AWARD NUMBER: W81XWH-19-1-0562

TITLE: Transcription Factor Analysis of SLE

PRINCIPAL INVESTIGATOR: Kathleen E. Sullivan

CONTRACTING ORGANIZATION: The Children's Hospital of Philadelphia
Philadelphia, PA

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PREPARED FOR: U.S. Army Medical Research and Development Command
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Transcription Factor Analysis of SLE

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**6. AUTHOR(S)**
Kathleen E Sullivan
E-Mail:

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
Purpose: Define transcription factors critical for SLE pathogenesis
Scope:

1) Expand chromatin marks to identify latent enhancers and regulatory regions relevant for SLE
2) Inhibit identified TF to define cellular effects

A key aspect was comparing different clinical subsets of SLE and using patients with rheumatoid arthritis as inflammatory controls
Major findings: Due to the pandemic, we only began our wet bench library preparation in January. We also suffered form the same pipeline supply chain issues as most molecular biology labs. Although we have data, we have not done any analysis.

**15. SUBJECT TERMS**
Lupus, epigenetics, transcription factors, chromatin

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
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| Unclassified | Unclassified | Unclassified | Unclassified | 12 | |

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## Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease affecting predominantly young women during their peak productive years. Typical manifestations of SLE include a photosensitive rash, glomerulonephritis, arthritis, and serositis. In the USA, 1.5 million people are thought to be affected with annual direct care costs of $15,000 and annual lost productivity costs of $9,000 per patient. In addition, people with lupus can have compromised fertility and have a 30% rate of disability, demonstrating the profound impact of this disease. Mortality is thought to be about 2.5 fold higher than the general population with some demographic groups (children, women of color, those living in poverty) having a poorer prognosis. Premature vascular disease and depression represent significant chronic medical burdens.

Our data have demonstrated a clearly altered epigenome in SLE patients, with both a global increase in H4 acetylation and higher peaks of H4ac at critical interferon-response genes. The transcriptome was also shown by us to be broadly altered, with increased splicing, expression of novel loci, as well as the expected increased expression of some interferon-responsive genes. Our ChIP-seq studies of SLE monocytes demonstrated a limited set of TF motifs, which gives us confidence in this approach and supports extension to B cells and T cells. We have proposed that the **altered epigenome contributes both to disease phenotype and also to persistence of disease** by facilitating and perpetuating pathologic gene expression in immunologically competent cells.

## Objective

Our ultimate objective is to cure SLE. Today it is considered a chronic disease with fairly established management options and the idea of cure is radical. Nevertheless, we foresee a time where the immune system can be re-set using epigenetic therapies. This proposal represents an early effort to innovate a new approach using rational targeting of the epigenome based on an unbiased analysis of transcription and epigenetic changes.

## Specific Aims

**Aim 1: We will expand the chromatin marks we previously examined to better identify latent enhancers. We will identify differences and commonalities among H3K4me3, H3K4me1, H3K27ac, and p300 patterns in CD4/CD45RO T cells, CD19 B cells, and CD14 monocytes from SLE patients. From the promoter and enhancer chromatin marks with differential peak height, we will derive a set of TFs via position weight matrix (PWM) analysis for further study.** Therapeutic efforts must address the broad range of dysfunction seen in immunologically competent cells in SLE. The goal is to identify altered TF and signaling pathways common to all three cell types.

**Aim 2: Inhibition of TFs using siRNA in cells from donors with SLE will be evaluated for effects on the epigenome.** The ultimate goal of our focus on TFs is to find a druggable pathway common to all three cell types in SLE. This Aim will test that hypothesis directly.

## Impact

SLE has not benefitted from the many therapeutic advances in targeting the immune system that have benefitted other patients in Rheumatology. A completely new approach is needed and we have robust data to support efforts directed at the epigenome.

