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1 **Ultra-sensitive serum interferon- $\alpha$  quantification during SLE remission identifies**  
2 **patients at risk for relapse.**

3 Running title: digital IFN- $\alpha$  predicts lupus flare

4 Alexis Mathian, MD, PhD\*<sup>1</sup> Suzanne Mouries-Martin, MD, MSc\*<sup>2</sup> Karim Dorgham, PhD\*<sup>3</sup>  
5 Hervé Devilliers, MD, PhD<sup>4</sup> Hans Yssel, PhD<sup>3</sup> Laura Garrido Castillo, MSc<sup>3</sup> Fleur Cohen-  
6 Aubart, MD, PhD<sup>1</sup> Julien Haroche, MD, PhD<sup>1</sup> Miguel Hie, MD, MSc<sup>5</sup> Marc Pineton de  
7 Chambrun, MD, MSc<sup>5</sup> Makoto Miyara, MD, PhD<sup>3</sup> Micheline Pha, MD, MSc<sup>5</sup> Flore  
8 Rozenberg, MD, PhD<sup>6</sup> Guy Gorochoy, MD, PhD<sup>†3</sup>, Zahir Amoura, MD, MSc<sup>†1</sup>

9 \*These authors contributed equally to this work.

10 †These authors contributed equally to this work.

11

12 <sup>1</sup>Sorbonne Université, Assistance Publique–Hôpitaux de Paris, Groupement Hospitalier Pitié–  
13 Salpêtrière, French National Referral Center for Systemic Lupus Erythematosus,  
14 Antiphospholipid Antibody Syndrome and Other Autoimmune Disorders, Service de  
15 Médecine Interne 2, Institut E3M, Inserm UMRS, Centre d’Immunologie et des Maladies  
16 Infectieuses (CIMI-Paris), Paris, France ;

17 <sup>2</sup>Centre Hospitalier Universitaire de Dijon, Hôpital François-Mitterrand, service de médecine  
18 interne et maladies systémiques (médecine interne 2), Dijon, France ;

19 <sup>3</sup>Sorbonne Université, Inserm UMRS, Centre d’Immunologie et des Maladies Infectieuses  
20 (CIMI-Paris), Assistance Publique–Hôpitaux de Paris, Groupement Hospitalier Pitié–  
21 Salpêtrière, Département d’Immunologie, Paris, France ;

22 <sup>4</sup>Centre Hospitalier Universitaire de Dijon, Hôpital François-Mitterrand, service de médecine  
23 interne et maladies systémiques (médecine interne 2) et Centre d’Investigation Clinique,  
24 Inserm CIC 1432, Dijon, France ;

25 <sup>5</sup>Assistance Publique–Hôpitaux de Paris, Groupement Hospitalier Pitié–Salpêtrière, French  
26 National Referral Center for Systemic Lupus Erythematosus, Antiphospholipid Antibody  
27 Syndrome and Other Autoimmune Disorders, Service de Médecine Interne 2, Institut E3M,  
28 Paris, France ;

29 <sup>6</sup>Sorbonne Paris Cité, Assistance Publique–Hôpitaux de Paris, Hôpital Cochin, Service de  
30 Virologie, Paris, France

31

32 **Correspondence to** Dr Alexis Mathian, Service de Médecine Interne 2, Institut E3M, Hôpital  
33 de la Pitié–Salpêtrière, 47–83, boulevard de l’Hôpital, 75651 Paris Cedex 13, France.

34 Tel: +33 (0)1 42 17 80 25; Fax: +33 (0)1 42 16 58 04; Email: [alexis.mathian@aphp.fr](mailto:alexis.mathian@aphp.fr)

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2 biomarker; relapse; flare

3

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8

9 **Competing interests** none

10

11 **Patient and Public Involvement** none

12

1 **ABSTRACT**

2 **Objectives** Maintenance of remission has become central in the management of systemic  
3 lupus erythematosus (SLE). The importance of interferon-alpha (IFN- $\alpha$ ) in the pathogenesis  
4 of SLE notwithstanding, its expression in remission has been poorly studied as yet. To study  
5 its expression in remission and its prognostic value in the prediction of a disease relapse,  
6 serum IFN- $\alpha$  levels were determined using an ultrasensitive single-molecule array (Simoa)  
7 digital immunoassay which enables the measurement of cytokines at physiological  
8 concentrations.

9 **Methods** A total of 254 SLE patients in remission, according to the Definition of Remission  
10 in SLE (DORIS) classification, were included in the study. Serum IFN- $\alpha$  concentrations were  
11 determined at baseline and patients were followed-up for one year. Lupus flares were defined  
12 according to the SELENA-SLEDAI Flare Index, whereas the Kaplan-Meier analysis and Cox  
13 regression analysis were used to estimate the time to relapse and to identify baseline factors  
14 associated with time to relapse, respectively.

15 **Results** Of all patients in remission, 26% displayed abnormally high IFN- $\alpha$  serum levels that  
16 were associated with the presence of antibodies specific for RNP, double stranded (ds)DNA  
17 and Ro/SSA60, as well as young age. Importantly, elevated-baseline IFN- $\alpha$  serum levels and  
18 remission duration were associated in an independent fashion, with shorter time to relapse,  
19 while low serum levels of C3 and anti-dsDNA Abs were not.

20 **Conclusion** Direct serum IFN- $\alpha$  assessment with highly sensitive digital immunoassay permit  
21 clinicians to identify a subgroup of SLE patients clinically in remission, but at higher risk of  
22 relapse.

23

1 **Key messages**

- 2 - serum IFN- $\alpha$  levels have been poorly studied in SLE patients in remission so far;
- 3 - the single-molecule array (Simoa) assay is an ultrasensitive assay that quantifies directly
- 4 IFN- $\alpha$  at attomolar concentrations;
- 5 - a quarter of SLE patients in remission display elevated serum IFN- $\alpha$  levels;
- 6 - elevated serum IFN- $\alpha$  levels constitute an independent predictive biomarker of lupus flare;
- 7 - adding serum IFN- $\alpha$  to the routine laboratory assessments in patient in remission could help
- 8 clinicians to identify a subgroup of SLE patients at higher risk of relapse.

9

## 1 INTRODUCTION

2 Systemic lupus erythematosus (SLE) is a chronic autoimmune disease of unknown  
3 incompletely known etiology characterized by the presence of anti-nuclear autoantibodies and  
4 inflammation in a wide spectrum of organs.[1] Despite the improvement of SLE prognosis in  
5 the last decades, SLE patients are still at high risk of disease-related complications and  
6 premature death.[2, 3] Several authors have shown that remission defined as the elimination  
7 of disease activity predicted a better disease outcome with a lower burden of damage and a  
8 lower risk of relapse.[4, 5] Thus, remission achievement and its maintenance have become  
9 central in the management of SLE patients.[6, 7] Many definitions have been used to better  
10 characterize remission states, but a single one has recently made consensus, the DORIS  
11 (Definitions Of Remission In SLE). For defining remission, the DORIS uses a clinical index,  
12 such as the clinical SLE disease activity index (SLEDAI)=0 with routine laboratory  
13 assessments including anti-double stranded DNA (anti-dsDNA) antibodies (Abs) and  
14 complement.[7] Serologically active patients, i.e. patients with an increase in serum anti-  
15 dsDNA Abs and/or a complement consumption, are much more likely to experience  
16 subsequent flares, as compared to those who are serologically inactive.[7] However, these two  
17 markers fail to reliably predict a lupus flare.[8, 9] Consequently, no consensus has yet been  
18 reached by the DORIS working group as to a reliable definition that enables the distinction of  
19 patients who are serologically active from those who are serologically inactive.[7]

20 Many authors consider the dysregulation of interferons (IFNs), especially IFN-alpha  
21 (IFN- $\alpha$ ), to be a central cause of the immunological abnormalities observed in SLE.[10-16]  
22 Transcriptome analysis using microarray technology revealed up-regulation of numerous  
23 IFN-stimulated genes (ISGs) in SLE patients' peripheral blood mononuclear cells,  
24 constituting an overall "IFN signature".[17, 18] Many reports showed patients' elevated

