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APPENDICES

APPENDIX A

SYSTEMIC LUPUS ERYTHEMATOSUS DISEASE ACTIVITY INDEX
SELENA MODIFICATION

Physicians Global Assessment.....

0	1	2	3
None	Mild	Med	Severe

SLEDAI SCORE

Check box: If descriptor is present at the time of visit or in the proceeding 10 days

Wt	Present	Descriptor	Definition
8	☐	Seizure	Recent onset. Exclude metabolic, infectious or drug cause
8	☐	Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behavior. Excluded uremia and drug causes.
8	☐	Organic Brain Syndrome	Altered mental function with impaired orientation, memory or other intelligent function, with rapid onset fluctuating clinical features. Include clouding of consciousness with reduced capacity to focus, and inability to sustain attention to environment, plus at least two of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious or drug causes.
8	☐	Visual Disturbance	Retinal changes of SLE. Include cytoid bodies, retinal hemorrhages, serious exodate or hemorrhages in the choroids, or optic neuritis. Exclude hypertension, infection, or drug causes.
8	☐	Cranial Nerve Disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8	☐	Lupus Headache	Severe persistent headache: may be migrainous, but must be nonresponsive to narcotic analgesia.
8	☐	CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis
8	☐	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual, infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis
4	☐	Arthritis	More than 2 joints with pain and signs of inflammation (i.e. tenderness, swelling, or effusion).
4	☐	Myositis	Proximal muscle aching/weakness, associated with elevated creatine

			phosphokinase/adolase or electromyogram changes or a biopsy showing myositis.
4	☐	Urinary Casts	Heme-granular or red blood cell casts
4	☐	Hematuria	>5 red blood cells/high power field. Exclude stone, infection or other cause.
4	☐	Proteinuria	>0.5 gm/24 hours. New onset or recent increase of more than 0.5 gm/24 hours.
4	☐	Pyuria	>5 white blood cells/high power field. Exclude infection.
2	☐	New Rash	New onset or recurrence of inflammatory type rash.
2	☐	Alopecia	New onset or recurrence of abnormal, patchy or diffuse loss of hair.
2	☐	Mucosal Ulcers	New onset or recurrence of oral or nasal ulcerations
2	☐	Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2	☐	Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram confirmation.
2	☐	Low Complement	Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory.
2	☐	Increased DNA binding	>25% binding by Farr assay or above normal range for testing laboratory.
2	☐	Fever	>38°C. Exclude infectious cause
1	☐	Thrombocytopenia	<100,000 platelets/mm3
1	☐	Leukopenia	<3,000 White blood cell/mm3. Exclude drug causes.

____ TOTAL SCORE (Sum of weights next to descriptors marked present)

Mild or Moderate Flare ☐	Severe Flare ☐
☐ Change in SLEDAI > 3 points	☐ Change in SLEDAI > 12
☐ New/worse discoid, photoscnsitive, profundus, cutaneous vasculitis, bullous lupus Nasopharyngeal ulcers Pleuritis Pericarditis Arthritis Fever (SLE)	☐ New/worse CNS-SLE Vasculius Nephritis Myositis Pk < 60.000 Home anemia: Hb <7% or decrease in Hb >3% Requiring: double prednisone Prednisone>0.5 mg/kg/day hospitalization
☐ Increase in Prednisone, but not to >0.5 mg/kg/day	☐ Prednisone >0.5 mg/kg/day
☐ Added NSAID or Plaquenil	☐ New Cytoxan, Azathioprine, Methotrexate, Hospitalization (SLE)
☐ ≥1.0 Increase in PGA, but not to more than 2.5	☐ Increase in PGA to > 2.5

