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TITLE: Biomarker-Based Prediction Models for Response to Treatment in Systemic Sclerosis-Related Interstitial Lung Disease

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14. ABSTRACT Systemic sclerosis (SSc-Scleroderma) is associated with substantial morbidity and mortality. Interstitial lung disease (ILD) is the leading cause of disease-related mortality. <u>Response to immunosuppression is highly variable in patients with SSc related ILD.</u> The currently available clinical markers are inadequate for identifying patients who are more likely to respond to treatment. The utilized treatments are also associated with potentially serious adverse events, and their use should be reserved for highly responsive patients, further underscoring the critical need for development of reliable prediction tools. Our goal is to develop prediction tools using a combination of serum biomarkers and whole blood/skin gene expression data with potential clinical predictors. As the first step, key inflammatory serum proteins have been determined in the baseline samples of the Scleroderma Lung Study II (SLS II). We are currently analyzing whether these serum proteins have predictive significance for response to treatment in SSc related ILD.					
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1. Introduction:

Systemic sclerosis (SSc-scleroderma) is associated with substantial morbidity and mortality. Interstitial lung disease (ILD) is the primary cause of disease related death. Immunosuppressive agents such as mycophenolate mofetil and cyclophosphamide are used for treatment of SSc-ILD. However, response to these treatments is highly variable and the clinical predictors cannot reliably identify the likely responders. In this project, we use the valuable samples collected in the Scleroderma Lung Study II (SLSII) clinical trial and the observational cohort, GENISOS to identify and verify molecular predictors of response to treatment. For this purpose, novel technologies and analytic approaches will be used to determine key serum protein levels and transcript signatures in whole blood and skin samples collected in the SLSII study. The identified candidate molecular predictors in the SLSII will be verified in the GENISOS cohort during the last year of study period.

2. Keywords:

Systemic sclerosis – scleroderma – interstitial lung disease – biomarker

3. Accomplishments:

What were the major goals of the project?

Major Task 1 : Institutional Review Board (IRB) and DOD Human Research Protection Office (HRPO) – 1 to 4 months

- Milestone Achieved #1: HRPO Approval was obtained on 1/9/2017

Major Task 2: Specific Aim 1: To determine the predictive significance of the peripheral blood type-I IFN signature – 5 to 24 months

- Subtask 2 A: Extract RNA from PAXgene samples collected at baseline and 12-month visits of SLSII: This subtask has been completed in Dr. Assassi's laboratory.

Major Task 3: Specific Aim 2: To define the predictive significance of the skin immune dysregulation transcript signature for response to treatment – 5 to 24 months

- **Subtask 3D: Immunohistochemistry (IHC) staining of skin samples:**
The slides for the IHC staining has been prepared and the staining protocols have been finalized at Boston University.

Major Task 4: Specific Aim 3: To characterize the predictive significance of key Th2 plasma cytokines for response to immunosuppression in SSc-ILD – 5 to 18 months

- Subtask 4A: Determine key plasma cytokine levels using V-plex platform in the baseline and month-12 SLSII samples: This subtask has been completed
- Subtask 4B: Analyze plasma cytokine data, correlate with clinical outcomes, identify predictor cytokines: This subtask is currently ongoing. The initial quality control of data has been completed. An estimated 30% progress has been reached.

Major Task 5: Specific Aim 4: To develop and validate multivariable prediction tools for SSc-ILD with identified clinical and molecular predictors – 25 to 36 months

What was accomplished under these goals?

HRPO approval: The proposal was submitted for Institution Review Board (IRB) Approval at the University of Texas Health Science Center at Houston, Boston University and the University of California Los Angeles. The portion of the project to be completed at the Boston University qualified for an exemption determination under the policies and procedures of the Human Research Protection Program. Upon obtaining local IRB approvals, the necessary documents were submitted for the

Human Research Protection Office (HRPO) review. All three study sites have obtained the HRPO approval.

Design of a sample repository data base: SLSII sample repository data base was created by combining sample information from 4 different resources on an Access platform. Subject ID, visit, record ID, sample type, presence of freezer sample, freezer location, sample aliquot vial ID, date and amount used, project used, PI have been recorded. Samples with inconsistent subject ID, visit time point, record ID and sample type were compared and verified individually based on shipment manifests and all discrepancies have been resolved. The database contains samples from 141 individuals from three study time points (baseline, 12 months and 24 months, a few individuals with 2-month and 6-month samples), and 5 sample types (serum, plasma, PaxRNA, skin and buffy coat), 6095 unique vials in total have been captured in the data base. This data base allows tracking of sample utilization and thaw/freeze cycles.