1 serum-IFN- $\alpha$  levels to be associated with SLE activity and severity, suggesting that  
2 monitoring this cytokine might help physicians to better evaluate disease activity,[17-35]  
3 although, unexpectedly, some clinically inactive patients have persistently elevated serum  
4 IFN- $\alpha$  levels.[19-23, 25, 35] Since serum concentrations of IFN- $\alpha$  are usually very low and  
5 often not detectable by classic immunoassays, monitoring the expression of ISGs is used to  
6 evaluate IFN serological activity. The resulting “IFN scores” are calculated based on the  
7 expression of several representative ISGs.[29, 36, 37] However, the low availability and high  
8 complexity of transcriptome-microarray technology implies that IFN scores are not  
9 standardized and therefore cannot be easily used in clinical practice. At present, IFN- $\alpha$   
10 overexpression has been poorly studied in patients in remission. The single-molecule array  
11 (Simoa) assay, or digital immunoassay, is an ultrasensitive assay based on enumeration of  
12 individual enzyme-labeled immunocomplexes of proteins captured on beads in single-  
13 molecule arrays. It enables direct IFN- $\alpha$  quantification at attomolar concentrations,[35, 38-40]  
14 corresponding to a 5,000-fold-increased sensitivity over classic ELISAs. We and others have  
15 shown that at physiological concentrations, the digital immunoassay is as sensitive as ISG  
16 expression as a means to quantify IFN- $\alpha$  levels and simpler to perform and standardize.[35,  
17 40] We thus conducted a study to determine the magnitude of serum IFN- $\alpha$  concentrations in  
18 SLE patients in remission using the DORIS-validated criteria.[7] Additionally, in order to  
19 improve the definition of remission, we determined the clinical and biological features  
20 associated with elevated IFN- $\alpha$  levels in patients experiencing a remission and we  
21 investigated whether high serum IFN- $\alpha$  levels at baseline in patients in remission were  
22 predictive of a flare in the ensuing year.

23

# 1 PATIENTS, MATERIALS AND METHODS

## 2 Study design and patients

3 We conducted this longitudinal study between September, 2014 and September 2017 at the  
4 National Referral Center for SLE, Paris, France. Serum samples were obtained at day 0 (=  
5 baseline) from consecutive patients diagnosed with SLE according to the 1997 American  
6 College of Rheumatology criteria for SLE classification, regardless of the activity of the  
7 disease.[41] Exclusion criteria were: 1) known or suspected infection or malignancy on the  
8 day blood was drawn; 2) increased Hydroxychloroquine (HCQ), prednisone and/or  
9 immunosuppressant (IS) during the 4 weeks preceding day 0. SLE clinical characteristics (see  
10 [online supplement](#)), SELENA-SLEDAI,[42-44] SLEDAI-2K,[45] class of lupus nephritis  
11 according to ISN/RPS-2003,[46] and therapeutic regimen were recorded on day 0. Lupus  
12 flares were defined according to the SELENA-SLEDAI Flare Index.[43, 44] The term  
13 "clinical" SLEDAI (cSLEDAI) refers to symptoms, signs and routine laboratory testing and  
14 disregards only the points that can be given for the presence of anti-dsDNA Abs and/or low  
15 complement.[7] Five exclusive disease activity statuses were defined, according to the  
16 DORIS [7] and following Wilhelm et al. [47] and Ugarte-Gil et al. [48] without physician  
17 global assessment (PGA) and serum C4 analysis:

- 18 - *Complete remission off treatment*: cSELENA-SLEDAI=0, no corticosteroids, no  
19 immunosuppressant (IS), no anti-dsDNA Abs and no C3 decrease;
- 20 - *Clinical remission off treatment*: cSELENA-SLEDAI=0, no corticosteroids, no IS.  
21 Anti-dsDNA Abs and/or C3 decrease present;
- 22 - *Complete remission on treatment*: cSELENA-SLEDAI=0, prednisone 1-5mg/day,  
23 IS allowed, no anti-dsDNA Abs and no C3 decrease;
- 24 - *Clinical remission on treatment*: cSELENA-SLEDAI=0, prednisone 1-5mg/day, IS



1 allowed. Anti-dsDNA Abs and/or C3 decrease present;  
2 - *Not in remission*: cSELENA-SLEDAI>0 and/or prednisone >5mg/day.  
3 HCQ was allowed in all groups. Duration of remission was recorded. Over a consecutive 5-  
4 year period of remission, the patient was considered in “prolonged remission”, as proposed by  
5 Steiman et al. [49] and treated in the statistical analysis as a 5-year remission. Alternatively,  
6 patients not in remission but fulfilling Lupus Low Disease Activity State (LLDAS) [48, 50]  
7 were included in the “*LLDAS without remission*” subgroup ([see online supplement](#)). Sera  
8 from age- and gender-matched healthy donors (n=68) were collected (Établissement Français  
9 du Sang, Île-de-France, Pitié–Salpêtrière Hospital, Paris, France) during the same time period.  
10 The local Ethics Committee of the Pitié-Salpêtrière Hospital approved this study (file N°  
11 30052012) and informed consent was obtained from all participants.

12

### 13 **Patient follow-up**

14 Patients in remission at day 0 were followed for one year ([see online supplement](#)).

15

### 16 **IFN- $\alpha$ digital immunoassay**

17 Serum IFN- $\alpha$  concentrations, expressed in fg/mL, were determined at day 0 with digital  
18 immunoassay technology (IFN- $\alpha$  Reagent Kit, Quanterix Simoa™, Lexington, MA, USA),  
19 based on a 3-step protocol ([see online supplement](#)) using the HD-1 Analyzer  
20 (Quanterix™).[38] The IFN- $\alpha$  digital ELISA positivity threshold was 136 fg/mL, which is 3  
21 SDs above the mean serum IFN- $\alpha$  concentration calculated from the sera samples from the 68  
22 healthy blood donors.

23

### 24 **Statistical analysis**

1 Statistical analyses were performed using GraphPad Prism, v5.0 software (GraphPad  
2 Software, San Diego, CA, USA) and SAS 9.4 software (Cary, NC, USA) ([see online](#)  
3 [supplement](#)).  
4

## 1 RESULTS

2

### 3 Patient characteristics and distribution according to disease activity

4 A total of 407 patients were included in the study, with 254 patients in remission (i.e.  
5 cSELENA-SLEDAI=0 and prednisone equal or less than 5 mg/day) and 153 not in remission.  
6 Patients' baseline characteristics are described in **Tables 1 and 2**. From the 254 patients in  
7 remission, 86 (33.9%) were in complete remission off treatment, 59 (23.2%) in complete  
8 remission on treatment, 47 (18.5%) in clinical remission off treatment and 62 (24.4%) in  
9 clinical remission on treatment. Sixty-seven (26.3%) patients were in remission for less than 1  
10 year, 101 (39.8%) for 1 to 5 years, and 86 (33.9%) for more than 5 years. The median (range)  
11 of remission duration was 5 years (0-5) for patients in complete remission off treatment, 1.6  
12 years (0-5) for patients in complete remission on treatment, 4.2 years (0.1-5) for clinical  
13 remission off treatment and 1.6 years (0-5) for clinical remission on treatment. Among the  
14 153 patients not in remission, 19 (12.4%) fulfilled the LLDAS criteria, defining the "LLDAS  
15 without remission" subgroup.

