High sensitivity measurement of circulating interferon proteins in systemic lupus erythematosus

Y. Huang[1,2], C.M. Bottomley[1], R.T. Maughan[1], A. Field[1], G.H.D. Leung[1], L. Lightstone[1,3], T.D. Cairns[3], T. Turner-Stokes[1,3], M.B. Condon[3], J.E. Peters[1,3], M. Botto[1,3], M.C. Pickering[1,3]

[1]Centre for Inflammatory Disease, Department of Immunology and Inflammation, Imperial College, London, United Kingdom; [2]School of Medicine, Chang Gung University, Taoyuan, Taiwan, and Division of Rheumatology, Allergy and Immunology, Chang Gung Memorial Hospital, Taoyuan, Taiwan; [3]Imperial Lupus Centre, Imperial College Healthcare NHS Trust, London, United Kingdom.

Abstract

Objective

Increased expression of type I interferon-induced genes is a hallmark of systemic lupus erythematosus (SLE). Measurement of interferon proteins in plasma has been challenging due to their low abundance. Here we utilise a new high sensitivity assay to evaluate levels of interferon proteins in SLE patients.

Methods

Seven interferon proteins (type I: IFNα1:IFNα13, IFNα2, IFNβ, IFNω; type II: IFNγ; type III: IFNλ1, IFNλ2:IFNλ3) were measured in 266 SLE patients using the NULISAseq Inflammation Panel 250 (Alamar Biosciences). IFN profiles (normal or high) were determined using the 95th percentile threshold in healthy controls for each IFN protein. Their relationship to disease activity and type I interferon-stimulated gene (ISG) scores was assessed.

Results

All seven IFN proteins were significantly increased in SLE patients compared to healthy controls and were higher in patients with anti-Sm, anti-Ro and anti-RNP antibodies. IFNα1:IFNα13, IFNα2 and IFNω strongly correlated with the ISG score whereas IFNβ did not. The median levels of IFNα1:IFNα13, IFNα2, IFNω, IFNλ1 and IFNλ2:IFNλ3 progressively increased with disease activity whereas this was not the case for either IFNβ or IFNγ. The most frequent IFN profiles were high type I+III (35%, n=93); normal levels of all IFN proteins (25%, n=67); high type I only (21%, n=56); and high type I+II+III (13%, n=34). The latter associated with serological activity (low complement and high dsDNA antibody titres) and nephritis.

Conclusion

Plasma levels of type I IFN proteins (IFNα1: IFNα13, IFNα2 and IFNω but not IFNβ) and type III IFN proteins (IFNλ1, IFNλ2:IFNλ3) were increased in active disease groups and ISG scores recapitulated this. Longitudinal intra-individual measures of these proteins are needed to explore their utility as biomarkers for SLE disease activity.

Key words

interferons, systemic lupus erythematosus, proteins

*Yun-Ju Huang, MD **
*Charlotte M. Bottomley, MSc**
*Robert T. Maughan, PhD **
Alex Field, MSc
Geoffrey H.D. Leung, BSc
Liz Lightstone, MD
Thomas D. Cairns, MD
Tabitha Turner-Stokes, MD, PhD
Marie B. Condon, MD
*James E. Peters, MD, PhD***
*Marina Botto, MD***
*Matthew C. Pickering, MD PhD***

**Equal first authors*
*** Co-senior authors*

Please address correspondence to:
Matthew Pickering
Centre for Inflammatory Disease
Department of Immunology and Inflammation
Imperial College London
Hammersmith Campus,
Commonwealth Building, 9th floor,
Du Cane Road,
London W12 0NN, United Kingdom.
E-mail: matthew.pickering@imperial.ac.uk

Received on May 1, 2025; accepted in revised form on September 10, 2025.

Funding: the authors acknowledge the funding from Chang Gung Memorial Hospital Research Program (CMRPG3N0761). This research was supported by the National Institute for Health and Care Research (NIHR) Imperial Biomedical Research Centre and by a Medical Research Foundation Fellowship to J.E. Peters (MRF-057-0003-RG-PETE-C0799). The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care.

Competing interests: none declared.

Background

Systemic lupus erythematosus (SLE) is a complex multifactorial autoimmune disease with diverse organ involvement including the skin, kidneys and nervous system. Manifestations range from mild to severe symptoms and the disease frequently follows a relapsing-remitting course (1, 2). The immunopathogenesis of SLE involves multiple immune pathways including B cell hyper-reactivity, elevated type I interferons (IFN) with increased expression of IFN-responsive genes (collectively referred to as the IFN signature) and autoantibodies directed against self-antigens (3).

The IFN protein family comprises type I IFN (consisting of 13 IFNα subtypes, IFNβ, IFNκ, IFNω, and IFNε); type II IFN (IFNγ); and type III IFN (consisting of IFN-λ1 [IL29], IFN-λ2 [IL28A], IFN-λ3 [IL28B] and IFN-λ4) (4). Elevated type I IFN proteins (5) together with a type I IFN gene signature (6) is a well-established feature of SLE and present in 60–80% of patients. Patients with high IFN gene signatures have more severe disease and a higher incidence of antibodies to Sm, Ro and RNP (7-11). Notably, the IFN gene signature is remarkably stable over time (9-11). In the largest longitudinal study to date, only very high dose glucocorticoids were associated with a reduction in the IFN gene signature with doses of <25 mg/day having no effect (10). Consequently, serial IFN gene signature measurement is not useful for assessing or monitoring disease activity. However, it has been suggested that a single baseline measurement can aid with prognosis and management, particularly since patients with high IFN gene signature achieve less time in lupus low disease activity state (10). There is also evidence that IFN activity is heritable as indicated by genetic studies (12-14) and studies showing higher activity in healthy relatives compared to controls (15).

Due to their low abundance the measurement of IFN proteins in the circulation is technically challenging. Consequently, most cohort studies in SLE have measured IFN activity indirectly, most commonly using the whole blood type I IFN gene signature or using cell reporter assays that can distinguish the effector functions driven by type I, II and III IFN groups but not the individual IFNs. However, using high-sensitivity protein assays, raised serum levels of IFNα were detected in SLE patients and associated with disease severity, flares and autoantibody levels (16-18). Levels of the type II IFNγ (18, 19) and the type III IFN-λ1 proteins (18) were also found increased in SLE with IFNγ associating with active disease (18, 19). Anifrolumab, a blocking antibody against the type I IFN receptor, as expected, reduced the type I IFN gene signature, but, interestingly, levels of the type III IFN protein, IFN-λ1 were also reduced (20). Some studies also found that different interferon profiles associate with specific disease manifestations (18, 21). For example, high IFNγ levels associated with arthritis, and high IFNα levels associated with mucocutaneous disease (18). Whilst high type I IFN activity associates with disease activity in many domains including nephritis and arthritis (18), a recent report defining IFN I, II and III activity using a different cell reporter assay found that skin involvement associated with type I IFN activity alone, whereas nephritis correlated with increased activity across all three IFN groups (21).

We utilised the nucleic-acid linked immuno-sandwich assay (NULISA) platform which has attomolar sensitivity (22) to determine the relative levels of four type I IFN proteins (IFNα1:IFNα13, IFNα2, IFNβ, IFNω); two type III IFN proteins (IFNλ1, IFNλ2:IFNλ3) and the type II IFN, IFNγ. We then examined the relationship between the IFN proteins, disease phenotype and the whole blood IFN gene score.

Methods

Study design

272 SLE patients were recruited from the Imperial Lupus Centre, Imperial College Healthcare NHS Trust Hammersmith Hospital in the United Kingdom between 2019 and 2024 together with n=39 healthy controls (Supplementary Fig. S1). All patients fulfilled the 1997 American College of Rheumatology (ACR) revised criteria (23) or the 2012 Systemic Lupus International Collaborating Clinics (SLICC) clas-

sification criteria (24) for SLE. Plasma samples were separated within 3 hours of blood collection and stored at -70°C. Whole blood was also collected at the same time in Tempus Blood RNA Tubes (Thermo Fisher Scientific) and stored at -70°C until RNA extraction for ISG score measurement. Anti-dsDNA titres were normalised relative to the laboratory reference thresholds to account for changes in the assays used over time. Proteinuria was defined as a urine protein-to-creatinine ratio (uPCR) >55 mg/mmol. Human samples used in this research project were obtained from the Imperial College Healthcare Tissue and Biobank (ICHTB). ICHTB is supported by the National Institute for Health Research Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London. ICHTB is approved by Wales REC3 to release human material for research (22/WA/0214). Ethical approval was provided by ICHTB (Human Tissue Authority Licensing number 12275; Project number R14042-3A; sub-collection IMM_MB_13_001) and informed consent obtained from all participants.

