

Association of the Interferon Signature Metric with serological disease manifestations but not global activity scores in multiple SLE patient cohorts

William P. Kennedy,¹ Romeo Maciuca,² Kristen Wolslegel,³ Wei Tew,³ Alexander R. Abbas,⁴ Christina Chaivorapol,⁴ Alyssa Morimoto,⁵ Jacqueline M. McBride,⁶ Paul Brunetta,⁷ Bruce C. Richardson,⁸ John C. Davis, Jr.,¹ Timothy W. Behrens,⁹ Michael J. Townsend³

¹Departments of Early Clinical Development, Genentech, South San Francisco, CA, USA

²Biostatistics, Genentech, South San Francisco, CA, USA

³ITGR Diagnostics Discovery, Genentech, South San Francisco, CA, USA

⁴Bioinformatics and Computational Biology, Genentech, South San Francisco, CA, USA

⁵Bioanalytical Sciences, Genentech, South San Francisco, CA, USA

⁶Pharmacodynamic Biomarkers ITGR, Genentech, South San Francisco, CA, USA

⁷Late Stage Immunology Product Development, Genentech, South San Francisco, CA, USA

⁹ITGR Human Genetics, Genentech, South San Francisco, CA, USA

⁸Department of Medicine, University of Michigan and the Ann Arbor VA Hospital, Ann Arbor, MI, USA

Corresponding author:

Michael J. Townsend, Ph.D.

ITGR Diagnostics Discovery

Genentech, Inc.

1 DNA Way

South San Francisco, CA 94080

Tel: 650-225-3239

Email: townsend.michael@gene.com

Key Words: Interferon Signature, SLE, lupus, disease activity, serological manifestations

SUPPLEMENTAL METHODS

SLE patient cohorts

University of Michigan SLE cohort

SLE patients (n=61) were enrolled at the University of Michigan into an observational cohort. Patient enrollment and sample collection were carried out under the supervision of the Institutional Review Board of the University of Michigan Medical School (IRBMED: protocol reference 2002-0339). Demographics and baseline disease characteristics are summarized in Supplemental Table 1. Healthy control subjects (n=20) were age-matched.

Rontalizumab Phase I trial¹⁶

Results of the trial have been published and are present on ClinicalTrials.gov (Identifier NCT00541749). Sixty patients were enrolled with mild disease activity as defined by SELENA-SLEDAI, and continued their pre-enrollment background medications, including nonsteroidal anti-inflammatory drugs (NSAIDs), anti-malarials, and steroids up to 20 mg/day prednisone equivalent, for lupus management.

EXPLORER: Assessment of flares in lupus patients enrolled in a Phase II/III study of rituximab¹⁷

Patients had moderate to severe active extra-renal lupus (ERL), with active disease defined according to the British Isles Lupus Disease Activity Group (BILAG) index, and stable use of 1 immunosuppressive drug at entry. Patients with severe central nervous system or organ-threatening lupus were excluded.

LUNAR: Lupus Nephritis Assessment with Rituximab¹⁸

Patients had Class III/IV LN according to the 2003 International Society of Nephrology/Renal Pathology Society criteria determined by renal biopsy within previous 12 months and presence of proteinuria.