16

### 17 Serum IFN- $\alpha$ in remission and LLDAS

18 The serum IFN- $\alpha$  levels according to the different states of remission are presented in  
19 **Table 2**. A total of 26.0% of patients in remission had still detectable serum IFN- $\alpha$  levels  
20 exceeding the positivity threshold. Patients in "clinical remission" had the highest rate of  
21 abnormal serum IFN- $\alpha$ : 41.9% for "clinical remission on treatment" and 38.3% for "clinical  
22 remission off treatment" [versus 16.3% for "complete remission off treatment",  $p = 0.0007$   
23 and 0.006, respectively]. Among patients in remission, patients in "clinical remission on  
24 treatment" had the highest concentration of IFN- $\alpha$  with a median (quartiles) of 109 fg/mL

1 (12-378) [versus 11 fg/mL (0-81) in patients in “complete remission off treatment”,  
2 p=0.0002]. Alternatively, we assessed serum IFN- $\alpha$  in different states of remission according  
3 to the definition of Zen et al., a definition of remission in which the role of corticosteroids is  
4 highlighted in comparison to immunosuppressive treatment.[5] As shown in **supplemental**  
5 **table 2**, we found similar results: patients in “clinical remission on or off corticosteroids” had  
6 the highest rate of abnormal serum IFN- $\alpha$  levels, as compared to those of patients in  
7 “complete remission”. Thus, a significant number of patients in remission had increased IFN-  
8  $\alpha$  levels in their serum. Eight (42.1%) patients of the “LLDAS without remission” subgroup  
9 displayed abnormal IFN- $\alpha$  serum concentration (OR 3.7 [95% CI 1.3-11.0] and a mean IFN- $\alpha$   
10 level of 111 fg/mL [0-1,647], p=0.02 and p=0.07, respectively, as compared with patients in  
11 “complete remission off treatment”).

12

### 13 **Parameters SLE characteristics associated with abnormal serum IFN- $\alpha$ levels in** 14 **remission**

15 Elevated IFN- $\alpha$  serum levels in remission were significantly associated, in multivariable  
16 analysis, with the presence of serum Abs specific for RNP, dsDNA and Ro/SSA60, young age  
17 and lower granulocyte counts (**Table 3**). In contrast, disease and remission duration,  
18 prednisone intake, immunosuppressant therapy, HCQ, and low C3 serum levels did not show  
19 a significant association with IFN- $\alpha$  serum levels.

20

### 21 **Elevated IFN- $\alpha$ serum levels in SLE patients in remission predicts a lupus flare**

22 Of the 254 patients in remission at day 0, 250 were followed for one year. Twenty-four  
23 (9.6%) patients experienced a flare. The median (range) time of the flare occurrence was 141  
24 (25-349) days. The type and severity of the flares are reported in **supplemental table 3**. A total

1 of 37.5% of the flares were severe. The most frequent type of relapses was arthritis (n=15)  
2 followed by cutaneous flare (n=7) and serositis (n=4). Unadjusted cox regression showed a  
3 significantly higher risk of relapse in patients who displayed at baseline elevated IFN- $\alpha$  (HR  
4 5.5 [95% CI 2.4-12.5], p<0.0001) or decreased C3 (HR 3.7 [95% CI 1.6-9.1], p=0.003) serum  
5 levels, respectively, but not in patients who had a positive Farr assay (HR 1.5 [95% CI 0.7-  
6 3.4], p=0.3) (Figure 1). The highest concentrations of IFN- $\alpha$  at baseline were associated with  
7 the greatest frequencies of relapse (supplemental figure 1). Other factors at baseline  
8 associated with the risk of relapse were prednisone intake 1-5 mg/day (HR 3.2 [95% CI 1.4-  
9 7.5], p=0.007), positive anti-RNP Abs (HR 3.1 [1.4-7.0], p=0.006), age <40 years (HR 0.4  
10 [95% CI 0.2-0.9], p=0.02) and disease duration <10 years (HR 0.2 [95% CI 0.1-0.6],  
11 p=0.003). The remission duration was a protective factor for the probability of flare (HR 0.6  
12 [95% CI 0.5-0.8] for each consecutive year of remission completed, p=0.0002).

13 Finally, in multivariable analysis, the factors independently associated with the risk of  
14 flare were abnormal serum IFN- $\alpha$  levels at baseline (HR 4.0 [95% CI 1.7-9.6], p=0.002) and  
15 remission duration (HR 0.7 [95% CI 0.5-0.9], p=0.02, for each year in remission). Low C3  
16 (HR 2.4 [95% CI 0.9-6.2], p=0.07) and prednisone intake (HR 2.4 [95% CI 0.9-5.9], p=0.06)  
17 were also kept in the model as-associated with a higher risk of flare but these associations  
18 were not statistically significant. We performed a sensitivity analysis using a lower  
19 concentration of IFN- $\alpha$  as the threshold of elevated IFN- $\alpha$  levels and found similar results  
20 (data not shown).

21 Additionally, the risks of relapse according to combined SLE biomarkers (low C3  
22 complement, positive Farr assay and positive IFN- $\alpha$ ) were independently analysed with a  
23 proportional risk cox model (supplemental table 4). In this model, isolated positive IFN- $\alpha$  was

1 a predictive factor of lupus flare (HR of 5.5 [95% CI 1.7-18.1],  $p = 0.005$ ). In contrast,  
2 isolated positive Farr assay and isolated low C3 were not predictive of lupus flare.

3

#### 4 **Digital immunoassay, complement C3 and Farr assay performances to predict a flare**

5 The time-dependent ROC area under the curve (AUC) (Figure 2) estimates for the IFN- $\alpha$   
6 digital immunoassay to predict a flare was 0.73, better than that of anti-dsDNA Abs (0.60,  
7  $p=0.055$ ), and complement C3 serum levels (0.56,  $p=0.2$ ). The time-dependent ROC AUCs  
8 for the three biomarkers were highest around day 90.

## 1 DISCUSSION

2 The significance of monitoring IFN- $\alpha$  in SLE for the assessment of remission and the risk to  
3 develop flares has been poorly documented as yet. In the present study, we show that a  
4 significant proportion of patients despite being in remission have high serum IFN- $\alpha$  levels that  
5 were found to independently predict the risk of subsequent SLE flares, thereby emphasizing  
6 the interest of measuring the production of this cytokine to monitor the course of disease.

7 Earlier publications have reported that between 3 and 32% of SLE patients without active  
8 disease present elevated IFN- $\alpha$  serum levels depending on the type of assay used to determine  
9 the presence of this cytokine.[19-23, 25, 35] However, because different definitions of disease  
10 inactivity were used in these studies, the results cannot directly be compared with our study  
11 that takes into account the recently formulated consensual definition of disease remission.[5,  
12 7, 47] Contrary to previous report,[51] we noted that serum concentrations of IFN- $\alpha$  above the  
13 positive threshold values varied between different remission subgroups. Patients in clinical  
14 remission, i.e. with serological activity, presented more frequently elevated serum levels of  
15 IFN- $\alpha$  than patients in complete remission. The presence of serum Abs specific for RNP,  
16 dsDNA and Ro/SSA60 were found to be independently associated with the magnitude of  
17 serum IFN- $\alpha$  levels. These associations have previously been shown in SLE [29-31, 52-57]  
18 but, to the best of our knowledge, never been reported for patients in remission. These data  
19 are in accordance with those from previous studies that showed a key role of DNA/RNA-  
20 associated immune complexes through the activation of TLR7 and TLR9 for the induction of  
21 type I IFN production.[58, 59]

22 The DORIS group has recently agreed that the subsequent occurrence of flares was  
23 among the most appropriate outcome variables for defining the prognostic value of remission  
24 and furthermore suggested the inclusion of serological criteria for this definition.[7] Results

