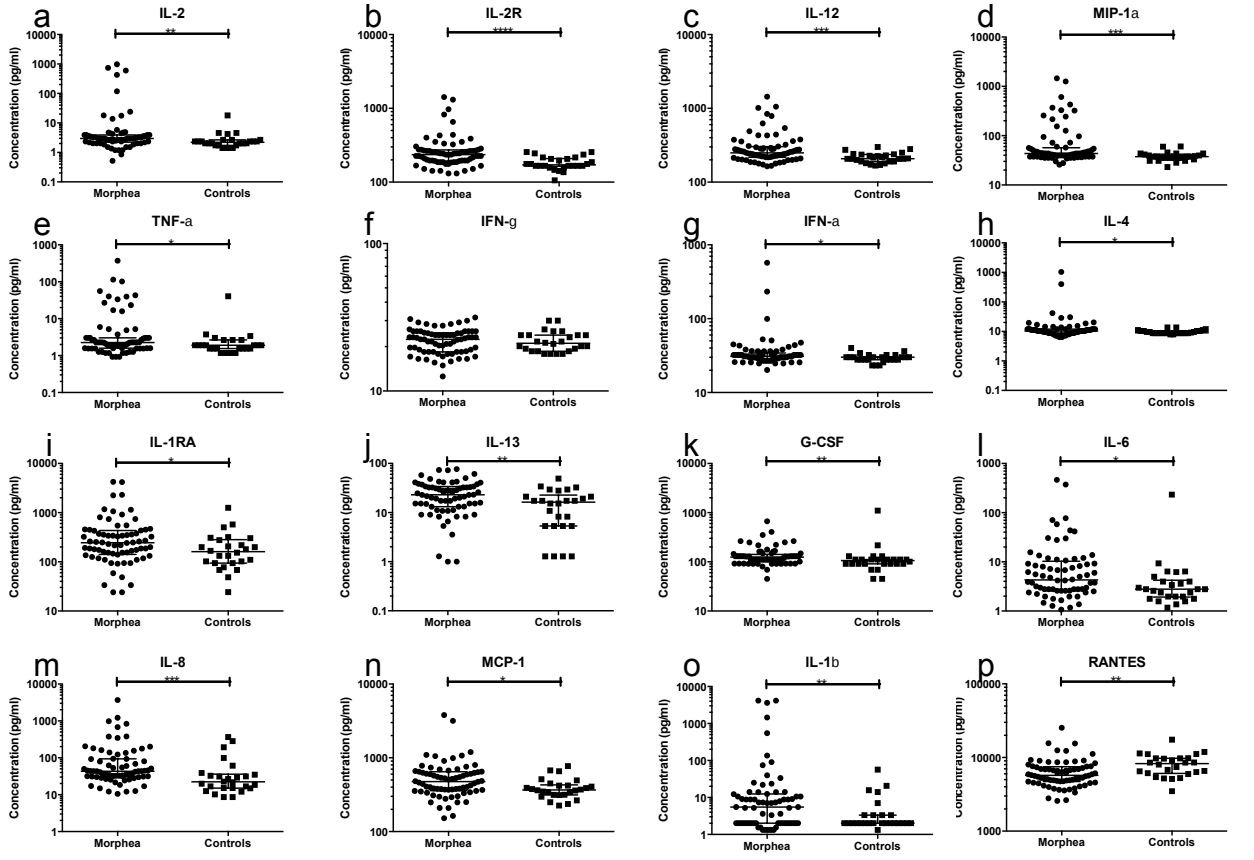
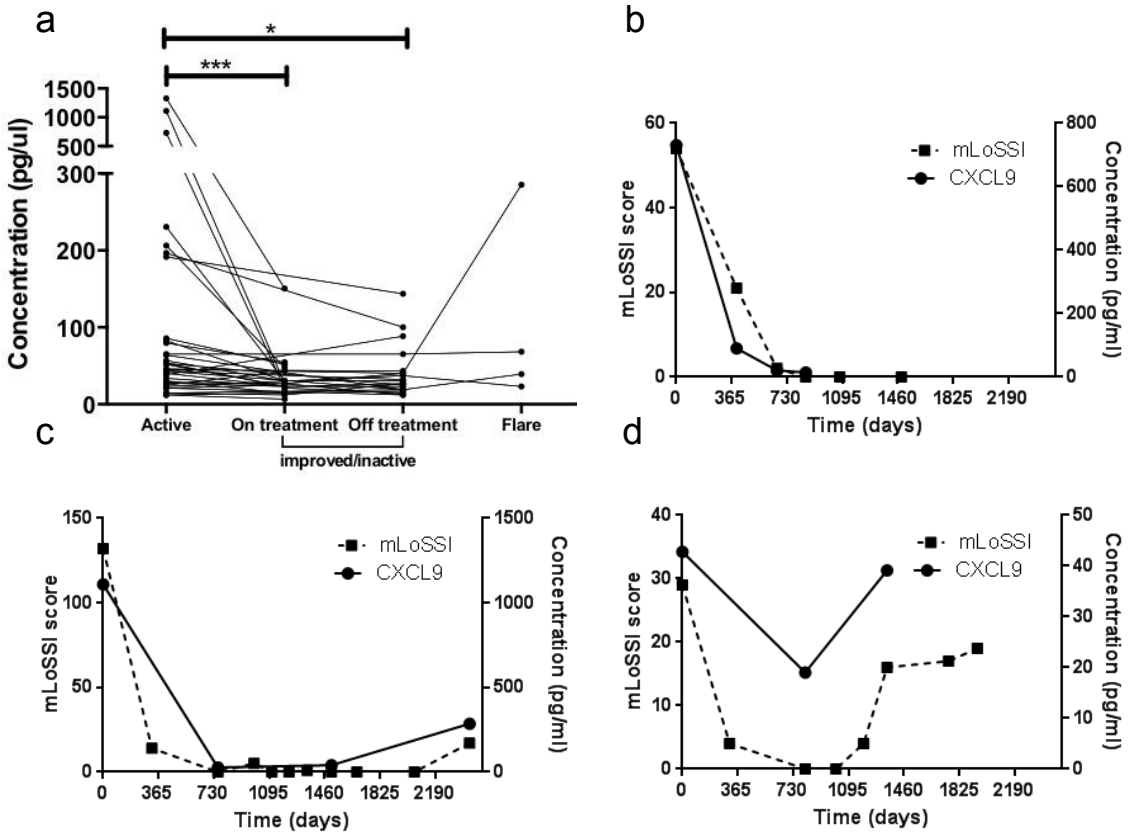
<u>Supplemental Figure 1.</u> Cytokines are elevated in morphea serum.