Whole blood IFN gene signature

Total RNA was isolated from whole blood samples using Tempus RNA isolation kits (Thermo Fisher Scientific) following the manufacturer's protocol. RNA quality and concentration were assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific) and samples with an A260/A280 ratio of 1.9–2 were carried forward for analysis. All RNA samples were stored at -70°C. RNA from each sample was reverse transcribed to cDNA using the iScript cDNA synthesis kit (Bio-Rad) with a final volume of 20 µl in a reaction comprising 25°C for 5 minutes, 42°C for 30 minutes, 85°C for 5 minutes and then held at 4°C until retrieval. The resulting cDNA was diluted with nuclease-free water to a final concentration of 2.5 ng/µl. Real-time qPCR reactions were conducted using Power SYBRTM Green Master Mix (Applied Biosystems) prepared according to the manufacturer's instructions. For each condition, 2 µl of cDNA and 8 µl of master mix were added to an optimal 384-well plate (Life Technologies). Gene expression was quantified utilising the ViiA 7 system (Life Technologies), and relative gene expression of *CMPK2*, *EPSTI1* and *HERC5* were calculated by subtracting the housekeeping gene *HPRT* using the ΔCt method (25). The ISG score is from the calculation of average of ΔCt from *CMPK2*, *EPSTI1* and *HERC5* genes *(-1). The ISG score status was defined as IFN-neg (ISG score ≤1), IFN-low (1 <ISG score ≤2) and IFN-high (ISG score >2) (25).

Disease activity groupings

We categorised SLE disease activity at the time of blood sampling into four groups on an ordinal scale: in remission, defined by the Definition of Remission in SLE (DORIS) (26); low disease activity, defined by the Lupus Low Disease Activity State (LLDAS) criteria (27); moderate disease activity (MDA), defined as SLEDAI-2K score between 4 and 9 ; and high disease activity (HDA), defined by a SLEDAI-2K score ≥10. In the LLDAS category, patients with chronic stable proteinuria due to glomerular damage and not active lupus nephritis were included. Clinical manifestations in the MDA and HDA groups are shown in Supplementary Table S1. The definition of disease flare was a clinical increase in disease activity in one or more organ systems as determined by the clinician.

Nucleic acid Linked Immuno-Sandwich Assay (NULISA)

Plasma samples were analysed using the NULISAseq Inflammation Panel 250 (Alamar Biosciences) (28). After normalisation using internal and inter-plate controls, and $\log_2$ transformation, the resulting values were designated as NULISA Protein Quantification (NPQ) units as the assay did not provide absolute quantification at this time. In addition to NULISA-specific control metrics, additional quality control (QC) was performed using principal component analysis (PCA), and relative log expression measures (Suppl. Fig. S2). Six proteins and eight plasma samples were excluded after failing QC analysis or, in the case of proteins, because of a high proportion of samples with values below lower limit of detection (Suppl. Fig. S2).

Statistical methods

The statistical analysis and graphical representations were conducted using GraphPad Prism software (v. 9.5.1, GraphPad Software, Inc., USA, 2023) and R software (v. 4.4.2, R Core Team, 2024). For the clinical variables, ISG score and protein measurements, differences were assessed using the Wilcoxon test or one-way ANOVA for continuous variables and the Chi-squared test for categorical variables. The differential abundance analysis was done by estimating the association of group status with each protein using linear models (lm function in R) *i.e.* NPQ ~ Group. *p*-values were adjusted for multiple comparisons using the Benjamini-Hochberg (BH) method. A BH-adjusted *p*-value <0.05 was used to define statistical significance. Since NPQ values are relative units on a $\log_2$ scale, the model estimates for the group coefficient correspond to the $\log_2$ fold change between cases and controls. To assess for proteomic associations with disease activity, we used a linear model to regress abundance of each protein on disease activity category, encoded as an ordinal variable according to increasing disease activity as follows: DORIS > LLDAS > MDA > HDA. This model returns a linear gradient that represents the relationship of each protein with increasing disease activity. Spearman correlations were used in correlation plots and correlograms. We used the 95th percentile of healthy controls for IFNα1–IFNα13, IFNγ, and IFNλ1 to classify SLE patients into different IFN groups. To explore the association of a particular IFN profile (*e.g.* high type I+III) with clinical or serological variables we calculated the odds ratio using logistic regression with the glm function in R comparing each IFN profile with all other profiles. A forest plot was used to display the odds ratios, 95% confidence intervals, and BH-adjusted *p*-values.

Results

Lupus cohort

The demographic and clinical details of the cohort are listed in Table I. The 266 patients with samples passing QC were predominantly female (89.5%), dsDNA antibody positive (63.5%),

Table I. Demographic and clinical data of single sample study cohort.

		Systemic lupus erythematosus				
	HC	**DORIS**	**LLDAS**	**MDA**	**HDA**	***p*-value**
n=305	39	129	38	88	11	
Female, n (%)	32 (82)	117 (91)	31 (82)	79 (90)	11 (100)	0.2591
Age, median [range]	33 [22,49]	44 [22,77]	47 [26,81]	39 [19,77]	33 [27,50]	0.0036**
Ethnicity, n (%)						
White	25 (64)	31 (24)	10 (26)	21 (24)	3 (27)	0.8544
Asian	11 (28)	34 (26)	7 (18)	24 (27)	3 (27)	
Black	2 (5)	32 (25)	9 (24)	26 (30)	4 (37)	
Other	1 (3)	32 (25)	12 (32)	17 (19)	1 (9)	
Autoantibody status, n (%)						
Anti-dsDNA +		68 (53)	26 (68)	65 (74)	10 (91)	0.0023**
Anti-Ro-60 and/or Ro-52 and/or La +		64 (50)	16 (42)	43 (49)	8 (73)	0.0725
Anti-Sm +		31 (24)	8 (21)	29 (33)	5 (45)	0.1978
Anti-RNP +		41 (32)	11 (29)	32 (36)	6 (54)	0.3905
Antiphospholipid antibodies +		12 (9)	2 (5)	7 (8)	1 (9)	0.8836
Laboratory features at sampling						
Elevated dsDNA (n, %)		45 (35)	17 (45)	48 (55)	10 (91)	0.0005***
dsDNA titre (iu /ml), median [range]		0.4 [0,42.4]	0.8 [0,23.9]	1.3 [0,1956]	6.7 [0.8,125]	0.1997
Low C3 (n, %)		13 (10)	4 (11)	20 (23)	7 (64)	<0.0001****
C3 level (g/L), median [range]		1.06 [0.35,2.04]	1.10 [0.57,2.07]	0.95 [0.38,2.04]	0.59 [0.38,1.04]	<0.0001****
Low C4 (n, %)		30 (23)	5 (13)	31 (35)	10 (91)	<0.0001****
C4 level (g/L), median [range]		0.21 [0,0.54]	0.25 [0.03,0.58]	0.19 [0.04,0.6]	0.1 [0.05,0.16]	<0.0001****
uPCR (mg/mmol), median [range]		18 [0,88]	95 [7,362]	63 [0,1286]	79 [0,306]	<0.0001****
Albumin (g/L), median [range]		41 [34,46]	39 [22,43]	38 [14,47]	36 [30,41]	<0.0001****
Creatinine (umol/L), median [range]		66 [47,180]	77 [43,393]	68 [40,595]	59 [49,284]	0.0020**
Lupus nephritis ever, n (%)		75 (58)	27 (71)	63 (72)	5 (45)	0.0866
ISG score available, n		120	34	84	11	
IFN-high, n (%)		72 (60)	21 (62)	57 (68)	10 (91)	0.4495
IFN-low, n (%)		17 (14)	4 (12)	7 (8)	0 (0)	
IFN-neg, n (%)		31 (26)	9 (26)	20 (24)	1 (9)	
Treatment at time of sampling, n (%)						
Hydroxychloroquine		95 (74)	21 (55)	62 (70)	6 (55)	0.1203
Prednisolone		26 (20)	17 (45)	51 (58)	5 (45)	<0.0001****
Prednisolone dose (mg/day), median [range]		4.5 [1,5]	3 [1,7.5]	10 [2.5,40]	10 [5,20]	<0.0001****
Mycophenolate mofetil		43 (33)	20 (53)	44 (50)	3 (27)	0.0289*
Azathioprine		13 (10)	4 (11)	12 (14)	0 (0)	0.5481
Immunosuppressant other		8 (6)	2 (5)	5 (6)	2 (18)	0.4371
Cyclophosphamide now		0 (0)	0 (0)	4 (5)	1 (9)	0.0214*
Cyclophosphamide ever		23 (18)	12 (32)	28 (32)	3 (27)	0.0837
Belimumab now		6 (5)	1 (3)	7 (8)	2 (18)	0.1985
Belimumab ever		6 (5)	1 (3)	12 (14)	5 (45)	<0.0001****
Anti-CD20 now		1 (1)	3 (8)	23 (26)	4 (36)	<0.0001****
Anti-CD20 ever		57 (44)	28 (74)	58 (66)	7 (64)	0.0012**
B cell count (cells/ul)**,** median [range]		197 [2,2012]	94 [2,1139]	67 [1,1533]	169 [3,903]	0.0058**