1 from previous reports on smaller SLE patient cohorts, based on the analysis of expression of  
2 ISGs in peripheral blood cells by microarray, failed to demonstrate an association between  
3 IFN scores and the longitudinal risk of relapse.[30, 31] It is to be noted that type I and type II  
4 IFNs largely overlap in the genes that they control, making it difficult to distinguish the  
5 signatures of IFN-gamma (IFN- $\gamma$ ) from IFN- $\alpha$  and IFN-beta (IFN- $\beta$ ). Indeed, the results from  
6 a modular repertoire analysis have emphasized that IFN signatures in SLE are not restricted to  
7 IFN- $\alpha$ , but also involve IFN- $\beta$  and IFN- $\gamma$ , thus underscoring the nonspecific nature of IFN  
8 scores.[60] Therefore, in a disease like SLE in which IFN- $\alpha$  appears to be central in the  
9 pathogenesis, it is likely that clinical activity better correlates with the values of IFN- $\alpha$  serum  
10 levels, directly measured by a highly specific digital ELISA, than with the less specific  
11 expression of IGSs. Moreover, the IFN scores are likely to be rather poorly sensitive to  
12 changes in IFN- $\alpha$  serum concentrations and therefore of little use in monitoring the risk of  
13 relapse of disease. The results of our study suggest that the measurement of additional  
14 interferons, including IFN- $\beta$ , - $\gamma$  and - $\lambda$  with ultrasensitive digital immunoassay, might help to  
15 determine whether the expression of other IFN members also correlates with disease activity  
16 or specific characteristics of the disease.[34, 61, 62] Nonetheless, our results corroborate  
17 those from an earlier study showing the usefulness of monitoring expression levels of certain  
18 IFN-regulated chemokines to predict future flares: serum levels of CXCL10, CCL2 and  
19 CCL19 chemokines were found to be linked with SLE activity, and performed better than  
20 other laboratory tests to predict a flare over the following year.[63] Yet, these chemokines can  
21 be induced by other SLE-associated cytokines beyond IFN- $\alpha$ , such as IFN- $\gamma$  and their  
22 monitoring remains difficult in routine practice. As reported by others, we did not find an  
23 association between the presence of anti-dsDNA Abs and the risk of flare. Not surprisingly,  
24 the duration of remission was significantly associated with a poor risk of flare in the



1 following year, thus identifying a subgroup of patients in prolonged remission with less  
2 relapsing risk and for whom clinical monitoring can probably be lightened.

3 The sustained presence of serum IFN- $\alpha$  in serum of SLE patients in remission may have  
4 pathological consequences by itself. IFN- $\alpha$  overexpression could be an explanation of chronic  
5 fatigue, depression and reduction of sleep secondary to the stimulation of dopamine  
6 metabolism by IFN- $\alpha$  in the central neurological system.[64-66] IFN- $\alpha$  plays also a prominent  
7 role in endothelial cell damage and up-regulation of the expression of scavenger receptors in  
8 monocyte and macrophages, leading to increased lipid uptake and foam cell formation, a  
9 process that may be at the origin of the accelerated atherosclerosis observed in SLE.[67-69]  
10 These data suggest that for patients in clinical remission, return of IFN- $\alpha$  to normal values  
11 could become one of the objectives of the treatment.

12 Our study has some limitations. It unfortunately lacks data on patients' ethnicity, which is  
13 known to bias serum IFN- $\alpha$  levels.[55] We also used a definition of remission and LLDAS in  
14 the absence of PGA that has not been assessed in our cohort. However, other teams have  
15 already adapted the definition of remission and LLDAS arguing that in the SLE Response  
16 Index, the SLEDAI is the variable with the highest impact on the definition of response and  
17 that even without PGA, a modified definition of remission or LLDAS is still entirely  
18 valid.[48, 70] **Finally, the serum IFN- $\alpha$  concentrations were not assessed during the  
19 longitudinal part of the study. Therefore information regarding the intra-individual variability  
20 over time of serum IFN- $\alpha$  levels is lacking. These important data will have to be studied in  
21 new studies.**

22 In conclusion, our data confirm that a large number of SLE patients in remission display  
23 an elevated serum IFN- $\alpha$  concentrations, especially in the presence of anti-dsDNA, anti-

1 ribonucleoprotein Abs (i.e. anti-Ro/SSA 60, anti-RNP), as well as young age. This  
2 overexpression is an independent predictive biomarker of lupus flare in the following year.  
3 Adding serum IFN- $\alpha$  measurement to the routine laboratory assessments in patient in  
4 remission could help clinicians to identify a subgroup of SLE patients clinically in remission  
5 but who still overexpress IFN- $\alpha$  and are at higher risk of relapse. These data suggest that the  
6 return of normalcy of serum IFN- $\alpha$  could become one of the objectives of the treatment. Our  
7 results must be validated in other independent cohorts.

8

9

10

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6 design of the study; SMM, AM, KD, FCA, JH, MH, MM, DS, MP, DBLTH, FR and ZA were  
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8 contributed to the analysis and interpretation of data. All authors contributed to drafting  
9 and/or revising the manuscript.

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## 1 REFERENCES

2

- 3 [1] Lisnevskaja L, Murphy G, Isenberg D. Systemic lupus erythematosus. *Lancet* 2014;384:1878-  
4 88.
- 5 [2] Bernatsky S, Boivin JF, Joseph L, Manzi S, Ginzler E, Gladman DD, *et al.* Mortality in systemic  
6 lupus erythematosus. *Arthritis Rheum* 2006;54:2550-7.
- 7 [3] Tektonidou MG, Lewandowski LB, Hu J, Dasgupta A, Ward MM. Survival in adults and  
8 children with systemic lupus erythematosus: a systematic review and Bayesian meta-analysis  
9 of studies from 1950 to 2016. *Ann Rheum Dis* 2017;76:2009-16.
- 10 [4] Nossent J, Kiss E, Rozman B, Pokorny G, Vlachoyiannopoulos P, Olesinska M, *et al.* Disease  
11 activity and damage accrual during the early disease course in a multinational inception  
12 cohort of patients with systemic lupus erythematosus. *Lupus* 2010;19:949-56.
- 13 [5] Zen M, Iaccarino L, Gatto M, Bettio S, Nalotto L, Ghirardello A, *et al.* Prolonged remission in  
14 Caucasian patients with SLE: prevalence and outcomes. *Ann Rheum Dis* 2015;74:2117-22.
- 15 [6] van Vollenhoven RF, Mosca M, Bertias G, Isenberg D, Kuhn A, Lerstrom K, *et al.* Treat-to-  
16 target in systemic lupus erythematosus: recommendations from an international task force.  
17 *Ann Rheum Dis* 2014;73:958-67.
- 18 [7] van Vollenhoven R, Voskuyl A, Bertias G, Aranow C, Aringer M, Arnaud L, *et al.* A framework  
19 for remission in SLE: consensus findings from a large international task force on definitions of  
20 remission in SLE (DORIS). *Ann Rheum Dis* 2017;76:554-61.
- 21 [8] Steiman AJ, Gladman DD, Ibanez D, Urowitz MB. Prolonged serologically active clinically  
22 quiescent systemic lupus erythematosus: frequency and outcome. *J Rheumatol*  
23 2010;37:1822-7.
- 24 [9] Gensous N, Marti A, Barnetche T, Blanco P, Lazaro E, Seneschal J, *et al.* Predictive biological  
25 markers of systemic lupus erythematosus flares: a systematic literature review. *Arthritis Res*  
26 *Ther* 2017;19:238.
- 27 [10] Theofilopoulos AN, Baccala R, Beutler B, Kono DH. Type I interferons (alpha/beta) in  
28 immunity and autoimmunity. *Annu Rev Immunol* 2005;23:307-36.
- 29 [11] Obermoser G, Pascual V. The interferon-alpha signature of systemic lupus erythematosus.  
30 *Lupus* 2010;19:1012-9.
- 31 [12] Ronnblom L, Elkon KB. Cytokines as therapeutic targets in SLE. *Nat Rev Rheumatol*  
32 2010;6:339-47.
- 33 [13] Niewold TB. Interferon alpha as a primary pathogenic factor in human lupus. *J Interferon*  
34 *Cytokine Res* 2011;31:887-92.
- 35 [14] Ronnblom L, Alm GV, Eloranta ML. The type I interferon system in the development of lupus.  
36 *Semin Immunol* 2011;23:113-21.
- 37 [15] Lauwerys BR, Ducreux J, Houssiau FA. Type I interferon blockade in systemic lupus  
38 erythematosus: where do we stand? *Rheumatology (Oxford)* 2014;53:1369-76.
- 39 [16] Kirou KA, Gkrouzman E. Anti-interferon alpha treatment in SLE. *Clin Immunol* 2013;148:303-  
40 12.
- 41 [17] Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, Banchereau J, *et al.* Interferon and  
42 granulopoiesis signatures in systemic lupus erythematosus blood. *J Exp Med* 2003;197:711-  
43 23.
- 44 [18] Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, *et al.* Interferon-  
45 inducible gene expression signature in peripheral blood cells of patients with severe lupus.  
46 *Proc Natl Acad Sci U S A* 2003;100:2610-5.