**Key words**
Lupus, epigenetics, histone marks, chromatin

**Accomplishments**

**Statement of work milestones**

| Task | Subtask | Subjects | Recruited | Prepared for library | Library runs |
|---|---|---|---|---|---|
| Aim 1A | ChIP-seq on SLE and controls | 40 SLE<br>(10 cutaneous, 10 nephritis)<br>20 RA<br>20 HC | 80 subjects- completed | 216 completed<br>24 subjects - pending | H3K4me3 138<br>H3K27ac 90<br>P300 90<br>GST 6 |
| | RNA-seq on SLE and controls | 40 SLE<br>(10 cutaneous, 10 nephritis)<br>20 RA<br>20 HC | 80- completes | 0 | 0 |
| | PWM matrix | 40 SLE<br>(10 cutaneous, 10 nephritis)<br>20 RA<br>20 controls | 80- completed | N/A | N/A |
| Aim 1B | ChIP assay confirmation | 20 SLE<br>20 controls | 40- completed | N/A | N/A |
| | qRT-PCR | 20 SLE<br>20 controls | 4- completed | N/A | N/A |
| Aim 1C | Compare clinical subsets | 10 Nephritis<br>10 Cutaneous | 20- completes | 60- completed | 0 |
| Aim 2A | IRF1 and ATF3 ChIP-seq | 10 SLE<br>10 controls | 20- completed | 0 | 0 |
| Aim 2B | IRF1 and ATF3 KD | 10 SLE<br>10 controls | 20- completed | N/A | N/A |
| Aim 2C | Other TF | 10 SLE<br>10 controls | 20- completed | N/A | N/A |

**Results and outcomes**

To date, our emphasis has been on completing recruitment and preparing samples as rapidly as possible. While the pandemic delayed our recruitment and our wet bench work due to supply chain issues faced by many in molecular biology, we have rapidly operationalized and have process all these samples just since January 2021.

We do not have any analyzed data at this time but project completion of sequencing runs in November, 2021. We had hoped to have a preliminary analysis at this point but have not been able to process sufficient data. Nevertheless, I believe that we will complete this study on time.

Training and professional development: Nothing to report.

Opportunities for training: The Research Associate working on library preparation has gained skills in epigenetics and improved her knowledge of chromatin dynamics.

Dissemination: Nothing to report

Plans for the next reporting period: At our current rate of ChIP preparation and library runs, we will complete the wet bench portion by November 2021. At that point, we will focus on data analysis of ChIP-seq runs and transcription factor knockdown studies.

**Impact**

Impact on principal disciplines: Nothing to report

Impact on the base of knowledge: Nothing to report

Impact on other disciplines: Nothing to report

Impact on technology transfer: Nothing to report

Impact on commercial technology: Nothing to report

Impact on society: Nothing to report

**Changes Problems**

This has surely been a period of major challenges. The pandemic was a huge detriment to our Program. We began our negotiations to have our IRB approved in January 2020. After revisions in March of 2020, right at the beginning of the pandemic, Johns Hopkins, our subject referral site, closed its clinics and CHOP, the site of the went bench work, closed its labs. We had our first patient recruitment in September 2020, just a year ago. In light of that delay and the many issues with pipette tips and kit availability, we have made enormous and impressive progress. We will still complete this study, although the timeline has shifted. The delay had an effect on our budget because we had to pay the subcontract for longer than anticipated. We therefore have less funds for library preparation.

**Products**

Publications: Nothing to report

Books or other non-periodicals: Nothing to report

Other publications: Nothing to report

Website: Nothing to report

Technologies or techniques: Nothing to report

Inventions, patens, licenses: Nothing to report

Other Products: Nothing to report.

Reportable outcomes: Nothing to report

**Participants and other collaborating organizations**

Name: Kathleen Sullivan
Role: PI
ORCID: 0000-0003-4018-1646
Nearest person months worked:
Contribution: Study design and execution
Other sources of funding: See following list

Name: Michelle Petri
Role: Co-investigator
ORCID: 0000-0003-1441-5373
Nearest person months worked:
Contribution: Recruitment and study design
Other sources of funding: See list

Has there been a change in the other support of the PI/key personnel? Yes. Please see listing below

**Sullivan, KE Other support**
Currently active and grants that have ended
Changes from beginning of DOD noted in Effort line

Department of Defense (PI: Sullivan) 9/15/19-9/14/22 0.6 cm
W81XWH-19-1-0562
Title: SLE Transcription Factor
*We are studying the role of ATF3 and other transcription factors in the etiology of SLE. Using ChIP-seq for chromatin marks and transcription factors in various cells, we will define pathways related to disease.*
Effort: Current project. Effort unchanged
No overlap in topic or studies