ROSE: ROntalizumab Phase II Trial in Systemic lupus Erythematosus¹⁹

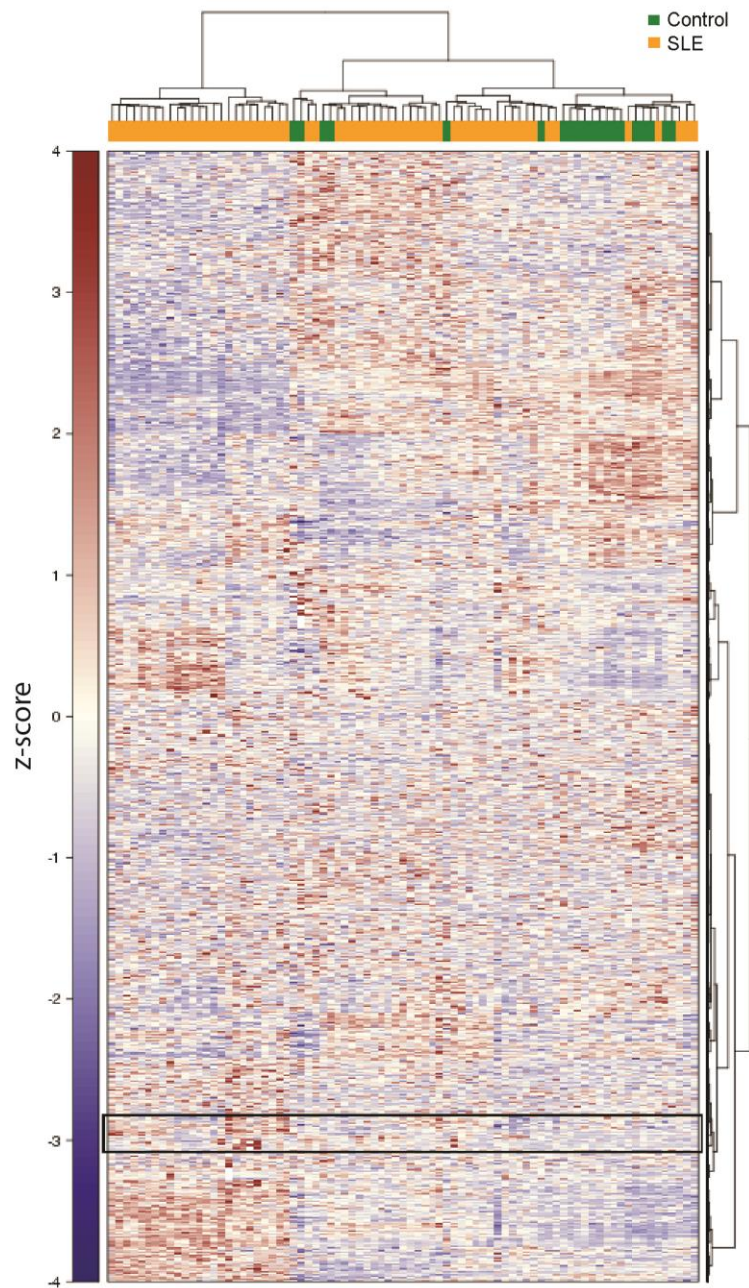
The trial is registered on ClinicalTrials.gov (identifier NCT00962832). Patients had moderate to severe active ERL (active disease defined according to the BILAG index; 1 BILAG A or 2 BILAG B domains of disease involvement and severity), and positive antinuclear antibody (ANA). Patients were excluded if they had lupus nephritis (LN), unstable neuropsychiatric SLE, or a recent history of severe anti-phospholipid antibody syndrome.

Blood RNA extraction and microarray analysis

Samples of RNA from all SLE cohorts were extracted from peripheral blood mononuclear cells (PBMCs) isolated by Ficoll gradient and/or from whole blood samples collected in PAXgene tubes using the RNeasy Mini Kit (Qiagen, Valencia, CA). PBMC RNA samples from the University of Michigan cohort were profiled on Affymetrix Human Genome U133 Plus 2.0 expression microarrays. The microarrays were MAS 5.0 normalized in R 3.0.0; then probe sets were filtered using the default settings of the featureFilter function in the geneFilter Bioconductor package²⁰ to result in one probe set per gene. Samples and filtered probe sets were hierarchically clustered using a Euclidean distance metric and Ward linkage method using hclust (R stats package). Twenty clusters were derived using cutree (k=20) on the probe set dendrogram. Heatmap plots were generated in R. Probe sets were mapped to genes using the hgu133plus2.db (v. 2.9.0) Bioconductor annotation package. Differential expression of genes was assessed by linear modeling via the Limma package (Bioconductor). Moderated *t*-statistics from modeling were used to calculate the adjusted *p* values, using the Benjamini-Hochberg correction. Differential expression was defined as gene expression greater than 1.5-fold and the adjusted *p* value for the difference in gene expression was less than 0.2. Microarray

data have been deposited in the Gene Expression Omnibus data repository (GEO accession number GSE50772).

Supplemental Figure 1: Unsupervised hierarchical clustering of microarray expression data from the University of Michigan SLE cohort. Filtered microarray probes generated from microarray analysis of 20 healthy control subjects and 61 SLE patients underwent unsupervised clustering. The left sided figure bar is the heatmap color scale and refers to z-score values. The location of the Interferon Regulated Gene cluster is demarcated with an open box. The bar above the heatmap refers to diagnosis (SLE in yellow, healthy control in green) for each subject sample (pertaining to each column of the heatmap).



