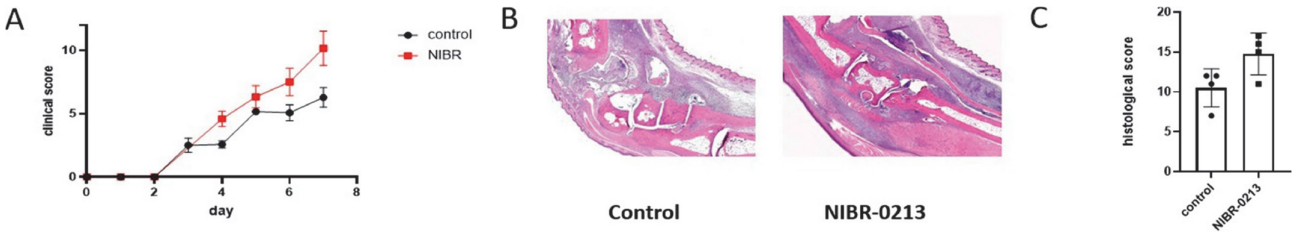

Abstract 1116 Figure 3 S1PR1 antagonist NIBR-0213 exacerbates immune complex mediated arthritis. (A) Clinical scores of mice subjected to serum induced arthritis (SIA) days 0-7. (B) Representative H&E stained sample of ankle obtained on day 7 after SIA. (C) Histological scoring of mice on day 7 after SIA.

cleavage of VE-cadherin, a key protector against vascular permeability that is highly expressed on renal ECs and is shed during inflammatory states. To further support the hypothesis that S1PR1 signaling is protective in immune complex mediated disease, we also examined the effects of a S1PR1 antagonist on serum induced arthritis, a relatively acute model of immune complex mediated arthritis to determine whether it increased disease severity.

Methods (1) Assessment of S1PR1 expression in human LN: renal biopsy samples from 15 SLE patients with class IV LN and kidney samples from age, sex and race matched controls were evaluated by immunohistochemistry and scored for the expression of S1PR1 in a blinded fashion. Staining of S1PR1 was assigned an arbitrary value of 0-4 with 4 corresponding to maximal expression and 0 corresponding to no signal above the isotype control antibody. (2) In vitro studies to test the hypothesis that S1PR1 antagonism induced VE-cadherin shedding: HUVEC were treated with an S1PR1 antagonist NIBR-0213 (1 uM) for 30, 60, or 180 min +/- the presence of a pan-metalloproteinase inhibitor marimastat (MM) and VE-cadherin shedding was assessed by western blotting. (3) EC resistance, a measure of barrier function, was measured in NIBR treated ECs +/- MM by Electric cell- substrate impedance sensing (ECIS). (4) In vivo studies to determine whether NIBR-0213 exacerbated injury in serum induced arthritis, a model of immune complex mediated injury: NIBR 30 mg/kg was administered once daily to WT C57BL/6 mice receiving 75 ul of K/BxN serum on days 0 and 2. Clinical scores were assessed daily and histological scoring was assessed on H&E stained paraffin embedded sections.

Results Assessment of S1PR1 staining in human renal tissues: 5/15 patients with lupus nephritis had EC S1PR1 expression scores of 0 in renal microvasculature compared to 0/10 controls and 10/15 patients had EC S1PR1 expression scores of 0-1 compared to 2/10 controls (figure 1).

In vitro mechanistic studies: acute blockade of S1PR1 signaling with the antagonist NIBR-0213 induced shedding of VE-cadherin in a metalloproteinase - dependent manner in as measured by increased C- terminal (remaining transmembrane fragment) and N-terminal VE-cadherin (cleaved extracellular domain) in HUVEC lysates and supernatants, respectively (Fig. 2). ECIS demonstrated that NIBR induced a drop in resistance (a measure of barrier function), as expected, but that metalloproteinase inhibition attenuated this drop in resistance suggesting that the increase in permeability induced by S1PR1 blockade requires shedding of VE-cadherin and/or other molecules that contribute to functional adherens junctions.

In vivo S1PR1 blockade in serum induced arthritis: NIBR-0213 treatment of mice subjected to serum induced arthritis exacerbated injury - based on clinical and histological assessments (Fig 3), suggesting S1PR1 signaling contributes to maintenance of EC barrier function and inhibition of S1PR1 signaling leads to vascular escape of mediators that contribute to tissue damage.