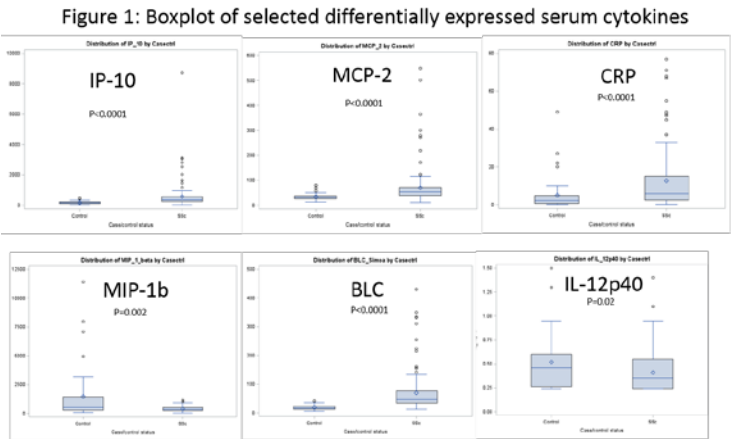
Serum protein determination: Key Th2 serum cytokines in addition to other important inflammatory proteins (a total of 59 proteins) were determined by Myriad Rule Based Medicine. The initial quality control analysis has been completed. These data have been transferred to Dr. Elashoff's group at the University of California Los Angeles. Table 1 shows the demographic characteristics of baseline

Table 1: Demographic characteristics of patients and controls in SLSII serum protein study		
Characteristic	Patients (n=135)	Controls (n=45)
Age, mean (SD)	52.6 (9.7)	52.3 (9.4)
Female gender, n (%)	100 (74.1)	33 (73.3)
Race		
White	94 (69.6)	31 (68.9)
Black	29 (21.5)	10 (22.2)
Other	12 (8.9)	4 (8.9)
Hispanic ethnicity, n (%)	18 (13.3)	5 (11.1)

patient samples and matched unaffected controls. Figure 1 shows a list of differentially expressed serum proteins in SSc versus control comparison.

RNA extraction from whole blood samples: RNA was extracted from PAXgene tubes (whole blood RNA samples)

collected at the baseline visit (n=135) and 12 month (n=101) visits. We are planning to complete the global gene expression studies in the second year of the funding cycle.



What opportunities for training and professional development has the project provided?

Dr. Assassi attended the Keystone Scientific Symposium, entitled “Type I Interferon: Friend and Foe Alike” in March 2017. Considering that the interferon signature is the most prominent transcript signature in the peripheral blood of patients with SSc, this symposium was an excellent platform to learn about the role of the interferons in the pathogenesis and severity of

autoimmune disease in general and in SSc in particular.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

Our goal is to complete the analysis of serum protein data and to summarize the results in a manuscript in the second year of funding cycle. Dr. Assassi will travel to Los Angeles in November to finalize the analysis of serum protein data and discuss the plans for the related manuscript. Also we will generate robust global gene expression data from skin and whole blood samples collected in the SLSII trial. Moreover, the analysis of the generated global gene expression data will be completed during this year of the funding period. The immunohistochemistry staining of skin samples by Dr. Browning at Boston University will be also completed.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

This project aims at developing clinically useful prediction models which will enable more focused and effective treatment of SSc related ILD. The analysis of the generated molecular data are currently ongoing.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. Changes/Problems:

Dr. Wenjin Zheng replaced Dr. Jeffrey Chang as the director of the Bioinformatics Core at the University of Texas Health Science Center at Houston. Therefore, Dr. Zheng was added as the co-investigator to this project. He will conduct the analysis of high through-put gene expression data. No other changes were necessary.

6. Products:

We are still generating and analyzing the molecular data. Therefore, no manuscripts or conference reports have been yet submitted or published.

7. Participants:

Dr. Shervin Assassi (PI; UTHealth):	No change
Dr. Jeffrey Chang (Co-I; UTHealth):	No change – replaced by Dr. Zheng
Julio Charles (Research Associate; UTHealth):	No change
Dr. Robert Elashoff (Co-I; UCLA):	No change

Name	Ning Li
Project Role	Biostatistician, UCLA
Nearest person month	1.77
Contribution to Project	Data analysis

Funding Support	National Institute of Child Health and Human Development UCLA Clinical and Translational Science Institute
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PREVIOUS, CURRENT AND PENDING SUPPORT

ASSASSI, SHERVIN

PREVIOUS

Title: Studies of HLA Region Genomics in Systemic Sclerosis and Ankylosing Spondylitis

Role: Co-investigator

Time Commitment: No Salary Support*

Supporting Agency: NIH/NIAID - 5U01AI09090-05 (PIs: Zhou, Reveille, Mayes)

Name and Address of the Funding Agency's Procuring Contracting/Grants Officer:

Gregory P. Smith, Lead Grants Management Specialist, NIAID, NIH, DHHS
5601 Fishers Lane, Room 4G70 MSC 9833, Bethesda, Maryland 20892-9833

Performance Period: 7/15/2010--6/30/2016 (NCE)

Level of Funding: \$1,892,757 (direct costs)

Goal: Aims to identify HLA and non-HLA disease-associated genes within the MHC region in SSc and AS in three different ethnic groups, and subsequently distinguish true causal disease associations from associations due to linkage disequilibrium (LD).

