Various combinations of cells and viruses have been reported (discussed in online supplemental text 6). The most common combination is the human lung carcinoma cell line A549 challenged with encephalomyocarditis virus (EMCV).

Criterion validity: All studies used IFN-I standards and further three additionally used neutralisation of IFN with anti-IFN-α antibodies.

Concurrent validity: Three reports provided evidence of concurrent validity against SiMoA and RIA IFN-α protein measurement.

Plaque reduction assay

Content: Evaluation of the ability of IFN-I in a biosample to prevent cell killing by a lytic virus in vitro. This assay was used in n=7 RMD studies. Many different combinations of cells, patient sample types and methods of readout have been published. The cell line used most frequently was WISH in combination with serum and vesicular stomatitis virus (VSV) (n=5). Two other studies used fibroblastic cell lines (human foreskin fibroblasts (HFF) ormammary stromal fibroblastic (MSF) cell lines) with VSV. All these studies were published in 1970s and 1980s. The last study identified, from 2011, reported using VERO cell line in combination with serum and EMCV.

Criterion validity: All eight studies reported using IFN standards (recombinant IFN-α mainly, IFN-β and IFN-γ in some cases) as positive controls, as well including negative controls. A neutralisation assay with anti-IFN antibodies was also performed in three cases.

Concurrent validity: nNne of studies showed results of concurrent validity

IHC

A single study reported the results of whole blood stained for MxA as semi-quantitative intensity of staining. This assay shares similar considerations to other assays for single IFN-stimulated proteins.

Feasibility

Key considerations in feasibility provided by the task force members after reviewing manuscripts is provided in online supplemental table 1.

Definitions of terms

The terminology used to describe aspects of IFN-I pathway activation was not consistent in the literature eligible for this SLR. This includes the distinction between signatures and scores, abbreviations for IFN subtypes and even the use of the term 'interferonopathy' (used to indicate either monogenic diseases, but also to refer to any polygenic RMDs in which increased IFN pathway activation was observed, especially SLE). Having identified key areas with inconsistent nomenclature and reporting, consensus terminology was agreed through an interactive compromise process with reference to the existing evidence. Recommended terms relating to IFN-I reporting in basic and clinical research is presented in table 2.

DISCUSSION

This SLR is the first summary of the entirety of the literature of IFN-I assays in the field of rheumatology. We identified a substantial body of literature supporting the value of these assays, but simultaneously several inconsistencies in the literature that are a barrier for the progression of these biomarkers into clinical practice. These findings provide a knowledge framework to guide assay selection and will inform the development of EULAR points to consider for the measurement, reporting and application

Table 2 Consensus terminology

Term	Abbreviation	Definition
Interferon	IFN	Proteins with antiviral activity; IFNs are mediators of an antiviral 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	ISGs	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 JAK/STAT signalling 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.

ISG, IFN-stimulated genes; RMDs, rheumatic musculoskeletal diseases.

of IFN-I pathway activation assays in clinical and research practice.

The assays described all measure different aspects of the complex IFN pathway. However, none of them can assess all relevant elements of the IFN-I activity in a human patient, and many of them are not specific for IFN-I. Hence, no single assay can evaluate the entirely of the IFN-I pathway activation. The most appropriate assay choice is contextual, depending on the specific research or clinical question as well as aspects of feasibility and reliability.

The complexity of this pathway presents an intrinsic problem in construct validity, that is, evidence that putative IFN-I assays measure IFN-I pathway activation specifically. Given that no assay can measure the entirety of IFN-I pathway activation, there is no 'Gold Standard' against which to evaluate new assays. Although SiMoA has increased the ability to measure IFN-I proteins themselves in the circulation more easily, this is only one component of the pathway. Further, non-circulating sources of IFN-I may be more important in many contexts. Molecular events downstream of the IFNAR may be more relevant to immunopathogenesis and thus, clinical applications. Meanwhile, assays for downstream molecules, such as assessment of ISG expression of IFN-inducible proteins, may be responsive to other IFNs or cytokines (Figure 6). Many of the papers reviewed presented concurrent validity (ie, correlation with another putative IFN-I assay) as evidence to support that they are IFN-I assays. This is weak evidence. The effects of IFN-I on the immune system and other aspects of its biology are so diverse that many parameters –ranging from acute-phase markers to symptom scores—may be found to correlate with IFN-I pathway activation. For this reason, methods for construct validity are likely to be measures of criterion validity, such as in vitro stimulation of cells with IFN-I as well as negative and positive controls before evaluating gene expression; or inhibition of reporter cell assays with anti-IFN-I antibodies. These methods do not represent autoimmunity in the complete organism. Regardless of these issues, representation of the complete IFN-I pathway may not be necessary for a clinically applicable biomarker.

An additional aspect of analytical validity that varied between reports was the analysis and reporting of results. Many papers reporting gene expression assays presented results as high or low, or positive or negative; and several cut-off criteria and score calculation practices were proposed. Although ISG expression is often bimodally distributed, this dichotomisation remains a dubious concept, since cytokine levels and cellular responses are naturally continuous. The bimodal distribution of results may differ in non-SLE RMDs, or in specific SLE populations. One study demonstrated that evaluation of IFN scores as continuous variables provided clinically relevant

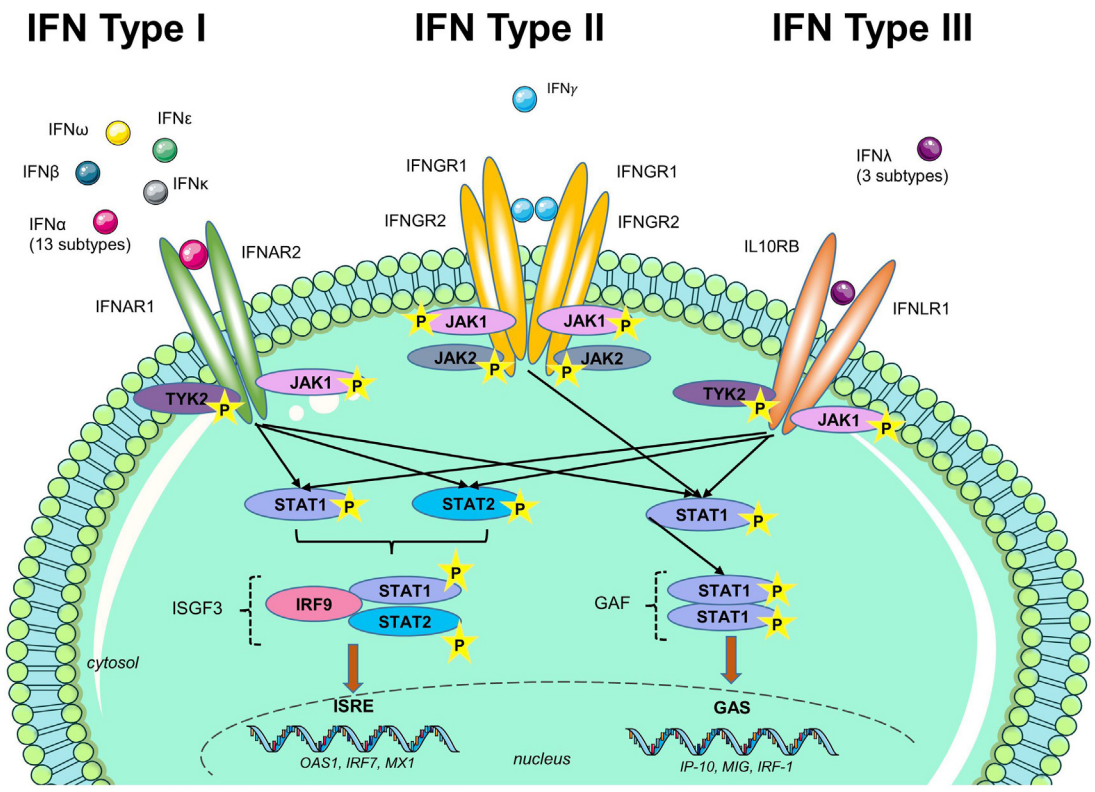

Figure 6 Overlap between types of IFNs. Although there are distinct receptors for type 1, 2 and 3 IFNs, there is substantial overlap in signalling and response elements. Assays measuring segments of the pathway downstream from the IFN receptor may not be specific for one subtype of IFN and some may preferentially reflect type II or III IFNs.

information in addition to high/low status (ie, very high expression has a different clinical significance to moderately high[50]).