NIH (PI: Sullivan) 9/20/18-6/30/22 1.2 cm
5U01HG010219-02
Title: UDN Clinical Site
*The UDN is a national network focused on the use of advanced technologies to diagnose patients with rare diseases. CHOP is one of the sites.*
Effort: decreased from 2.4 to 1.2 calendar months.
No overlap in topic or studies

Primary Immune Deficiency Treatment Consortium (PI: Puck) 9/13/2019-8/31/2024 0.24 cm
5U54AI082973-12
Title: Primary Immune Deficiency Treatment Consortium
This support will be used to support patient advocacy group involvement in the PIDTC and to support development of PIDTC clinical research protocols.
Effort: No changes to effort
No overlap in topic or studies

National Institute of Diabetes and Digestive and Kidney Diseases (PI: Kelsen) 7/01/2021 – 7/01/26 0.6cm
R01DK127044-01A1
Title: Integration of genomics and transcriptomics to investigate biological pathways in very early onset IBD
The major goal of this project is to integrate genomics and transcriptomics to characterize the underlying drivers of very early onset inflammatory bowel disease.
Effort: New project
No overlap in topic or studies. This grant studies IBD.

NIH (PI: Kathleen Sullivan) 6/12/18-5/31/21 1.2cm
5R21AI130967-02
Title: Persistent Rubella
Effort: Project ended. No salary support currently

NIH (PI: Sullivan) 4/1/2015 - 3/31/2020 1.2cm
5U24AI086037-10
Title: Resources to Assist Investigations in Primary Immunodeficiency Diseases
Effort: In NCE without salary currently

Lupus Foundation of America (PI: Kathleen Sullivan) 10/1/14- 12/30/20 1.8 cm
Title: HER2 in Lupus Nephritis
Effort: Project ended. No salary support currently.

Alliance for Lupus Research 7/1/16-6/30/19 0.6 cm
BRISC DUB activity as a Novel Target for Lupus
Role: Co-Investigator
Effort: Project ended. No salary support currently

**Michelle Petri Other support**
Currently active and grants that have ended
Changes from beginning of DOD noted in Effort line

Department of Defense- The Children's Hospital of Philadelphia (PI: Sullivan) 7/1/19-6/30/22 .60 cm
W81XWH-19-1-0562
Title: Transcription Factor Analysis of SLE
Effort: Current Project. No change in effort.
Overlap: Current project.

NIH (PI: Petri) 7/1/16-6/30/22 4.2 cm
R01 AR069572
Title: Hopkins Lupus Cohort
Effort: No change in effort.
Overlap: This cohort represents the base population for patient recruitment and the clinical data will be used in analyses. The effort does not overlap because the DOD grant effort supports data analysis of the project data.

NIH (PI: Petri) 9/4/14-5/31/22 1.2 cm
UH2AR067679
Title: Accelerating Medicines Partnership in RA and Lupus: Network Research Sites (UH2/UH3)
Effort: No change
Overlap: This study is focused on biomarkers of outcome in SLE. No overlap.

Astra Zeneca (PI: Petri) 12/1/17-10/31/22 .24 cm
N/A
Title: Systemic Lupus Erythematosus (SLE) Prospective Observational
Effort: no change
Overlap: This supports an observational study of interferon signature and QOL. No overlap.

APS Action (PI: Petri) 11/1/12-9/5/25 .12 cm
N/A
Title: Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking Database and Repository
Effort: No change in effort

Overlap: This supports development of a registry and database for APS. No overlap.

Exagen Diagnostics (PI: Petri) 12/1/16-2/14/26 .12 cm
N/A
Title: Exagen: prospective analysis of complement on red blood cells
Effort: No change in effort
Overlap: This study supports measuring complement on red cells. No overlap.

Eli Lilly (PI: Petri) 9/15/12-12/31/22 .12 cm
N/A
Title: Disease Progression, Treatment Patterns and Outcomes of a SLE US Cohort
Effort: No change in effort
Overlap: This study defines burden of disease in SLE. No overlap.

