

AWARD NUMBER: W81XWH-13-1-0452

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

PRINCIPAL INVESTIGATOR: Dr. Maureen Mayes

CONTRACTING ORGANIZATION: The University of Texas Health Science Center
Houston, TX 77030

REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE October 2015		2. REPORT TYPE Annual		3. DATES COVERED 23Sep2014 - 22Sep2015	
4. TITLE AND SUBTITLE Predicting Disease Progression in Scleroderma with Skin and Blood Biomarkers				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-13-1-0452	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Maureen D. Mayes, M.D., M.P.H. E-Mail: mmayes@uth.tmc.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Texas Health Science Center at Houston 7000 Fannin St., UCT 1006 Houston, TX 77030-5401				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Scleroderma (Systemic Sclerosis, SSc) is a chronic, incurable autoimmune disease associated with high morbidity and mortality primarily due to lung disease. There is a large variability in individual patients' courses and current predictors of disease progression are inadequate. The overall objective of the proposed research is to develop reliable predictors for clinical outcomes in scleroderma, utilizing the biospecimens and longitudinal clinical data in the GENISOS cohort combining data from multiple areas to develop robust prediction models for ILD progression. In this second year, we have focused on the measurement of serum analytes, skin gene expression studies and preparation for genotyping of GENISOS samples. We have 6 abstracts accepted for presentation at the annual American College of Rheumatology meeting Nov 7-11, 2015 in San Francisco. In addition, for the current year, we have published 9 papers with data on gene expression, genotyping and cytokine studies involving data from the GENISOS cohort that was generated through this award. We are on track with all proposed activities.					
15. SUBJECT TERMS Scleroderma, Systemic Sclerosis, GENISOS (Genes versus Environment in Scleroderma Outcome Study), Interstitial Lung Disease, cytokines, DNA, RNA, skin biopsy					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	17	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	1
2. Keywords.....	1
3. Overall Project Summary.....	1
4. Key Research Accomplishments.....	9
5. Conclusion.....	9
6. Publications, Abstracts, and Presentations.....	10
7. Inventions, Patents and Licenses.....	12
8. Reportable Outcomes.....	12
9. Other Achievements.....	13
10. References.....	13
11. Appendices.....	13

**ANNUAL PROGRESS REPORT for W81XWH-13-1-0452
for period 9/23/2014 through 9/22/2015
“Predicting Disease Progression in Scleroderma with Skin and Blood
Biomarkers”**

1. Introduction:

Scleroderma (Systemic Sclerosis, SSc) is a chronic, incurable autoimmune disease associated with high morbidity and mortality primarily due to SSc-lung disease (1, 2). There is a large variability in individual patients' courses and current predictors of disease progression are inadequate. The overall objective of the proposed research is to develop reliable predictors for clinical outcomes, particularly interstitial lung disease, in scleroderma, utilizing the biospecimens and longitudinal clinical data in the GENISOS cohort to perform an analysis combining data from multiple areas to develop robust prediction models for ILD progression. The model will include genotypic data, gene expression profiling and cytokine/analyte levels, in addition to clinical parameters of pulmonary function tests and chest CAT (computer assisted tomography) scans. In the first year we have focused on patient recruitment, clinical characterization, specimen collection (DNA, RNA, skin biopsies, serum, plasma, monocytes).

In this second year, we have focused on the measurement and analysis of serum analytes, gene expression studies in skin and preparation of genotyping of GENISOS samples. We have 6 abstracts accepted for presentation at the annual American College of Rheumatology meeting Nov 7-11, 2015 in San Francisco. Regarding follow-up of our Progress Report from last year, there were 3 abstracts presented at the annual American College of Rheumatology meeting. Of these three, two have been published as full manuscripts and the 3rd is under revision for resubmission. In addition for the current year, we have published 9 papers with data on gene expression, genotyping and cytokine studies involving data from the GENISOS cohort that was generated through this grant award. We are on track with all proposed activities.

2. Keywords

Scleroderma, Systemic Sclerosis, GENISOS (Genes versus Environment in Scleroderma Outcome Study), Interstitial Lung Disease, cytokines, DNA, RNA, skin biopsy

3. Overall Project Summary

STATEMENT OF WORK from original application followed by responses. All Tasks that were to be done within the first year are highlighted.

Task 1: Institutional Review Board (IRB) and DOD Human Research Protection Office (HRPO)

1.a. Local IRB Approval (months 1-2)

“Skin and blood samples are currently collected in the GENISOS cohort based on the existing protocols approved by the UTHealth (Houston, TX) IRB. We will modify the study protocol based on the proposed research and obtain approval from the local IRB at UTHealth.”

1.b. DOD HRPO Approval (month 3)

“The study protocols and consent forms approved by the local IRB will be submitted to DOD HRPO for review and approval.”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: The initial approval has been completed; annual reviews are up-to-date and will be ongoing throughout the study. The HRPO has been kept informed of the annual reviews and approvals.