Immunoassays are often used to assess directly IFN levels in biosamples from patients with RMDs. However, sensitivity of these methods has been questioned in the past since IFN-I may be below detection[55] when assayed using ELISA. This method is also influenced by low reproducibility and shows rather variable correlation with bioassays[55 56] possibly because of other subtypes of IFN or because the assay can detect a similar epitope on a non-IFN-α protein, a stable but biologically inactive IFN-α protein degradation product[56] or the presence of other IFN subtypes. Of note, these limitations must be regarded as antibody specificity-related rather than to the assay itself. While the SiMoA technology can measure proteins with an increased sensitivity a limitation is the lack of a commercially available kit to detect all IFN-I subtypes. And levels of IFN in serum may not be the only relevant IFN protein influencing IFN pathway activation. Clinical validation to demonstrate any superior properties as a biomarker is still required.

Three main types of assay to evaluate downstream cellular responses to IFN-I had the most substantial body of literature; immunoassays for soluble IFN-stimulated proteins, assays for IFN-stimulated genes and flow cytometric assays for IFN-stimulated cell surface proteins. For all of these, confirmation that they pertain specifically to IFN-I is critical and not always provided. However, these assays generally appeared feasible in routine practice. One additional flow cytometry marker was published after completion of the SLR, which is evaluable on any cell subset.[4]

Functional assays or bioassays are among the oldest in the literature with more modern adaptations in later papers. These assays have obvious interest in terms of biological significance, but most appeared to have poor feasibility for routine clinical practice.

This SLR has some limitations. Due to the heterogeneity in the methods of reporting, it was not possible to perform meta-analysis. The presentation format of the results representing the same assay was so variable that did not allow for comparison of data between studies. Moreover, assays were evaluated on the basis of their methodologies and technical aspects, whereas their clinical associations were the focus of other SLR (reference to SLR2). Although some papers reported more than one assay or method (2–4), these did not necessarily provide concordant results. Some studies described multiple assays as independent assays of IFN-I pathway activation. Reports also varied by sample types and study designs, and assay usage differed across RMDs. Furthermore, there is a lack of standardised instruments for comparative analyses and specific technical aspects, such as feasibility. For these reasons, we relied on expert commentary in this SLR.

This SLR is the first step in a programme designed to facilitate translation of IFN-I assays in clinical research

and practice. Together with an analysis of their associations with clinical outcomes, these will inform the EULAR points to consider for the measurement, reporting and application of IFN-I pathway activation assays in clinical and research practice. We believe this programme will ultimately lead harmonisation and to widespread adoption of these promising assays to improve patient care. Although genetic studies were not included in this review, we consider that our task force will also facilitate progress in the characterisation of genetic variants. Moreover, due to the broad involvement of IFN in human disease, our analysis may be also applicable beyond the field of rheumatology.

Author affiliations
[1]Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds & NIHR Leeds Biomedical Research Centre, Leeds, UK
[2]University of Oviedo, Area of Immunology, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain
[3]Charité University Medicine Berlin, Department of Rheumatology, Berlin, Germany
[4]Erasmus MC, University Medical Center Rotterdam, Laboratory Medical Immunology, Department of Immunology, Rotterdam, Netherlands Immunology, Rotterdam, The Netherlands
[5]Uppsala University, Department of Medical Sciences, Rheumatology, Uppsala, Sweden
[6]Unit of Immunology, Rheumatology, Allergy and Rare Diseases, Vita-Salute San Raffaele University, Milan, Italy
[7]EULAR, PARE Patient Research Partners, Amsterdam, Netherlands
[8]University of Crete, Medical School, Department of Internal Medicine, Heraklion, Greece
[9]University of Crete, Medical School, Department of Rheumatology-Clinical Immunology, Heraklion, Greece
[10]Karolinska Institutet, Division of Rheumatology, Stockholm, Sweden
[11]Broegelmann Research Laboratory, Department of Clinical Science, University of Bergen, Norway
[12]Medical Research Council Human Immunology Unit, Medical Research Council Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK
[13]Université de Paris Cité, Hôpital Necker-Enfants Malades, Immuno-Hématologie et Rhumatologie pédiatriques, Paris, France
[14]Hospital for Special Surgery, Weill Cornell Medical College, Mary Kirkland Center for Lupus Research, New York, USA
[15]Erasmus MC, Department of Immunology, Rotterdam, The Netherlands

Twitter Javier Rodríguez-Carrio @javierrcarrio, Robert Biesen @BiesenRobert, Marie-Louise Frémond @MLFremond and Ed Vital @edvital

Acknowledgements PGC and EV are supported in part by the UK National Institute for Health and Care Research (NIHR) Leeds Biomedical Research Centre.

Contributors AB, JR-C, PGC, MVe and EV designed the protocol. AB and JR-C performed the literature search and data extraction supervised by PGC, EV and MVi. AB and JR-C drafted the manuscript. All authors contributed and approved the final version of the manuscript. PGC, EV and MVi edited the manuscript draft. EV is guarantor for this manuscript.

Funding This work was funded by the European Alliance of Associations for Rheumatology (EULAR) (grant number SCI019).

Competing interests MKC has received consulting fees from AstraZeneca, Bristol Meyers Squibb, Lilly and Shannon Pharmaceuticals, as well as grant/research support from Gilead. LR has received consulting fees from AstraZeneca. EV 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. The rest of the authors have no conflict of interest to declare.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

ORCID iDs
Javier Rodríguez-Carrio http://orcid.org/0000-0002-0011-5102
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
PG Conaghan http://orcid.org/0000-0002-3478-5665

REFERENCES

1 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.
2 Crow MK, Olferiev M, Kirou KA. Type I interferons in autoimmune disease. *Annu Rev Pathol Mech Dis* 2019;14:369–93.
3 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.
4 El-Sherbiny YM, Md Yusof MY, Psarras A, *et al*. B cell tetherin: a flow cytometric cell-specific assay for response to type I interferon predicts clinical features and flares in systemic lupus erythematosus. *Arthritis Rheumatol* 2020;72:769–79.
5 Barrat FJ, Crow MK, Ivashkiv LB. Interferon target-gene expression and epigenomic signatures in health and disease. *Nat Immunol* 2019;20:1574–83.
6 Pervolaraki K, Rastgou Talemi S, Albrecht D, *et al*. Differential induction of interferon stimulated genes between type I and type III interferons is independent of interferon receptor abundance. *PLOS Pathog* 2018;14:e1007420.
7 Psarras A, Alase A, Antanaviciute A, *et al*. Functionally impaired plasmacytoid dendritic cells and non-haematopoietic sources of type I interferon characterize human autoimmunity. *Nat Commun* 2020;11:6149.
8 Rönnblom L, Leonard D. Interferon pathway in SLE: one key to unlocking the mystery of the disease. *Lupus Sci Med* 2019;6:e000270.
9 Psarras A, Emery P, Vital EM. Type I interferon-mediated autoimmune diseases: pathogenesis, diagnosis and targeted therapy. *Rheumatology (Oxford)* 2017;56:1662–75.
10 Rönnblom L. The type I interferon system in the etiopathogenesis of autoimmune diseases. *Ups J Med Sci* 2011;116:227–37.
11 Furie RA, Morand EF, Bruce IN, *et al*. Type I interferon inhibitor anifrolumab in active systemic lupus erythematosus (TULIP-1): a randomised, controlled, phase 3 trial. *The Lancet Rheumatology* 2019;1:e208–19.
12 Furie R, Morand EF, Askanase AD, *et al*. Anifrolumab reduces flare rates in patients with moderate to severe systemic lupus erythematosus. *Lupus* 2021;30:1254–63.
13 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.
14 Llibre A, Bondet V, Rodero MP, *et al*. n.d. Development and validation of an ultrasensitive single molecule array digital enzyme-linked immunosorbent assay for human interferon-α. *JoVE*
15 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.
16 Gao L, OConnell M, Allen M, *et al*. Bone marrow mesenchymal stem cells from patients with SLE maintain an interferon signature during in vitro culture. *Cytokine* 2020;132.