- 1 [19] Hooks JJ, Moutsopoulos HM, Geis SA, Stahl NI, Decker JL, Notkins AL. Immune interferon in  
2 the circulation of patients with autoimmune disease. *N Engl J Med* 1979;301:5-8.
- 3 [20] Preble OT, Black RJ, Friedman RM, Klippel JH, Vilcek J. Systemic lupus erythematosus:  
4 presence in human serum of an unusual acid-labile leukocyte interferon. *Science*  
5 1982;216:429-31.
- 6 [21] Ytterberg SR, Schnitzer TJ. Serum interferon levels in patients with systemic lupus  
7 erythematosus. *Arthritis Rheum* 1982;25:401-6.
- 8 [22] Friedman RM, Preble O, Black R, Harrell S. Interferon production in patients with systemic  
9 lupus erythematosus. *Arthritis Rheum* 1982;25:802-3.
- 10 [23] Shi SN, Feng SF, Wen YM, He LF, Huang YX. Serum interferon in systemic lupus  
11 erythematosus. *Br J Dermatol* 1987;117:155-9.
- 12 [24] Kanayama Y, Kim T, Inariba H, Negoro N, Okamura M, Takeda T, *et al.* Possible involvement  
13 of interferon alfa in the pathogenesis of fever in systemic lupus erythematosus. *Ann Rheum*  
14 *Dis* 1989;48:861-3.
- 15 [25] Vallin H, Blomberg S, Alm GV, Cederblad B, Ronnblom L. Patients with systemic lupus  
16 erythematosus (SLE) have a circulating inducer of interferon-alpha (IFN-alpha) production  
17 acting on leucocytes resembling immature dendritic cells. *Clin Exp Immunol* 1999;115:196-  
18 202.
- 19 [26] Bengtsson AA, Sturfelt G, Truedsson L, Blomberg J, Alm G, Vallin H, *et al.* Activation of type I  
20 interferon system in systemic lupus erythematosus correlates with disease activity but not  
21 with antiretroviral antibodies. *Lupus* 2000;9:664-71.
- 22 [27] Dall'era MC, Cardarelli PM, Preston BT, Witte A, Davis JC, Jr. Type I interferon correlates with  
23 serological and clinical manifestations of SLE. *Ann Rheum Dis* 2005;64:1692-7.
- 24 [28] Kirou KA, Lee C, George S, Louca K, Peterson MG, Crow MK. Activation of the interferon-  
25 alpha pathway identifies a subgroup of systemic lupus erythematosus patients with distinct  
26 serologic features and active disease. *Arthritis Rheum* 2005;52:1491-503.
- 27 [29] Feng X, Wu H, Grossman JM, Hanvivadhanakul P, FitzGerald JD, Park GS, *et al.* Association of  
28 increased interferon-inducible gene expression with disease activity and lupus nephritis in  
29 patients with systemic lupus erythematosus. *Arthritis Rheum* 2006;54:2951-62.
- 30 [30] Petri M, Singh S, Tesfayone H, Dedrick R, Fry K, Lal P, *et al.* Longitudinal expression of type I  
31 interferon responsive genes in systemic lupus erythematosus. *Lupus* 2009;18:980-9.
- 32 [31] Landolt-Marticorena C, Bonventi G, Lubovich A, Ferguson C, Unnithan T, Su J, *et al.* Lack of  
33 association between the interferon-alpha signature and longitudinal changes in disease  
34 activity in systemic lupus erythematosus. *Ann Rheum Dis* 2009;68:1440-6.
- 35 [32] Rose T, Grutzkau A, Hirseland H, Huscher D, Dahnrich C, Dzionek A, *et al.* IFNalpha and its  
36 response proteins, IP-10 and SIGLEC-1, are biomarkers of disease activity in systemic lupus  
37 erythematosus. *Ann Rheum Dis* 2013;72:1639-45.
- 38 [33] Rose T, Grutzkau A, Klotsche J, Enghard P, Flechsig A, Keller J, *et al.* Are interferon-related  
39 biomarkers advantageous for monitoring disease activity in systemic lupus erythematosus? A  
40 longitudinal benchmark study. *Rheumatology (Oxford)* 2017;56:1618-26.
- 41 [34] Oke V, Brauner S, Larsson A, Gustafsson J, Zickert A, Gunnarsson I, *et al.* IFN-lambda1 with  
42 Th17 axis cytokines and IFN-alpha define different subsets in systemic lupus erythematosus  
43 (SLE). *Arthritis Res Ther* 2017;19:139.
- 44 [35] Mathian A, Mouries-Martin S, Dorgham K, Devilliers H, Barnabei L, Ben Salah E, *et al.*  
45 Monitoring Disease Activity in Systemic Lupus Erythematosus With Single-Molecule Array  
46 Digital Enzyme-Linked Immunosorbent Assay Quantification of Serum Interferon-alpha.  
47 *Arthritis Rheumatol* 2019;71:756-65.
- 48 [36] Yao Y, Higgs BW, Morehouse C, de Los Reyes M, Trigona W, Brohawn P, *et al.* Development  
49 of Potential Pharmacodynamic and Diagnostic Markers for Anti-IFN-alpha Monoclonal