Task 2: Collection of DNA samples, genotyping and analysis of genetic data (Specific Aim 1)

2.a. Collection of DNA samples (months 1-36)

“Forty new patients will be enrolled annually by Drs. Mayes and Assassi into the GENISOS cohort at UTHealth. DNA will be extracted from baseline blood samples by the Research Assistant in the laboratories of Division of Rheumatology at UTHealth.”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: Forty-three new patients have been enrolled in the past year for a cumulative total of 83 subjects since the start of this project; baseline blood samples have been collected – see Tables 1 (proposed sample collection schedule) and Table 2 (actual sample collection schedule). We are on target with this.

2.b. Genotyping by Taqman Assay (months 25-30)

“Genotyping data are available on the majority of patients in the GENISOS cohort through genome wide association study and Immunochip efforts. Genotyping by Taqman assays will be performed for selected susceptibility loci in the newly enrolled patients (120 by the end of funding period). The assays will be completed by the Research Assistant under Dr. Mayes’s supervision in the laboratories of Division of Rheumatology at UTHealth.”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: In preparation for this task, we have sent samples on GENISOS subjects not previously genotyped to a long-time collaborator (Dr. Javier Martin, Granada, Spain) who will be able to perform some of the genotyping. Data from these subjects are expected to be available early in the New Year. Based on these results, we will perform genotyping by Taqman assay on the top “hits” from this most recent analysis in conjunction with our previously identified hits from GWAS and Immunochip studies.

2.c Analysis of genetic data (months 31-32)

“The predictive significance of selected susceptibility loci for disease severity will be examined. The analysis will be performed by Drs. Pedroza (UTHealth) and Gorlova (UT M.D. Anderson Cancer Center, Houston, TX).”

Not applicable to the first two years (months 1-24); included here for the sake of transparency and completeness.

2.d. Manuscript on genetic predictors of disease progression (months 33-34)

“A manuscript on genetic predictors of disease progression will be prepared by Drs. Mayes and Assassi (UTHealth) as well as Dr. Gorlova (UT M.D. Anderson Cancer Center).”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: Although this is not applicable to the first two years (months 1-24), we have analyzed our current and most recent genotype data on GENISOS subjects, in collaboration with other similar international cohorts and identified the following new genetic loci as SSc-susceptibility loci:

a) TYK2 gene variants in the IL12 immune pathway are associated with SSc (publication # 3 in the Publications section of peer-reviewed articles);

b) CCR6 gene variants as a risk factor for antitopoisomerase I antibodies in SSc (publication # 6 below)

c) IRF5-TNP03 is a common susceptibility locus shared with SLE as well as SSc (publication # 8).

d) Presentation of the first Genome-Wide Association Study (GWAS) in Hispanic cases. (Abstract # 2 accepted for presentation at the 2015 ACR meeting in San Francisco).

e) An analysis of genetic susceptibility loci of idiopathic interstitial pneumonia do NOT represent risk for SSc – an important finding that helps to differentiate other forms of lung disease from SSc-related interstitial lung disease (ILD (publication # 5).

Taken together, these are exciting results that help to explain the genetic underpinnings of SSc and that have strong implications for pathogenesis.

Task 3: Collection of skin samples, RNA extraction, gene expression analysis (Specific Aim 2)

3.a. Collection of skin biopsy samples (months 1-24)

“Skin biopsy samples will be collected from newly enrolled patients and a subgroup of patients seen at year 1 visit. 80 skin biopsy samples have been already collected. Drs. Mayes and Assassi will perform 60 skin biopsies per year at UTHealth. The skin biopsy samples are stored in RNAlater in -80 freezer. The sample collection and study design will be conducted in consultation with Dr. Michael Whitfield at Dartmouth Medical School (Dartmouth, NH).”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: Over the past two years, 187 skin biopsy samples have been collected (71 at baseline and 116 at follow-up visits - see Table 2 below). All samples have been processed per protocol and stored at -80 freezer in RNAlater solution. Conversations with Dr. Whitfield have been ongoing throughout the year – both electronically and in person at the November 2014 American College of Rheumatology (ACR) annual meeting and at the Scleroderma Research Workshop in Cambridge, England in August 2015. A meeting is also planned for the November 2015 (Nov 7-11, 2015) ACR annual meeting in San Francisco. We are on track for this task.

3.b. RNA extraction and global gene expression study in skin samples (months 25-26)

“Purified RNA will be extracted from stored skin biopsy samples by the Research Assistant using commercially available kits in the laboratories of Division of Rheumatology at UTHealth. The RNA quantity and quality will be assessed by Nanodrop and Bionalyzer in the CTSA Microarray Core Laboratories at UTHealth. Global gene expression profiling will also be performed in the Microarray Core Laboratories.”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: Although this task was not scheduled to start until month 25, we have successfully performed global gene expression studies from 61 GENISOS subjects (see analysis results below).