17 Hoffman RW, Merrill JT, Alarcón-Riquelme MME, *et al*. Gene expression and pharmacodynamic changes in 1,760 systemic lupus erythematosus patients from two phase III trials of BAFF blockade with tabalumab. *Arthritis Rheumatol* 2017;69:643–54.
18 Weix J, Häupl T, Raio L, *et al*. The physiologic increase in expression of some type I IFN-inducible genes during pregnancy is not associated with improved disease activity in pregnant patients with rheumatoid arthritis. *Transl Res* 2013;161:505–12.
19 Sun WC, Sun YC, Lin H, *et al*. Dysregulation of the type I interferon system in adult-onset clinically amyopathic dermatomyositis has a potential contribution to the development of interstitial lung disease. *Br J Dermatol* 2012;167:1236–44.
20 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.
21 Oke V, Brauner S, Larsson A, *et al*. Ifn-λ1 with Th17 axis cytokines and IFN-α define different subsets in systemic lupus erythematosus (SLE). *Arthritis Res Ther* 2017;19:139.
22 Rose T, Grützkau A, Hirseland H, *et al*. Ifnα and its response proteins, IP-10 and SIGLEC-1, are biomarkers of disease activity in systemic lupus erythematosus. *Ann Rheum Dis* 2013;72:1639–45.
23 von Wussow P, Jakschies D, Hartung K, *et al*. Presence of interferon and anti-interferon in patients with systemic lupus erythematosus. *Rheumatol Int* 1988;8:225–30.
24 Shiozawa S, Chihara K, Shiozawa K, *et al*. A sensitive radioimmunoassay for alpha-interferon: circulating alpha-interferon-like substance in the plasma of healthy individuals and rheumatoid arthritis patients. *Clin Exp Immunol* 1986;66:77–87.
25 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.
26 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.
27 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.
28 Reynolds JA, Briggs TA, Rice GI, *et al*. Type I interferon in patients with systemic autoimmune rheumatic disease is associated with haematological abnormalities and specific autoantibody profiles. *Arthritis Res Ther* 2019;21:147.
29 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.
30 Thanarajasingam U, Muppirala AN, Jensen MA, *et al*. Type I interferon predicts an alternate immune system phenotype in systemic lupus erythematosus. *ACR Open Rheumatol* 2019;1:499–506.
31 van den Hoogen LL, van Roon JAG, Mertens JS, *et al*. Galectin-9 is an easy to measure biomarker for the interferon signature in systemic lupus erythematosus and antiphospholipid syndrome. *Ann Rheum Dis* 2018;77:1810–4.
32 Tydén H, Lood C, Gullstrand B, *et al*. Endothelial dysfunction is associated with activation of the type I interferon system and platelets in patients with systemic lupus erythematosus. *RMD Open* 2017;3:e000508.
33 Reed AM, Peterson E, Bilgic H, *et al*. Changes in novel biomarkers of disease activity in juvenile and adult dermatomyositis are sensitive biomarkers of disease course. *Arthritis Rheum* 2012;64:4078–86.
34 McBride JM, Jiang J, Abbas AR, *et al*. Safety and pharmacodynamics of rontalizumab in patients with systemic lupus erythematosus: results of a phase I, placebo-controlled, double-blind, dose-escalation study. *Arthritis Rheum* 2012;64:3666–76.
35 Maria NI, Brkic Z, Waris M, *et al*. Mxa as a clinically applicable biomarker for identifying systemic interferon type I in primary Sjogren's syndrome. *Ann Rheum Dis* 2014;73:1052–9.
36 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.
37 Baechler EC, Bauer JW, Slattery CA, *et al*. An interferon signature in the peripheral blood of dermatomyositis patients is associated with disease activity. *Mol Med* 2007;13:59–68.
38 Assassi S, Wang X, Chen G, *et al*. Myeloablation followed by autologous stem cell transplantation normalises systemic sclerosis molecular signatures. *Ann Rheum Dis* 2019;78:1371–8.
39 Oliveira JJ, Karrar S, Rainbow DB, *et al*. The plasma biomarker soluble SIGLEC-1 is associated with the type I interferon transcriptional signature, ethnic background and renal disease in systemic lupus erythematosus. *Arthritis Res Ther* 2018;20:152.
40 Nielsen CT, Lood C, Ostergaard O, *et al*. Plasma levels of galectin-3-binding protein reflect type I interferon activity and are increased in patients with systemic lupus erythematosus. *Lupus Sci Med* 2014;1:e000026.
41 Li Y, Lee PY, Kellner ES, *et al*. Monocyte surface expression of Fcgamma receptor RI (CD64), a biomarker reflecting type-I interferon levels in systemic lupus erythematosus. *Arthritis Res Ther* 2010;12:R90.
42 de Oliveira DB, Almeida GM de F, Guedes ACM, *et al*. Basal activation of type I interferons (alpha2 and beta) and 2' ''OAS genes: insights into differential expression profiles of interferon system components in systemic sclerosis. *Int J Rheumatol* 2011;2011:275617.
43 York MR, Nagai T, Mangini AJ, *et al*. A macrophage marker, siglec-1, is increased on circulating monocytes in patients with systemic sclerosis and induced by type I interferons and Toll-like receptor agonists. *Arthritis Rheum* 2007;56:1010–20.
44 Piper CJM, Wilkinson MGLI, Deakin CT, *et al*. CD19+CD24hiCD38hi B cells are expanded in juvenile dermatomyositis and exhibit a pro-inflammatory phenotype after activation through Toll-like receptor 7 and interferon-α. *Front Immunol* 2018;9.
45 Imgenberg-Kreuz J, Sandling JK, Almlöf JC, *et al*. Genome-Wide DNA methylation analysis in multiple tissues in primary Sjögren's syndrome reveals regulatory effects at interferon-induced genes. *Ann Rheum Dis* 2016;75:2029–36.
46 Imgenberg-Kreuz J, Sandling JK, Björk A, *et al*. Transcription profiling of peripheral B cells in antibody-positive primary Sjögren's syndrome reveals upregulated expression of CX3CR1 and a type I and type II interferon signature. *Scand J Immunol* 2018;87:e12662.
47 Wither J, Johnson SR, Liu T, *et al*. Presence of an interferon signature in individuals who are anti-nuclear antibody positive lacking a systemic autoimmune rheumatic disease diagnosis. *Arthritis Res Ther* 2017;19:41.
48 Bodewes ILA, Al-Ali S, van Helden-Meeuwsen 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.
49 de Jong TD, Vosslamber S, Mantel E, *et al*. Physiological evidence for diversification of ifnaalpha- and ifnbeta-mediated response programs in different autoimmune diseases. *Arthritis Res Ther* 2016;18:1.
50 El-Sherbiny YM, Psarras A, Md Yusof MY, *et al*. Publisher correction: A novel two-score system for interferon status segregates autoimmune diseases and correlates with clinical features. *Sci Rep* 2018;8:14846.
51 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.
52 Karageorgas T, Fragioudaki S, Nezos A, *et al*. Fatigue in primary Sjögren's syndrome: clinical, laboratory, psychometric, and biologic associations. *Arthritis Care Res (Hoboken)* 2016;68:123–31.
53 Nezos A, Gravani F, Tassidou A, *et al*. Type I and II interferon signatures in sjogren's syndrome pathogenesis: contributions in distinct clinical phenotypes and sjogren's related lymphomagenesis. *J Autoimmun* 2015;63:47–58.
54 Liu M, Liu J, Hao S, *et al*. Higher activation of the interferon-gamma signaling pathway in systemic lupus erythematosus patients with a high type I IFN score: relation to disease activity. *Clin Rheumatol* 2018;37:2675–84.
55 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.
56 Niewold TB, Hua J, Lehman TJA, *et al*. High serum IFN-α activity is a heritable risk factor for systemic lupus erythematosus. *Genes Immun* 2007;8:492–502.
57 Abdel Galil SM, El-Shafey AM, Abdul-Maksoud RS, *et al*. Interferon alpha gene expression and serum level association with low vitamin D levels in Egyptian female patients with systemic lupus erythematosus. *Lupus* 2018;27:199–209.
58 Båve U, Nordmark G, Lövgren T, *et al*. Activation of the type I interferon system in primary Sjögren's syndrome: a possible etiopathogenic mechanism. *Arthritis Rheum* 2005;52:1185–95.
59 Becker-Merok A, Østli-Eilersten G, Lester S, *et al*. Circulating interferon-α2 levels are increased in the majority of patients with systemic lupus erythematosus and are associated with disease activity and multiple cytokine activation. *Lupus* 2013;22:155–63.
60 Bengtsson AA, Sturfelt G, Truedsson L, *et al*. Activation of type I interferon system in systemic lupus erythematosus correlates