- 1 Antibody Trials in Systemic Lupus Erythematosus. *Hum Genomics Proteomics*  
2 2009;doi:10.4061/2009/374312.
- 3 [37] Yao Y, Higgs BW, Richman L, White B, Jallal B. Use of type I interferon-inducible mRNAs as  
4 pharmacodynamic markers and potential diagnostic markers in trials with sifalimumab, an  
5 anti-IFNalpha antibody, in systemic lupus erythematosus. *Arthritis Res Ther* 2010;12 Suppl  
6 1:S6.
- 7 [38] Rissin DM, Kan CW, Campbell TG, Howes SC, Fournier DR, Song L, *et al.* Single-molecule  
8 enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar  
9 concentrations. *Nat Biotechnol* 2010;28:595-9.
- 10 [39] Wilson DH, Rissin DM, Kan CW, Fournier DR, Piech T, Campbell TG, *et al.* The simoa HD-1  
11 analyzer: a novel fully automated digital immunoassay analyzer with single-molecule  
12 sensitivity and multiplexing. *J Lab Autom* 2016;21:533-47.
- 13 [40] Rodero MP, Decalf J, Bondet V, Hunt D, Rice GI, Werneke S, *et al.* Detection of interferon  
14 alpha protein reveals differential levels and cellular sources in disease. *J Exp Med*  
15 2017;214:1547-55.
- 16 [41] Hochberg MC. Updating the American College of Rheumatology revised criteria for the  
17 classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725.
- 18 [42] Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A  
19 disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis*  
20 *Rheum* 1992;35:630-40.
- 21 [43] Buyon JP, Petri MA, Kim MY, Kalunian KC, Grossman J, Hahn BH, *et al.* The effect of combined  
22 estrogen and progesterone hormone replacement therapy on disease activity in systemic  
23 lupus erythematosus: a randomized trial. *Ann Intern Med* 2005;142:953-62.
- 24 [44] Petri M, Kim MY, Kalunian KC, Grossman J, Hahn BH, Sammaritano LR, *et al.* Combined oral  
25 contraceptives in women with systemic lupus erythematosus. *N Engl J Med* 2005;353:2550-8.
- 26 [45] Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index  
27 2000. *J Rheumatol* 2002;29:288-91.
- 28 [46] Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, *et al.* The  
29 classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int*  
30 2004;65:521-30.
- 31 [47] Wilhelm TR, Magder LS, Petri M. Remission in systemic lupus erythematosus: durable  
32 remission is rare. *Ann Rheum Dis* 2017;76:547-53.
- 33 [48] Ugarte-Gil MF, Wojdyla D, Pons-Estel GJ, Catoggio LJ, Drenkard C, Sarano J, *et al.* Remission  
34 and Low Disease Activity Status (LDAS) protect lupus patients from damage occurrence: data  
35 from a multiethnic, multinational Latin American Lupus Cohort (GLADEL). *Ann Rheum Dis*  
36 2017;76:2071-74.
- 37 [49] Steiman AJ, Urowitz MB, Ibanez D, Papneja A, Gladman DD. Prolonged clinical remission in  
38 patients with systemic lupus erythematosus. *J Rheumatol* 2014;41:1808-16.
- 39 [50] Franklyn K, Lau CS, Navarra SV, Louthrenoo W, Lateef A, Hamijoyo L, *et al.* Definition and  
40 initial validation of a Lupus Low Disease Activity State (LLDAS). *Ann Rheum Dis* 2016;75:1615-  
41 21.
- 42 [51] Steiman AJ, Gladman DD, Ibanez D, Noamani B, Landolt-Marticorena C, Urowitz MB, *et al.*  
43 Lack of Interferon and Proinflammatory Cyto/chemokines in Serologically Active Clinically  
44 Quiescent Systemic Lupus Erythematosus. *J Rheumatol* 2015;42:2318-26.
- 45 [52] Hua J, Kirou K, Lee C, Crow MK. Functional assay of type I interferon in systemic lupus  
46 erythematosus plasma and association with anti-RNA binding protein autoantibodies.  
47 *Arthritis Rheum* 2006;54:1906-16.
- 48 [53] Niewold TB, Hua J, Lehman TJ, Harley JB, Crow MK. High serum IFN-alpha activity is a  
49 heritable risk factor for systemic lupus erythematosus. *Genes Immun* 2007;8:492-502.

- 1 [54] Niewold TB, Kelly JA, Flesch MH, Espinoza LR, Harley JB, Crow MK. Association of the IRF5 risk  
2 haplotype with high serum interferon-alpha activity in systemic lupus erythematosus  
3 patients. *Arthritis Rheum* 2008;58:2481-7.
- 4 [55] Weckerle CE, Franek BS, Kelly JA, Kumabe M, Mikolaitis RA, Green SL, *et al.* Network analysis  
5 of associations between serum interferon-alpha activity, autoantibodies, and clinical features  
6 in systemic lupus erythematosus. *Arthritis Rheum* 2011;63:1044-53.
- 7 [56] Ko K, Koldobskaya Y, Rosenzweig E, Niewold TB. Activation of the Interferon Pathway is  
8 Dependent Upon Autoantibodies in African-American SLE Patients, but Not in European-  
9 American SLE Patients. *Front Immunol* 2013;4:309.
- 10 [57] Connelly KL, Kandane-Rathnayake R, Hoi A, Nikpour M, Morand EF. Association of MIF, but  
11 not type I interferon-induced chemokines, with increased disease activity in Asian patients  
12 with systemic lupus erythematosus. *Sci Rep* 2016;6:29909.
- 13 [58] Banchereau J, Pascual V. Type I interferon in systemic lupus erythematosus and other  
14 autoimmune diseases. *Immunity* 2006;25:383-92.
- 15 [59] Bengtsson AA, Ronnblom L. Role of interferons in SLE. *Best Pract Res Clin Rheumatol*  
16 2017;31:415-28.
- 17 [60] Chiche L, Jourde-Chiche N, Whalen E, Presnell S, Gersuk V, Dang K, *et al.* Modular  
18 transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal  
19 distinct type I and type II interferon signatures. *Arthritis Rheumatol* 2014;66:1583-95.
- 20 [61] Munroe ME, Lu R, Zhao YD, Fife DA, Robertson JM, Guthridge JM, *et al.* Altered type II  
21 interferon precedes autoantibody accrual and elevated type I interferon activity prior to  
22 systemic lupus erythematosus classification. *Ann Rheum Dis* 2016;75:2014-21.
- 23 [62] Oke V, Gunnarsson I, Dorschner J, Eketjall S, Zickert A, Niewold TB, *et al.* High levels of  
24 circulating interferons type I, type II and type III associate with distinct clinical features of  
25 active systemic lupus erythematosus. *Arthritis Res Ther* 2019;21:107.
- 26 [63] Bauer JW, Petri M, Batliwalla FM, Koeth T, Wilson J, Slattery C, *et al.* Interferon-regulated  
27 chemokines as biomarkers of systemic lupus erythematosus disease activity: a validation  
28 study. *Arthritis Rheum* 2009;60:3098-107.
- 29 [64] Raison CL, Borisov AS, Broadwell SD, Capuron L, Woolwine BJ, Jacobson IM, *et al.* Depression  
30 during pegylated interferon-alpha plus ribavirin therapy: prevalence and prediction. *J Clin*  
31 *Psychiatry* 2005;66:41-8.
- 32 [65] Raison CL, Rye DB, Woolwine BJ, Vogt GJ, Bautista BM, Spivey JR, *et al.* Chronic interferon-  
33 alpha administration disrupts sleep continuity and depth in patients with hepatitis C:  
34 association with fatigue, motor slowing, and increased evening cortisol. *Biol Psychiatry*  
35 2010;68:942-9.
- 36 [66] Felger JC, Li L, Marvar PJ, Woolwine BJ, Harrison DG, Raison CL, *et al.* Tyrosine metabolism  
37 during interferon-alpha administration: association with fatigue and CSF dopamine  
38 concentrations. *Brain Behav Immun* 2013;31:153-60.
- 39 [67] Kaplan MJ, Salmon JE. How does interferon-alpha insult the vasculature? Let me count the  
40 ways. *Arthritis Rheum* 2011;63:334-6.
- 41 [68] Denny MF, Thacker S, Mehta H, Somers EC, Dodick T, Barrat FJ, *et al.* Interferon-alpha  
42 promotes abnormal vasculogenesis in lupus: a potential pathway for premature  
43 atherosclerosis. *Blood* 2007;110:2907-15.
- 44 [69] Li J, Fu Q, Cui H, Qu B, Pan W, Shen N, *et al.* Interferon-alpha priming promotes lipid uptake  
45 and macrophage-derived foam cell formation: a novel link between interferon-alpha and  
46 atherosclerosis in lupus. *Arthritis Rheum* 2011;63:492-502.
- 47 [70] Navarra SV, Guzman RM, Gallacher AE, Hall S, Levy RA, Jimenez RE, *et al.* Efficacy and safety  
48 of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-  
49 controlled, phase 3 trial. *Lancet* 2011;377:721-31.

- 1 [71] Uno H, Cai T, Pencina MJ, D'Agostino RB, Wei LJ. On the C-statistics for evaluating overall
- 2 adequacy of risk prediction procedures with censored survival data. *Stat Med* 2011;30:1105-
- 3 17.
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1 **Figures legends**

2 **Figure 1. Baseline positive serum IFN- $\alpha$  levels and low complement C3 identify patients**

3 **with elevated risk for future SLE flares.** Kaplan-Meier curves. IFN- $\alpha$  = interferon alpha.

4 Serum IFN- $\alpha$  (positivity threshold of 136 fg/mL), anti-dsDNA Abs by Farr assay (cut-off

5 value: 9.0 IU/mL) and complement C3 levels (cut-off value: 0.78g/L) were assessed at day 0.