Overlap: None

* Dr. Assassi did not receive any salary support for this project because of the budgetary requirement in his K23 award.

Title: Genetics and Ankylosing Spondylitis (AS) Pathogenesis (Project 3)

Role: Co-Investigator

Time Commitment: 0.6 calendar months

Supporting Agency: NIH - 5P01AR052915-08 (PI: Reveille)

Name and Address of the Funding Agency's Procuring Contracting/Grants Officer:

Teresa Do, Grants Management Specialist, 1 AMS Circle, Building: Democracy I, Room 823,
Bethesda, MD 20892-3675

Performance Period: 07/01/2005 – 6/30/2017

Level of Funding: \$160,462 (direct costs)

Goal: Examine the clinical and genetic disease heterogeneity in the first degree relatives of the AS patients.

Overlap: None

* Dr. Assassi did not receive any salary support for this project because of the budgetary requirement in his K23 award.

Title: Molecular Profiling in Early Diffuse Systemic Sclerosis

Role: Primary PI of a Multi-PI Project

Time Commitment: 3.00 calendar months

Supporting Agency: Scleroderma Foundation Collaborative Research (SCORE) Grant

Name and Address of the Funding Agency's Procuring Contracting/Grants Officer:

Tracey Sperry, National Director of Development and Research, Scleroderma Foundation
300 Rosewood Drive, Suite 105, Danvers, MA 01923

Performance Period: 1/15/2015 – 12/31/2016

Level of Funding: \$199,062 (direct costs)

Brief Description of Project Goals: This study aims at examining the SSc molecular dysregulations in blood and skin samples of early diffuse SSc patients (disease duration < 2years) enrolled in the multicenter Prospective Registry of Early Systemic Sclerosis (PRESS)

Overlap: None

Title: Molecular Markers for Progression of Pulmonary Fibrosis in Systemic Sclerosis

Role: Principal investigator

Time Commitment: No Salary Support during NCE period

Supporting Agency: NIH/NIAMS – 5K23AR061436-04

Name and Address of the Funding Agency's Procuring Contracting/Grants Officer:

Teresa Do, Grants Management Specialist, 1 AMS Circle, Building: Democracy I, Room 823,
Bethesda, MD 20892-3675

Performance Period: 8/15/2011-7/31/2016 (NCE)

Level of Funding: \$457,623 (direct costs).

Brief Description of Project Goals: The aim of this study is to identify interferon induced chemokines and gene expression profiles that predict the course of SSc related interstitial lung disease in the established GENISOS Cohort.

Overlap: None

Title: Molecular Changes following Treatment with Cyclophosphamide or Autologous Hematopoietic Cell Transplantation in Patients with SSc

Role: PI

Time Commitment: No Salary Support during NCE

Supporting Agency: Karen Brown Scleroderma Foundation

Name and Address of the Funding Agency's Procuring Contracting/Grants Officer:

Michael Brown, The Karen Brown Scleroderma Foundation, P.O. Box 261671 Encino, CA 91426-1671

Performance Period: 9/1/2012-12/31/2015 (NCE)

Level of Funding: \$96,548 (direct costs)

Brief Description of Project Goals: The aim of this study to identify the molecular changes at the gene expression and cytokine levels resulting from treatment with autologous stem cell transplantation in comparison to cyclophosphamide in SSc patients.

Overlap: None

Title: Career Development Bridge Funding Award: R Bridge

Role: PI

Time Commitment: 6.90 calendar months

Supporting Agency: Rheumatology Research Foundation

Name and Address of the Funding Agency's Procuring Contracting/Grants Officer:

Amy Kane, Director, Awards and Grants, Rheumatology Research Foundation
2200 Lake Boulevard NE, Atlanta, GA 30319

Performance Period: 1/15/2015-1/14/2017

Level of Funding: \$99,902 (direct costs)

Brief Description of Project Goals: The main purpose of this award is to provide sufficient protected time to an investigator to generate preliminary data for subsequent strong NIH/R01 grant application

Overlap: None

Title: Predicting Disease Progression in Scleroderma with Skin and Blood Biomarkers

Role: Co-investigator

Time Commitment: No Salary Support*

Supporting Agency: Department of Defense - W81XWH-13-1-0452

Name and Address of Funding Agency's Procuring Contracting/Grants Officer: Kevin Moore, U.S. Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick, MD 21702-5014

Performance Period: 7/1/2013 – 9/22/2016

Level of Funding: \$224,545 (direct costs)

Brief Description of Project Goals: Aims to examine predictive significance of HLA/non-HLA genetic susceptibility loci, cytokines/chemokines, as well as gene expression signatures, of SSc patients enrolled in the established GENISOS Cohort for disease progression. This project focuses on predictors of natural history of disease (regardless of treatment regimen).