3.c. Analysis of skin global gene expression data (months 27-30)

“The analysis of skin gene expression data will be completed by Drs. Assassi (UTHealth) and Gorlova (UT M.D. Anderson Cancer Center) in consultation with Dr. Michael Whitfield at Dartmouth Medical School (Dartmouth, NH).”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: Although this task was not scheduled to start until month 27, we have analyzed a subset of GENISOS subjects and reported the results in a manuscript now in press (Publication # 4 below). We analyzed skin biopsies for 61 GENISOS subjects and 36 unaffected controls. This is the largest such study to date in SSc. We found a “keratin” signature that had not been reported previously and this signature was associated with shorter disease duration and interstitial lung disease, while higher fibro-inflammatory scores were associated with worse skin disease. These findings – which need to be verified in an subsequent cohort and which we will be doing in this current year – may be useful in stratifying patients for different therapies.

3.d. Manuscript on skin gene expression predictors of disease progression (months 30-32)

“A manuscript on skin gene expression predictors of disease progression will be prepared by Drs. Mayes and Assassi (UTHealth).”

Not applicable to the first two years (months 1-24); included here for the sake of transparency and completeness. Note that publication #6 with the analysis of 61 GENISOS subjects did not provide data on disease progression which will be the goal of our next publication.

Task 4: Collection of monocyte samples, RNA extraction, gene expression analysis (Specific Aim 2)

4.a. Collection of monocyte samples (months 1-24)

“Blood samples will be collected in CPT tubes from newly enrolled patients and patients seen at year 1 visit. 80 monocyte samples have been already collected. Drs. Mayes and Assassi will conduct the GENISOS visit and collect the clinical data. The monocyte samples will be purified and the RNA will be extracted by the Research

Assistant in the laboratories of Division of Rheumatology at UTHealth. The purified RNA samples will be stored in -80 freezer.”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: Blood samples for monocyte collection have been obtained from 69 out of 83 newly enrolled samples. Not all subjects provided a blood sample. Follow-up samples have been collected from 208 subjects, as noted in Table 2. Monocytes have been purified, RNA extracted and stored per protocol. Although the number of blood samples from newly enrolled subjects is not quite up to the numbers proposed, we have “over-sampled” individuals for follow-up and thus we will be able to compare changes over time.

4.b. Global gene expression study in monocyte samples (month 24)

“The RNA quantity and quality will be assessed by Nanodrop and Bionalyzer in the CTSA Microarray Core Laboratories at UTHealth. Global gene expression profiling will also be performed in the Microarray Core Laboratories.”

Not applicable to the first two years (months 1-24); this will be initiated in the next quarter.

4.c. Analysis of monocyte global gene expression data (months 25-28)

“The analysis of monocyte gene expression data will be completed by Drs. Assassi (UTHealth) and Gorlova (UT M.D. Anderson Cancer Center).”

Not applicable to the first two years (months 1-24); This will also be initiated this coming quarter.

4.d. Manuscript on monocyte gene expression predictors of disease progression (months 29-30)

“A manuscript on monocyte gene expression predictors of disease progression will be prepared by Drs. Mayes and Assassi (UTHealth).”

Not applicable to the first two years (months 1-24); included here for the sake of transparency and completeness

Task 5: Cytokine level determination and data analysis (Specific Aim 3)

5.a. Multiplex assays in rapid/slow progressor groups (months 6-10)

“Human DiscoveryMAP v 1.0 multiplex assays for the analyte determination in already collected 62 samples (rapid and slow progressor samples-Stage I of Specific Aim 3) will be performed by Myriad Rule Based Medicine (Austin, TX).”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: We have identified a group of patients within the GENISOS cohort who have interstitial lung disease (ILD) verified by imaging. We studied these patients to identify potential clinical or cytokine predictors of early ILD progression. A total of 92 patients were included. So far we studied CCL18 also known as pulmonary activation-regulated chemokine (PARC) as we previously observed that PARC had a significant correlation with short-term decline in functional vital capacity (FVC) as a surrogate of ILD progression. In this cohort of patients with verified ILD there was

no statistical significant relationship between PARC levels and FVC progression ($p=0.45$). We then analyzed whether antibodies and other various clinical and demographic variables could predict FVC decline and observed that Scl-70 performed by immunodiffusion was the only variable that predicted faster FVC decline in patients with SSc related ILD. Interestingly, the same antibody performed by a chemoluminescent or a line-blot immunoassay were not predictive of FVC decline. The discrepancy observed between different methods of Scl-70 determination is important as it might have relevant implications for enrichment strategies in clinical trials of SSc-ILD. These data were submitted as an abstract for the Scleroderma World Congress in 2016 (abstract selection has not yet occurred).

We will also send serum samples collected from 70 SSc patients and 70 matched unaffected controls to Rule-Based Medicine next week (October, 2015). They will determine levels of over 250 cytokines using proprietary DiscoveryMAP250+. Based on the results of this study, we will identify cytokines that are differentially expressed in SSc. In the next step, we will work with Rule-Based Medicine to develop a focused multiplex panel that can be applied to the above mentioned group of GENISOS patients with verified ILD. This study is a potential source of biomarkers for ILD progression.