NIH/NIAID (PI: Andrade) 2/1/20-1/31/22 .60 cm
R21 AI147598-01A1
Title: Autoimmunity to LINE-1-encoded antigens in SLE pathogenesis
Goal: The goal of this project is to understand the significance of antibodies to LINE-1 encoded antigens in SLE
Effort: New Project
Overlap: This study evaluates antibodies to LINE elements in SLE. There is no overlap in topic or studies

GlaxoSmithKline (PI: Petri) 5/1/19-2/1/22 .12 cm
N/A
Title: Urinary Proteomics as a Marker of Lupus Nephritis Outcome in the GSK Belimumab Trial
Effort: No change in effort
Goal: the analysis of potential urinary biomarkers of lupus nephritis using the Human Kiloplex Quantitative Proteomics
Overlap: The analysis will be done on GSK urine samples from the BLISS-LN Phase 3 clinical trial, assessing biomarkers of nephritis. There is no overlap.

FNIH (PI: Petri) 12/13/19-12/31/21 .12 cm
N/A
Title: AMP RA SLE_FNIH
Effort: new project.
Goal: This grant funds the analysis of potential urinary biomarkers of lupus nephritis using the Human Kiloplex Quantitative Proteomics Array and then validate these proteins as biomarkers for lupus nephritis outcomes. The analysis will be done on AMP Phase 2 urine samples
New Project
There is no overlap in topic or studies

Janssen (PI: Petri) 3/1/20-4/30/22 .20cm
N/A
Title: Relationship Between Remission, Lupus Low Disease Activity State, and Disease Flares on Healthcare Resource Utilization and Mortality in Individuals with Systemic Lupus Erythematosus (SLE): Results from the Hopkins SLE Cohort Study
Effort: New Project
Overlap: This study looks at the impact of disease activity reduction on direct healthcare resource utilization and mortality. There is no overlap in topic or studies

Thermo Fischer Scientific Phadia (PI: Petri) 3/1/20-1/22/22 .20 cm
N/A
Title: Urine Biomarkers for Lupus Nephritis
Effort: new project.
Overlap: Determine if the titers of two urine proteins can serve as biomarkers that can predict an upcoming LN flare. There is no overlap in topic or studies

University of Alabama (PI: Brown) 9/1/18-8/30/23 .24 cm
5R01AR073850
Title: Characterization of the lupus nephritis microRNAome

Effort: No change
Overlap: Define genetics of renal involvement and organ damage associated with systemic lupus erythematosus (SLE). There is no overlap in topic or studies

University of Houston (PI: Mohan) 3/8/19-2/29/24 .60 cm
R01 AR074096
Title: Monitoring Disease in Lupus
Effort: New project
Overlap: Identify biomarkers that can be used to monitor disease course in lupus nephritis. There is no overlap in topic or studies.

Systemic Lupus International Clinics Consortium (PI: Petri) 10/1/11-12/31/25 .12 cm
N/A
Title: Cancer Risk in Systemic Lupus
Effort: No change
Overlap: Establish an international consortium of sites to facilitate research studies in the field of lupus and related diseases. There is no overlap in topic or studies

GlaxoSmithKline (PI: Petri) 9/25/19-8/31/22 .12 cm
N/A
Title: The Long-Term Clinical Outcomes of Lupus Nephritis - Reanalysis of the Dataset
Effort: New project.
Overlap: Updates to BLISS-LN (BEL114054) trial Primary Endpoint. There is no overlap in topic or studies

Hospital for Sick Children (Canada) (PI: Petri) 12/9/19-9/30/21 .10cm
N/A
Title: Identifying risk factors for decline of kidney function in SLE
Effort: New project
Overlap: Collection of DNA samples to establish a longitudinal multiethnic cohort of SLE patients. There is no overlap in topic or studies

GlaxoSmithKline (PI: Petri) 10/3/20-10/2/21 .10 cm
N/A
Title: The link between SLE disease control, remission, LLDAS and long-term outcomes: Johns Hopkins Lupus cohort
Effort: New project
Overlap: The purpose of this study is to better understand disease activity and long-term outcomes such as organ damage. There is no overlap in topic or studies