with disease activity but not with antiretroviral antibodies. *Lupus* 2000;9:664–71.
61 Björkander S, Bremme K, Persson J-O, *et al*. Pregnancy-Associated inflammatory markers are elevated in pregnant women with systemic lupus erythematosus. *Cytokine* 2012;59:392–9.
62 Duan H, Fleming J, Pritchard DK, *et al*. Combined analysis of monocyte and lymphocyte messenger RNA expression with serum protein profiles in patients with scleroderma. *Arthritis Rheum* 2008;58:1465–74.
63 Fernández Matilla M, Grau García E, Fernández-Llanio Comella N, *et al*. Increased interferon-1α, interleukin-10 and blys concentrations as clinical activity biomarkers in systemic lupus erythematosus. *Med Clin (Barc)* 2019;153:225–31.
64 Fragoso-Loyo H, Atisha-Fregoso Y, Núñez-Alvarez CA, *et al*. Utility of interferon-α as a biomarker in central neuropsychiatric involvement in systemic lupus erythematosus. *J Rheumatol* 2012;39:504–9.
65 Grenn RC, Yalavarthi S, Gandhi AA, *et al*. Endothelial progenitor dysfunction associates with a type I interferon signature in primary antiphospholipid syndrome. *Ann Rheum Dis* 2017;76:450–7.
66 Horai Y, Koga T, Fujikawa K, *et al*. Serum interferon-α is a useful biomarker in patients with anti-melanoma differentiation-associated gene 5 (MDA5) antibody-positive dermatomyositis. *Mod Rheumatol* 2015;25:85–9.
67 Jönsen A, Bengtsson AA, Nived O, *et al*. The heterogeneity of neuropsychiatric systemic lupus erythematosus is reflected in lack of association with cerebrospinal fluid cytokine profiles. *Lupus* 2003;12:846–50.
68 Kim T, Kanayama Y, Negoro N, *et al*. Serum levels of interferons in patients with systemic lupus erythematosus. *Clin Exp Immunol* 1987;70:562–9.
69 Krol P, Krystufkova O, Polanska M, *et al*. Serum levels of interferon do not correlate with disease activity in patients with dermatomyositis/polymyositis. *Annals of the Rheumatic Diseases* 2011;70:879–80.
70 Lood C, Amisten S, Gullstrand B, *et al*. Platelet transcriptional profile and protein expression in patients with systemic lupus erythematosus: up-regulation of the type I interferon system is strongly associated with vascular disease. *Blood* 2010;116:1951–7.
71 Luan JJ, Xing GQ. Pathogenesis of antimicrobial peptides LL-37 and cpg-ODN in ANCA associated vasculitis. *J Nephrol* 2017;30:63–71.
72 Mozo L, López P, Caminal-Montero L, *et al*. Anti-Ribosomal P antibodies are associated with elevated circulating IFNα and IL-10 levels in systemic lupus erythematosus patients. *Lupus* 2014;23:1477–85.
73 Pay S, Simsek I, Erdem H, *et al*. Dendritic cell subsets and type I interferon system in Behçet's disease: does functional abnormality in plasmacytoid dendritic cells contribute to Th1 polarization? *Clin Exp Rheumatol* 2007;25(4 Suppl 45):S34–40.
74 Pollard RPE, Abdulahad WH, Bootsma H, *et al*. Predominantly proinflammatory cytokines decrease after B cell depletion therapy in patients with primary Sjogren's syndrome. *Ann Rheum Dis* 2013;72:2048–50.
75 Postal M, Sinicato NA, Peliçari KO, *et al*. Clinical and serological manifestations associated with interferon-α levels in childhood-onset systemic lupus erythematosus. *Clinics (Sao Paulo)* 2012;67:157–62.
76 Rodríguez-Carrio J, de Paz B, López P, *et al*. Ifnα serum levels are associated with endothelial progenitor cells imbalance and disease features in rheumatoid arthritis patients. *PLoS ONE* 2014;9:e86069.
77 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.
78 Schneider L, Colar da Silva AC, Werres Junior LC, *et al*. Vitamin D levels and cytokine profiles in patients with systemic lupus erythematosus. *Lupus* 2015;24:1191–7.
79 Shahin D, El-Refaey AM, El-Hawary AK, *et al*. Serum interferon-alpha level in first degree relatives of systemic lupus erythematosus patients: correlation with autoantibodies titers. *Egyptian Journal of Medical Human Genetics* 2011;12:139–46.
80 Shi SN, Feng SF, Wen YM, *et al*. Serum interferon in systemic lupus erythematosus. *Br J Dermatol* 1987;117:155–9.
81 Shiozawa S, Shiozawa K, Shimizu S, *et al*. Immunoreactive circulating alpha-interferon is low in Sjögren's syndrome. *Br J Rheumatol* 1990;29:50–2.
82 Wildenberg ME, van Helden-Meeuwsen 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.
83 Willis R, Seif AM, McGwin G Jr, *et al*. Effect of hydroxychloroquine treatment on pro-inflammatory cytokines and disease activity in SLE patients: data from LUMINA (LXXV), a multiethnic US cohort. *Lupus* 2012;21:830–5.
84 Ye H, Wang X, Wang L, *et al*. Full high-throughput sequencing analysis of differences in expression profiles of long noncoding RNAs and their mechanisms of action in systemic lupus erythematosus. *Arthritis Res Ther* 2019;21:1.
85 Yilmaz S, Cinar M, Pekel A, *et al*. The expression of transmembrane and soluble CXCL16 and the relation with interferon-alpha secretion in patients with Behçet's disease. *Clin Exp Rheumatol* 2013;31(3 Suppl 77):84–7.
86 Yin Z, Huang J, He W, *et al*. Serum level of eight cytokines in Han Chinese patients with systemic lupus erythematosus using multiplex fluorescent microsphere method. *Cent Eur J Immunol* 2014;39:228–35.
87 Zecevic L, Karamehic J, Coric J, *et al*. Potential immune biomarkers in diagnosis and clinical management for systemic lupus erythematosus. *J Med Biochem* 2018;37:163–71.
88 Zhang Y, Shi W, Tang S, *et al*. The influence of cathelicidin LL37 in human anti-neutrophils cytoplasmic antibody (ANCA) -associated vasculitis. *Arthritis Res Ther* 2013;15:R161.
89 Zheng L, Zhang Z, Yu C, *et al*. Association between IFN-alpha and primary Sjogren's syndrome. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;107:e12–8.
90 Zhuang H, Narain S, Sobel E, *et al*. Association of anti-nucleoprotein autoantibodies with upregulation of type I interferon-inducible gene transcripts and dendritic cell maturation in systemic lupus erythematosus. *Clin Immunol* 2005;117:238–50.
91 Blomberg S, Eloranta ML, Cederblad B, *et al*. Presence of cutaneous interferon-alpha producing cells in patients with systemic lupus erythematosus. *Lupus* 2001;10:484–90.
92 Fong KY, Boey ML, Koh WH, *et al*. Cytokine concentrations in the synovial fluid and plasma of rheumatoid arthritis patients: correlation with bony erosions. *Clin Exp Rheumatol* 1994;12:55–8.
93 Hashad DI, Abdelmagid MH, Elsherif SH. MicroRNA146a expression in lupus patients with and without renal complications. *J Clin Lab Anal* 2012;26:35–40.
94 Kanakoudi-Tsakalidou F, Farmaki E, Tzimouli V, *et al*. Simultaneous changes in serum HMGB1 and IFN-α levels and in LAIR-1 expression on plasmatoid dendritic cells of patients with juvenile SLE. new therapeutic options? *Lupus* 2014;23:305–12.
95 Lee MT, Hooper LC, Kump L, *et al*. Interferon-Beta and adhesion molecules (E-selectin and s-intracellular adhesion molecule-1) are detected in sera from patients with retinal vasculitis and are induced in retinal vascular endothelial cells by Toll-like receptor 3 signalling. *Clin Exp Immunol* 2007;147:71–80.
96 Lepore L, Pennesi M, Saletta S, *et al*. Study of IL-2, IL-6, TNF alpha, IFN gamma and beta in the serum and synovial fluid of patients with juvenile chronic arthritis. *Clin Exp Rheumatol* 1994;12:561–5.
97 Ma C, Jiao Y, Zhang J, *et al*. Elevated plasma level of HMGB1 is associated with disease activity and combined alterations with IFN-α and TNF-α in systemic lupus erythematosus. *Rheumatol Int* 2012;32:395–402.
98 Mandal M, Tripathy R, Panda AK, *et al*. Vitamin D levels in Indian systemic lupus erythematosus patients: association with disease activity index and interferon alpha. *Arthritis Res Ther* 2014;16:R49.
99 Pacheco Y, Barahona-Correa J, Monsalve DM, *et al*. Cytokine and autoantibody clusters interaction in systemic lupus erythematosus. *J Transl Med* 2017;15:239.
100 Robak E, Smolewski P, Wozniacka A, *et al*. Relationship between peripheral blood dendritic cells and cytokines involved in the pathogenesis of systemic lupus erythematosus. *Eur Cytokine Netw* 2004;15:222–30.
101 Takeuchi Y, Seki T, Kobayashi N, *et al*. Analysis of serum IL-38 in juvenile-onset systemic lupus erythematosus. *Mod Rheumatol* 2018;28:1069–72.
102 Wirestam L, Enocsson H, Skogh T, *et al*. Interferon-A coincides with suppressed levels of pentraxin-3 (PTX3) in systemic lupus erythematosus and regulates leucocyte PTX3 in vitro. *Clin Exp Immunol* 2017;189:83–91.
103 Yoshio T, Okamoto H, Kurasawa K, *et al*. Il-6, IL-8, IP-10, MCP-1 and G-CSF are significantly increased in cerebrospinal fluid but not in sera of patients with central neuropsychiatric lupus erythematosus. *Lupus* 2016;25:997–1003.
104 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.