In summary – although we are “behind” in this task, we are “ahead” in several other tasks and we are on track to complete this task in the next few months.

5.b. Analysis data of Human DiscoveryMAP multiplex assays (months 10-16)

“The data from Human DiscoveryMAP multiplex assays will be conducted by Dr. Pedroza at UTHealth. A limited number of cytokines will be identified that differentiate the fast progressor group from slow progressors.”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: This analysis is well underway as noted in the paragraph above.

5.c. Design of custom-made multiplex assay (months 16-20)

“Custom-made multiplex assays will be designed in collaboration with Myriad Ruled Based Medicine (Austin, TX) based on the identified cytokines in the Subtask 5.b.”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: This analysis is underway as noted in Task 5.a. above.

5.d. Determination of cytokine levels by custom-made assays (months 23-24)

“The levels of selected cytokines will be determined in all collected baseline samples (currently available 331 + newly enrolled 80= 411) utilizing custom-made assays. These assays will be performed by Myriad Ruled Based Medicine (Austin, TX).”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: This analysis is underway as noted in Task 5.a. above.

5.e. Manuscript based on the cytokine data (months 25-27)

“The predictive significance of determined cytokine levels in Subtask 5.d. will be examined for disease progression. This analysis will be performed by Dr. Pedroza (UTHealth). A manuscript will be prepared based on these results by Drs. Mayes, Assassi, and Pedroza (UTHealth)”

Not applicable to the first two years (months 1-24); however, we have analyzed the chemokine/cytokine CXCL4 as a predictor of progression of SSc-related interstitial lung disease. Although initially promising, our data indicate that there is no correlation of CXCL4 with severity or progression of SSc-related ILD (Abstract to be presented at the 2015 ACR meeting in San Francisco.)

Task 6: Multivariable models with identified clinical and molecular predictors (Specific Aim 4)

6.a. Random forest and longitudinal analyses (months 32-34)

“The identified molecular predictors in Tasks 2-5 will be analyzed along with clinical predictors. Random forest analysis will be used for data reduction. Multivariable joint analysis of longitudinal measurements and survival data will be performed. These analyses will be performed by Drs. Pedroza (UTHealth) and Gorlova (UT M.D. Anderson Cancer Center).”

Not applicable to the first two years (months 1-24); included here for the sake of transparency and completeness

6.b Manuscript on multivariable models predictive of disease progression (months 35-36)

“A manuscript reporting on multivariable predictors of disease progression will be prepared by Drs. Mayes, Assassi, Pedroza (UTHealth) and Dr Gorlova (UT M.D. Anderson Cancer Center).”

Not applicable to the first two years (months 1-24); included here for the sake of transparency and completeness

Task 7: Calculation of fibrosis score of high resolution chest CTs (months 0-36)

“Dr. Ferguson will determine the fibrosis score on the high resolution chest CTs obtained in the GENISOS cohort”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: Dr. Emma Ferguson (study radiologist) has scored 76 chest high resolution cat scans (HRCTs) over the course of the two years. In addition she has presented these data to our monthly scleroderma research conference. This is an integral part of our outcome analysis. We are on track with this.

Task 8: Expansion of the GENISOS cohort (months 0-36)

“The baseline and follow-up GENISOS visits will be conducted by Drs. Mayes and Assassi at UTHealth. This is necessary for capturing the longitudinal clinical data and collecting the biospecimens for the proposed research.”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: We have reached, and actually

exceeded, our goal of 80 newly recruited subjects as we have enrolled a total of 83 cases. All clinical data and biospecimens have been collected per protocol (see Tables 1 and 2 below).

Task 9: Maintenance and expansion of the GENISOS data base (months 0-36)

“The Data Base Manager at UTHealth in close collaboration with Mr. Tony Mattar (Computer Task Force, Inc, Troy, MI) will maintain the GENISOS data base. The data base will also be expanded to accommodate the genetic and cytokine data. They also will establish an interface for connecting the gene expression data with the clinical data base.”

ANNUAL PROGRESS REPORT RESPONSES - Current Objectives, Results, Progress and Accomplishments, Discussion: Tracking of all skin biopsies and blood samples has been added to the database, all visit data has been checked for quality (through our continuous data quality measures) and entered.

SUMMARY TABLE 1. 8th Quarter Sample Collection Report

8th Quarter Sample Collection for GENISOS patients from 19 JUN 2015 through 19 OCT 2015

QUARTERLY – 8th Quarter Proposed Sample Collection – GENISOS Patients								
Quarter	Time/ Months	Skin bx. Baseline	Skin bx. F/U	Monocyte Baseline	Monocyte F/U	DNA Baseline	Serum/ PAXgene Baseline	Serum/ PAXgene F/U
8	22-25	10	5	10	5	10	10	22
QUARTERLY – 8th Quarter ACTUAL Sample Collection – GENISOS Patients								
8	22-25	7	26	7	45	7	7 Serum 7 PAXgene	45 Serum 45 PAXgene

F/U = Follow-Up

Bx = biopsy

Note: 9 subjects were recruited this quarter but only 7 had a skin biopsy done due to time constraints of patients who could not wait to get the biopsy done or who declined biopsy at this visit. Total newly recruited patients in this quarter = 9; total return visits in this quarter = 52. Skin biopsies were done on 26 total subjects and blood samples for DNA/plasma/ and/or RNA-PAXgene tubes were drawn on a total of 45 subjects. (Note that not all patients get skin biopsies performed or blood drawn for all studies at every visit.)

