AWARD NUMBER: **W81XWH-19-1-0319**

TITLE: Using Affinity-Based Proteomics to Identify Diagnostic and Plasma Biomarkers for Endometriosis

PRINCIPAL INVESTIGATOR: Towia Libermann, PhD

CONTRACTING ORGANIZATION: **Beth Israel Deaconess Medical Center**

REPORT DATE: **October 2020**

TYPE OF REPORT: **Annual Report**

PREPARED FOR: U.S. Army Medical Research and Development Command

Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2020	Annual Report	09/01/2019-08/31/2020

4. TITLE AND SUBTITLE

Using affinity-based proteomics to identify diagnostic and plasma biomarkers for endometriosis

5a. CONTRACT NUMBER
W81XWH-19-1-0319

5b. GRANT NUMBER
PR 181 444P1

5c. PROGRAM ELEMENT NUMBER
PE 0602787A

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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8. PERFORMING ORGANIZATION REPORT NUMBER
61484

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: Endometriosis, which is characterized by pain and infertility, is the most frequent reproductive health diagnosis among female veterans along with menstrual disorders. Notoriously difficult to diagnose, the time between symptom onset and endometriosis diagnosis averages seven years, resulting in prolonged pain symptoms leading to decreased activity and poor mental health, greatly impacting women physically, psychologically, and economically over the life-course. Identifying diagnostic and prognostic biomarkers would enable earlier intervention and prevent progression to severe pain and infertility. However, identification of endometriosis biomarkers has been limited by the heterogeneity of the disease, inappropriate control groups, and lack of prospectively collected samples. Furthermore, progression of endometriosis is not well understood. Discovery of non-invasive diagnostic and prognostic biomarkers for endometriosis has the potential to revolutionize current medical practice, leading to earlier diagnosis and interventions as well as better clinical care that could significantly impact improvement in clinical outcomes of endometriosis. We hypothesize that endometriosis development and progression will lead to altered circulating protein profiles related to systemic inflammation and immunity years before emergence of symptoms and the clinical diagnosis of endometriosis that will be detectable through the novel proteomics technology, SOMAscan, enabling early diagnosis of endometriosis. In addition, alteration of inflammation and immune proteins in systemic environments will be greater among women who do not experience pain remediation after surgical treatment.

15. SUBJECT TERMS

NONE LISTED

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
Unclassified	Unclassified	Unclassified	Unclassified	30	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION

Endometriosis, which is characterized by pain and infertility, is the most frequent reproductive health diagnosis among female veterans along with menstrual disorders. Notoriously difficult to diagnose, the time between symptom onset and endometriosis diagnosis averages seven years, resulting in prolonged pain symptoms leading to decreased activity and poor mental health, greatly impacting women physically, psychologically, and economically over the life-course. Identifying diagnostic and prognostic biomarkers would enable earlier intervention and prevent progression to severe pain and infertility. However, identification of endometriosis biomarkers has been limited by the heterogeneity of the disease, inappropriate control groups, and lack of prospectively collected samples. Furthermore, progression of endometriosis is not well understood. Discovery of non-invasive diagnostic and prognostic biomarkers for endometriosis has the potential to revolutionize current medical practice, leading to earlier diagnosis and interventions as well as better clinical care that could significantly impact improvement in clinical outcomes of endometriosis. We hypothesize that endometriosis development and progression will lead to altered circulating protein profiles related to systemic inflammation and immunity years before emergence of symptoms and the clinical diagnosis of endometriosis that will be detectable through the novel proteomics technology, SOMAscan, enabling early diagnosis of endometriosis. In addition, alteration of inflammation and immune proteins in systemic environments will be greater among women who do not experience pain remediation after surgical treatment. We will utilize data and specimens from the two population-based cohort studies, the Nurses' Health Study II (NHSII), a prospective cohort study with blood samples collected months to years before endometriosis diagnosis, and the Women's Health Study: Adolescent to Adulthood (A2A), a deeply phenotyped longitudinal cohort of endometriosis patients, to identify non-invasive diagnostic and prognostic protein biomarkers for endometriosis. We urgently need endometriosis biomarkers to reduce the delay to treatment and reveal new potential therapeutic targets to improve treatment outcomes and quality of life in these patients. Our unique resources will enable us to identify novel diagnostic and prognostic blood protein biomarkers for endometriosis. **Short-term**, the proteomic data generated in our study will provide clinically applicable non-invasive diagnostic and prognostic biomarkers for endometriosis and improve treatment outcomes. **Long-term**, our study will provide biological insight to the heterogeneity and different pathogenesis by types of endometriosis and progression from the aspect of inflammation, immune dysregulation, and angiogenesis, which could lead to potential prevention strategies and development of novel therapeutic targets including immunotherapies.