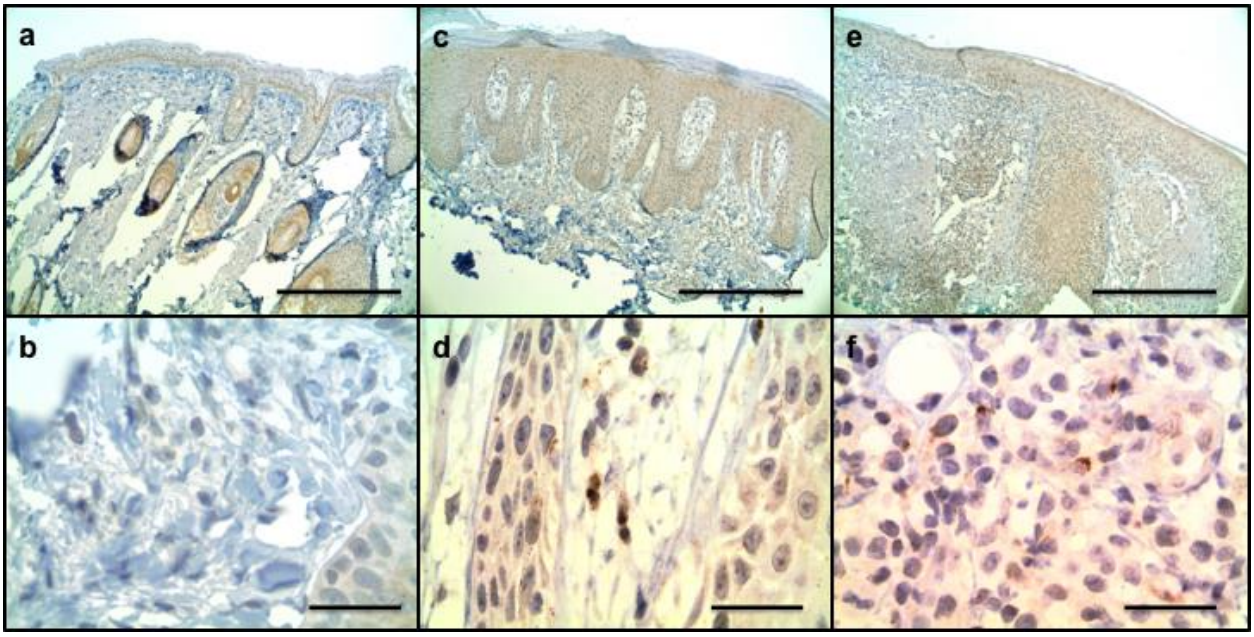
IL-2 (a), IL-2R (b), IL-12 (c), MIP-1α (d), TNF-α (e), IFN-α (g), IL-4 (h), IL-1RA (i), IL-13 (j), G-CSF (k), IL-6 (l), IL-8 (m), MCP-1 (n), and IL-1β (o) were increased in morphea serum. IFN-γ (f) was not elevated. RANTES (p) was decreased in morphea serum.