HC: healthy control; DORIS: definition of remission in SLE; LLDAS: lupus low disease activity state; MDA: mild disease activity; HDA: high disease activity. dsDNA titre is normalised relative to laboratory reference threshold due to a change in measurement method. Elevated dsDNA is a dsDNA titre greater than laboratory normal range threshold. Low C3 is <0.7 g/L, Low C4 is <0.16 g/L. Albumin normal range is 35–50 g/L, Creatinine normal range is 55–110 umol/L. A uPCR >55 mg/mmol is considered as proteinuria. Other immunosuppressants include methotrexate and tacrolimus. Note that no patients were treated with anifrolumab. Cyclophosphamide now includes patients undergoing cyclophosphamide treatment within the last 12 weeks before sampling. Anti-CD20 now includes patients who have had a cycle of rituximab or ofatumumab within the last 6 months before sampling. B cell count normal range is 100–500 cells/ul. Testing for differences was performed by one-way ANOVA for continuous variables and Chi-squared test for categorical variables.

and had a history of lupus nephritis (63.9%). The median age of the cohort was 42 years (range 19–81 years). The most frequently prescribed drugs at the time of sampling were hydroxychloroquine (69.2%); mycophenolate mofetil (MMF, 41.3%) and prednisolone (37.2%). Patients stratified by disease activity at the time of the collection comprised: DORIS (n=129, 48.5%), LLDAS (n=38, 14.3%), MDA (n=88, 33.1%) and HDA (n=11, 4.1%) (Table I). As expected hypocomplementaemia and elevated dsDNA antibody titres were more frequent in the MDA and HDA groups. As previously reported the majority of SLE patients displayed a high ISG score irrespective of the disease activity with HDA group reaching 91% (Table I). Of note, the median ISG score was significantly higher in the HDA compared to the DORIS group (Fig. 1A) but it did not markedly differ between the other disease activity groups (Fig. 1A) confirming that the IFN signature is not a reliable biomarker of disease activity.

Plasma IFN levels in SLE patients and correlation with the whole blood ISG score

We performed differential abundance analysis comparing the 266 SLE sam-

ples with 39 healthy control samples. Of the 244 proteins analysed, 157 were significantly increased in the SLE patients and 3 were significantly reduced (Fig. 1B). All seven IFN proteins measured in the NULISAseq Inflammation Panel were among the significantly increased proteins. Notably, the three proteins with the largest effect sizes (log_2 fold changes >3) were all type 1 IFN proteins (IFNα1:IFNα13, IFNα2 and IFNω) (Fig. 1B). The other type 1 IFN protein measured, IFNβ, had a much smaller log_2 fold change. Importantly, the levels of IFNα1:IFNα13, IFNα2 and IFNω strongly correlated (ρ=0.74, 0.72 and 0.73 respectively) with the whole blood ISG score, whereas IFNβ correlated only weakly (ρ=0.38) (Fig. 2). For the type III IFNs (IFNλ1 and IFNλ2:IFNλ3), correlation with the ISG score was intermediate (ρ=0.57 and 0.48 respectively) and weak for IFNγ (ρ=0.40) (Fig. 2). We next explored the extent to which variation in each IFN protein's level explained the variance in ISG score. For IFNα1:IFNα13, IFNα2 and IFNω the variance explained (R^2) was 0.58, 0.51 and 0.53, respectively. In contrast, for IFNλ1, IFNλ2:IFNλ3, IFNβ and IFNγ, R^2 was 0.14, 0.17, 0.11 and 0.14 respectively. Thus, the largest contribution to the variance in ISG score was from the IFNα1:IFNα13, IFNα2 and IFNω proteins.

We next investigated the correlation of each IFN protein with all other proteins measured in the panel and identified 22 proteins that had a Spearman correlation coefficient of ≥0.5 with at least one IFN protein (Suppl. Fig. S3). The correlation was again strongest for the type I IFN proteins: IFNα1:IFNα13, IFNα2, IFNω and the type III proteins, but notably weaker for both IFNβ and IFNγ. As expected, these 22 proteins included the IFN-induced protein BST2, chemokines CXCL9 and CXCL10 which are known to be induced by IFNγ, and CXCL11 known to be induced by IFNβ (29, 30). IFNγ correlated most strongly with CXCL10 (ρ=0.63) and IFNβ correlated most strongly with CXCL11 (ρ=0.56) of all the proteins measured in the panel. Notably, all six of the proteins (BST2, CXCL10, LAG3, CD80, GRN, LGALS9) with ρ≥0.6 with type I IFN have been shown to be downregulated on anifrolumab treatment (20).

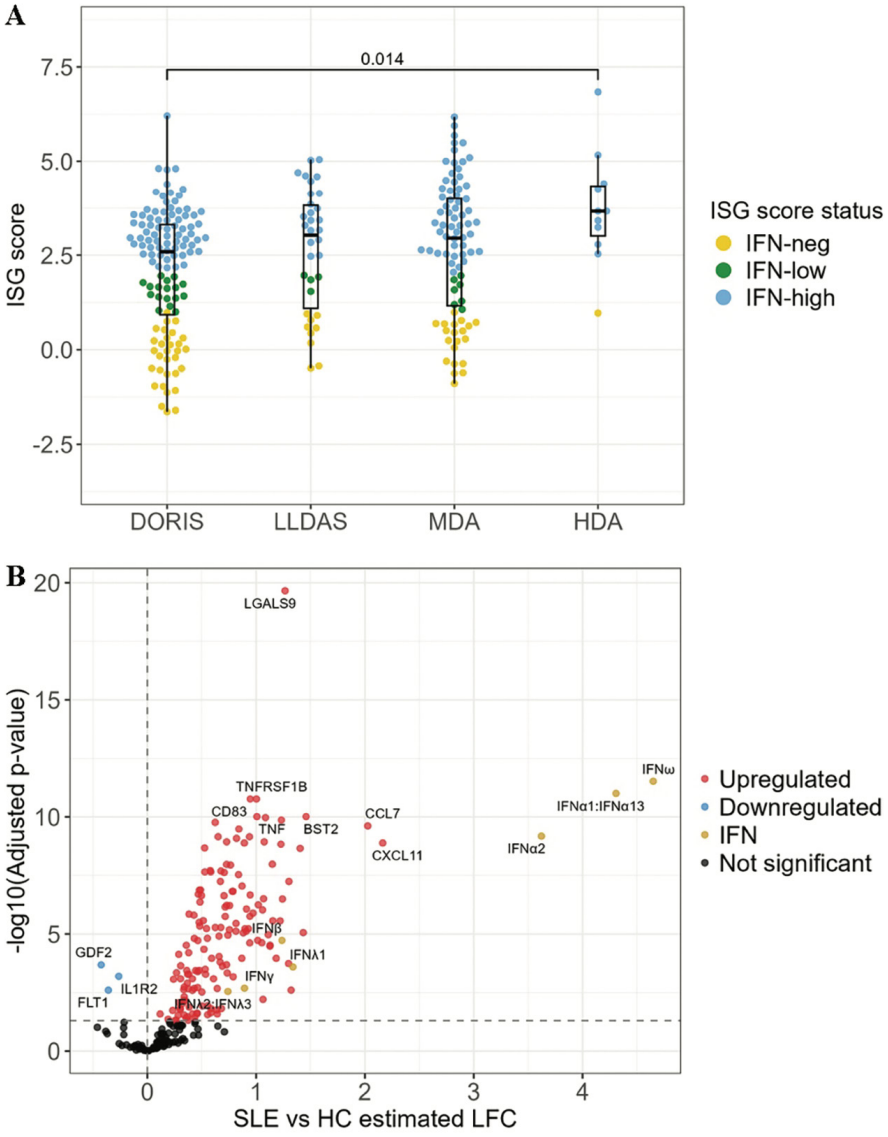

Fig. 1. Whole blood IFN gene signature and IFN proteins.
A: Type I IFN stimulated gene (ISG) score across SLE disease activity groups. ISG score status defined as IFN-neg (ISG ≤ 1), IFN-low (1 < ISG ≤2), IFN-high (ISG >2). One-way ANOVA with Tukey's *post-hoc* test, only significant differences (p<0.05) shown. Boxplots represent median and IQR.
B: Volcano plot showing differentially abundant proteins in SLE *vs*. healthy controls (HC). Differential protein abundance analysis in SLE patients (n=266) *vs*. healthy controls (n=39). -log10 (Adjusted p-value) = $-\log_{10}$ Benjamini-Hochberg adjusted p-value. LFC = $\log_2$ fold-change. Yellow highlights IFN proteins, red and blue indicate proteins that are significantly (P_{adj} <0.05) upregulated and downregulated, respectively.