APPENDIX B

THE TABLE OF CLINICAL AND GENE EXPRESSION RAW DATA

No.	Patho_no.	Age	UPCI	RBC	Prot_24Hr.	Serum_Cr
1	SP47-00517	35.00	999.00	30.00	3.97	2.30
2	SP47-00652	22.00	3.70	25.00	0.72	0.60
3	SP46-01081	41.00	999.00	999.00	1.05	1.70
4	SP47-00455	41.00	999.00	12.00	1.17	2.20
5	SP46-00406	34.00	999.00	8.00	1.84	999.00
6	SP46-01081	30.00	999.00	30.00	2.19	0.70
7	SP46-01009	35.00	0.44	3.00	0.69	1.00
8	SP47-00241	26.00	999.00	40.00	6.03	999.00
9	SP46-00739	18.00	999.00	2.00	999.00	0.90
10	SP47-00383	18.00	999.00	60.00	999.00	0.50
11	SP46-00845	22.00	999.00	20.00	0.97	0.90
12	SP46-00595	36.00	4.58	3.00	5.34	3.40
13	SP46-00879	34.00	999.00	20.00	999.00	0.60
14	SP47-00186	34.00	1.15	999.00	1.25	0.50
15	SP46-00895	26.00	999.00	20.00	2.27	0.90
16	SP46-00963	49.00	999.00	40.00	1.21	0.90
17	SP46-00678	24.00	999.00	999.00	2.50	1.00
18	SP46-00115	30.00	999.00	1.00	3.16	4.30
19	SP46-00826	22.00	999.00	15.00	7.74	0.90
20	SP46-00406	29.00	999.00	30.00	0.11	1.00
21	SP46-00264	31.00	999.00	50.00	999.00	999.00
22	SP46-00419	35.00	4.13	5.00	4.13	0.70
23	SP46-00187	23.00	999.00	0.00	3.58	4.20
24	SP46-00600	23.00	11.30	510.00	999.00	4.90
25	SP47-00584	23.00	999.00	100.00	2.33	5.10
26	SP46-00624	22.00	5.04	50.00	999.00	0.60
27	SP47-00281	22.00	4.00	90.00	999.00	0.80
28	SP46-00803	38.00	999.00	1.00	2.00	1.50
29	SP47-00455	18.00	999.00	12.00	999.00	0.70
30	SP47-00115	23.00	0.74	0.00	3.12	0.70
31	SP46-00943	27.00	999.00	1.00	1.18	0.80
32	SP47-00077	31.00	999.00	1.00	0.20	1.30
33	SP46-00553	23.00	999.00	1.00	1.83	1.00
34	SP47-00207	23.00	999.00	999.00	0.29	1.00
35	SP46-00141	32.00	999.00	80.00	0.40	0.70
36	SP46-00670	38.00	999.00	100.00	2.92	1.30
37	SP46-00600	29.00	3.08	999.00	999.00	5.00
38	SP47-00269	19.00	999.00	15.00	1.74	0.80
39	SP47-00360	27.00	999.00	1.00	3.25	0.80
40	SP46-00251	21.00	1.25	10.00	999.00	0.70
41	SP46-00278	32.00	999.00	80.00	9.10	2.20