6 Kaplan-Meier plots show the percentage of patients who flared in any organ system. Vertical

7 tick marks along each curve represent patients who remained flare-free but did not have a full

8 year of clinical follow-up (censored data). Curves were compared using Log-Rank tests.

9 Crude Hazard Ratios (HR) were calculated using proportional risk cox model **A.** The red

10 dashed line represents the 64 patients with positive IFN- $\alpha$  serum level at day 0. The

11 continuous blue line represents the 186 patients with negative IFN- $\alpha$  serum level at day 0. **B.**

12 The red dashed line represents the 99 patients with positive Farr assay at day 0. The

13 continuous blue line represents the 151 patients with negative Farr assay at day 0. **C.** The red

14 dashed line represents the 28 patients with low C3 level at day 0. The continuous blue line

15 represents the 216 patients with normal C3 level at day 0.

16

17 **Figure 2. Cumulative time-dependent receiver operating characteristics (ROC) curves.**

18 IFN- $\alpha$  = interferon alpha; C3 = complement C3. Serum IFN- $\alpha$  digital immunoassay, anti-

19 dsDNA Abs by Farr assay and complement C3 levels were assessed at baseline (day 0). SLE

20 patients in remission were followed for one year. Lupus flares were defined using the

21 SELENA-SLEDAI Flare Index. Patients who had a flare in any organ system were recorded.

22 The diagnostic performances of the baseline serum-IFN- $\alpha$  digital immunoassay (red line),

23 Farr assay (blue line) and complement C3 (green line) to predict SLE flare were investigated

24 by computing cumulative time-dependent ROC curves, with lupus flares serving as the gold

1 standard for those analyses. The dashed line represents the upper and the lower 95%  
2 confidence interval (CI). The cumulative time-dependent ROC area under the curve (AUC)  
3 estimated to predict a flare are given and were compared based on Uno *et al.*[71]

1 **Table 1. Disease parameters-characteristics in SLE patients at baseline**

	<b>Patients (N=407)</b>
Women	365 (90)
Age, years, mean±SD	40.1±12.9
Disease duration, years, mean±SD	11.2±9.4
SELENA-SLEDAI score, median (range)	2 (0–41)
SELENA-SLEDAI score ≥4	131 (32)
Mild/moderate flare*	31 (8)
Severe flare*	76 (19)
<b>Clinical involvement</b>	
Fever	37 (9)
Weight loss or anorexia	21 (5)
Lymphadenopathy	25 (6)
Any constitutional signs	47 (12)
Active cutaneous lupus	48 (12)
Active lupus serositis	23 (6)
Active lupus arthritis	67 (16)
Active lupus nephropathy	31 (8)
proliferative nephropathy	15 (4)
membranous nephropathy	16 (4)
Active neuropsychiatric lupus	8 (2)
Cytopenia	34 (8)
<b>Treatment regimen</b>	
Hydroxychloroquine use	347 (85)
Prednisone use	205 (50)
Prednisone ≥10 mg/j	56 (14)
Immunosuppressive agent use†	96 (24)
<b>Biological tests</b>	

Positive Farr test	198 (49)
Positive anti-RNP Abs	118 (29)
Positive anti-Sm Abs	51 (13)
Positive anti-Ro/SSA 52 Abs	97 (24)
Positive anti-Ro/SSA 60 Abs	151 (37)
Positive anti-La/SSB Abs	42 (10)
Low C3	98/398‡ (25)
Positive IFN- $\alpha$	163 (40)

1 Values are expressed as n (%), unless stated otherwise.

2 \* Defined using SELENA flare index [43, 44]

3 † Excluding antimalarials and prednisone. Immunosuppressant therapy was mycophenolate mofetil (MMF) for 43 (44%)  
4 patients, methotrexate (MTX) for 39 (41%), azathioprine for 13 (14%) and cyclophosphamide for 1 (1%). Two patients were  
5 receiving calcineurin inhibitor in addition to MMF and three patients were receiving belimumab in addition to MTX.

6 ‡ Positive assay/number of patients assessed.

7 SD, standard deviation; SELENA-SLEDAI, Safety of Estrogens in Lupus Erythematosus: National Assessment version of the  
8 Systemic Lupus Erythematosus Disease Activity Index.

9

10

**Table 2. Serum IFN- $\alpha$  levels at baseline in SLE patients in remission or not in remission (according to Wilhelm et al. [47] modified).**

	<b>Abnormal serum IFN-<math>\alpha</math> level</b>	<b>Odds Ratio [95% CI]</b>	<b>p value<sup>a</sup></b>	<b>Serum IFN-<math>\alpha</math> fg/mL, median (Q1, Q3)</b>	<b>p value<sup>b</sup></b>
<b>Remission (n=254)</b>	<b>66 (26.0)</b>				
complete remission off treatment (n=86)*	14 (16.3)	1 [Ref.]	Ref.	11 (0-81)	Ref.
complete remission on treatment (n=59)	8 (13.6)	0.8 [0.3-2.1]	NS	15 (0-51)	NS
clinical remission off treatment (n=47)	18 (38.3)	3.2 [1.4-7.2]	0.006	18 (0-314)	NS
clinical remission on treatment (n=62)	26 (41.9)	3.7 [1.7-8.0]	0.0007	109 (12-378)	0.0002
<b>Not in remission (n=153)</b>	<b>97 (63.4)</b>				
prednisone >5 mg/day (n=33)	10 (30.3)	2.2 [0.9-5.7]	NS	78 (0-296)	0.04
cSLEDAI >0 (n=74)	51 (68.9)	11.4 [5.4-24.3]	<0.0001	519 (68-3,087)	<0.0001
prednisone >5 mg/day and cSLEDAI >0 (n=46)	36 (78.3)	18.5 [7.5-45.8]	<0.0001	2,054 (455-8,751)	<0.0001

Values are expressed as n (%), unless stated otherwise.

\* reference group for statistical analysis

<sup>a</sup> bivariable analysis using Mann–Whitney U-test compared to “complete remission off treatment” as the reference.

<sup>b</sup> bivariable comparison using Fisher’s exact test compared with patients in “complete remission off treatment” as the reference.

NS, Non Significant; IFN- $\alpha$ , interferon alpha; fg/mL, femtogram/milliliter; cSLEDAI, clinicalSLEDAI; SD, standard deviation.

**Table 3. Parameters-Disease characteristics associated with serum IFN- $\alpha$  levels at baseline in SLE patients in remission**

	IFN- $\alpha$		Bivariable p value <sup>a</sup>	Multivariable p value <sup>b</sup>	Adjusted odds ratio <sup>b</sup> [95% CI]
	Normal	elevated			
	N=188	N=66			
Women	165 (88)	61 (92)	0.30	ND	
Age, years, mean $\pm$ SD	44.0 $\pm$ 13.3	37.5 $\pm$ 10.8	<0.001	0.002	0.96 [0.93-0.98]
Disease duration, years, mean $\pm$ SD	13.4 $\pm$ 10.0	10.4 $\pm$ 8.3	0.029	NS	
Remission duration, years, median (Q1-Q3)	3.4 (1.1-5.0)	1.8 (0.5-5.0)	0.024	NS	
Hydroxychloroquine use	165 (88)	63 (95)	0.08	ND	
Prednisone use	72 (38)	28 (42)	0.55	ND	
Prednisone use, mg/d, median (Q1-Q3)	0 (0-5)	0 (0-5)	0.65	ND	
Immunosuppressive agent use <sup>†</sup>	31 (16)	15 (23)	0.26	ND	
Positive Farr test	59 (31)	42 (64)	<0.001	0.02	2.3 [1.1-4.6]
Positive anti-RNP Abs	21 (11)	29 (44)	<0.001	0.0002	4.6 [2.1-10.2]
Positive anti-Ro/SSA 52 Abs	34 (18)	22 (33)	0.01	NS	
Positive anti-Ro/SSA 60 Abs	52 (28)	31 (47)	0.004	0.01	2.6 [1.3-5.4]
Positive anti-La/SSB Abs	14 (7)	8 (12)	0.25	ND	
Positive anti-Sm Abs	5 (3)	13 (20)	<0.001	NS	
Low C3	14/184 <sup>‡</sup> (8)	16 (25)	<0.001	NS	
Lymphocytes, G/L, median (Q1-Q3)	1.5 (1.2-2.1)	1.2 (0.9-1.5)	0.0001	NS	
Granulocytes, G/L, median (Q1-Q3)	3.9 (2.9-5.5)	3.1 (2.4-3.7)	<0.001	0.001	0.7 [0.5-0.8]
Thrombocytes, G/L, median (Q1-Q3)	249 (215-284)	232 (205-282)	0.5	ND	
Haemoglobin, g/dL, median (Q1-Q3)	13.3 (12.6-14.1)	12.6 (11.8-13.7)	<0.001	NS	

Values are expressed as n (%), unless stated otherwise.