Conclusion Our studies indicate that EC S1PR1 signaling maintains barrier function, in part by restraining MMP-dependent cleavage of VE-cadherin, and thereby may protect against immune complex mediated injury in experimental models. In some patients with LN, markedly decreased EC S1PR1 expression may result in loss of barrier integrity and increased vulnerability to glomerular injury. We identify a potential new approach to attenuate renal immune complex driven glomerular injury – enhancement of EC barrier function through S1PR1 signaling. Future studies will test the role of EC S1PR1 signaling in a mouse model of lupus nephritis.

Lupus Nephritis

1117 NEUTROPHIL EXTRACELLULAR TRAPS AS A BIOMARKER TO PREDICT OUTCOMES IN LUPUS NEPHRITIS

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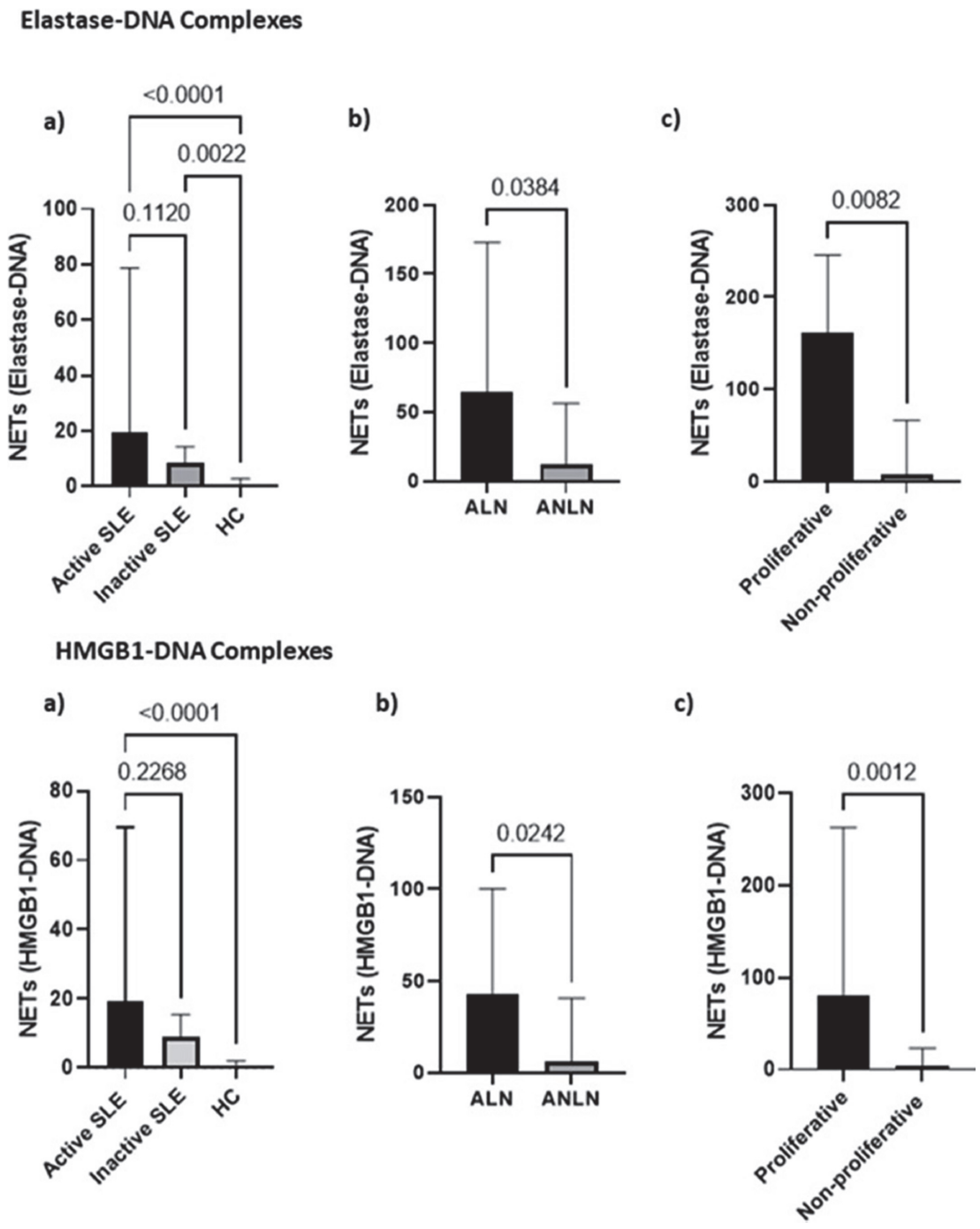
Background Neutrophil Extracellular Traps (NETs) have been implicated in Lupus Nephritis (LN) pathogenesis. SLE neutrophils release High Mobility Group Box-1 (HMGB1) protein, in turn, HMGB1 in NETs correlates with histologic findings of Active LN (ALN). The aim was to determine if the amount of NET complexes (Elastase-DNA and HMGB1-DNA) in serum at the time of a LN flare predicts renal outcomes in the following 24 months.