SUMMARY TABLE 2. Cumulative Sample Collection Report:

Cumulative Sample Collection for GENISOS patients from 23 Sep 2013 through 19 Oct 2015

CUMULATIVE 1STthrough 8h Quarter Proposed Sample Collection – GENISOS Patients								
Quarters	Time/ Months	Skin bx Baseline	Skin bx F/U	Monocyte Baseline	Monocyte F/U	DNA Baseline	Serum/ PAXgene Baseline	Serum/ PAXgene F/U
1 thru 8	0-25	80	40	80	40	80	80	176
CUMULATIVE 1STthrough 8th Quarter ACTUAL Sample Collection – GENISOS Patients								
1 thru8	0-25	71	116	69	208	81	68 serum/ 69 PAXgene	214 serum/ 207 PAXgene

F/U = Follow-Up

Bx = biopsy

As can be seen from Summary Table 2, we are on track for our sample collection, either meeting or exceeding our goals in some categories. These are fairly minor discrepancies which will “even out” over the subsequent quarters of this project.

4. Key Research Accomplishments (bullets ‘a through e’ represent accomplishments in Year 2 and bullets ‘f through h’ related to Year 1)

- a. The collection of the largest longitudinal sample repository in scleroderma that will permit completion of all proposed experiments.
- b. TYK2 gene variants in the IL12 immune pathway are associated with SSc (publication # 3 in the Publications section of peer-reviewed articles);
- c. CCR6 gene variants as a risk factor for antitopoisomerase I antibodies in SSc (publication # 6 below)
- d. IRF5-TNP03 is a common susceptibility locus shared with SLE as well as SSc (publication # 8).
- e. Presentation of the first Genome-Wide Association Study (GWAS) in Hispanic cases. (Abstract # 2 accepted for presentation at the 2015 ACR meeting in San Francisco).
- f. Results of our cytokine analysis (reported in abstract #1 listed below entitled “The Global miRNA Whole Blood Profile in Systemic Sclerosis and Its Correlation with Serum Cytokine Levels”) suggest a link of miRNA to the pathogenesis of SSc and could have important ramifications for future drug and biomarker development
- g. The analysis of our gene expression data (reported in abstract #2 listed below entitled “Dissecting the Heterogeneity of Skin Gene Expression Patterns in Systemic Sclerosis”) showed a prominent keratin signature in addition to the fibro-inflammatory signature indicating that the dysregulation in SSc skin is not confined to the dermis (as currently believed) but also involves other cell compartments. This is a novel finding and potentially useful for stratifying patients for interventions.
- h. Analysis of genetic risk factors for interstitial lung disease in the GENISOS cohort (reported in abstract # 3 listed below entitled “Genetic Susceptibility Loci of Idiopathic Interstitial Pneumonitis do not Represent Risk for Systemic Sclerosis”) indicating that these 2 conditions, although phenotypically similar, have quite distinct genetic risk factors.

5. Conclusion

In summary, this second year has seen the production of research results, the publication of several manuscripts, the completion of most tasks on time. In addition we are on track in terms of increasing the GENISOS cohort and performing follow-up visits which are essential to our goal of predicting worsening, stability or improvement of disease.

Future plans will include continued recruitment, follow-up, data and sample collection from the GENISOS cohort. The third year will be devoted to the analysis of changes over time in these parameters with emphasis on predictors of disease progression (or lack thereof).

6. Publications, Abstracts, and Presentations

a. Manuscripts:

a.1. Lay Press: Nothing to report.

a.2. Peer-Reviewed Scientific Journal Publications (8 from year 2 and 1 from Year 1):