IFN protein levels across disease activity groups

We then examined the relationship between plasma IFN protein levels and disease activity. The median levels of three of the type I IFNs (IFNα1:IFNα13, IFNα2 and IFNω) and the type III IFN proteins (IFNλ1, IFNλ2:IFNλ3) progressively increased with disease activity (Fig. 3). For example, levels of IFNα1:IFNα13, IFNα2 and IFNω were significantly higher in the HDA group compared to patients in either the LLDAS or DORIS groups. In contrast, IFNβ and IFNγ levels did not differ between the groups. To further investigate the association of these proteins with disease activity, an ordinal linear model was used to account for the four groups with increasing disease activity. This revealed that IFNα1: IFNα13, IFNα2 and IFNω,

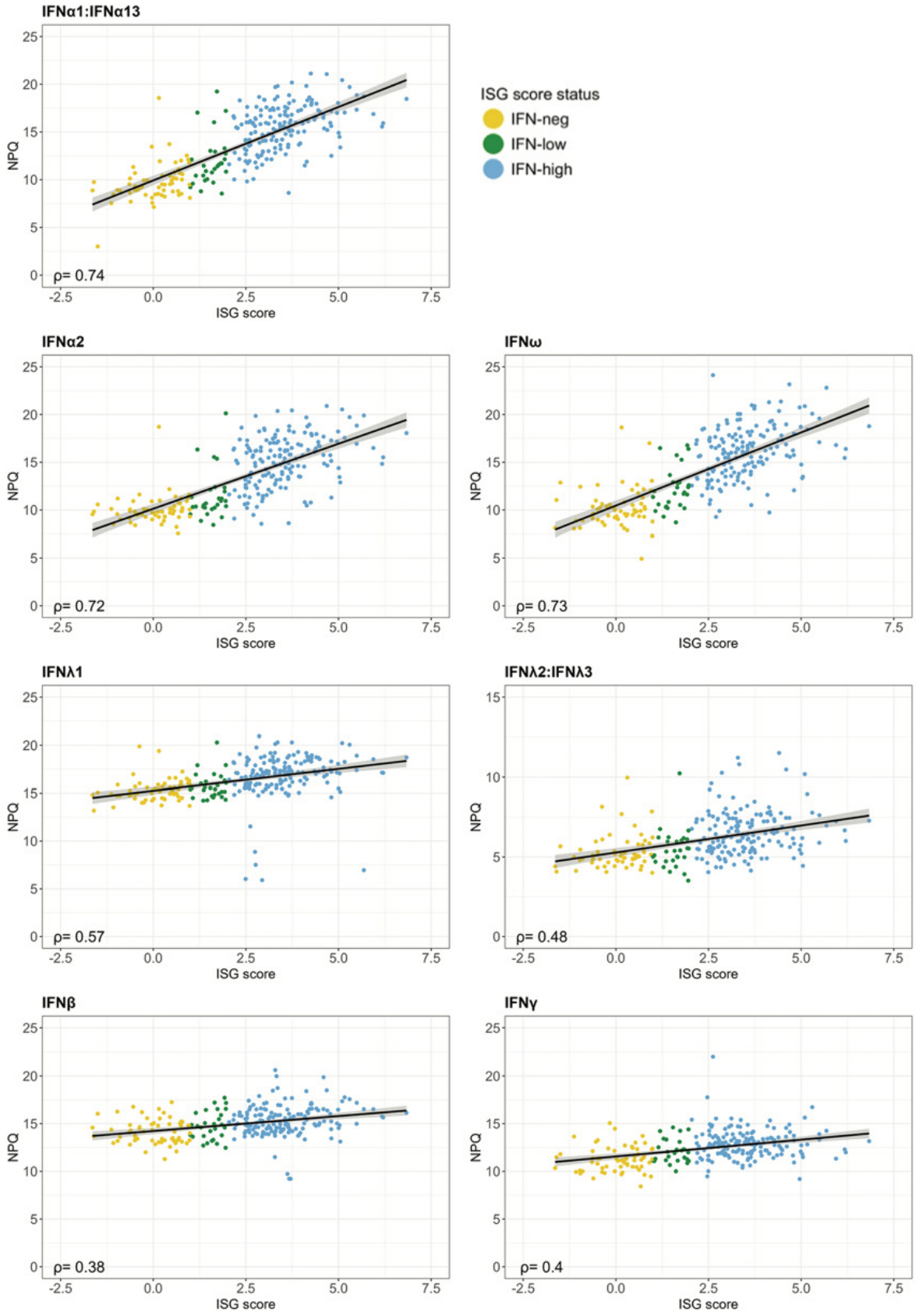

Fig. 2. Correlation of IFN proteins with ISG score.
Data points coloured by ISG score status, defined as IFN-neg (ISG ≤ 1), IFN-low (1 < ISG ≤ 2), IFN-high (ISG > 2). NPQ = NULISA Protein Quantification. Type I IFNs (IFNα1:IFNα13, IFNα2, IFNω, IFNβ), Type II IFN (IFNγ), Type III IFNs (IFNλ1, IFNλ2:IFNλ3). Line with shading represents linear regression with 95% confidence interval. ρ=Spearman correlation coefficient.

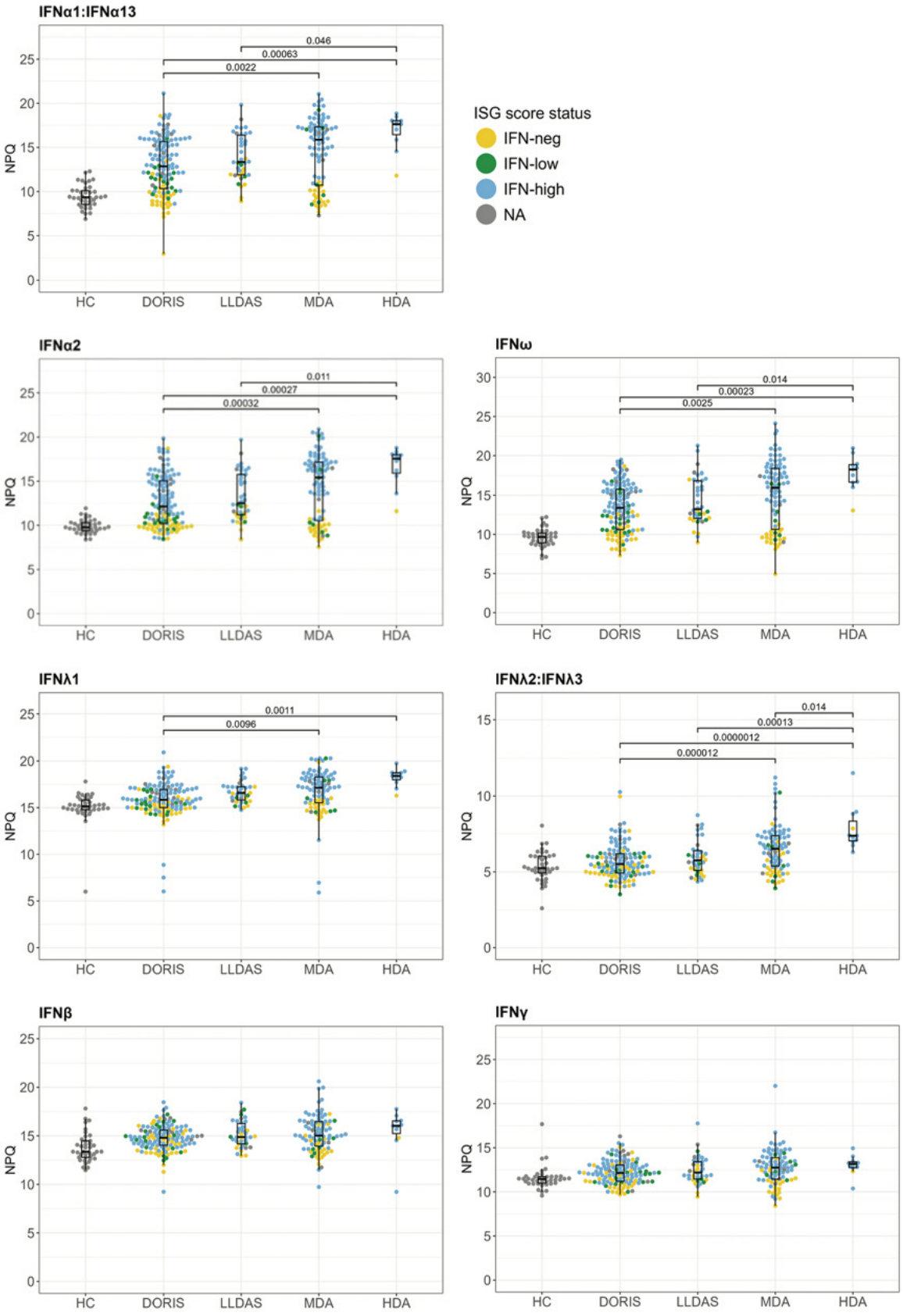

Fig. 3. Circulating IFN protein levels across SLE disease activity groups.
Data points coloured by ISG score status, defined as IFN-neg (ISG ≤1), IFN-low (1 < ISG ≤2), IFN-high (ISG >2). NPQ = NULISA Protein Quantification. Type I IFNs (IFNα1:IFNα13, IFNα2, IFNω, IFNβ), Type II IFN (IFNγ), Type III IFNs (IFNλ1, IFNλ2:IFNλ3). One-way ANOVA with Tukey's *post-hoc* test, only significant differences ($p<0.05$) shown, excluding differences between HC and disease activity groups. Boxplots represent median and IQR.

and the type III IFN proteins IFNλ1, IFNλ2:IFNλ3 were significantly associated with disease activity while IFNβ and IFNγ were not (Suppl. Fig. S4).