No.	Patho_no.	Total_Glom	Percent interstitial infiltrate	Percent interstitial fibrosis	Percent tubular atrophy
1	SP47-00517	12.00	40.00	40.00	20.00
2	SP47-00652	11.00	0.00	0.00	0.00
3	SP46-01081	5.00	5.00	30.00	30.00
4	SP47-00455	16.00	30.00	50.00	50.00
5	SP46-00406	16.00	5.00	4.00	4.00
6	SP46-01081	22.00	0.00	0.00	0.00
7	SP46-01009	4.00	0.00	4.00	4.00
8	SP47-00241	24.00	15.00	10.00	10.00
9	SP46-00739	16.00	4.00	4.00	4.00
10	SP47-00383	1.00	10.00	10.00	10.00
11	SP46-00845	999.00	999.00	999.00	999.00
12	SP46-00595	20.00	35.00	75.00	75.00
13	SP46-00879	14.00	5.00	5.00	5.00
14	SP47-00186	14.00	4.00	4.00	4.00
15	SP46-00895	999.00	999.00	999.00	999.00
16	SP46-00963	12.00	0.00	0.00	0.00
17	SP46-00678	5.00	4.00	4.00	4.00
18	SP46-00115	16.00	10.00	26.67	26.60
19	SP46-00826	35.00	10.00	10.00	10.00
20	SP46-00406	8.00	0.00	0.00	0.00
21	SP46-00264	13.00	0.00	0.00	0.00
22	SP46-00419	15.00	5.00	4.00	4.00
23	SP46-00187	3.00	4.00	4.00	4.00
24	SP46-00600	15.00	15.00	15.00	15.00
25	SP47-00584	30.00	30.00	80.00	80.00
26	SP46-00624	4.00	50.00	4.00	4.00
27	SP47-00281	1.00	10.00	10.00	10.00
28	SP46-00803	6.00	30.00	30.00	30.00
29	SP47-00455	28.00	4.00	5.00	5.00
30	SP47-00115	18.00	5.00	10.00	10.00
31	SP46-00943	9.00	0.00	5.00	4.00
32	SP47-00077	18.00	10.00	26.67	26.60
33	SP46-00553	9.00	0.00	10.00	10.00
34	SP47-00207	49.00	40.00	45.00	45.00
35	SP46-00141	9.00	4.00	4.00	4.00
36	SP46-00670	999.00	999.00	999.00	999.00
37	SP46-00600	7.00	15.00	90.00	90.00
38	SP47-00269	999.00	999.00	999.00	999.00
39	SP47-00360	20.00	4.00	4.00	4.00
40	SP46-00251	5.00	0.00	0.00	0.00
41	SP46-00278	11.00	80.00	4.00	4.00

No.	Patho_no.	Percent wireloop	Percent glomPNMs>=2	Percent thrombotic microangiopathy	Percent crescent formation
1	SP47-00517	50.00	33.33	8.33	25.00
2	SP47-00652	0.00	0.00	0.00	0.00
3	SP46-01081	0.00	20.00	0.00	0.00
4	SP47-00455	0.00	18.75	0.00	0.00
5	SP46-00406	62.50	25.00	0.00	6.25
6	SP46-01081	31.82	13.64	0.00	4.55
7	SP46-01009	0.00	25.00	0.00	0.00
8	SP47-00241	66.67	66.67	0.00	20.83
9	SP46-00739	100.00	0.00	0.00	12.50
10	SP47-00383	100.00	0.00	0.00	0.00
11	SP46-00845	999.00	999.00	999.00	999.00
12	SP46-00595	20.00	#NULL!	15.00	20.00
13	SP46-00879	0.00	14.29	0.00	35.71
14	SP47-00186	0.00	0.00	0.00	0.00
15	SP46-00895	999.00	999.00	999.00	999.00
16	SP46-00963	0.00	8.33	0.00	0.00
17	SP46-00678	0.00	0.00	0.00	0.00
18	SP46-00115	18.75	6.25	0.00	0.00
19	SP46-00826	80.00	20.00	0.00	11.43
20	SP46-00406	0.00	0.00	0.00	0.00
21	SP46-00264	53.85	0.00	0.00	0.00
22	SP46-00419	26.67	13.33	0.00	0.00
23	SP46-00187	66.67	100.00	0.00	66.67
24	SP46-00600	0.00	6.67	13.33	26.67
25	SP47-00584	0.00	20.00	3.33	13.33
26	SP46-00624	25.00	25.00	0.00	25.00
27	SP47-00281	100.00	0.00	0.00	0.00
28	SP46-00803	0.00	0.00	0.00	0.00
29	SP47-00455	3.57	10.71	0.00	10.71
30	SP47-00115	0.00	11.11	0.00	0.00
31	SP46-00943	0.00	0.00	0.00	0.00
32	SP47-00077	0.00	0.00	0.00	0.00
33	SP46-00553	0.00	0.00	0.00	0.00
34	SP47-00207	0.00	0.00	0.00	2.04
35	SP46-00141	0.00	0.00	0.00	0.00
36	SP46-00670	999.00	999.00	999.00	999.00
37	SP46-00600	14.29	14.29	0.00	14.29
38	SP47-00269	999.00	999.00	999.00	999.00
39	SP47-00360	0.00	5.00	0.00	0.00
40	SP46-00251	0.00	40.00	0.00	20.00
41	SP46-00278	18.18	45.45	0.00	81.82