1. Wodkowski M, Hudson M, Proudman S, Walker J, Stevens W, Nikpour M, Assassi S, Mayes MD, Tatibouet S, Wang M; Canadian Scleroderma Research Group (CSRG); Australian Scleroderma Interest Group (ASIG); Genetics versus Environment in Scleroderma Outcome Study (GENISOS), Baron M, Fritzler MJ. Clinical correlates of monospecific anti-PM75 and anti-PM100 antibodies in a tri-nation cohort of 1574 systemic sclerosis subjects. Autoimmunity. 2015 Sep 3:1-10. [Epub ahead of print] PMID: 26334795
2. Wodkowski M, Hudson M, Proudman S, Walker J, Stevens W, Nikpour M, Assassi S, Mayes M, Wang M, Baron M, Fritzler M. Monospecific antioRo52/TRIM21 antibodies in a tri-nation cohort of 1574 systemic sclerosis subjects – Evidence of an association with interstitial lung disease and worse survival. Clin Exp Rheumatol. 2015 Jul-Aug;33(4 Suppl 91):S131-5. Epub 2015 Aug 27. PMID: 26315678
3. López-Isac E, Campillo D, Bossini-Castillo L, Guerra SG, Assassi S, Simeón CP, Carreira P, Ortego-Centeno N, García de la Peña P, the Spanish Scleroderma Group, Beretta L, Lunardi C, Moroncini G, Riemekasten G, Witte T, Hunzelmann N, Kreuter A, Distler JHW, Voskuyl AE, de Vries-Bouwstra J, Herrick A, Worthington J, Denton C, Fonseca C, Radstake TRDJ, Mayes MD, Martín J. Influence of TYK2 in Systemic Sclerosis susceptibility: a new *locus* in the IL-12 pathway. Ann Rheum Dis. 2015 Sep 2. pii: annrheumdis-2015-208154. doi: 10.1136/annrheumdis-2015-208154. [Epub ahead of print] PMID: 26338038
4. Assassi S, Swindell WR, Wu M, Tan FK, Khanna D, Furst DE, Tashkin DP, Jahan-Tigh RR, Mayes MD, Gudjonsson JE, Chang JT. Dissecting the heterogeneity of skin gene expression patterns in systemic sclerosis. Arthritis Rheumatol. 2015 Aug 3. Doi: 10.1002/art.39289. [Epub ahead of print]
5. Wu M, Assassi S, Salazar GA, Pedroza C, Gorlova OY, Chen WV, Charles J, Taing ML, Wigley FM, Hummers LK, Shah AA, Hinchcliff M, Khanna D, Schiopu E, Phillips K, Furst DE, Steen V, Baron M, Hudson M, Zhou X, Pope J, Jones N, Docherty P, Khalidi NA, Robinson D, Simms RW, Silver RM, Frech TM, Fessler BJ, Fritzler MJ, Molitor JA, Segal BM, Movahedian M, Martin J, Varga J, **Mayes MD**. Genetic susceptibility loci of idiopathic interstitial pneumonia do not represent risk for systemic sclerosis. *Revised and Re-submitted to Arthritis Rheum* 2015.
6. Ochoa E, Martin JE, Assassi S, Beretta L, Carreira P, Simeón CP, Koumakis E, Dieude P, Allanore Y, García-Hernández FJ, Espinosa G, Castellvi I, Trapiella JL, Rodriguez L, González-Gay MA, Egurbide MV, Sáez L, Callejas-Rubio JL, Vargas JA, Hunzelmann N, Riemekasten G, Witte T, Distler JHW, Kreuter A, Lunardi C, Santaniello A, Tan FK, Arnett FC, Shiels PG, Herrick A, Worthington J, Vonk MC, Koeleman BP, Radstake TRDJ, Mayes MD, Martin J. Confirmation of CCR6 as a risk factor for antitopoisomerase I antibodies in