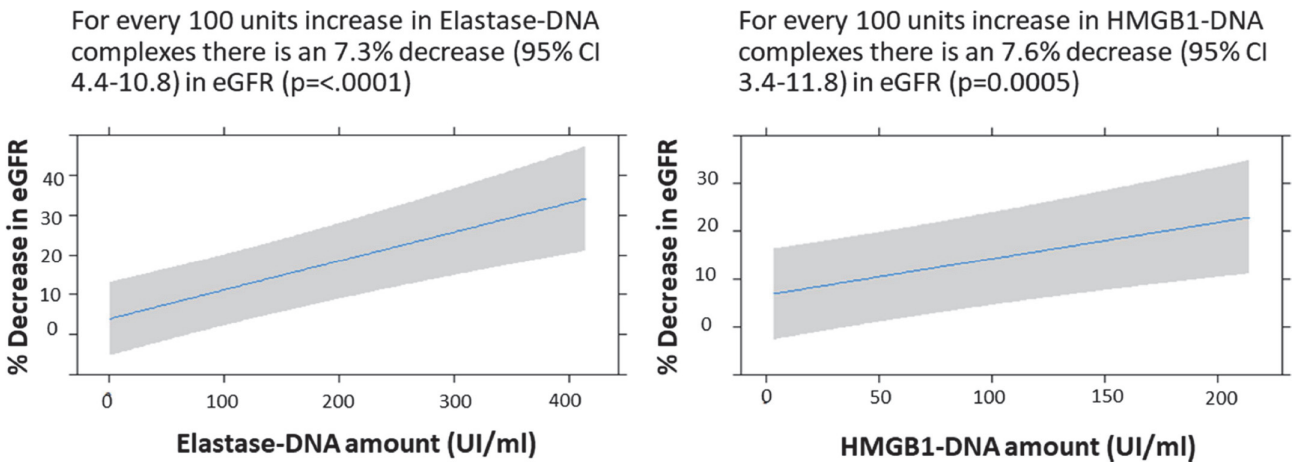
Methods The study had a 2-staged approach. In an exploratory cohort composed of active SLE (clinical SLEDAI $\geq$ 1), inactive SLE and healthy controls (HC), we assessed the association between our in-house ELISA assays for Elastase-DNA

and HMGB1-DNA complexes and ALN. A separate LN cohort was then used to determine the utility of NET complexes to predict renal outcomes over the subsequent 24 months. All patients had ALN, defined as a 24-hour urine protein >500mg with a subsequent modification in therapy by the treating physician, a baseline eGFR >30ml/min (3 months prior to the flare), stored serum sample ±3 months from the renal flare, and at least 2- years follow-up. The following outcomes were ascertained: Complete response (CR) at 12 and 24 months after flare (proteinuria <500mg/day and a serum creatinine within 15% of the baseline); severe renal impairment (eGFR≤30ml/min) at 12 and 24 months after flare; and the percentage decline in the eGFR over the 24 months after flare.

Results Ninety-two individuals were included in the exploratory cohort (49 active SLE, 23 inactive SLE and 20 HC). NET complexes were significantly higher in SLE patients compared to HC and tended to be higher in active SLE compared



Abstract 1117 Figure 1 Comparison of NET complex levels (Elastase-DNA and HMGB1-DNA) between (a) active SLE (n=49), inactive SLE (n=23) and HC (n=20); (b) ALN (n=18) and active non LN (ANLN, n=31); (c) Proliferative (n=7) and non-Proliferative LN (n=6). All graphs represent the media with IQR. Kruskal- Wallis Test was used to assess the differences in NET complex levels between Active SLE, Inactive SLE and HC and Mann-Whitney test to assess the differences between ALN and ANLN and between Proliferative and non-Proliferative LN. Units for all graphs are in UI/ml.