Supplemental Table 1: Demographics and clinical characteristics of the University of Michigan SLE observational cohort

Parameter	University of Michigan SLE cohort		
	ISM-Low (n=28)	ISM-High (n=33)	Total (N=61)
Age (years), mean (SD)	39 (9.7)	37.6 (11.05)	38.2 (10.4)
SLE duration (years), mean (SD)	8.2 (8.3)	11.6 (8.3)	9.7 (8.3)
Female, %	96	97	97
Race/Ethnicity, %			
Black	29	33	31
Hispanic	4	0	2
White	64	67	66
Other	3	0	1
SELENA-SLEDAI, mean (SD)	5.8 (3.1)	5.6 (3.2)	5.7 (3.1)
Proteinuria (> 0.5 g), %	13.6	14.3	14
Serum creatinine (mg/dL), mean (SD)	1.03 (0.43)	0.85 (0.28)	0.94 (0.37)
Prednisone usage (mg), mean (SD)	20.3 (21.3)	18.7 (17.7)	19.4 (19.3)

Supplemental Table 2: Baseline peripheral blood populations in SLE trials stratified by ISM status

Parameter	Trial	ISM-Low	ISM-High	p-value
White Blood Cell Count (x10 ⁹ /L)	LUNAR Trial (N=80)	9.88 (2.6)	7.07 (3.6)	0.0012
	EXPLORER Trial (N=135)	9.04 (4.2)	6.47 (3.5)	0.0003
	ROSE Trial (N=238)	7.20 (2.8)	5.54 (2.5)	<0.0001
	Phase I Rontalizumab Trial (N=60)	6.37 (2.2)	5.26 (1.9)	0.0323
Lymphocytes (absolute counts x10 ⁹ /L)	LUNAR Trial (N=80)	2.46 (1.7)	1.37 (0.9)	0.0158
	EXPLORER Trial (N=135)	1.54 (1.0)	1.09 (0.6)	0.0154
	ROSE Trial (N=238)	1.76 (0.8)	1.15 (0.6)	<0.0001
	Phase I Rontalizumab Trial (N=60)	1.99 (0.8)	1.59 (0.6)	0.0608
Neutrophils (absolute counts x10 ⁹ /L)	LUNAR Trial (N=80)	6.78 (1.8)	5.22 (3.0)	0.0107
	EXPLORER Trial (N=135)	7.02 (3.9)	4.99 (3.2)	0.0036
	ROSE Trial (N=238)	4.94 (2.6)	4.0 (2.2)	0.0087
	Phase I Rontalizumab Trial (N=60)	3.87 (1.6)	3.19 (1.5)	0.0781
Monocytes (absolute counts x10 ⁹ /L)	LUNAR Trial (N=80)	0.45 (0.19)	0.34 (0.2)	0.0149
	EXPLORER Trial (N=135)	0.34 (0.2)	0.30 (0.3)	0.0341
	ROSE Trial (N=238)	0.34 (0.1)	0.28 (0.2)	0.0007
	Phase I Rontalizumab Trial (N=60)	0.32 (0.1)	0.32 (0.1)	0.81
Eosinophils (absolute counts x10 ⁹ /L)	LUNAR Trial (N=80)	0.09 (0.08)	0.08 (0.07)	0.5662
	EXPLORER Trial (N=135)	0.08 (0.08)	0.04 (0.06)	0.001
	ROSE Trial (N=238)	0.10 (0.07)	0.07 (0.06)	<0.0001
	Phase I Rontalizumab Trial (N=60)	0.14 (0.11)	0.11 (0.09)	0.14

Data shown as mean (SD) for baseline values as defined by last pre-treatment value. P values calculated using the Wilcoxon test.

Supplemental Table 3: Multivariate linear model for the ISM

Intercept refers to the free term of the linear regression model:

Predicted ISM = -4.045 -0.0013 * CD4 + 0.810 * (anti-ENA status = Positive) + 0.706*Ln (BAFF)
+ 0.205*Ln (anti-dsDNA) - 0.049 *(SLE duration)

Parameter	Coefficient Estimate	t statistic	p-value
Intercept	-4.045	-2.68	0.0079
CD4 counts (/μL)	-0.0013	-4.48	<0.0001
anti-ENA status (Positive vs. Negative)	0.810	3.71	0.0003
Ln(BAFF (IU/mL))	0.706	3.86	0.0002
Ln(anti-dsDNA (IU/mL))	0.205	3.10	0.0022
SLE duration (years)	-0.049	-3.16	0.0018

The t-statistic equals the coefficient estimate divided by the standard error used to derive a p-value for each coefficient.