Human Genome Sciences INC (GSK) (PI: Petri) 11/1/05-12/31/21 .10 cm
LBSL99
Title: A Multi-Center, Open-Label, Continuation Trial of LymphoStat-B™ Antibody (Monoclonal Anti-BLyS Antibody) in Subjects with Systemic Lupus Erythematosus (SLE) who Completed the Phase 2 Protocol LBSL02 (LBSL-99)
Effort: Was listed as completed but no cost extension was granted and project is active.
Overlap: The goal of this trial was to evaluate the long-term safety of LymphoStat. The trial ended several years ago. There is no overlap in topic or studies

Immunarray (PI: Petri) 11/1/12-10/30/20 .10 cm
N/A
Title: Immunarray: validation of new biomarkers in lupus
Effort: Project ended. No salary support currently.
Overlap: Immunarray will test serum samples from SLE patients to determine if a new blood test, “SLE Key”, will correlate with a disease activity index (SLEDAI) and can thus serve as a biomarker for lupus disease activity. No overlap.

Department of Defense (Stojan) 9/1/19-8/31/20
W81XWH-19-1-0793
Title: A spatial temporal analysis of organ-specific lupus flares in relation to atmospheric and environmental factors
Effort: Moved from pending, grant was awarded and is now completed

Overlap: Understanding lupus disease heterogeneity through cluster analysis. There is no overlap.

**Special reporting requirements**

Quad chart

# Transcription Factor Analysis of SLE

LR180127

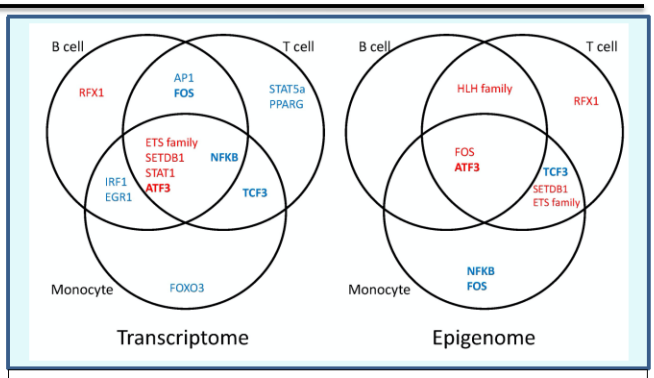
**PI:** Kathleen Sullivan **Org:** Children's Hospital of Philadelphia **Award Amount:** $524,610

**Study/Product Aim(s)**

- Examine chromatin marks in T cells, B cells and monocytes in SLE
- Identify putative transcription factors related to altered chromatin
- Test the role of the transcription factors in RNA expression

**Approach**

We will identify differences and commonalities among H3K4me3, H3K4me1, H3K27ac, and p300 patterns in CD4/CD45RO T cells, CD19 B cells, and CD14 monocytes from SLE patients. From the promoter and enhancer chromatin marks with differential peak height, we will derive a set of TFs via position weight matrix (PWM) analysis for further study. Inhibition of TFs using siRNA in cells from donors with SLE will be evaluated for effects on the epigenome.




This forms the basis of our approach. To date, we have completed patient recruitment and have prepped most of the libraries.

**Timeline and Cost**

| Activities CY | 19 | 20 | 21 | 22 |
|---|---|---|---|---|
| Patient recruitment | | | | |
| ChIP-seq for chromatin | | | | |
| ChIP-seq for transcription factors | | | | |
| TF inhibition/overexpression | | | | |
| **Estimated Budget ($K)** | **$000** | **$000** | **$000** | **$000** |

**Updated: September 3, 2021**

**Goals/Milestones (Example)**

**CY20 Goal** – Patient recruitment
Pipeline established, IRB in place
**CY20 Goals** – Begin ChIP-seq for histone marks
Antibodies quality check completed
**CY20 Goal** – Begin ChIP-seq for transcription factors
Immunoprecipitation done, testing completed
Libraries prepared and run
**CY21 Goal** – Transcription factor testing
Over-expression and under-expression in vitro

**Comments/Challenges/Issues/Concerns**

- We will complete all tasks but the start was delayed due to COVID.

**Budget Expenditure to Date**

Projected Expenditure: 350,000
Actual Expenditure: 372,205