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PRINCIPAL INVESTIGATOR: Chandra Mohan

CONTRACTING ORGANIZATION: University of Houston System
Houston TX 77004

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14. ABSTRACT

Excessive accumulation of collagen and skin-thickening are hallmark features of Systemic sclerosis (SSc). The subjectiveness and the lack of precision associated with the Rodnan skin score, the current gold standard, calls for an improved detection method. Technologies such as magnetic resonance imaging, ultrasound, and optical coherence tomography have been tested in SSc, with varying success. Here, we advance a novel contact-independent noninvasive technique capable of micrometer/nanometer spatial resolution called optical coherence elastography (OCE) for monitoring skin disease in SSc. We have established the feasibility of using this technology to identify elasticity changes caused by SSc *in vivo* in murine skin. For the proposed human studies in Aim 2, the assessment of mRSS score and the OCE examination has been completed in 2 participants (one patient with limited systemic sclerosis and one age-, race-, and gender-matched control). The generated raw data have been analyzed and shows promising preliminary results. We have been actively screening our clinic patients for additional participants. Four additional participants have been scheduled to participate in this study in December 2017. For the proposed murine studies in Aim 1, the animal model of SSc has been successfully re-established. In addition, animals are being scheduled for the proposed treatment and monitoring study.

15. SUBJECT TERMS

Systemic Sclerosis, Imaging, Skin, Diagnostics, Animal Models, OCT, OCE

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1. INTRODUCTION: Excessive accumulation of collagen and skin-thickening are hallmark features of Systemic sclerosis (SSc). The subjectiveness and the lack of precision associated with the Rodnan skin score, the current gold standard, calls for an improved detection method. Technologies such as magnetic resonance imaging, ultrasound, and optical coherence tomography have been tested in SSc, with varying success. Here, we advance a novel contact-independent noninvasive technique capable of micrometer/nanometer spatial resolution called optical coherence elastography (OCE) for monitoring skin disease in SSc. We have established the feasibility of using this technology to identify elasticity changes caused by SSc *in vivo* in murine skin. In Aim 1, we propose to extend these findings to a longitudinal model, where OCE assessed elasticity will be monitored in a murine model of SSc following drug treatment, and compared to traditional disease measures. In Aim 2, we will extend these studies to humans. Specifically, a cross-sectional analysis of skin from patients with limited cutaneous and diffuse SSc, and healthy controls, will be performed using OCE and compared to conventional metrics including the Rodnan skin score and histopathology.

2.KEYWORDS: *Provide a brief list of keywords (limit to 20 words)*

Keywords: Systemic Sclerosis, Imaging, Skin, Diagnostics, Animal Models, OCT, OCE

3.ACCOMPLISHMENTS:

- **What were the major goals of the project?**

The goals of Aim 1, as outlined in the SOW were:

1.1 Acquiring and Aging of Mice, Acquiring drugs
1.2 Treatment/Scanning Studies (batch 1)
1.3 Treatment/Scanning Studies (batch 2)
1.4 Phenotyping/scanning of mice for disease
1.5 Histopathology analysis
1.6 Data Analysis
1.7 Publish paper

The goals of Aim 2, as outlined in the SOW were:

2.1 Patients & Control recruitment
2.2 mRSS and OCE of subjects
2.3 Histopathology analysis
2.4 Data Analysis

2.5 Publish paper

- **What was accomplished under these goals?**

General note: Although the official grant commencement date might indicate that the 1-year annual report is due, it took several revisions before the animal and human subjects approvals were finally sanctioned and we were given the green light to commence these studies. Hence progress made is modest, as detailed below.

Major Activities: For the human studies described in Aim 2, the necessary Institutional Review Board approvals have been obtained. The OCE machine has been set up by Dr. Larin's group in the research room of the McGovern Medical School Rheumatology Clinic, at the University of Texas. Patient recruitment has commenced. For the animal studies described in Aim 1, the animal use protocol was finally approved by the funding agency only recently. We have spent efforts in importing mice and re-establishing the murine SSc model, before we can proceed to the actual experiments as proposed.