Supplemental Table 4: Reproducibility of ISM^a categorization over 36 weeks in the placebo arm of the ROSE study

ISM Value	Patients (n)	P_{rep} %
≤ -0.5	11	97
$> -0.5, \leq 1$	6	81
$> 1 \text{ to } \leq 2$	11	85
$> 2 \text{ to } \leq 3$	19	98
≥ 3	14	100
All patients	61	94 (95% CI; 91 to 97)

CI, confidence interval; P_{rep} , probability of reproducibility

^a Data generated using a research-grade assay

Supplemental Table 5: Identity of Affymetrix probes for 128 Interferon-regulated gene cluster identified in the University of Michigan SLE cohort PBMC microarray analysis

Probe Set ID	Gene Symbol	Gene Title
1552623_at	HSH2D	hematopoietic SH2 domain containing
1555251_a_at	OTOF	otoferlin
1556643_at	LOC100507535	uncharacterized LOC100507535
1568592_at	TRIM69	tripartite motif containing 69
200814_at	PSME1	proteasome (prosome, macropain) activator subunit 1 (PA28 alpha)
200887_s_at	STAT1	signal transducer and activator of transcription 1, 91kDa
200923_at	LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein
200986_at	SERPING1	serpin peptidase inhibitor, clade G (C1 inhibitor), member 1
201315_x_at	IFITM2	interferon induced transmembrane protein 2
201641_at	BST2	bone marrow stromal cell antigen 2
201649_at	UBE2L6	ubiquitin-conjugating enzyme E2L 6
201762_s_at	PSME2	proteasome (prosome, macropain) activator subunit 2 (PA28 beta)
201786_s_at	ADAR	adenosine deaminase, RNA-specific
202086_at	MX1	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)
202145_at	LY6E	lymphocyte antigen 6 complex, locus E
202307_s_at	TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)
202411_at	IFI27	interferon, alpha-inducible protein 27
202446_s_at	PLSCR1	phospholipid scramblase 1
202688_at	TNFSF10	tumor necrosis factor (ligand) superfamily, member 10
202869_at	OAS1	2'-5'-oligoadenylate synthetase 1, 40/46kDa
203153_at	IFIT1	interferon-induced protein with tetratricopeptide repeats 1
203236_s_at	LGALS9	lectin, galactoside-binding, soluble, 9
203258_at	DRAP1	DR1-associated protein 1 (negative cofactor 2 alpha)
203595_s_at	IFIT5	interferon-induced protein with tetratricopeptide repeats 5
203773_x_at	BLVRA	biliverdin reductase A
203882_at	IRF9	interferon regulatory factor 9
203964_at	NMI	N-myc (and STAT) interactor
204279_at	PSMB9	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)
204415_at	IFI6	interferon, alpha-inducible protein 6
204439_at	IFI44L	interferon-induced protein 44-like
204698_at	ISG20	interferon stimulated exonuclease gene 20kDa

204804_at	TRIM21	tripartite motif containing 21
204858_s_at	TYMP	thymidine phosphorylase
204972_at	OAS2	2'-5'-oligoadenylate synthetase 2, 69/71kDa
204994_at	MX2	myxovirus (influenza virus) resistance 2 (mouse)
205098_at	CCR1	chemokine (C-C motif) receptor 1
205126_at	VRK2	vaccinia related kinase 2
205241_at	SCO2	SCO cytochrome oxidase deficient homolog 2 (yeast)
205483_s_at	ISG15	ISG15 ubiquitin-like modifier
205569_at	LAMP3	lysosomal-associated membrane protein 3
205660_at	OASL	2'-5'-oligoadenylate synthetase-like
206513_at	AIM2	absent in melanoma 2
207777_s_at	SP140	SP140 nuclear body protein
208436_s_at	IRF7	interferon regulatory factor 7
208966_x_at	IFI16	interferon, gamma-inducible protein 16
209417_s_at	IFI35	interferon-induced protein 35
209546_s_at	APOL1	apolipoprotein L, 1
209568_s_at	RGL1	ral guanine nucleotide dissociation stimulator-like 1
209593_s_at	TOR1B	torsin family 1, member B (torsin B)
209762_x_at	SP110	SP110 nuclear body protein
210873_x_at	APOBEC3 A	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A
211012_s_at	PML	promyelocytic leukemia
211138_s_at	KMO	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)
211267_at	HESX1	HESX homeobox 1
212203_x_at	IFITM3	interferon induced transmembrane protein 3
212380_at	FTSJD2	FtsJ methyltransferase domain containing 2
212657_s_at	IL1RN	interleukin 1 receptor antagonist
213051_at	ZC3HAV1	zinc finger CCCH-type, antiviral 1
213261_at	TRANK1	tetratricopeptide repeat and ankyrin repeat containing 1
213293_s_at	TRIM22	tripartite motif containing 22
213294_at	EIF2AK2	eukaryotic translation initiation factor 2-alpha kinase 2
213361_at	TDRD7	tudor domain containing 7
214022_s_at	IFITM1	interferon induced transmembrane protein 1
214453_s_at	IFI44	interferon-induced protein 44
214511_x_at	FCGR1B	Fc fragment of IgG, high affinity lb, receptor (CD64)
217933_s_at	LAP3	leucine aminopeptidase 3
217986_s_at	BAZ1A	bromodomain adjacent to zinc finger domain, 1A
218076_s_at	ARHGAP 17	Rho GTPase activating protein 17
218231_at	NAGK	N-acetylglucosamine kinase
218400_at	OAS3	2'-5'-oligoadenylate synthetase 3, 100kDa
218429_s_at	C19orf66	chromosome 19 open reading frame 66