Correlation of IFN levels with serological markers of SLE

We then explored the correlation between plasma IFN protein levels and complement C3 and C4 levels and anti-dsDNA titre, which are widely utilised serological markers of disease activity (31) (Fig. 4). All IFN proteins measured and the ISG score positively correlated with anti-dsDNA antibody levels and negatively correlated with complement levels but, notably, the correlation for IFNβ and IFNγ was weak (Fig. 4). Strong positive correlations within the type I IFN family were observed between IFNα1:IFNα13, IFNα2 and IFNω but not IFNβ (Fig. 4). The type I IFN signature and particularly IFNα activity has been associated with anti-Sm, anti-RNP and anti-La antibodies (18, 32). Consistent with these observations, we found significantly higher IFN levels in patients with anti-Sm, anti-Ro and anti-RNP antibodies (Suppl. Fig. S5) corroborating the interferogenic nature of antibodies against extractable nuclear antigens (ENA) (8, 33).

IFN profiles and SLE disease activity

Next, we examined the relationship between clinical features and IFN profiles since these have been reported to provide additional information (18, 21) (Fig. 5). We used the 95th percentile of levels in controls as the upper limit of normal for each protein. We first checked the correlations between the three type I IFN proteins (IFNα1:IFNα13, IFNα2 and IFNω) and between the two type III IFN proteins (IFNλ1, IFNλ2:IFNλ3). Since the intra-class correlations were very strong, we selected IFNα1:IFNα13 and IFNλ1 to determine type I and III status (high or normal) respectively (Suppl. Fig. S6). Using this approach 72%, 18% and 49% of samples had elevated levels of type I, II and III interferons respectively (Fig. 5A). In our cohort the IFN profiles from most frequent to least were: high type I+III (35%, n=93); normal levels of all IFN proteins (25%, n=67); high type I only (21%, n=56); and high type I+II+III (13%, n=34). No patients had high II+III and the remaining combinations were very rare: high I+II (3%, n=8); high II (2%, n=5); and high III (1%, n=3) (Fig. 5B). In comparison to the other groups, the high type I+II+III group had an increased odds ratio of high dsDNA and low complement levels at the time of sampling (Fig. 5C) or ever (Suppl. Fig. S7). This group also had an increased odds ratio of lupus nephritis within 2-years (Fig. 5C).

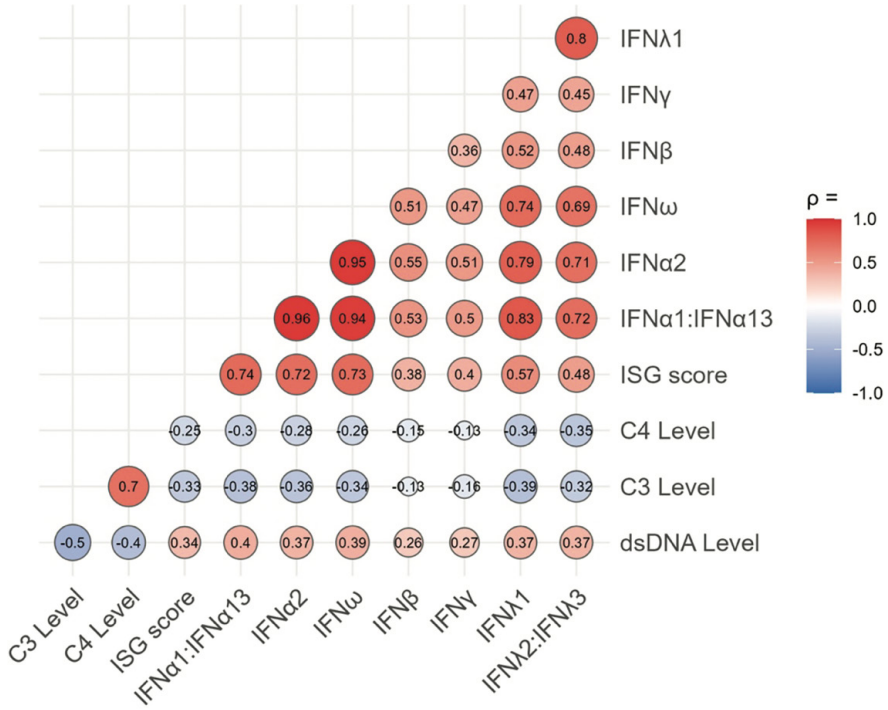

Fig. 4. Correlations of IFN proteins and ISG score with complement levels and normalised dsDNA titre. dsDNA level is the dsDNA titre normalised relative to laboratory reference threshold due to changes in the assays used. Circles contain Spearman correlation coefficients; all were significant $p<0.001$.

Interferon proteins and flare rates over time

Patients in remission or with low disease activity that have elevated IFNα1 have been reported to be at higher risk of disease flares (34). In our cohort, within the 12 months following blood sampling, there were 11 (6.6%) flares in patients meeting DORIS (n=129) or LLDAS (n=38) criteria at the time of recruitment. One-year flare rates did not differ according to the baseline sampling ISG status (negative *vs.* low/high), anti-dsDNA antibody status, complement levels or whether the individual measured interferon proteins were above those seen in the healthy controls (Table II). The time-to-flare was shorter in patients with high IFNλ2:IFNλ3 levels compared to those with low levels (4.3 *vs.* 8.6 months, $p=0.035$).

We had longitudinal sampling for two patients with active disease treated with therapies targeting B cells (Fig. 6). In one patient (P1) the levels of type I IFN proteins (IFNα1:IFNα13, IFNα2 and IFNω) did not change despite fluctuations in SLEDAI score, dsDNA antibody titre and C3 levels (Fig. 6). In another patient (P2), the level of type I IFN proteins decreased after treatment matching improvements in SLEDAI score and C3 level but did not reach the levels seen in healthy controls (Fig. 6). Further data is needed to understand the temporal relationship between plasma IFN levels and disease activity.

Discussion

Our data showed that the type I IFNs, IFNα1:IFNα13, IFNα2 and IFNω, and the type III interferons, IFNλ1 and IFNλ2:IFNλ3, displayed strong association with SLE disease activity, whole blood ISG score, complement levels and autoantibodies. In contrast this was not the case for IFNβ or for IFNγ. IFNα has long been noted to be elevated in SLE