Specific Objectives: For the human studies in Aim 2, the specific objectives for this study period were (a) to ensure that the new imaging technology is operational in the hospital where patients are seen, (b) to begin recruiting patients and controls, and (c) to begin applying the novel imaging platform as well as the mRSS scores to the patients. For the animal studies in Aim 1, the specific objectives for this study period were to re-establish the murine SSc model, and then to commence the proposed treatment studies.

3) Significant results or key outcome. For the human studies in Aim 2, the assessment of mRSS score and the OCE examination has been completed in 2 participants (one patient with limited systemic sclerosis and one age-, race-, and gender-matched control). The generated raw data have been analyzed and shows promising preliminary results. Dr. Assassi has been actively screening his clinic patients for additional participants. Four additional participants have been scheduled to participate in this study in December 2017. They consist of two patients with diffuse cutaneous involvement and two age-, gender-, and ethnicity matched controls. With respect to the listed SOW (see above), action items 2.1 and 2.2 have commenced. For the murine studies proposed in Aim 1, the animal model of SSc has been successfully re-established. Please see Figure 1. In addition, animals are being scheduled for the proposed treatment and monitoring study.

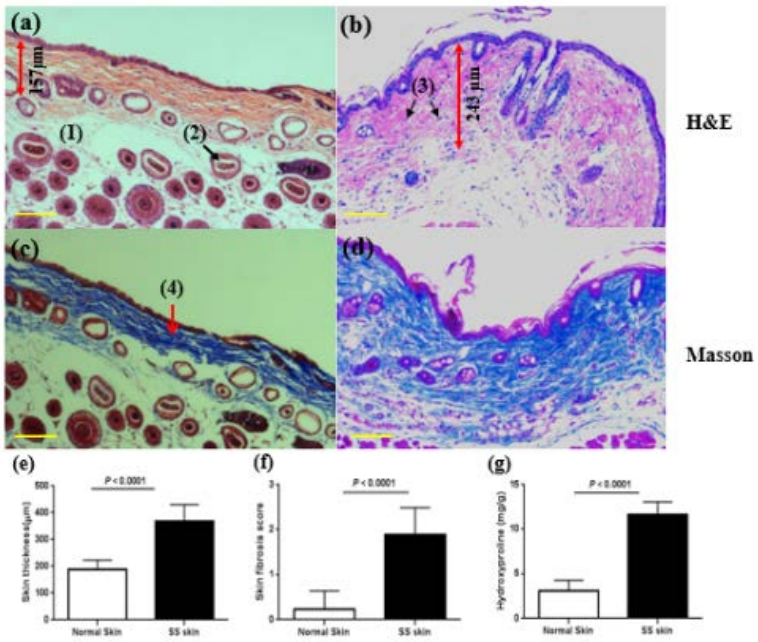

Fig. 1: Murine SSc was successfully induced using bleomycin, as indicated by the skin thickening (b), increased collagen deposition (d), and elevated biochemical readouts of SSc (black bars

in e-g). Control mice are shown in (a), (c), and white bars in (e) - (g).

Major achievements: 1►. The OCT/OCE imaging apparatus has been successfully established in the hospital. Though this was not stated as a "SOW" this turned out to be a herculean task. Having established this technology in the hospital now allows this technology to be tested in multiple other diseases, beyond the scope of this funded project. 2►. Although only limited subjects have been studied thus far, this technology is clearly safe, easy and convenient for patient use, and the preliminary data are encouraging and support continued testing of this technology. 3►. Additional patients have been lined up for this testing. 4►. All required methodologies for both Aims have been successfully established.

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

  This project has provided training opportunities for one graduate student, Manmohan Singh, and one junior faculty, Dr. Yong Du. The subject of training was the use of optical imaging modalities to monitor common chronic diseases, with a focus on systemic sclerosis. Their training is ongoing.