105 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.
106 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.
107 Alunno A, Caneparo V, Carubbi F, *et al*. Interferon gamma-inducible protein 16 (IFI16) and anti-IFI16 antibodies in primary Sjögren's syndrome: findings in serum and minor salivary glands. *Reumatismo* 2015;67:85–90.
108 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.
109 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;5:1.
110 Connelly KL, Kandane-Rathnayake R, Hoi A, *et al*. Association of MIF, but not type I interferon-induced chemokines, with increased disease activity in Asian patients with systemic lupus erythematosus. *Sci Rep* 2016;6:29909:29909.:.
111 Connelly KL, Kandane-Rathnayake R, Huq M, *et al*. Longitudinal association of type 1 interferon-induced chemokines with disease activity in systemic lupus erythematosus. *Sci Rep* 2018;8.
112 Guo X, Higgs BW, Bay-Jensen AC, *et al*. Suppression of T cell activation and collagen accumulation by an anti-IFNAR1 mab, anifrolumab, in adult patients with systemic sclerosis. *J Invest Dermatol* 2015;135:2402–9.
113 James JA, Guthridge JM, Chen H, *et al*. Unique sjogren's syndrome patient subsets defined by molecular features. *Rheumatology* 2019:08.
114 Lee J-R, Haddon DJ, Wand HE, *et al*. Multiplex giant magnetoresistive biosensor microarrays identify interferon-associated autoantibodies in systemic lupus erythematosus. *Sci Rep* 2016;6:27623.
115 López De Padilla CM, Crowson CS, Hein MS, *et al*. Interferon-Regulated chemokine score associated with improvement in disease activity in refractory myositis patients treated with rituximab. *Clin Exp Rheumatol* 2015;33:655–63.
116 Mariotti B, Servaas NH, Rossato M, *et al*. The long non-coding RNA NRIR drives IFN-response in monocytes: implication for systemic sclerosis. *Front Immunol* 2019;10.
117 Olsen NJ, McAloose C, Carter J, *et al*. Clinical and immunologic profiles in incomplete lupus erythematosus and improvement with hydroxychloroquine treatment. *Autoimmune Dis* 2016;2016:8791629.
118 Quartier P, Allantaz F, Cimaz R, *et al*. A multicentre, randomised, double-blind, placebo-controlled trial with the interleukin-1 receptor antagonist anakinra in patients with systemic-onset juvenile idiopathic arthritis (ANAJIS trial). *Ann Rheum Dis* 2011;70:747–54.
119 Reed AM, Crowson CS, Hein M, *et al*. Biologic predictors of clinical improvement in rituximab-treated refractory myositis. *BMC Musculoskelet Disord* 2015;16:257:257.:.
120 Rose T, Szelinski F, Lisney A, *et al*. SIGLEC1 is a biomarker of disease activity and indicates extraglandular manifestation in primary Sjögren's syndrome. *RMD Open* 2016;2:e000292.
121 Wahadat MJ, Bodewes ILA, Maria NI, *et al*. Type I IFN signature in childhood-onset systemic lupus erythematosus: a conspiracy of DNA- and RNA-sensing receptors? *Arthritis Res Ther* 2018;20.
122 al-Masri AN, Werfel T, Jakschies D, *et al*. Intracellular staining of Mx proteins in cells from peripheral blood, bone marrow and skin. *Molecular Pathology* 1997;50:9–14.
123 De Andrea M, De Santis M, Caneparo V, *et al*. Serum IFI16 and anti-IFI16 antibodies in psoriatic arthritis. *Clin Exp Immunol* 2019;199:30:88–96.:.
124 Yamamoto M, Takano K, Kamekura R, *et al*. Stage classification of IgG4-related Dacryoadenitis and sialadenitis by the serum cytokine environment. *Modern Rheumatology* 2018;28:1004–8.
125 Zhu H, Mi W, Luo H, *et al*. Whole-Genome transcription and DNA methylation analysis of peripheral blood mononuclear cells identified aberrant gene regulation pathways in systemic lupus erythematosus. *Arthritis Res Ther* 2016;18:1.
126 Alexander T, Sarfert R, Klotsche J, *et al*. The proteasome inhibitior bortezomib depletes plasma cells and ameliorates clinical manifestations of refractory systemic lupus erythematosus. *Ann Rheum Dis* 2015;74:1474–8.
127 Biesen R, Demir C, Barkhudarova 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 & Rheumatism* 2008;58:1136–45.
128 Wilhelm TR, Taddeo A, Winter O, *et al*. Siglec-1-positive plasmacytoid dendritic cells (pdcs) in human peripheral blood: A semi-mature and myeloid-like subset imbalanced during protective and autoimmune responses. *Clin Immunol* 2016;163:42–51.
129 Aranow C, Kamen DL, Dall'Era M, *et al*. Randomized, double-blind, placebo-controlled trial of the effect of vitamin D 3 on the interferon signature in patients with systemic lupus erythematosus. *Arthritis & Rheumatology* 2015;67:1848–57.
130 Banchereau R, Hong S, Cantarel B, *et al*. Personalized immunomonitoring uncovers molecular networks that stratify lupus patients. *Cell* 2016;165:1548–50.
131 Chiche L, Jourde-Chiche N, Whalen E, *et al*. Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. *Arthritis Rheumatol* 2014;66:1583–95.
132 Flint SM, Jovanovic V, Teo BW, *et al*. Leucocyte subset-specific type 1 interferon signatures in SLE and other immune-mediated diseases. *RMD Open* 2016;2:e000183.
133 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.
134 Petri M, Fu W, Ranger A, *et al*. Association between changes in gene signatures expression and disease activity among patients with systemic lupus erythematosus. *BMC Med Genomics* 2019;12:4.
135 Olferiev M, Jacek E, Kirou KA, *et al*. Novel molecular signatures in mononuclear cell populations from patients with systemic lupus erythematosus. *Clin Immunol* 2016;172:34–43.
136 Assassi S, Mayes MD, Arnett FC, *et al*. Systemic sclerosis and lupus: points in an interferon-mediated continuum. *Arthritis Rheum* 2010;62:589–98.
137 Bienkowska J, Allaire N, Thai A, *et al*. Lymphotoxin-LIGHT pathway regulates the interferon signature in rheumatoid arthritis. *PLoS ONE* 2014;9:e112545.
138 Cantaert T, van Baarsen LG, Wijbrandts CA, *et al*. Type I interferons have no major influence on humoral autoimmunity in rheumatoid arthritis. *Rheumatology (Oxford)* 2010;49:156–66.
139 Christmann RB, Hayes E, Pendergrass S, *et al*. Interferon and alternative activation of monocyte/macrophages in systemic sclerosis-associated pulmonary arterial hypertension. *Arthritis Rheum* 2011;63:1718–28.
140 Goldberg A, Geppert T, Schiopu E, *et al*. Dose-Escalation of human anti-interferon-α receptor monoclonal antibody MEDI-546 in subjects with systemic sclerosis: a phase 1, multicenter, open label study. *Arthritis Res Ther* 2014;16:R57.
141 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.
142 Higgs BW, Zhu W, Morehouse C, *et al*. A phase 1B clinical trial evaluating sifalimumab, an anti-IFN-α monoclonal antibody, shows target neutralisation of a type I IFN signature in blood of dermatomyositis and polymyositis patients. *Ann Rheum Dis* 2014;73:256–62.
143 Higgs BW, Zhu W, Richman L, *et al*. Identification of activated cytokine pathways in the blood of systemic lupus erythematosus, myositis, rheumatoid arthritis, and scleroderma patients. *Int J Rheum Dis* 2012;15:25–35.
144 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.
145 Kennedy WP, Maciuca 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.
146 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.
147 Petri M, Singh S, Tesfasyone H, *et al*. Longitudinal expression of type I interferon responsive genes in systemic lupus erythematosus. *Lupus* 2009;18:980–9.
148 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.
149 Serikawa KA, Jacobsen S, Lundsgaard D, *et al*. Detection of gene expression signatures related to underlying disease and treatment in rheumatoid arthritis patients. *Mod Rheumatol* 2013;23:729–40.
150 Yao Y, Richman L, Higgs BW, *et al*. Neutralization of interferon-alpha/beta-inducible genes and downstream effect in a phase I trial