No.	Patho_no.	Percent endocapillary proliferation	Percent global sclerosis	Percent segmental sclerosis	Percent necrosis
1	SP47-00517	58.33	33.33	999.00	16.67
2	SP47-00652	9.09	0.00	0.00	0.00
3	SP46-01081	40.00	20.00	0.00	0.00
4	SP47-00455	12.50	50.00	12.50	6.25
5	SP46-00406	87.50	0.00	0.00	6.25
6	SP46-01081	40.91	0.00	0.00	4.55
7	SP46-01009	0.00	0.00	50.00	0.00
8	SP47-00241	100.00	0.00	0.00	29.17
9	SP46-00739	0.00	6.25	25.00	0.00
10	SP47-00383	100.00	0.00	0.00	0.00
11	SP46-00845	999.00	999.00	999.00	999.00
12	SP46-00595	45.00	25.00	20.00	0.00
13	SP46-00879	35.71	0.00	14.29	7.14
14	SP47-00186	0.00	21.43	14.29	0.00
15	SP46-00895	999.00	999.00	999.00	999.00
16	SP46-00963	0.00	0.00	0.00	0.00
17	SP46-00678	20.00	0.00	0.00	0.00
18	SP46-00115	25.00	75.00	25.00	0.00
19	SP46-00826	48.57	14.29	0.00	14.29
20	SP46-00406	0.00	12.50	0.00	0.00
21	SP46-00264	23.08	0.00	0.00	0.00
22	SP46-00419	53.33	20.00	999.00	0.00
23	SP46-00187	100.00	0.00	0.00	33.33
24	SP46-00600	53.33	6.67	26.67	0.00
25	SP47-00584	30.00	56.67	999.00	6.67
26	SP46-00624	100.00	0.00	0.00	25.00
27	SP47-00281	100.00	0.00	0.00	0.00
28	SP46-00803	0.00	16.67	33.33	0.00
29	SP47-00455	17.86	7.14	10.71	0.00
30	SP47-00115	0.00	22.22	11.11	0.00
31	SP46-00943	0.00	11.11	22.22	0.00
32	SP47-00077	0.00	22.22	33.33	0.00
33	SP46-00553	0.00	0.00	0.00	0.00
34	SP47-00207	0.00	10.20	4.08	0.00
35	SP46-00141	0.00	11.11	0.00	0.00
36	SP46-00670	999.00	999.00	999.00	999.00
37	SP46-00600	28.57	57.14	14.29	0.00
38	SP47-00269	#NULL!	999.00	999.00	999.00
39	SP47-00360	15.00	0.00	30.00	0.00
40	SP46-00251	80.00	0.00	20.00	0.00
41	SP46-00278	90.91	0.00	0.00	27.27