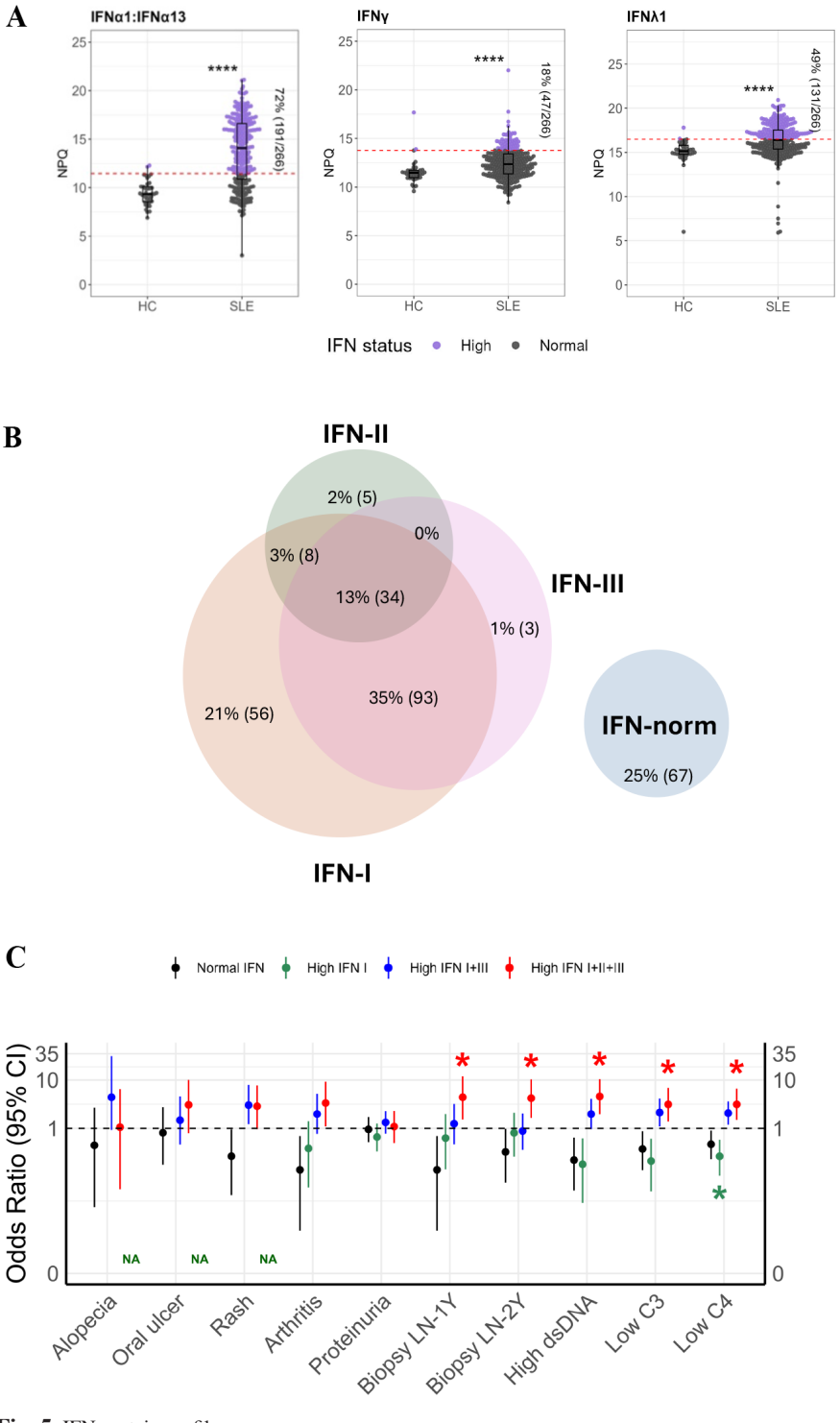

Fig. 5. IFN protein profiles.
A: IFNα1:IFNα13, IFNγ and IFNλ1 levels in SLE and healthy controls. Measurements are annotated as either high (purple) or normal (grey) based on the 95th percentile in healthy controls (11.45, 13.78 and 16.49 for IFNα1:IFNα13, IFNγ and IFNλ1 respectively). Percentage of high values in the SLE groups with numbers are shown. *p*-value derived from Mann-Whitney test.
B: Venn diagram showing the IFN combinations across the cohort.
C: Relationship between clinical manifestations at the time of sampling and the four most frequent IFN profiles high type I+III (35%, n=93, blue); normal levels of all IFN proteins (25%, n=67, black); high type I only (21%, n=56, green); and high type I+II+III (13%, n=34, red). Odds ratio with 95% confidence interval (CI) using these four groups. Asterisks denote significance using BH-adjusted *p*-value <0.05. Biopsy LN-1Y, Biopsy LN-2Y - kidney biopsy for suspected lupus nephritis within 1-year and 2-years of sampling respectively. NA – not applicable since these features (alopecia, oral ulcers, rash) were not present in the high IFN I group.

(reviewed in [35]) and IFNα1:IFNα13 and IFNα2 were markedly increased in our study. Using quantitative assays, the abnormal serum IFNα level threshold value was 136 fg/ml (derived from healthy control samples), whilst the threshold associated with active disease was 266 fg/ml (17). Plasma IFNβ was increased in a subset of patients with aggregates of mitochondrial antiviral signalling protein in PBMCs and, as we found, did not correlate with disease activity (36). IFNβ influences the production of IFNk from keratinocytes in response to ultraviolet B light suggesting a role in cutaneous lupus (37). Plasma IFNω levels were increased in our study and TLR7 stimulation in plasmacytoid dendritic cells *in vitro* results in upregulation of IFNω in addition to IFNα and IFNβ (38). However, the role of IFNω in SLE remains unclear.

The roles of the type III interferons (IFN-λ1, IFN-λ2, IFN-λ3 and IFN-λ4) and the type II IFN, IFNγ, in SLE remain unclear. In longitudinal studies type II IFN dysregulation appears to precede elevated IFNα in SLE (39). Consistent with our findings, plasma IFNλ1 correlates with disease activity, anti-dsDNA autoantibodies and glomerulonephritis (40). Arthritis and nephritis are associated with increased type I+II+III activity rather than type I activity alone, suggesting a synergistic effect between IFN proteins (40). Puzzlingly, in longitudinal measurements of SLE patients treated with anifrolumab, which blocks the type I IFN receptor, levels of IFN-λ1 significantly decreased compared to placebo whereas IFNα1:IFNα13 levels did not change (20), suggesting some cross-talk between the signalling pathways mediated by these IFNs. In an SLE cohort (SPARE), elevation of only type I IFN was associated with skin disease (rash and alopecia); systemic disease with elevation of all three IFN types and no clinical activity was associated with elevation of type II+III IFN (21). We did not reproduce these findings using groupings defined by protein measurement. The sub-groupings were different, most notably 25% of the SPARE cohort had high II+III IFN, whereas none of our patients did. However, in

Table II. Flare-up rates and time-to-flare up in SLE patients with DORIS and LLDAS after one-year follow-up.

ISG score	**ISG score low/high** (n=114)	**ISG score negative** (n=40)	***p*-value**
Flare-up rate, (%)	8 (7.0)	2 (5.0)	0.785
Time-to-flare up (months), mean	6.6	10	0.231
IFN proteins	**High IFNα1:IFNα13** (n=139)	**Low IFNα1:IFNα13** (n=27)	
Flare-up rate, (%)	9 (6.5)	2 (7.4)	0.957
Time-to-flare up (months), mean	6.3	10	0.188
	High IFNα2 (n=133)	**Low IFNα2** (n=34)	
Flare-up rate, (%)	8 (6)	3 (8.8)	0.841
Time-to-flare up (months), mean	6.5	8.3	0.407
	High IFNω (n=144)	**Low IFNω** (n=23)	
Flare-up rate, (%)	9 (6.3)	2 (8.7)	0.612
Time-to-flare up (months), mean	6.3	10	0.188
	High IFNβ (n=112)	**Low IFNβ** (n=55)	
Flare-up rate, (%)	7 (6.3)	4 (7.3)	0.911
Time-to-flare up (months), mean	5.1	10.3	0.055
	High IFNγ (n=91)	**Low IFNγ** (n=76)	
Flare-up rate, (%)	7 (7.7)	4 (5.3)	0.249
Time-to-flare up (months), mean	7.3	6.5	0.773
	High IFNλ1 (n=104)	**Low IFNλ** (n=63)	
Flare-up rate, (%)	7 (6.7)	4 (6.3)	0.702
Time-to-flare up (months), mean	5.7	9.3	0.124
	High IFNλ2:IFNλ3 (n=75)	**Low IFNλ2:IFNλ3** (n=92)	
Flare-up rate, (%)	4 (5.3)	7 (7.6)	0.243
Time-to-flare up (months), mean	4.3	8.6	*0.035
Disease activity markers	**Anti dsDNA Ab positive** (n=62)	**Anti dsDNA Ab negative** (n=105)	
Flare-up rate, (%)	5 (8.1)	6 (5.7)	0.62
Time-to-flare up (months), mean	7.4	6.7	1
	Low C3 (n=17)	**Normal C3** (n=150)	
Flare-up rate, (%)	1 (5.9)	10 (6.7)	0.737
Time-to-flare up (months), mean	3	7.4	0.1481
	Low C4 (n=35)	**Normal C4** (n=132)	
Flare-up rate, (%)	1(2.9)	10 (7.6)	0.289
Time-to-flare up (months), mean	3	7.4	0.1481

This table included a total of 166 SLE patients composed of DORIS (n=129) and LLDAS (n=38). Flare-up is defined as the increase in disease activity in one or more organ systems involving new or worse clinical signs and symptoms and/or laboratory measurements within one year and considered clinically significant by the clinicians. ISG score positive was defined as SLE patients with an ISG score ≥1, while ISG score negative was defined as SLE patients with an ISG score <1.

The cut-off levels for individual interferon protein levels were derived from the upper 95% confidence intervals of 39 healthy controls. These levels were as follows: IFNα1; α13: 9.88, IFNα2: 10.14, IFNω: 9.98, IFNβ: 14.25, IFNγ: 12.00, IFNλ1: 15.60, and IFNλ2; λ3: 5.69 (units: NPQ). Categorical variables were analysed using the Chi-square test, and continuous variables were evaluated using the Wilcoxon test.

both studies patients with high I+II+III (13% in our cohort and 26% in SPARE) had increased serological activity (high dsDNA and low complement) and kidney involvement.