of an anti-interferon-alpha monoclonal antibody in systemic lupus erythematosus. *Arthritis Rheum* 2009;60:1785–96.
151 Becker AM, Dao KH, Han BK, *et al*. Sle peripheral blood B cell, T cell and myeloid cell transcriptomes display unique profiles and each subset contributes to the interferon signature. *PLoS ONE* 2013;8:e67003.
152 Bennett L, Palucka AK, Arce E, *et al*. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J Exp Med* 2003;197:711–23.
153 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.
154 Dey-Rao R, Sinha AA. Genome-wide transcriptional profiling of chronic cutaneous lupus erythematosus (CCLE) peripheral blood identifies systemic alterations relevant to the skin manifestation. *Genomics* 2015;105:90–100.
155 Dolcino M, Ottria A, Barbieri A, *et al*. Gene expression profiling in peripheral blood cells and synovial membranes of patients with psoriatic arthritis. *PLoS ONE* 2015;10:e0128262.
156 Emamian ES, Leon JM, Lessard CJ, *et al*. Peripheral blood gene expression profiling in Sjögren's syndrome. *Genes Immun* 2009;10:285–96.
157 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.
158 Kimoto O, Sawada J, Shimoyama K, *et al*. Activation of the interferon pathway in peripheral blood of patients with Sjogren's syndrome. *J Rheumatol* 2011;38:310–6.
159 Kyogoku C, Smiljanovic B, Grün JR, *et al*. Cell-specific type I IFN signatures in autoimmunity and viral infection: what makes the difference? *PLoS ONE* 2013;8:e83776.
160 Lee H-M, Mima T, Sugino H, *et al*. Interactions among type I and type II interferon, tumor necrosis factor, and β-estradiol in the regulation of immune response-related gene expressions in systemic lupus erythematosus. *Arthritis Res Ther* 2009;11:R1.
161 Li Q-Z, Zhou J, Lian Y, *et al*. Interferon signature gene expression is correlated with autoantibody profiles in patients with incomplete lupus syndromes. *Clin Exp Immunol* 2010;159:281–91.
162 Lugar PL, Love C, Grammer AC, *et al*. Molecular characterization of circulating plasma cells in patients with active systemic lupus erythematosus. *PLoS ONE* 2012;7:e44362.
163 Morimoto AM, Flesher DT, Yang J, *et al*. Association of endogenous anti-interferon-α autoantibodies with decreased interferon-pathway and disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum* 2011;63:2407–15.
164 Nikpour M, Dempsey AA, Urowitz MB, *et al*. Association of a gene expression profile from whole blood with disease activity in systemic lupus erythaematosus. *Ann Rheum Dis* 2008;67:1069–75.
165 Puccetti A, Fiore PF, Pelosi A, *et al*. Gene expression profiling in behcet's disease indicates an autoimmune component in the pathogenesis of the disease and opens new avenues for targeted therapy. *J Immunol Res* 2018;2018:4246965.
166 Raterman HG, Vosslamber 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.
167 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 & Rheumatology* 2014;66:1421–31.
168 Sharma S, Jin Z, Rosenzweig E, *et al*. Widely divergent transcriptional patterns between SLE patients of different ancestral backgrounds in sorted immune cell populations. *J Autoimmun* 2015;60:51–8.
169 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.
170 van Baarsen LGM, Bos WH, Rustenburg F, *et al*. Gene expression profiling in autoantibody-positive patients with arthralgia predicts development of arthritis. *Arthritis Rheum* 2010;62:694–704.
171 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.
172 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.
173 Vosslamber 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.
174 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.
175 Ye S, Pang H, Gu Y-Y, *et al*. Protein interaction for an interferon-inducible systemic lupus associated gene, IFIT1. *Rheumatology (Oxford)* 2003;42:1155–63.
176 Li Q-Z, Karp DR, Quan J, *et al*. Risk factors for ANA positivity in healthy persons. *Arthritis Res Ther* 2011;13:R38.
177 Perez-Sanchez C, Barbarroja N, Messineo S, *et al*. Gene profiling reveals specific molecular pathways in the pathogenesis of atherosclerosis and cardiovascular disease in antiphospholipid syndrome, systemic lupus erythematosus and antiphospholipid syndrome with lupus. *Ann Rheum Dis* 2015;74:11:1441–9.:.
178 Smiljanovic B, Grün JR, Biesen R, *et al*. The multifaceted balance of TNF-α and type I/II interferon responses in SLE and RA: how monocytes manage the impact of cytokines. *J Mol Med (Berl)* 2012;90:1295–309.
179 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.
180 Castañeda-Delgado JE, Bastián-Hernandez Y, Macias-Segura N, *et al*. Type I interferon gene response is increased in early and established rheumatoid arthritis and correlates with autoantibody production. *Front Immunol* 2017;8:285.
181 Joseph S, George NI, Green-Knox B, *et al*. Epigenome-wide association study of peripheral blood mononuclear cells in systemic lupus erythematosus: identifying DNA methylation signatures associated with interferon-related genes based on ethnicity and SLEDAI. *J Autoimmun* 2019;96:147–57.
182 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.
183 O'Connor KA, Abbott KA, Sabin B, *et al*. MxA gene expression in juvenile dermatomyositis peripheral blood mononuclear cells: association with muscle involvement. *Clin Immunol* 2006;120:319–25.
184 Rodríguez-Carrio J, López P, Alperi-López M, *et al*. Irf4 and irgs delineate clinically relevant gene expression signatures in systemic lupus erythematosus and rheumatoid arthritis. *Front Immunol* 2019;9.
185 Tang J, Gu Y, Zhang M, *et al*. Increased expression of the type I interferon-inducible gene, lymphocyte antigen 6 complex locus E, in peripheral blood cells is predictive of lupus activity in a large cohort of chinese lupus patients. *Lupus* 2008;17:805–13.
186 Komatsuda A, Wakui H, Iwamoto K, *et al*. Up-Regulated expression of Toll-like receptors mRNAs in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. *Clin Exp Immunol* 2008;152:482–7.
187 Li D, Song L, Fan Y, *et al*. Down-Regulation of TIPE2 mRNA expression in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. *Clin Immunol* 2009;133:422–7.
188 Bilgic H, Ytterberg SR, Amin S, *et al*. Interleukin-6 and type I interferon-regulated genes and chemokines mark disease activity in dermatomyositis. *Arthritis Rheum* 2009;60:3436–46.
189 Blokland SLM, van den Hoogen LL, Leijten EFA, *et al*. Increased expression of Fas on group 2 and 3 innate lymphoid cells is associated with an interferon signature in systemic lupus erythematosus and Sjögren's syndrome. *Rheumatology (Oxford)* 2019;58:1740–5.
190 Bodewes ILA, Gottenberg J-E, van Helden-Meeuwsen CG, *et al*. Hydroxychloroquine treatment downregulates systemic interferon activation in primary sjögren's syndrome in the JOQUER randomized trial. *Rheumatology (Oxford)* 2020;59:107–11.
191 Braunstein I, Klein R, Okawa J, *et al*. The interferon-regulated gene signature is elevated in subacute cutaneous lupus erythematosus and discoid lupus erythematosus and correlates with the cutaneous lupus area and severity index score. *British Journal of Dermatology* 2012;166:971–5.
192 Brkic Z, Corneth OB, van Helden-Meeuwsen CG, *et al*. T-Helper 17 cell cytokines and interferon type I: partners in crime in systemic lupus erythematosus? *Arthritis Res Ther* 2014;16:R62.
193 Brkic Z, Maria NI, van Helden-Meeuwsen 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.