- **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?**

  Planned studies for Aim 1 are as follows:

1.1 Acquiring and Aging of Mice, Acquiring drugs
1.2 Treatment/Scanning Studies (batch 1)
1.3 Treatment/Scanning Studies (batch 2)
1.4 Phenotyping/scanning of mice for disease
1.5 Histopathology analysis
1.6 Data Analysis
1.7 Publish paper

  Planned studies for Aim 2 are as follows:

2.1 Patients & Control recruitment - **This will be continued further.**
2.2 mRSS and OCE of subjects - **This will be continued further.**
2.3 Histopathology analysis

2.4 Data Analysis
2.5 Publish paper

▪

4.IMPACT:

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

  Nothing to Report.

- **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:

- **Changes in approach and reasons for change**

  Nothing to Report.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

  Nothing to Report.

- **Changes that had a significant impact on expenditures**

  Nothing to Report.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

  Nothing to Report.

- **Significant changes in use or care of human subjects**

  Nothing to Report.

- **Significant changes in use or care of vertebrate animals.**

  Nothing to Report.

- **Significant changes in use of biohazards and/or select agents**

  Nothing to Report.

6.PRODUCTS:

Publications, conference papers, and presentations- *Nothing to Report.*

Journal publications. *Nothing to Report.*

Books or other non-periodical, one-time publications. *Nothing to Report.*

Other publications, conference papers, and presentations. *Nothing to Report.*

Website(s) or other Internet site(s) - *Nothing to Report.*

Technologies or techniques - *Nothing to Report.*

Inventions, patent applications, and/or licenses - *Nothing to Report.*

Other Products - *Nothing to Report.*

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. **What individuals have worked on the project?**

Name:	*Chandra Mohan*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-5992-5687*
Nearest person month worked:	*1*
Contribution to Project:	*Overall planning of studies and organizing the fine details of the murine studies.*
Funding Support:	*No Change*

Name:	*Kirill Larin*
Project Role:	*Co-PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-5532-5027*
Nearest person month worked:	*1*
Contribution to Project:	*Overall planning of studies and organizing the fine details of OCT/OCE equipment usage.*
Funding Support:	*No Change*

Name:	*Shervin Assassi*
Project Role:	*Co-PI*
Researcher Identifier (e.g. ORCID ID):	sassassi
Nearest person month worked:	*1*
Contribution to Project:	*Overall planning of studies and organizing the fine details of the human studies*
Funding Support:	*No Change*

Name:	*Yong Du*
Project Role:	Junior Investigator
Researcher Identifier (e.g. ORCID ID):	*YDU*
Nearest person month worked:	*12*
Contribution to Project:	*organizing the fine details of all murine studies and executing the murine studies*
Funding Support:	*No Change*

Name:	*Manmohan Singh*
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	*None*
Nearest person month worked:	*12*
Contribution to Project:	*optimizing the fine details of OCT/OCE machine usage*
Funding Support:	*No Change*

b. **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

i. *There has been a change in support for Dr. Mohan, Dr. Assassi and Dr. Larin.*

The following grants of Dr. Mohan are no longer active:

1. R01 DK81872 NIH (PI: Mohan)
07/01/2009 – 08/31/15 (1 year no-cost extension till 08/31/16)
FOREBODING LUPUS NEPHRITIS IN MINORITY WOMEN
The goals of this study are to study SLE patients longitudinally to determine if selected molecules in the urine may have the capacity to predict impending renal disease or flares. In addition, the proposed studies will evaluate whether these selected urinary markers are specific for lupus nephritis.
Role: PI

2. Novel Research Grant, Lupus Research Institute (PI: Mohan)
01/01/2013 – 12/31/15
NOVEL INSIGHTS INTO CEREBRAL LUPUS
The major goals of this proposal are to use a combination of cross-sectional and longitudinal studies to ascertain if the levels of selected novel markers in the serum or cerebrospinal fluid could help predict neuropsychiatric events in patients with cerebral lupus.
Role: PI