Overlap: None

* Dr. Assassi did not receive any salary support for this project because of the budgetary requirement in his K23 award.

Title: Cadherin-11 Regulation of Dermal Fibrosis and Macrophage Function

Role: Co-investigator

Time Commitment: No Salary Support*

Supporting Agency: NIH/NIAMS - R01-AR062056 (Subcontract from Baylor College of Medicine)

Name and Address of the Funding Agency's Procuring Contracting/Grant Officer: Laura K. Moen, 6701 Democracy Boulevard, Ste 800, Bethesda MD, 20892

Performance Period: 9/17/2012-8/31/2016

Level of Funding: \$20,114 (direct costs)

Brief Description of Project Goals: The primary goal is to understand the role of Cadherin-11 in the regulation of skin fibrosis and macrophage function using mouse models and human tissue.

Overlap: None

* Dr. Assassi did not receive any salary support for this project because of the budgetary requirement in his K23 award.

CURRENT

Title: A Randomized, Double Blind, Placebo Controlled Phase II Study to Investigate the Efficacy and Safety of Riociguat in patients with Diffuse Cutaneous Systemic Sclerosis

Role: PI

Time Commitment: 0.24 calendar mos

Supporting Agency: Bayer Health Clinic

Name and Address of the Funding Agency's Procuring Contracting/Grant Officer: Jinnell Brown, 2400 Spring Stuebner Rd, Spring, TX 77389

Performance Period: 2/24/15-2/23/20

Level of Funding: \$36,519 (direct costs)

Brief Description of Project Goals: The primary goal of the project is to assess the efficacy and safety of riociguat in patients with diffuse cutaneous systemic sclerosis in a clinical trial.

Overlap: None

Title: Longitudinal analysis of SSc skin morphology and correlation to gene expression signatures

Role: PI

Time Commitment: 0.60 calendar mos

Supporting Agency: Biogen MA

Name and Address of the Funding Agency's Procuring Contracting/Grant Officer: Juan Chavez, 225 Binney St, Cambridge, MA 02142

Performance Period: 10/5/15-10/4/18

Level of Funding: \$75,273 (direct costs)

Brief Description of Project Goals: The primary goal of the project is to conduct longitudinal analysis of scleroderma skin morphology and correlation to gene expression signatures.

Overlap: None

Title: Optical Elastography of Systemic Sclerosis Skin

Role: Co-Investigator

Time Commitment: 0.60 calendar mos

Supporting Agency: University of Houston (Department of Defense grant W81XWH-16-1-0140)

Name and Address of the Funding Agency's Procuring Contracting/Grant Officer: Kimberly Jordan, 4302 University Drive, Houston, TX 77204

Performance Period: 9/1/16-2/28/18

Level of Funding: \$37,020 (direct costs)

Brief Description of Project Goals: In collaboration with University of Houston, the goal is to assess the effectiveness of the OCE technology in patients with systemic sclerosis.

Overlap: None

Title: Collaborative Project to Analyze Systemic Sclerosis Patient-Derived Data

Role: PI

Time Commitment: 0.24 calendar mos

Supporting Agency: Momenta Pharmaceuticals, Inc

Name and Address of the Funding Agency's Procuring Contracting/Grant Officer: Momenta Pharmaceuticals, 675 West Kendall St, Cambridge, MA 02142

Performance Period: 3/31/17-3/30/18

Level of Funding: \$20,000 (direct costs)

Brief Description of Project Goals: To expand analysis of existing scleroderma patient gene expression database and to generate orthogonal bio-characterization data sets.

Overlap: None

PENDING

Title: CFIm25 mediated alternative polyadenylation regulates fibrosis in systemic sclerosis

Role: Co-PI (multi-PI project)

Time Commitment: 2.40 calendar months

Supporting Agency: National Institutes of Health - NIAMS

Name and Address of the Funding Agency's Procuring Contracting/Grant Officer: Melinda Nelson, 6701 Democracy Blvd, Bethesda, MD 20892

Performance Period: 04/01/2018 – 03/31/20/23

Level of Funding: \$1,249,954 (direct cost)

Brief Description of Project Goals: This project can ultimately lead to discovery of new targets for treatment of fibrosis in persons with systemic sclerosis.

Overlap: None