194 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.
195 Brohawn P, Streicher K, Higgs BW, *et al*. Type I interferon gene signature test–low and –high patients with systemic lupus erythematosus have distinct gene expression signatures. *Lupus* 2019;28:1524–33.
196 Cooles FAH, Anderson AE, Lendrem DW, *et al*. The interferon gene signature is increased in patients with early treatment-naive rheumatoid arthritis and predicts a poorer response to initial therapy. *J Allergy Clin Immunol* 2018;141:445–8.
197 Davies R, Sarkar I, Hammenfors D, *et al*. Single cell based phosphorylation profiling identifies alterations in toll-like receptor 7 and 9 signaling in patients with primary sjögren's syndrome. *Front Immunol* 2019;10:281.
198 de Jong TD, Blits M, de Ridder S, *et al*. Type I interferon response gene expression in established rheumatoid arthritis is not associated with clinical parameters. *Arthritis Res Ther* 2016;18:290.
199 de Jong TD, Lübbers J, Turk S, *et al*. The type I interferon signature in leukocyte subsets from peripheral blood of patients with early arthritis: a major contribution by granulocytes. *Arthritis Res Ther* 2016;18:165.
200 de Jong TD, Vosslamber 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.
201 Dominguez-Gutierrez PR, Ceribelli A, Satoh M, *et al*. Elevated signal transducers and activators of transcription 1 correlates with increased C-C motif chemokine ligand 2 and C-X-C motif chemokine 10 levels in peripheral blood of patients with systemic lupus erythematosus. *Arthritis Res Ther* 2014;16:R20.
202 Dominguez-Gutierrez PR, Ceribelli A, Satoh M, *et al*. Reduced levels of CCL2 and CXCL10 in systemic lupus erythematosus patients under treatment with prednisone, mycophenolate mofetil, or hydroxychloroquine, except in a high STAT1 subset. *Arthritis Res Ther* 2014;16:R23.
203 Dominguez-Gutierrez PR, Ceribelli A, Satoh M, *et al*. Positive correlation of STAT1 and miR-146a with anemia in patients with systemic lupus erythematosus. *J Clin Immunol* 2014;34:171–80.
204 Ekholm L, Vosslamber S, Tjärnlund A, *et al*. Autoantibody specificities and type I interferon pathway activation in idiopathic inflammatory myopathies. *Scand J Immunol* 2016;84:100–9.
205 Feng X, Chen W, Xiao L, *et al*. Artesunate inhibits type I interferon-induced production of macrophage migration inhibitory factor in patients with systemic lupus erythematosus. *Lupus* 2017;26:62–72.
206 Feng X, Huang J, Liu Y, *et al*. Identification of interferon-inducible genes as diagnostic biomarker for systemic lupus erythematosus. *Clin Rheumatol* 2015;34:71–9.
207 Feng X, Wu H, Grossman JM, *et al*. Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. *Arthritis Rheum* 2006;54:2951–62.
208 Furie R, Khamashta M, Merrill JT, *et al*. Anifrolumab, an anti-interferon-α receptor monoclonal antibody, in moderate-to-severe systemic lupus erythematosus. *Arthritis & Rheumatology* 2017;69:376–86.
209 Furie R, Werth VP, Merola JF, *et al*. Monoclonal antibody targeting BDCA2 ameliorates skin lesions in systemic lupus erythematosus. *J Clin Invest* 2019;129:1359–71.
210 Hasni S, Gupta S, Davis M, *et al*. Safety and tolerability of omalizumab: a randomized clinical trial of humanized anti-IgE monoclonal antibody in systemic lupus erythematosus. *Arthritis Rheumatol* 2019;71:1135–40.
211 Hillen MR, Pandit A, Blokland SLM, *et al*. Plasmacytoid DCs from patients with Sjögren's syndrome are transcriptionally primed for enhanced pro-inflammatory cytokine production. *Front Immunol* 2019;10.
212 Hua J, Kirou K, Lee C, *et al*. Functional assay of type I interferon in systemic lupus erythematosus plasma and association with anti-RNA binding protein autoantibodies. *Arthritis Rheum* 2006;54:1906–16.
213 Jin Z, Fan W, Jensen MA, *et al*. Single-Cell gene expression patterns in lupus monocytes independently indicate disease activity, interferon and therapy. *Lupus Sci Med* 2017;4:e000202.
214 Kalunian KC, Merrill JT, Maciuca R, *et al*. A phase II study of the efficacy and safety of rontalizumab (rhumab interferon-α) in patients with systemic lupus erythematosus (rose). *Ann Rheum Dis* 2016;75:196–202.
215 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.
216 Landolt-Marticorena C, Bonventi G, Lubovich A, *et al*. Lack of association between the interferon-alpha signature and longitudinal changes in disease activity in systemic lupus erythematosus. *Ann Rheum Dis* 2009;68:1440–6.
217 Landolt-Marticorena C, Wither R, Reich H, *et al*. Increased expression of B cell activation factor supports the abnormal expansion of transitional B cells in systemic lupus erythematosus. *J Rheumatol* 2011;38:642–51.
218 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.
219 Lübbers J, Vosslamber S, van de Stadt LA, *et al*. B cell signature contributes to the prediction of RA development in patients with arthralgia. *Ann Rheum Dis* 2015;74:1786–8.
220 Maria NI, Steenwijk EC, IJpma AS, *et al*. Contrasting expression pattern of RNA-sensing receptors TLR7, RIG-I and MDA5 in interferon-positive and interferon-negative patients with primary Sjögren's syndrome. *Ann Rheum Dis* 2017;76:721–30.
221 Maria NI, van Helden-Meeuwsen CG, Brkic Z, *et al*. Association of increased Treg cell levels with elevated indoleamine 2,3-dioxygenase activity and an imbalanced kynurenine pathway in interferon-positive primary Sjögren's syndrome. *Arthritis Rheumatol* 2016;68:1688–99.
222 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.
223 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.
224 Olsson P, Bodewes ILA, Nilsson AM, *et al*. Associations of cigarette smoking with disease phenotype and type I interferon expression in primary Sjögren's syndrome. *Rheumatol Int* 2019;39:1575–84.
225 Palli E, Kravvariti E, Tektonidou MG. Type I interferon signature in primary antiphospholipid syndrome: clinical and laboratory associations. *Front Immunol* 2019;10:487.
226 Pisetsky DS, Thompson DK, Wajdula J, *et al*. Variability in antinuclear antibody testing to assess patient eligibility for clinical trials of novel treatments for systemic lupus erythematosus. *Arthritis Rheumatol* 2019;71:1534–8.
227 Quartuccio L, Mavragani CP, Nezos A, *et al*. Type I interferon signature may influence the effect of belimumab on immunoglobulin levels, including rheumatoid factor in sjögren's syndrome. *Clin Exp Rheumatol* 2017;35:719–20.
228 Steiman AJ, Gladman DD, Ibañez D, *et al*. Lack of interferon and proinflammatory cyto/chemokines in serologically active clinically quiescent systemic lupus erythematosus. *J Rheumatol* 2015;42:2318–26.
229 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 & Rheumatism* 2010;62:3607–14.
230 Ugolini-Lopes MR, Torrezan GT, Gândara APR, *et al*. Enhanced type I interferon gene signature in primary antiphospholipid syndrome: association with earlier disease onset and preeclampsia. *Autoimmunity Reviews* 2019;18:393–8.
231 van den Hoogen LL, Fritsch-Stork RDE, Versnel MA, *et al*. Monocyte type I interferon signature in antiphospholipid syndrome is related to proinflammatory monocyte subsets, hydroxychloroquine and statin use. *Ann Rheum Dis* 2016;75:e81.
232 Van Den Hoogen LL, Van Der Heijden EHM, Hillen MR, *et al*. Galectin-9 reflects the interferon signature and correlates with disease activity in systemic autoimmune diseases. *Ann Rheum Dis* 2018.
233 Wang B, Higgs BW, Chang L, *et al*. Pharmacogenomics and translational simulations to bridge indications for an anti-interferon-α receptor antibody. *Clin Pharmacol Ther* 2013;93:483–92.
234 Bodewes ILA, Huijser E, van Helden-Meeuwsen CG, *et al*. Tbk1: a key regulator and potential treatment target for interferon positive sjögren's syndrome, systemic lupus erythematosus and systemic sclerosis. *J Autoimmun* 2018;91:97–102.
235 Kellner ES, Lee PY, Li Y, *et al*. Endogenous type-I interferon activity is not associated with depression or fatigue in systemic lupus erythematosus. *J Neuroimmunol* 2010;223:13–9.
236 Merrill JT, Immermann F, Whitley M, *et al*. The biomarkers of lupus disease study: a BOLD approach may mitigate interference