- systemic sclerosis. *Clin Exp Rheumatol*. 2015 Jul-Aug;33(4 Suppl 91):S31-55. PMID: 26314374
7. Salazar GA, Assassi S, Wigley F, Hummers L, Varga J, Hinchcliff M, Khanna D, Schiopu E, Phillips K, Furst DE, Steen V, Baron M, Hudson M, Taillefer SS, Pope J, Jones N, Docherty P, Khalidi NA, Robinson D, Simms RW, Silver RM, Frech TM, Fessler BJ, Molitor JA, Fritzler MJ, Segal BM, Al-Kassab F, Perry M, Yang J, Zamanian S, Reveille JD, Arnett FC, Pedroza C, Mayes MD. Antinuclear antibody-negative systemic sclerosis. *Semin Arthritis Rheum*. 2015
 8. Kottyan LC, Zoller EE, Bene J, Lu X, Kelly JA, Rupert AM, Lessard CJ, Vaughn SE, Marion M, Weirauch MT, Namjou B, Adler A, Rasmussen A, Glenn S, Montgomery CG, Hirschfield GM, Xie G, Coltescu C, Amos C, Li H, Ice JA, Nath SK, Mariette X; Simon Bowman for UK primary Sjögren's syndrome registry, Rischmueller M, Lester S, Brun JG, Gøransson LG, Harboe E, Omdal R, Cunninghame-Graham DS, Vyse T, Miceli-Richard C, Brennan MT, Lessard JA, Wahren-Herlenius M, Kvarnström M, Illei GG, Witte T, Jonsson R, Ericksson P, Nordmark G, Ng W-F for UK primary Sjögren's syndrome registry, Anaya JM, Rhodus NL, Segal BM, Merrill JT, James JA, Guthridge JM, Scofield RH, Alarcon-Riquelme M, Bae SC, Boackle SA, Criswell LA, Gilkeson G, Kamen DL, Jacob CO, Kimberly R, Brown E, Edberg J, Alarcón GS, Reveille JD, Vilá LM, Petri M, Ramsey-Goldman R, Freedman BI, Niewold T, Stevens AM, Tsao BP, Ying J, Mayes MD, Gorlova OY, Wakeland W, Radstake T, Martin E, Martin J, Simonovitch K, Moser Sivils KL, Gaffney PM, Langefeld CD, Harley JB, Kaufman KM. The IRF5-TNPO3 association with systemic lupus erythematosus (SLE) has two components that other autoimmune disorders variably share. *Hum Mol Genet*. 2015 Jan 15;24(2):582-96. PMID: 25205108 PMCID: PMC4275071
 9. Assassi S, Swindell WR, Wu M, Tan FD, Khanna D, Furst DE, Tashkin DP, Jahan-Tigh RR, Mayes MD, Gudjonsson JE, Chang JT. Dissecting the Heterogeneity of Skin Gene Expression Patterns in Systemic Sclerosis. *Arthritis Rheumatol*. 2015 Aug 3. doi: 10.1002/art.39289. [Epub ahead of print] PMID: 26238292
 10. Wiese AB, Berrocal VJ, Furst DE, Seibold JR, Merkel PA, **Mayes MD**, Khanna D. Correlates and responsiveness to change of measures of skin and musculoskeletal disease in early diffuse systemic sclerosis. *Arthritis Care Res (Hoboken)*. 2014 Apr 1. doi: 10.1002/acr.22339. [Epub ahead of print] PMID:24692361
- a.3. Invited Articles (1 in Year 1 and 1 in Year 2):
1. Wu M, Mayes MD. Insights into the genetic basis of systemic sclerosis: Immunity in human disease and SSc mouse models. *Adv Genomics Genet*. 2014, 4:143-151.
 2. Salazar G, Mayes MD. Genetics, epigenetics and genomics of Systemic Sclerosis. *Rheum Dis Clin North Am*. 2015 Aug;41(3):345-66. doi: 0.1016/j.rdc.2015.04.001. Epub 2015 May 20. Review. PMID: 26210123

a.4. Abstracts (5 for Year 2 and 3 for Year 1):

1. Lopez-Isac E, Bossini-Castillo L, Guerra SG, Assassi S, Simeón CP, Carreira PE, Ortego-Centeno N, García de la Peña P, Beretta L, Santaniello A, Bellocchi C, Lunardi C, Moroncini G, Gabrielli A, Riemekasten G, Witte T, Hunzelmann N, Kreuter A, Jorg HW, Distler O, Voskuyl AE, de Vries-Bouwstra JK, Herrick AL, Worthington J, Denton CP, Fonseca C, Radstake CRDJ, Mayes MD, Martín J. TYK2 and Systemic Sclerosis Susceptibility: a New Associated Locus in the IL-12 Pathway
2. Bossini-Castillo L, Raya E, Reveille JD, Assassi S, Gorlova OY, Khanna D, Furst DE, Kafaja S, Simms RW, Lafyatis R, Frech TM, Steen VD, Fessler BJ, Molitor JA, Alarcón-Riquelme M, Martín J, Mayes MD. A Genome-Wide Association Study of a Hispanic Systemic Sclerosis Cohort
3. Salazar G, Hagan J, Wu M, Guo X, Zhou X, Charles J, Mayes MD, Assassi S, The Global MicroRNA Profile of Systemic Sclerosis Whole Skin/Dermal Fibroblasts and the Role of the Xq26.3 miRNA Cluster As a TGF- β Pathway Positive Feedback Mechanism
4. Volkmann ER, Tashkin DP, Roth M, Tseng C-h, LeClair H, Clements PJ, Furst DE, Mayes MD, Charles J, Khanna D, Elashoff R, Assassi A, Scleroderma Lung Study II Group. CXCL4 Does Not Predict Extent or Progression of Interstitial Lung Disease in Systemic Sclerosis
5. Assassi S, Mayes MD, Pedroza C, Chang JT, Furst DE, Crofford LJ, Nash R, McSweeney P, Csuka ME, Goldmuntz E, Keyes-Elstein L, Wallace P, Sullivan K. Immunoablation Followed By Autologous Stem Cell Transplantation in Systemic Sclerosis Patients Decreases Significantly the Interferon Signature
6. Salazar GA, Hagan J, Wu M, Mayes MD, Reveille HD, Assassi S. The Global miRNA Whole Blood Profile in Systemic Sclerosis and Its Correlation with Serum Cytokine Levels. American College of Rheumatology Annual Meeting, 11/2014.
7. Assassi S, Swindell WR, Wu M, Tan FK, Khanna D, Furst DEF, Tashkin DP, Jahan-Tigh RR, Mayes MD, Gudjonsson JE, Chang JT. Dissecting the Heterogeneity of Skin Gene Expression Patterns in Systemic Sclerosis. American College of Rheumatology Annual Meeting, 11/2014.
8. Wu M, Assassi S, Salazar GA, Gorlova OY, Chen W, Charles J, Wigley F, Hummers L, Varga J, Hinchcliff M, Khanna D, Schioppa E, Phillips K, Furst DE, Steen V, Baron M, Hudson M, Taillefer SS, Pope J, Jones N, Markland J, Docherty P, Khalidi NA, Robinson D, Simms R, Silver R, Frech TM, Fessler B, Molitor J, Fritzler M, Segal B, Al-Kassab F, Yang J, Mayes MD. Genetic Susceptibility Loci of Idiopathic Interstitial Pneumonitis Do not Represent Risk for Systemic Sclerosis. American College of Rheumatology Annual Meeting, 11/2014.