3. Target Identification in Lupus Grant, Alliance for Lupus Research (PI: Mohan)
02/01/2014 – 2/28/16
BRADYKININS IN LUPUS
The major goals of this proposal are to ascertain the role of bradykinins and their receptors in lupusnephritis.
Role: PI

The following new support is now available to Dr. Mohan:

1. Project Title: **PLK1, a potential novel therapeutic target of lupus** (PI: Wu)
Supporting Agency: Lupus Research Institute
Performance Period: 01/01/2016-12/31/2018
Goals: The goals of this project are to test the efficacy of a PLK inhibitor in mouse models of lupus.
Overlap: None

2. Project Title: **Negative Regulation of Lupus by the VISTA Pathway** (PI: Noelle)
Supporting Agency: Dartmouth/NIH
Performance Period: 09/01/16 – 08/31/21
The goals of this grant are to define how VISTA impacts murine lupus and nephritis.
Overlap: None

3. Project Title: **Salivary biomarkers in SLE** (PI: Mohan)
Supporting Agency: NIH R21
Performance Period: 09/22/17 – 07/31/19
The goal of this study is to identify novel autoantibodies and biomarker candidate proteins that are elevated in the saliva of SLE and lupus nephritis patients, and establish their association with disease activity.
Overlap: None

4. Project Title: **Novel Point of Care assays for Urinary Diagnostics of Nephritis** (PI: Willson)
Supporting Agency: NIH
Performance Period: 09/22/17 – 07/31/21
The goal of this study is to develop quantitative lateral flow tests for urinary markers in nephritis and to integrate multiple marker testing into a user-friendly system with software error-catching, barcode reading, and quality controls, for point-of-care applications.
Overlap: None

The following grants of Dr. Assassi are no longer active:

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

The following new support is now available to Dr. Assassi:

1. **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

2. **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

The following new support is now available to Dr. Larin:

1. 1R01HD086765 (MPI: Larin, Miranda) 01/01/16-12/31/20
NIH Annual Direct Costs: $238,339

Optical Coherence Tomography to Study Effect of Poly - Drug Exposure on Fetal Brain Development
The overall objective of this study is to develop an optical coherence tomography (OCT) based high-resolution mouse embryonic brain imaging and analysis approach, and to use this method in correlation with molecular analysis to understand the interplay between ethanol (EtOH) and nicotine (NIC) effects on embryonic brain development.
Lead institution: University of Houston
Role: PI
OVERLAP: None

2. 1R01HL130804 (MPI: Larin, Martin) 06/01/16-03/31/20
NIH Annual Direct Costs: $145,000
Optical elastography for assessment of myocardial regeneration
Heart failure due to cardiomyocyte loss and pump dysfunction after ischemic heart disease is the leading cause of death in the United States. If a way could be found to facilitate cardiac regeneration after heart damage, then survival rates would improve. Limited endogenous adult cardiomyocyte regenerative potential in the face of acute damage is thought to result from inadequate adult cardiomyocyte proliferative capacity. Our goal is to develop new methods to regenerate cardiomyocytes.
Role: PI
OVERLAP: None

c. **What other organizations were involved as partners?**
i. *Nothing to Report.*
ii. **Organization Name:** *Nothing to Report.*
iii. **Location of Organization:** *Nothing to Report.*
iv. **Partner's contribution to the projec**t *Nothing to Report.*
v. **Financial support:** *Nothing to Report.*
vi. **In-kind support**: *Nothing to Report.*
vii. **Facilities**: *Nothing to Report.*
viii. **Collaboration**: *Nothing to Report.*
ix. **Personnel exchanges**: *Nothing to Report.*
x. **Other:** *Nothing to Report.*

8. SPECIAL REPORTING REQUIREMENTS
a. **COLLABORATIVE AWARDS:** *Nothing to Report.*
b. **QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Nothing to Report.*