† Excluding antimalarials and prednisone

‡Positive assay/number of patients assessed

<sup>a</sup> estimated by Mann-Whitney test, Fisher's exact or  $\chi^2$  test

<sup>b</sup> evaluated by multivariable logistic regression.

SD, standard deviation; ND, Not Done; NS: Non Significant.

Figure 1

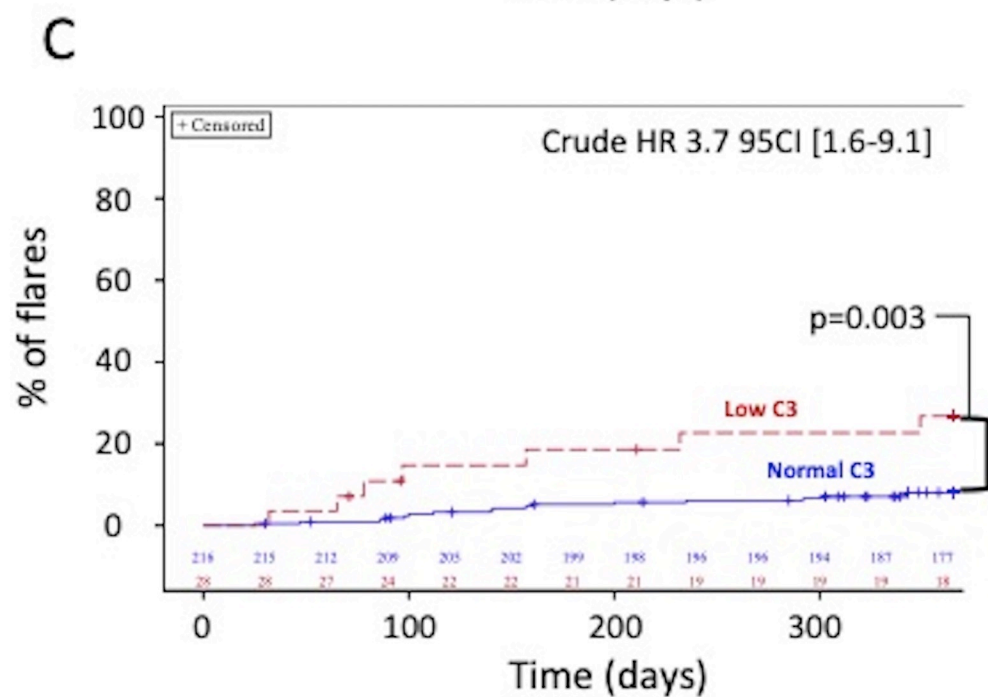
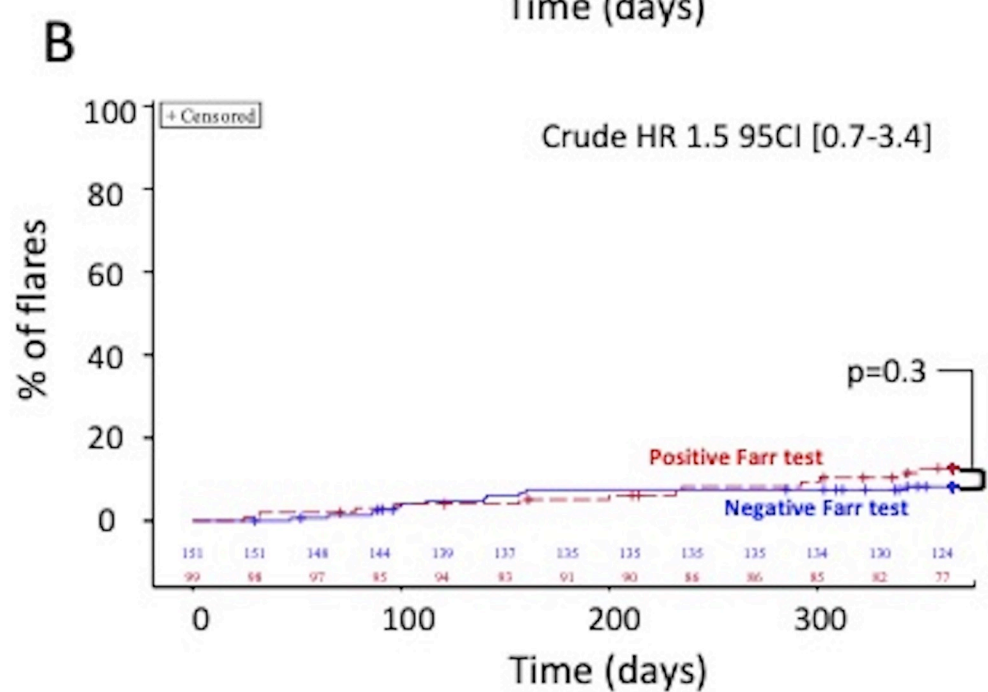
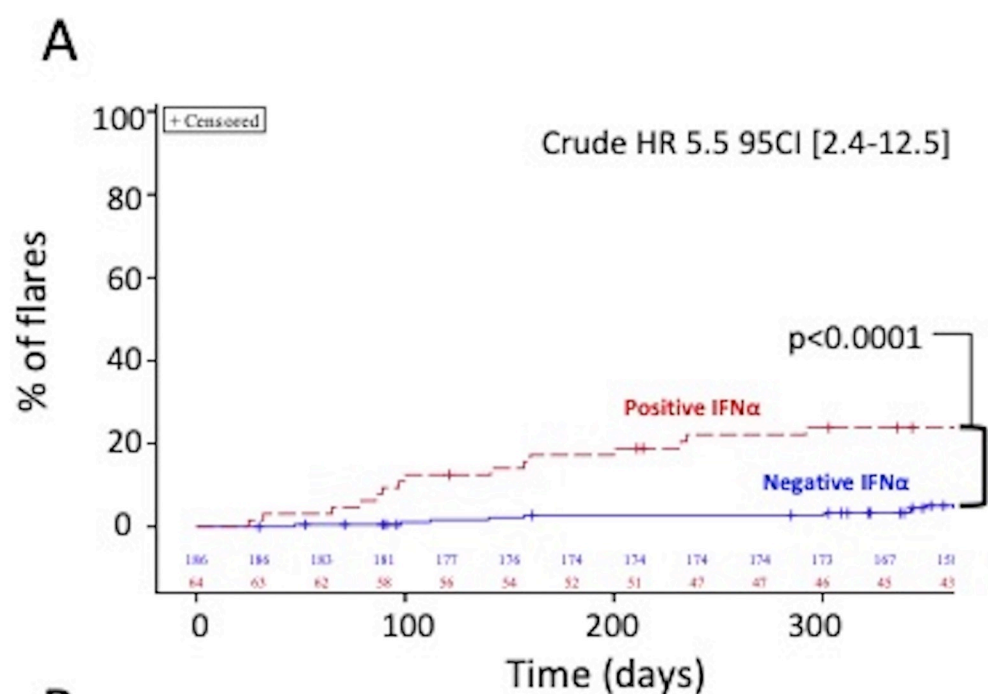




Figure 2