No.	Patho_no.	Activity score	Chronicity score	Class Dx	Log VEGF_expression
1	SP47-00517	16.00	6.00	4.00	-0.73
2	SP47-00652	1.00	0.00	3.00	-0.17
3	SP46-01081	4.00	5.00	4.00	-0.82
4	SP47-00455	7.00	9.00	3.00	-0.64
5	SP46-00406	12.00	0.00	4.00	-1.01
6	SP46-01081	9.00	0.00	3.00	-3.22
7	SP46-01009	2.00	0.00	3.00	-0.16
8	SP47-00241	16.00	2.00	4.00	-0.36
9	SP46-00739	5.00	2.00	3.00	-0.44
10	SP47-00383	7.00	2.00	4.00	-0.48
11	SP46-00845	999.00	999.00	999.00	-0.13
12	SP46-00595	12.00	8.00	4.00	-0.66
13	SP46-00879	9.00	1.00	3.00	-0.36
14	SP47-00186	0.00	2.00	4.00	-0.30
15	SP46-00895	999.00	999.00	999.00	-0.45
16	SP46-00963	0.00	0.00	5.00	-0.11
17	SP46-00678	999.00	1.00	3.00	-2.85
18	SP46-00115	6.00	7.00	4.00	-0.86
19	SP46-00826	11.00	3.00	4.00	-0.82
20	SP46-00406	0.00	1.00	2.00	-2.16
21	SP46-00264	3.00	0.00	3.00	-0.70
22	SP46-00419	6.00	1.00	4.00	-0.33
23	SP46-00187	19.00	0.00	4.00	-0.68
24	SP46-00600	8.00	5.00	4.00	-1.01
25	SP47-00584	11.00	10.00	4.00	-0.82
26	SP46-00624	17.00	0.00	4.00	-0.43
27	SP47-00281	7.00	2.00	4.00	-0.04
28	SP46-00803	2.00	7.00	3.00	-0.52
29	SP47-00455	5.00	2.00	3.00	-0.46
30	SP47-00115	1.00	4.00	5.00	0.04
31	SP46-00943	0.00	2.00	3.00	-0.50
32	SP47-00077	999.00	7.00	3.00	-0.73
33	SP46-00553	0.00	2.00	5.00	-0.33
34	SP47-00207	0.00	6.00	5.00	-1.32
35	SP46-00141	0.00	1.00	999.00	-0.34
36	SP46-00670	999.00	999.00	4.00	-0.27
37	SP46-00600	12.00	9.00	4.00	-1.05
38	SP47-00269	999.00	999.00	999.00	-0.30
39	SP47-00360	999.00	2.00	3.00	-0.92
40	SP46-00251	5.00	2.00	4.00	-0.96
41	SP46-00278	19.00	0.00	4.00	-0.95

APPENDIX C

IMMUNOHISTOCHEMISTRY REAGENTS

1. Phosphate Buffered Saline (PBS), 30x conc.

NaCl	526	g
$NaH_2PO_4H_2O$	82.8	g
5N NaOH	120	ml
DW maker up to	2000	ml

1. DAB Tris-HCl Buffer, pH 7.4

- Tri-HCl buffer, pH 7.4

Stock A: 2.42 g Trizma base (Sigma T-1503) in 100 ml DW

Stock B: 1.7 ml conc. HCl in 100 ml DW

To make Tris-HCl buffer, pH 7.4

Stock A	2.5	ml
Stock B	2.07	ml

Make up to 10 ml with DW

- DAB (Sigma D-5637) (3.3 -Diaminobenzidine tetrahydrochloride, anhydrous)

DAB 50 mg/ ml in DW, aliquot to microtube 100 ul/tube (store at 0°)

- Working DAB Tris-HCl buffer, pH 7.4 (made up fresh)

DAB	1 microtube
Tris-HCl buffer	10 ml
30% H_2O_2	10 ul

2. Working PBS pH 7.4

30X PBS	70 ml
DW	2030 ml

3. 3% NHS in PBS pH7.4

Working PBS pH7.4	100 ml
NHS	3 ml

4. 0.1% NHS+0.01% TritonX-100 in PBS

Working PBS pH7.4	1000 ml
NHS	1 ml
TritonX-100	100 ul

5. 10mM Sodium Citrate Buffer pH6.0

Tri-sodium citrate (dehydrate)	2.94 g
Distilled water	1000 ml

Mix to dissolve. Adjust pH to 6.0 with 1N HCl and then add 0.5 ml of Tween 20 and mix well. Store this solution at 4°c.

6. Tris-EDTA buffer, pH9.0 (10mM Tris, 1mM EDTA, pH9.0) in DW 10 Liters

Tris	12.11 g
EDTA	3.72 g

7. Coated Slides

Reagent 2% 3-aminopropyltriethoxysilane (sigma A3648) in acetone

1. Rinse slides in acetone 2 changes
2. Rinse slides in 2% 3-aminopropytricthoxysilane 10 seconds.
3. Quick rinse in acetone
4. Rinse in deionized water
5. Air dried or in oven

8. 1M Imidazole

Imidazole (MW 68.08)	6.808 g
DW	100 ml

9. DAB with 1M Imidazole

Tris-HCl pH7.4	10 ml
30% H_2O_2	10 ul

DAB	5 mg
1 M Imidazole	100 ul

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Original articles

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