<u>Supplemental Figure 2.</u> CXCL9 mirrors disease activity over time.



In longitudinally-followed participants, CXCL9 serum levels were increased in active disease, at normal levels while inactive on treatment and after treatment was discontinued, and increased with flare in some participants (a). Representative concentrations of CXCL9 (solid line) and mLoSSI score (dashed line) are pictured (b-d).

Supplemental Figure S3. CXCL9 immunohistochemistry in normal and inflamed skin.



CXCL9 staining is absent in the dermis of normal skin controls (a, b). CXCL9 staining is present in inflamed psoriasis (c, d) and mycosis fungoides (e, f) skin. Inflamed skin immunohistochemistry demonstrates the typical perinuclear staining pattern for CXCL9. Scale bar (a, c, e) = 100 μm; scale bar (b, d, f) = 25 μm.

Supplemental Materials and Methods:

Whole Blood Transcriptional Profiling

Peripheral blood was drawn in PAXgene™ tubes (PreAnalytiX) via phlebotomy. Total-RNA extraction was performed with the PAXgene™ Blood RNA kit (PreAnalytiX). For skin, 4 mm punch biopsies were taken from the inflammatory border of active morphea lesions. Collected tissue was stored in RNALater® solution and frozen at -20°C. RNA was extracted with the RNeasy Lipid Tissue kit (Qiagen, Hilden, Germany) and stored at -80°C. All samples were hybridized to microarray bead-chips (Human WG6 v3.0, Illumina) at the UT Southwestern Microarray Core facility. Gene expression analysis was performed with GeneSpring GX (Agilent Technologies) Version 11.0.2 with significant genes considered to be those with a fold change of at least 1.5 and p-value of <0.05.

Lesional skin PCR

Whole RNA extracted from lesional and site-matched non-lesional skin biopsies obtained from morphea participants was preserved in RNALater. Primers for genes of interest (CXCL9 and CXCL10) and the housekeeping gene GADPH were used. RNA extraction was conducted by the UT Southwestern Core with RNA quantity was measured using UV spectroscopy. The quality was determined using the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). Only samples with a RNA Integrity Number (RIN) ≥ 7 were used for analysis with RT-PCR. Samples were run in triplicate. The extracted RNA was converted to cDNA using Bio-Rad iScript cDNA Synthesis Kits (Bio-Rad Hercules, CA, USA). Real time PCR was run using Bio-Rad SYBER Green assays and a Bio-Rad CFX PCR Detection System was used to run the PCR. Cycle threshold (Ct) values were obtained from the RT-PCR, which were then used to calculate the delta Ct values of each of the genes of interest from the housekeeping gene GAPDH. Gene

expression was normalized to the housekeeping gene GAPDH and average fold changes were determined.

Immunohistochemistry protocol

Formalin-fixed paraffin-embedded tissues (multi-tumor sandwich blocks) (Miller, 1993) were sectioned at 4 microns and mounted on adhesive slides, along with case tissue being studied. After drying, the slides were deparaffinized in xylene and rehydrated in graded alcohols to distilled water. Endogenous peroxidase activity was quenched for 10 minutes at room temperature, using 0.3% H_2O_2 with 0.1% Sodium Azide added.

For all immunostains, epitope retrieval was performed by placing the slides in 1mM EDTA, pH 8.5 for 30 minutes in a household vegetable steamer, followed by a 10 minute cool-down time in the steamer. After rinsing the slides in phosphate buffered saline (PBS) buffer, primary antibody incubation with anti-CD4 (rabbit monoclonal, clone EPR6855, 1:100, Abcam), anti-CD8 (mouse monoclonoal, clone 4B11, 1:50, Leica), anti-CD20 (mouse monoclonal, clone L26, 1:120, Leica), anti-CD68 (mouse monoclonal, clone PG-M1, 1:30, Thermo/Neomarkers), anti-CD34 (rabbit monoclonal, clone EP373Y, 1:800, Abcam), anti-CD123 (mouse monoclonal, clone BR4MS, 1:100, Leica), anti-SMA (mouse monoclonal, clone 1A4, 1:200, Thermo/Neomarkers), anti-CXCL9 (rabbit polyclonal, 1:1000, Abcam), and anti-CXCR3/CD183, (mouse monoclonal, clone 1C6/CXCR3, 1:10, BD Biosciences) was performed for 50 minutes at room temperature, using gentle orbital rotation at 40 rpm (Butz, 1994). Following another rinse in PBS, incubation with anti-mouse or anti-rabbit horseradish peroxidase-conjugated polymer (PowerVision Poly-HRP anti-Mouse IgG or PowerVision Poly-HRP anti- Rabbit IgG, Leica) (Shi et al., 1999) was performed for 45 minutes at 25˚C, using

gentle orbital rotation at 40 rpm. The slides were then immersed for 8 minutes in 25˚C diaminobenzidine (DAB) (Invitrogen) to develop the brown colored staining.