Abstract 1117 Figure 2 Linear regression analysis showing a linear relationship between the amount of Elastase-DNA and HMGB1-DNA complexes and the decline in kidney function in the following 2 years. *Adjusted to age, sex, ethnicity and immunosuppressive therapy received in the prior 3 months

Abstract 1117 Table 1 Logistic Regression analysis*. Higher baseline levels of NET complexes increase the odds of non-response to therapy and severe renal impairment in the following 2 years after the LN flare **(N=109)**

	12 months after renal flare		24 months after renal flare	
Variables (at baseline, ±3 months from the LN flare)	**Odds Ratio (95% CI)**	**P value**	**Odds Ratio (95% CI)**	**P value**
Failure to achieve complete renal response				
Elastase-DNA	1.68 (0.98-2.88)	0.056	2.00 (1.00-3.79)	**0.05**
HMGB1-DNA	2.17 (1.10-4.26)	**0.02**	1.93 (1.04-3.60)	**0.03**
24-hour proteinuria	2.03 (0.95-4.32)	0.06	1.11 (0.55-2.24)	0.75
Anti dsDNA ab	1.71 (0.79-3.65)	0.16	1.55 (0.75-3.21)	0.23
C3	0.77 (0.46-1.27)	0.31	0.71 (0.43-1.17)	0.18
C4	0.96 (0.58-1.79)	0.88	0.87 (0.50-1.49)	0.62
Presence of severe renal impairment (eGFR≤30ml/min)				
Elastase-DNA	1.34 (0.96-1.87)	0.08	1.68 (1.15-2.45)	**0.006**
HMGB1-DNA	1.77 (0.90-3.45)	0.09	1.86 (1.03-2.38)	**0.03**
Proteinuria	4.00 (1.25-12.87)	**0.02**	1.96 (0.75-5.06)	0.16
Anti dsDNA ab	1.20 (0.41-3.49)	0.73	2.32 (0.96-5.51)	0.056
C3	1.93 (0.59-3.41)	0.53	0.76 (0.63-1.61)	0.48
C4	1.52 (0.77-2.97)	0.22	0.98 (0.45-2.09)	0.96

*The Logistic Regression analysis for each of the variables of interest was adjusted for age, sex, ethnicity and immunosuppressive therapy received in the prior 3 months.

to inactive patients. Patients with ALN (36.7%) had significantly higher levels of NET complexes compared to active SLE without LN. Furthermore, patients with proliferative LN had higher levels of NET complexes compared to non-proliferative LN (figure 1).

The LN cohort included 109 ALN patients. The median (IQR) age was 29 (23-41) years, 84% were women, and disease duration was 6.4 (0.8-10.5) years. 37.9% were Caucasian, 22.2% Black and 17.5% Asian, the baseline eGFR was 112 (97-127) ml/min. 77.9% had a kidney biopsy at the time of the LN flare, of whom 55.9% had a proliferative or mixed class, 17.4% class V, and 4.5% class I or II. 39.4% and 50.5% of the ALN patients achieved CR at 12 and 24 months, respectively and 11% had an eGFR ≤ 30ml/min after 24 months.

Similar to the results from the exploratory cohort, proliferative LN had higher levels of NET complexes compared to non-proliferative LN patients (Elastase-DNA: 111.7 vs 25.9, p=0.0003; HMGB1-DNA: 85.2 vs 25.4, p=0.002, proliferative vs non-proliferative, respectively). Patients with higher baseline levels of NET complexes had higher odds of not achieving CR and of having severe renal impairment after 24 months of the flare. NET complexes outperformed conventional biomarkers (table 1). There was a linear relationship between the amount of baseline Elastase-DNA and HMGB1-DNA complexes and the decline in renal function in the subsequent 24 months (figure 2).

Conclusions Elastase-DNA and HMGB1-DNA complexes predicted renal outcomes, including response to therapy and decline in kidney function at 2 years after the LN flare.

1201 IMPACT STUDY: PRELIMINARY RESULTS OF A TRIAL WITH A BIOLOGIC TO PREVENT PREECLAMPSIA IN WOMEN WITH ANTIPHOSPHOLIPID SYNDROME

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Background Pregnant women with antiphospholipid antibodies and/or lupus have higher rates of adverse pregnancy outcomes (APOs), such as fetal loss and preterm birth due to severe preeclampsia (PE) or placental insufficiency (PI). The presence of lupus anticoagulant (LAC) is the strongest predictor of an APO. At present, there is no effective treatment for women with these high-risk pregnancies, but in an animal model that mimics this human condition we found that TNF-α was a critical downstream effector of abnormal placental development and fetal damage, and that TNF-α blockade normalized placentation and spiral artery remodeling, and rescued pregnancies. We sought to determine whether TNF-α blockade during pregnancy, added to a regimen of heparin and low dose aspirin, reduces the rate of APOs in women with clinical APS and LAC.