In agreement with previous studies (9-11) more than 60% of the patients in our cohort displayed a high ISG score (IFN-high status) irrespective of the disease activity. In a transcriptomic study of 102 SLE patients, the IFN signature and gene modules related to chromatin structure modification were found to remain consistent across disease activity states further exemplifying persistent activation of the type I IFN pathway (41). These observations also indicate that most treatment modalities do not impact the IFN signature. Only very high doses of glucocorticoids appear able of modulating the IFN signature (10, 42). Blockade of the type I IFN receptor with anifrolumab, as might be expected, reduced the IFN signature but not IFNα protein levels (20). Interestingly, in patients achieving cutaneous remission, anifrolumab normalised only discrete subsets of interferon-stimulated genes (43). Recently, CD19 CAR T cell therapy has also been reported to reduce IFN activity in SLE (44).

Limitations of our study include: (1) it is a single-centre cohort study so we cannot be certain as to the generalisability of our findings; (2) it was a cross-sectional recruitment of patients with wide variation in disease duration, current and previous therapies; (3) we did not have longitudinal measurements of IFN levels across the cohort so cannot make any conclusions on how IFN proteins might change over time either in response to therapy or disease activity; (4) the protein panel did not include all IFN proteins; (5) the protein measurement platform we used did not provide absolute quantification; and (6) whilst a separate cohort study reported that DORIS patients with elevated IFNα levels exhibit a higher flare-up rate and shorter time to flare-up (34) and we could not reproduce this, our flare rate over the observed period was too low to draw any firm conclusions.

In summary, 72%, 18% and 49% of our cohort had elevated levels of type I, II

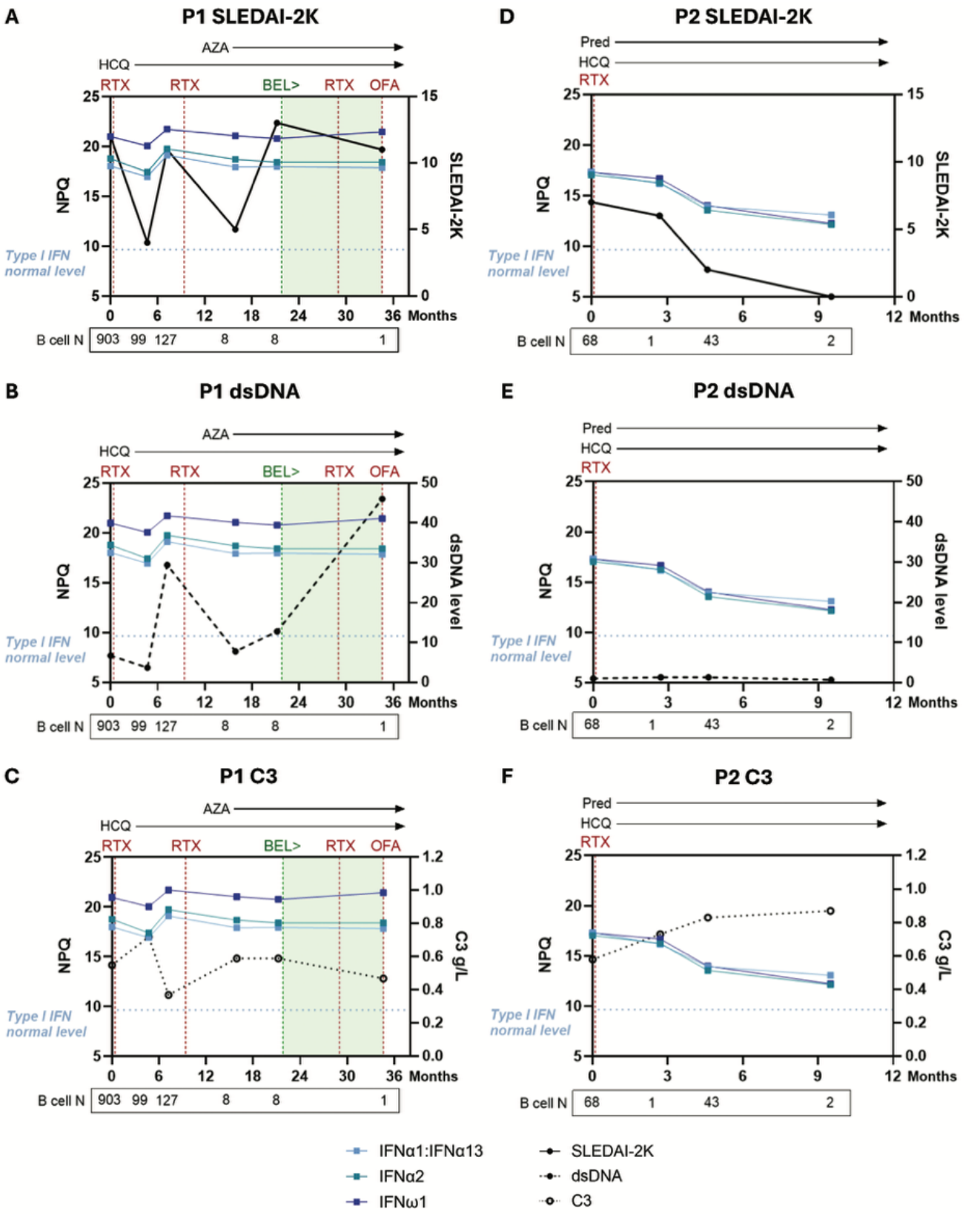

Fig. 6. Longitudinal analysis of two SLE patients treated with therapies targeting B cells. All graphs show levels of the Type I IFNs IFNα1:IFNα13, IFNα2 and IFNω (blue shades) measured by NULISA over time post index sampling, with the Type I IFN normal level indicated by dashed line, calculated as the mean of IFNα1:IFNα13, IFNα2 and IFNω in HC samples. Patient medications are indicated above and B cell number indicated below the graph. Panels **A-C** show data from patient P1, and panels **D-F** show data from patient P2. **(A** and **D)** SLEDAI-2K score (black solid line). **(B** and **E)** dsDNA level (black dashed line) normalised to laboratory threshold value (black dashed line). **(C** and **F)** C3 level (g/L).
HCQ: hydroxychloroquine; AZA: azathioprine; Pred: prednisolone; RTX; rituximab; BEL: belimumab; OFA: ofatumumab.

and III interferons respectively. Type I IFN levels, with the notable exception of IFNβ, correlated with the whole blood ISG score. IFNα1:IFNα13, IFNα2 and IFNω and the type III IFN proteins (IFNλ1, IFNλ2:IFNλ3) were higher in active disease. Further studies are needed to understand how these levels change longitudinally and if this has clinical utility, for example in disease activity monitoring or treatment response. As previously suggested (10), the current clinical relevance of IFN activity measurement in SLE is likely to be in early disease where it can contribute, albeit broadly, to disease prognosis.

Acknowledgements

The authors acknowledge the funding from Chang Gung Memorial Hospital Research Program (CMRPG3N0761). We thank the patients and healthy volunteers who participated in the study and the support of the Community Partners of the Immunology Theme of the National Institute for Health and Care Research (NIHR) Imperial Biomedical Research Centre. We acknowledge the support of the health professionals in the Imperial Lupus Centre, Imperial College Healthcare NHS Trust.