7. Inventions, Patents and Licenses – Nothing to report**8. Reportable Outcomes – Scientific Advances:**

- a. Identification of the keratin signature as being important in SSc-related skin gene expression.
- b. Publication of the largest skin gene-expression in SSc with implications for stratifying subjects by disease pathway and potentially as a means to determine response to therapy.
- c. Publication of the first microRNA whole blood profiling in SSc as described in Abstract #1 above.
- d. The discovery of the keratin signature in SSc skin described in Abstract # 2 above.
- e. The discovery that the susceptibility genes of idiopathic interstitial lung disease are quite distinct from those of SSc-related ILD.

9. Other Achievements

A repository of DNA, RNA, serum and plasma has resulted from the sample collection for this study. This repository is linked to longitudinal clinical data for ready analysis of predictors of outcome.

10. References

1. Elhai M, Meune C, Avouac J, Kahan A, Allanore Y: Trends in mortality in patients with systemic sclerosis over 40 years: a systematic review and meta-analysis of cohort studies. *Rheumatology (Oxford)* 2011.
2. Thomas E, Symmons DP, Brewster DH, Black RJ, Macfarlane GJ: National study of cause-specific mortality in rheumatoid arthritis, juvenile chronic arthritis, and other rheumatic conditions: a 20 year followup study. *J Rheumatol* 2003, 30: 958-965.

11. Appendices – Nothing to report



Predicting Disease Progression in Scleroderma with Skin and Blood Biomarkers

Proposal Log Number PR120687 (HRPO Log Number A-17770.1)

Award Number W81XWH-13- 1-0452



PI: Maureen D. Mayes, M.D., M.P.H.

Org: University of Texas Health Science Center at Houston

Award Amount: \$1,127,763.00

Study/Project Aim(s)

- Aim 1. To study genetic susceptibility variants (DNA) (identified from our previous GWAS, Immunochip and HLA studies) as predictors of progressive disease, in the GENISOS cohort
- Aim 2. To identify blood and skin gene expression profiles (RNA) predictive of progressive disease
- Aim 3. To identify the cytokines/analytes (protein) predictive of disease course utilizing multiplex assays
- 4. To build multivariable models with identified clinical and molecular predictors, utilizing advanced variable reduction and longitudinal analysis strategies

Approach - Funding for this project started 23 Sep 2013. The GENISOS (early scleroderma disease) cohort provides the subjects from which sample collection (DNA, RNA [PAXgene], skin biopsies, monocytes, and serum), clinical characterization and autoantibodies are obtained. Data collection is captured in an electronic database for analysis.



SCLERODERMA RESEARCH PROGRAM

In the 8th quarter, we have enrolled **9 new** cases and conducted follow-up visits on **52** additional subjects (cumulatively 83 new cases). Sample collection is on track as is clinical characterization, data collection and entry as well as chest CAT-scan fibrosis scoring.

Timeline and Cost

Activities	CY	13	14	15	16
Text (Major aim/study/milestone)					
Text (Major aim/study/milestone)					
Text (Major aim/study/milestone)					
Text (Major aim/study/milestone)					
Estimated Budget (\$K)		\$85.3	\$85.3	\$85.3	

Goals/Milestones (relevant to this period):

Task 1: IRB & DOD HRPO approvals – **Completed initial approvals, ongoing annual reviews up to date**

Task 2a: Collection of DNA samples: **81 samples to date**

Task 3a: Collection of Skin biopsy samples: **187 biopsies to date**

Task 4a: Collection of monocyte samples: **278 samples to date**

Task 7: Calculation of fibrosis score of HRCTs (months 0-36) **Dr. Ferguson has reviewed and scored a total of 76 HRCTs.**

Task 8: Expansion of the GENISOS cohort: Cumulative **83 new enrollees, 263 follow-up visits.**

Task 9: Maintenance and expansion of the GENISOS data base (months 0-36) **tracking of all skin biopsies and blood samples has been added to the database, all visit data has been checked for quality (through our data quality measures) and entered.**

Budget Expenditure to Date

Projected Expenditure: **\$392,746.00** (Year 02)

Actual Expenditure: **\$322,188.49** (23 Sept 2014 thru 22 Sept 2015)

Updated: 21 Oct. 2015