Next, the slides were placed back in a 0.3% H2O2/0.1% Sodium Azide solution for 10 minutes at room temperature to quench endogenous peroxidase activity, as described above. Epitope retrieval was again performed, this time by placing the slides in a 0.25M Tris solution, pH 9.0, for 48 minutes in a pressure cooker (Miller, 1995). After rinsing the slides in phosphate buffered saline (PBS) buffer, antibody incubation (Rabbit polyclonal anti-MIG/CXCL9, diluted 1:1000, Abcam) was performed for 50 minutes at room temperature, using gentle orbital rotation at 40 rpm. Following another rinse in PBS, incubation with anti-rabbit horseradish peroxidase-conjugated polymer was performed for 45 minutes at RT as described above. The slides were treated with VIP chromagen (Vector Labs) for 8 minutes at 25˚C to develop the purple colored staining. Lastly, the stains were enhanced with 0.5% copper sulfate in phosphate buffered saline (PBS) for 1 minute at 25˚C, counterstained in hematoxylin, dehydrated in graded alcohols, cleared in xylene, and coverslipped.

REFERENCES

Butz WC, KC; Miller, RT. Improved Manual Immunohistochemistry Employing Orbital Mixing of Reagents During Incubations. Appl Immunohistochem 1994;2(1):65-7.

Miller R. Multitumor "Sandwich" Blocks in Immunohistochemistry. Simplified Method of Preparation and Practical Uses. Appl Immunohistochem 1993;1(2):156-9.

Miller RE, CE. Heat-Induced Epitope Retrieval with a Pressure Cooker: Suggestions for Optimal Use. Appl Immunohistochem 1995;3(3):190-3.

Shi S-R, Guo J, Cote RJ, Young LL, Hawes D, Shi Y, et al. Sensitivity and Detection Efficiency of a Novel Two-Step Detection System (PowerVision) for Immunohistochemistry. Applied Immunohistochemistry & Molecular Morphology 1999;7(3):201.

Supplemental Table 1. Participant demographics and characteristics.

	All participants with morphea		Luminex cohort		Healthy controls		Blood transcriptional profiling		Skin transcriptional profiling	
Number	87*		72		26		19		7	
Age, mean (SD)	47.5	(21.1)	49.7	(20.2)	52.0	(17.8)	44.5	(21.6)	55.7	(16.0)
Sex, No. (%)										
Female	70	(80%)	57	(79%)	19	(73%)	14	(74%)	7	(100%)
Male	17	(20%)	15	(21%)	7	(27%)	5	(26%)	0	(0%)
Ethnicity, No. (%)										
Caucasian	61	(70%)	50	(69%)	18	(69%)	13	(68%)	5	(71%)
Hispanic	17	(20%)	14	(19%)	5	(19%)	3	(16%)	2	(28%)
African American	4	(5%)	4	(6%)	1	(4%)	1	(5%)	0	(0%)
Other	5	(6%)	4	(6%)	2	(8%)	2	(11%)	0	(0%)
Subtype, No. (%)										
Generalized	49	(56%)	43	(60%)	N/A		13	(68%)	5	(71%)
Linear	28	(32%)	21	(29%)	N/A		6	(32%)	1	(14%)
Plaque	8	(9%)	6	(8%)	N/A		0	(0%)	1	(14%)
Mixed	2	(2%)	2	(3%)	N/A		0	(0%)	0	(0%)
Disease features, No. (%)										
Adult onset	75	(86%)	64	(89%)	N/A		16	(84%)	6	(86%)
Pediatric onset	12	(14%)	8	(11%)	N/A		3	(16%)	1	(14%)
Deep involvement	39	(45%)	35	(49%)	N/A		6	(32%)	2	(28%)
Functional impairment	21	(24%)	20	(28%)	N/A		3	(16%)	2	(28%)
Clinical scores, median (IQR)										
mLoSSI	18	(7-27)	19	(10-29)	N/A		18	(6-46)	16	(12-26)
LoSDI	11	(7-20)	11	(8-23)	N/A		15	(7-19)	15	(10-60)
PGA-A	50	(25-70)	45	(30-70)	N/A		60	(50-90)	40	(10-50)
PGA-D	20	(10-40)	20	(10-40)	N/A		35	(19-50)	10	(10-20)

* Nine participants in whole blood microarray were included in the Luminex panel.