References

1. FAVA A, PETRI M: Systemic lupus erythematosus: Diagnosis and clinical management. *J Autoimmun* 2019; 96: 1-13. https://doi.org/10.1016/j.jaut.2018.11.001
2. SCHILIRO D, SILVAGNI E, CIRIBE B *et al*.: Systemic lupus erythematosus: one year in review 2024. *Clin Exp Rheumatol* 2024; 42: 583-92. https://doi.org/10.55563/clinexprheumatol/mnvmvo
3. ARNAUD L, CHASSET F, MARTIN T: Immunopathogenesis of systemic lupus erythematosus: An update. *Autoimmun Rev* 2024; 23: 103648. https://doi.org/10.1016/j.autrev.2024.103648
4. CAIELLI S, WAN Z, PASCUAL V: Systemic lupus erythematosus pathogenesis: interferon and beyond. *Annu Rev Immunol* 2023; 41: 533-60. https://doi.org/10.1146/annurev-immunol-101921-042422
5. HOOKS JJ, MOUTSOPOULOS HM, GEIS SA, STAHL NI, DECKER JL, NOTKINS AL: Immune interferon in the circulation of patients with autoimmune disease. *N Engl J Med* 1979; 301: 5-8. https://doi.org/10.1056/nejm197907053010102
6. 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 USA* 2003; 100: 2610-15. https://doi.org/10.1073/pnas.0337679100
7. 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. https://doi.org/10.1002/art.22044
8. KIROU KA, LEE C, GEORGE S, LOUCA K, PETERSON MG, CROW MK: 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. https://doi.org/10.1002/art.21031
9. 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: 1440-46. https://doi.org/10.1136/ard.2008.093146
10. NORTHCOTT M, JONES S, KOELMEYER R *et al*.: Type 1 interferon status in systemic lupus erythematosus: a longitudinal analysis. *Lupus Sci Med* 2022; 9. https://doi.org/10.1136/lupus-2021-000625
11. PETRI M, SINGH S, TESFASYONE H *et al*.: Longitudinal expression of type I interferon responsive genes in systemic lupus erythematosus. *Lupus* 2009; 18: 980-89. https://doi.org/10.1177/0961203309105529
12. NIEWOLD TB, KELLY JA, FLESCH MH, ESPINOZA LR, HARLEY JB, CROW MK: Association of the IRF5 risk haplotype with high serum interferon-alpha activity in systemic lupus erythematosus patients. *Arthritis Rheum* 2008; 58: 2481-87. https://doi.org/10.1002/art.23613
13. NIEWOLD TB, KELLY JA, KARIUKI SN *et al*.: IRF5 haplotypes demonstrate diverse serological associations which predict serum interferon alpha activity and explain the majority of the genetic association with systemic lupus erythematosus. *Ann Rheum Dis* 2012; 71: 463-68. https://doi.org/10.1136/annrheumdis-2011-200463
14. PSARRAS A, WITTMANN M, VITAL EM: Emerging concepts of type I interferons in SLE pathogenesis and therapy. *Nat Rev Rheumatol* 2022; 18: 575-90. https://doi.org/10.1038/s41584-022-00826-z
15. NIEWOLD TB, HUA J, LEHMAN TJ, HARLEY JB, CROW MK: High serum IFN-alpha activity is a heritable risk factor for systemic lupus erythematosus. *Genes Immun* 2007; 8: 492-502. https://doi.org/10.1038/sj.gene.6364408
16. 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. https://doi.org/10.1191/096120300674499064
17. 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-alpha. *Arthritis Rheumatol* 2019; 71: 756-65. https://doi.org/10.1002/art.40792
18. 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. https://doi.org/10.1186/s13075-019-1878-y
19. VIALLARD JF, PELLEGRIN JL, RANCHIN V *et al*.: Th1 (IL-2, interferon-gamma (IFN-gamma)) and Th2 (IL-10, IL-4) cytokine production by peripheral blood mononuclear cells (PBMC) from patients with systemic lupus erythematosus (SLE). *Clin Exp Immunol* 1999; 115: 189-95. https://doi.org/10.1046/j.1365-2249.1999.00766.x
20. BAKER T, SHARIFIAN H, NEWCOMBE PJ *et al*.: Type I interferon blockade with anifrolumab in patients with systemic lupus erythematosus modulates key immunopathological pathways in a gene expression and proteomic analysis of two phase 3 trials. *Ann Rheum Dis* 2024; 83: 1018-27. https://doi.org/10.1136/ard-2023-225445
21. GOMEZ-BANUELOS E, GOLDMAN DW, ANDRADE V, DARRAH E, PETRI M, ANDRADE F: Uncoupling interferons and the interferon signature explains clinical and transcriptional subsets in SLE. *Cell Rep Med* 2024; 5: 101569. https://doi.org/10.1016/j.xcrm.2024.101569
22. FENG W, BEER JC, HAO Q *et al*.: NULISA: a proteomic liquid biopsy platform with attomolar sensitivity and high multiplexing. *Nat Commun* 2023; 14:7238. https://doi.org/10.1038/s41467-023-42834-x
23. TAN EM, COHEN AS, FRIES JF *et al*.: The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; 25: 1271-77. https://doi.org/10.1002/art.1780251101
24. PETRI M, ORBAI AM, ALARCON GS *et al*.: Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012; 64: 2677-86. https://doi.org/10.1002/art.34473
25. BUANG N, TAPENG L, GRAY V, SARDINI A, WHILDING C, LIGHTSTONE L *et al*.: Type I interferons affect the metabolic fitness of CD8(+) T cells from patients with systemic lupus erythematosus. *Nat Commun* 2021; 12: 1980. https://doi.org/10.1038/s41467-021-22312-y
26. VAN VOLLENHOVEN RF, BERTSIAS G, DORIA A *et al*.: 2021 DORIS definition of remission in SLE: final recommendations from an international task force. *Lupus Sci Med* 2021; 8. https://doi.org/10.1136/lupus-2021-000538
27. FRANKLYN K, LAU CS, NAVARRA SV *et al*.: Definition and initial validation of a Lupus Low Disease Activity State (LLDAS). *Ann Rheum Dis* 2016; 75: 1615-21. https://doi.org/10.1136/annrheumdis-2015-207726
28. FENG W, BEER JC, HAO Q *et al*.: NULISA: a proteomic liquid biopsy platform with attomolar sensitivity and high multiplexing. *Nat Commun* 2023; 14: 7238. https://doi.org/10.1038/s41467-023-42834-x
29. HAN JH, SUH CH, JUNG JY *et al*.: Elevated circulating levels of the interferon-gamma-induced chemokines are associated with disease activity and cutaneous manifestations in adult-onset Still's disease. *Sci Rep* 2017; 7: 46652. https://doi.org/10.1038/srep46652
30. TOKUNAGA R, ZHANG W, NASEEM M *et al*.:

CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - A target for novel cancer therapy. *Cancer Treat Rev* 2018; 63:40-7. https://doi.org/10.1016/j.ctrv.2017.11.007
31. TOUMA Z, GLADMAN DD, SU J, ANDERSON N, UROWITZ MB: A novel lupus activity index accounting for glucocorticoids: SLE-DAI-2K glucocorticoid index. *Rheumatology* (Oxford) 2018; 57: 1370-76. https://doi.org/10.1093/rheumatology/key103
32. 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. https://doi.org/10.1002/art.30187
33. 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. https://doi.org/10.1016/j.clim.2005.07.009
34. MATHIAN A, MOURIES-MARTIN S, DORGHAM K *et al*.: Ultrasensitive serum interferon-alpha quantification during SLE remission identifies patients at risk for relapse. *Ann Rheum Dis* 2019; 78: 1669-76. https://doi.org/10.1136/annrheumdis-2019-215571
35. SIM TM, ONG SJ, MAK A, TAY SH: Type I interferons in systemic lupus erythematosus: a journey from bench to bedside. *Int J Mol Sci* 2022; 23. https://doi.org/10.3390/ijms23052505
36. SHAO WH, SHU DH, ZHEN Y *et al*.: Prion-like aggregation of mitochondrial antiviral signaling protein in lupus patients is associated with increased levels of type I interferon. *Arthritis Rheumatol* 2016; 68: 2697-707. https://doi.org/10.1002/art.39733
37. XU B, MUSAI J, TAN YS *et al*.: A critical role for IFN-beta signaling for IFN-kappa induction in keratinocytes. *Front Lupus* 2024; 2. https://doi.org/10.3389/flupu.2024.1359714
38. BIRMACHU W, GLEASON RM, BULBULIAN BJ *et al*.: Transcriptional networks in plasmacytoid dendritic cells stimulated with synthetic TLR 7 agonists. *BMC Immunol* 2007; 8: 26. https://doi.org/10.1186/1471-2172-8-26
39. MUNROE ME, LU R, ZHAO YD *et al*.: Altered type II interferon precedes autoantibody accrual and elevated type I interferon activity prior to systemic lupus erythematosus classification. *Ann Rheum Dis* 2016; 75: 2014-21. https://doi.org/10.1136/annrheumdis-2015-208140
40. WU Q, YANG Q, LOURENCO E, SUN H, ZHANG Y: Interferon-lambda1 induces peripheral blood mononuclear cell-derived chemokines secretion in patients with systemic lupus erythematosus: its correlation with disease activity. *Arthritis Res Ther* 2011; 13:R88. https://doi.org/10.1186/ar3363
41. WANG FQ, SHAO L, DANG X *et al*.: Unraveling transcriptomic signatures and dysregulated pathways in systemic lupus erythematosus across disease states. *Arthritis Res Ther* 2024; 26: 99. https://doi.org/10.1186/s13075-024-03327-4
42. BANCHEREAU R, HONG S, CANTAREL B *et al*.: Personalized immunomonitoring uncovers molecular networks that stratify lupus patients. *Cell* 2016; 165: 551-65. https://doi.org/10.1016/j.cell.2016.03.008
43. CARTER LM, WIGSTON Z, LAWS P, VITAL EM: Rapid efficacy of anifrolumab across multiple subtypes of recalcitrant cutaneous lupus erythematosus parallels changes in discrete subsets of blood transcriptomic and cellular biomarkers. *Br J Dermatol* 2023; 189: 210-18. https://doi.org/10.1093/bjd/ljad089
44. WILHELM A, CHAMBERS D, MULLER F *et al*.: Selective CAR T cell-mediated B cell depletion suppresses IFN signature in SLE. *JCI Insight* 2024; 9. https://doi.org/10.1172/jci.insight.179433