218543_s_at	PARP12	poly (ADP-ribose) polymerase family, member 12
218943_s_at	DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58
218986_s_at	DDX60	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60
219014_at	PLAC8	placenta-specific 8
219062_s_at	ZCCHC2	zinc finger, CCHC domain containing 2
219209_at	IFIH1	interferon induced with helicase C domain 1
219211_at	USP18	ubiquitin specific peptidase 18
219352_at	HERC6	HECT and RLD domain containing E3 ubiquitin protein ligase family member 6
219356_s_at	CHMP5	charged multivesicular body protein 5
219364_at	DHX58	DEXH (Asp-Glu-X-His) box polypeptide 58
219371_s_at	KLF2	Kruppel-like factor 2 (lung)
219519_s_at	SIGLEC1	sialic acid binding Ig-like lectin 1, sialoadhesin
219684_at	RTP4	receptor (chemosensory) transporter protein 4
219863_at	HERC5	HECT and RLD domain containing E3 ubiquitin protein ligase 5
220146_at	TLR7	toll-like receptor 7
221680_s_at	ETV7	ets variant 7
221816_s_at	PHF11	PHD finger protein 11
221827_at	RBCK1	RanBP-type and C3HC4-type zinc finger containing 1
222154_s_at	SPATS2L	spermatogenesis associated, serine-rich 2-like
222986_s_at	SHISA5	shisa homolog 5 (<i>Xenopus laevis</i>)
223220_s_at	PARP9	poly (ADP-ribose) polymerase family, member 9
223322_at	RASSF5	Ras association (RalGDS/AF-6) domain family member 5
223501_at	TNFSF13B	tumor necrosis factor (ligand) superfamily, member 13b
223599_at	TRIM6	tripartite motif containing 6
224701_at	PARP14	poly (ADP-ribose) polymerase family, member 14
225076_s_at	ZNFX1	zinc finger, NFX1-type containing 1
225291_at	PNPT1	polyribonucleotide nucleotidyltransferase 1
225344_at	NCOA7	nuclear receptor coactivator 7
225415_at	DTX3L	deltex 3-like (<i>Drosophila</i>)
225636_at	STAT2	signal transducer and activator of transcription 2, 113kDa
226603_at	SAMD9L	sterile alpha motif domain containing 9-like
226702_at	CMPK2	cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial
226748_at	LYSMD2	LysM, putative peptidoglycan-binding, domain containing 2
226757_at	IFIT2	interferon-induced protein with tetratricopeptide repeats 2
227609_at	EPSTI1	epithelial stromal interaction 1 (breast)
228152_s_at	DDX60L	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60-like
228230_at	PRIC285	peroxisomal proliferator-activated receptor A interacting complex 285
228439_at	BATF2	basic leucine zipper transcription factor, ATF-like 2

228531_at	SAMD9	sterile alpha motif domain containing 9
228617_at	XAF1	XIAP associated factor 1
229350_x_at	PARP10	poly (ADP-ribose) polymerase family, member 10
229450_at	IFIT3	interferon-induced protein with tetratricopeptide repeats 3
230166_at	KIAA1958	KIAA1958
230405_at	C5orf56	chromosome 5 open reading frame 56
231455_at	LINC00487	long intergenic non-protein coding RNA 487
231577_s_at	GBP1	guanylate binding protein 1, interferon-inducible
231747_at	CYSLTR1	cysteinyl leukotriene receptor 1
231769_at	FBXO6	F-box protein 6
232222_at	C18orf49	chromosome 18 open reading frame 49
236285_at	KLHDC7B	kelch domain containing 7B
238327_at	ODF3B	outer dense fiber of sperm tails 3B
238439_at	ANKRD22	ankyrin repeat domain 22
242020_s_at	ZBP1	Z-DNA binding protein 1
242625_at	RSAD2	radical S-adenosyl methionine domain containing 2
32069_at	N4BP1	NEDD4 binding protein 1
35254_at	TRAFD1	TRAF-type zinc finger domain containing 1
38269_at	PRKD2	protein kinase D2