of background immunosuppressants in clinical trials. *Arthritis Rheumatol* 2017;69:1257–66.
237 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.
238 Fu Q, Chen X, Cui H, *et al*. Association of elevated transcript levels of interferon-inducible chemokines with disease activity and organ damage in systemic lupus erythematosus patients. *Arthritis Res Ther* 2008;10:R112.
239 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.
240 Xu Z, Wang X, Zheng Y. Screening for key genes and transcription factors in ankylosing spondylitis by RNA-seq. *Exp Ther Med* 2018;15:1394–402.
241 Altorok N, Coit P, Hughes T, *et al*. Genome-wide DNA methylation patterns in naive CD4+ T cells from patients with primary sjögren's syndrome. *Arthritis & Rheumatology* 2014;66:731–9.
242 Coit P, Jeffries M, Altorok N, *et al*. Genome-Wide DNA methylation study suggests epigenetic accessibility and transcriptional poising of interferon-regulated genes in naïve CD4+ T cells from lupus patients. *Journal of Autoimmunity* 2013;43:78–84.
243 Ding W, Pu W, Wang L, *et al*. Genome-Wide DNA methylation analysis in systemic sclerosis reveals hypomethylation of IFN-associated genes in CD4+ and CD8+ T cells. *Journal of Investigative Dermatology* 2018;138:1069–77.
244 Yeung KS, Chung BH-Y, Choufani S, *et al*. Genome-Wide DNA methylation analysis of Chinese patients with systemic lupus erythematosus identified hypomethylation in genes related to the type I interferon pathway. *PLoS ONE* 2017;12:e0169553.
245 Absher DM, Li X, Waite LL, *et al*. Genome-Wide DNA methylation analysis of systemic lupus erythematosus reveals persistent hypomethylation of interferon genes and compositional changes to CD4+ T-cell populations. *PLoS Genet* 2013;9:e1003678.
246 Coit P, Yalavarthi S, Ognenovski M, *et al*. Epigenome profiling reveals significant DNA demethylation of interferon signature genes in lupus neutrophils. *J Autoimmun* 2015;58:59–66.
247 Imgenberg-Kreuz J, Almlöf JC, Leonard D, *et al*. Shared and unique patterns of DNA methylation in systemic lupus erythematosus and primary sjögren's syndrome. *Front Immunol* 2019;10:1686.
248 Lanata CM, Paranjpe I, Nititham J, *et al*. A phenotypic and genomics approach in A multi-ethnic cohort to subtype systemic lupus erythematosus. *Nat Commun* 2019;10:3902.
249 Mok A, Solomon O, Nayak RR, *et al*. Genome-wide profiling identifies associations between lupus nephritis and differential methylation of genes regulating tissue hypoxia and type 1 interferon responses. *Lupus Sci Med* 2016;3:e000183.
250 Ulff-Møller CJ, Asmar F, Liu Y, *et al*. Twin DNA methylation profiling reveals flare-dependent interferon signature and B cell promoter hypermethylation in systemic lupus erythematosus. *Arthritis Rheumatol* 2018;70:878–90.
251 Weeding E, Coit P, Yalavarthi S, *et al*. Genome-wide DNA methylation analysis in primary antiphospholipid syndrome neutrophils. *Clin Immunol* 2018;196:110–6.
252 Zhao M, Zhou Y, Zhu B, *et al*. IFI44L promoter methylation as a blood biomarker for systemic lupus erythematosus. *Ann Rheum Dis* 2016;75:1998–2006.
253 Andrade D, Kim M, Blanco LP, *et al*. Interferon-A and angiogenic dysregulation in pregnant lupus patients who develop preeclampsia. *Arthritis Rheumatol* 2015;67:977–87.
254 Balboni I, Niewold TB, Morgan G, *et al*. Interferon-A induction and detection of anti-Ro, anti-La, anti-Sm, and anti-RNP autoantibodies by autoantigen microarray analysis in juvenile dermatomyositis. *Arthritis Rheum* 2013;65:2424–9.
255 Dastmalchi M, Grundtman C, Alexanderson H, *et al*. A high incidence of disease flares in an open pilot study of infliximab in patients with refractory inflammatory myopathies. *Ann Rheum Dis* 2008;67:1670–7.
256 Ekholm L, Kahlenberg JM, Barbasso Helmers S, *et al*. Dysfunction of endothelial progenitor cells is associated with the type I IFN pathway in patients with polymyositis and dermatomyositis. *Rheumatology (Oxford)* 2016;55:1987–92.
257 Iwamoto T, Dorschner J, Jolly M, *et al*. Associations between type I interferon and antiphospholipid antibody status differ between ancestral backgrounds. *Lupus Sci Med* 2018;5:e000246.
258 Kirou KA, Lee C, George S, *et al*. Coordinate overexpression of interferon-alpha-induced genes in systemic lupus erythematosus. *Arthritis Rheum* 2004;50:3958–67.
259 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.
260 Mavragani CP, Niewold TB, Moutsopoulos NM, *et al*. Augmented interferon-alpha pathway activation in patients with Sjögren's syndrome treated with etanercept. *Arthritis Rheum* 2007;56:3995–4004.
261 Mohan S, Barsalou J, Bradley TJ, *et al*. Endothelial progenitor cell phenotype and function are impaired in childhood-onset systemic lupus erythematosus. *Arthritis Rheumatol* 2015;67:2257–62.
262 Wampler Muskardin T, Vashisht 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.
263 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.
264 Niewold TB, Kariuki SN, Morgan GA, *et al*. Elevated serum interferon-alpha activity in juvenile dermatomyositis: associations with disease activity at diagnosis and after thirty-six months of therapy. *Arthritis Rheum* 2009;60:1815–24.
265 Oke V, Gunnarsson I, Dorschner J, *et al*. High levels of circulating interferons type I, type II and type III associate with distinct clinical features of active systemic lupus erythematosus. *Arthritis Res Ther* 2019;21:107.
266 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.
267 Weckerle CE, Franek BS, Kelly JA, *et al*. Network analysis of associations between serum interferon-α activity, autoantibodies, and clinical features in systemic lupus erythematosus. *Arthritis Rheum* 2011;63:1044–53.
268 Weckerle CE, Mangale D, Franek BS, *et al*. Large-Scale analysis of tumor necrosis factor α levels in systemic lupus erythematosus. *Arthritis Rheum* 2012;64:2947–52.
269 Wuttge DM, Lood C, Tufvesson E, *et al*. Increased serum type I interferon activity in early systemic sclerosis patients is associated with antibodies against Sjögren's syndrome antigens and nuclear ribonucleoprotein antigens. *Scand J Rheumatol* 2013;42:235–40.
270 Kariuki SN, Crow MK, Niewold TB. The PTPN22 C1858T polymorphism is associated with skewing of cytokine profiles toward high interferon-alpha activity and low tumor necrosis factor alpha levels in patients with lupus. *Arthritis Rheum* 2008;58:2818–23.
271 Lood C, Tydén H, Gullstrand B, *et al*. Type I interferon-mediated skewing of the serotonin synthesis is associated with severe disease in systemic lupus erythematosus. *PLoS ONE* 2015;10:e0125109.
272 Niewold TB, Adler JE, Glenn SB, *et al*. Age- and sex-related patterns of serum interferon-α activity in lupus families. *Arthritis Rheum* 2008;58:2113–9.
273 Niewold TB, Rivera TL, Buyon JP, *et al*. Serum type I interferon activity is dependent on maternal diagnosis in anti-SSA/ro–positive mothers of children with neonatal lupus. *Arthritis Rheum* 2008;58:541–6.
274 Biswas PS, Aggarwal R, Levesque MC, *et al*. Type I interferon and T helper 17 cells co-exist and co-regulate disease pathogenesis in lupus patients. *Int J Rheum Dis* 2015;18:646–53.
275 Dall'Era MC. Type I interferon correlates with serological and clinical manifestations of SLE. *Annals of the Rheumatic Diseases* 2005;64:1692–7.
276 Kato Y, Park J, Takamatsu H, *et al*. Apoptosis-derived membrane vesicles drive the cGAS-STING pathway and enhance type I IFN production in systemic lupus erythematosus. *Ann Rheum Dis* 2018;77:1507–15.
277 Preble OT, Black RJ, Friedman RM, *et al*. Systemic lupus erythematosus: presence in human serum of an unusual acid-labile leukocyte interferon. *Science* 1982;216:429–31.
278 Hertzog PJ, Emery P, Cheetham BF, *et al*. Interferons in rheumatoid arthritis: alterations in production and response related to disease activity. *Clin Immunol Immunopathol* 1988;48:192–201.
279 Yee AM, Yip YK, Fischer HD, *et al*. Serum activity that confers acid lability to alpha-interferon in systemic lupus erythematosus: its association with disease activity and its independence from circulating alpha-interferon. *Arthritis Rheum* 1990;33:563–8.
280 Suit BE, Axelrod D, Moutsopoulos HM, *et al*. Detection of anti-interferon antibodies in systemic lupus erythematosus. *Clin Exp Rheumatol* 1983;1:133–5.
281 Sibbitt WL, Gibbs DL, Kenny C, *et al*. Relationship between circulating interferon and anti-interferon antibodies and impaired

natural killer cell activity in systemic lupus erythematosus. *Arthritis Rheum* 1985;28:624–9.
282 Rich SA, Owens TR, Anzola MC, *et al*. Induction of lupus inclusions by sera from patients with systemic lupus erythematosus. *Arthritis Rheum* 1986;29:501–7.
283 Lackovic V, Borecký L, Rovenský J, *et al*. Periodicity of interferon appearance in serum of patients with systemic lupus erythematosus. *Arthritis Rheum* 1984;27:597–8.
284 Hervier B, Beziat V, Haroche J, *et al*. Phenotype and function of natural killer cells in systemic lupus erythematosus: excess interferon-γ production in patients with active disease. *Arthritis Rheum* 2011;63:1698–706.
285 Husby G, Williams RC, Ramirez F, *et al*. Absence of interferons-alpha and -gamma in renal lesions of systemic lupus erythematosus and membranous glomerulonephritis. *Clin Immunol Immunopathol* 1986;39:68–80.
286 Ytterberg SR, Schnitzer TJ. Serum interferon levels in patients with systemic lupus erythematosus. *Arthritis Rheum* 1982;25:401–6.
287 Hooks JJ, Jordan GW, Cupps T, *et al*. Multiple interferons in the circulation of patients with systemic lupus erythematosus and vasculitis. *Arthritis Rheum* 1982;25:396–400.
288 Hooks JJ, Moutsopoulos HM, Geis SA, *et al*. Immune interferon in the circulation of patients with autoimmune disease. *N Engl J Med* 1979;301:5–8.
289 Cesario TC, Andrews BS, Martin DA, *et al*. Interferon in synovial fluid and serum of patients with rheumatic disease. *J Rheumatol* 1983;10:647–50.
290 Arvin AM, Miller JJ. Acid labile alpha-interferon in sera and synovial fluids from patients with juvenile arthritis. *Arthritis Rheum* 1984;27:582–5.