KEYWORDS

Endometriosis, proteomics, biomarkers, plasma, SOMAscan, diagnosis, pathophysiology, systems biology, predictor, risk model, pain

ACCOMPLISHMENTS:

The major goals of the project are as follows:

Major Task 1. Generating proteomics data

a. Local IRB Approval: IRB Approved 11/14/19 and 12/23/19
b. Milestone #1: HRPO Approval obtained 4/3/20

Specific Aim 1: In prospectively collected samples from 200 NHSII participants with laparoscopically confirmed endometriosis and 200 without, identify proteins that differentiate women who will be diagnosed with laparoscopically confirmed endometriosis from controls.

Major Task 2. Generating proteomics data on NHSII samples

a. Subtask 1. Identify appropriate cases and controls in NHSII. Retrieve and aliquot plasma samples: Due to the Covid-19 pandemic all labs were shut down for 4 months which delayed the identification and

retrieval of samples from NHSII. Nevertheless, the samples were identified, retrieved from storage, aliquoted, and delivered to the Libermann lab by 8/31/20.

b. Subtask 2. Create quality control (QC) samples and plan how to align the blinded QCs and samples for proteomics assay: 50% complete. Blinded QCs have been created and integrated into the sample. Additional laboratory QCs still need to be created. Completion by 10/1/20
c. Subtask 3. Measure 1,305 proteins, check quality control for variation within and between plates on NHSII samples: Planned to be assayed between 12/1/20-2/2/21
d. Milestone #2: Generating proteomics NHSII data

Major Task 3. Identify proteins that differentiate endometriosis cases from controls using proteomics data from 200 cases and 200 controls in the NHSII

a. Subtask 1. Identify proteins that differentiate endometriosis cases from controls using the prospective samples in NHS: Planned completion by 8/31/21
b. Subtask 2. Manuscript preparation: Planned completion by 3/31/22
c. Milestone #3: Publish proteomics data predictive of endometriosis

Specific Aim 2: In plasma from 150 deeply phenotyped cases and 50 matched controls from the A2A study, determine whether proteins differ between subtypes.

Major Task 4. Identify proteins that differentiate endometriosis subtypes using proteomics data from 150 cases and 50 controls in the A2A

a. Subtask 1. Identify proteins that differentiate endometriosis cases from controls in A2A: Completed SOMAscan run on 7/20/20. Proteomics Data analysis will be completed by 10/31/21.
b. Subtask 2. Evaluate whether proteins identified perform better than CA125 to discriminate cases from controls: Planned completion by 3/31/22
c. Subtask 3. Manuscript preparation: Planned completion by 8/31/22
d. Milestone #4: Publish protein performance compared to CA125 and by endometriosis subtype

Specific Aim 3: In preoperative samples from 100 women with endometriosis from the A2A study, identify proteins and pathways that discriminate between those who have progressive disease, characterized by chronic pain and poor quality of life, and those who improve after surgery.

Major Task 5. Identify proteins associated with progression of endometriosis

a. Subtask 1. Identify proteins associated with persistent pain and/or poor quality of life after surgical treatment of endometriosis in the A2A progression study: Completed SOMAscan run on 7/20/20. Proteomics Data analysis will be completed by 12/31/21
b. Subtask 2. Use systems biology to identify pathways relevant to progression of endometriosis: Planned completion by 5/31/22
c. Subtask 3. Manuscript preparation: Planned completion by 8/31/22
d. Milestone #5: Publish proteins associated with endometriosis progression

What was accomplished under these goals?

1) Major Activities

In the first three months of the current funding period IRB approval was sought and obtained. The next four months the HRPO application was completed, reviewed, revised, and finally approved.

For Aim 1 (major tasks 2), we were able to identify and select samples for appropriate endometriosis cases and controls within the NHSII study, but then the biorespository was shutdown due to the Covid-19 pandemic lab. The biorepository reopenend this summer and these samples have finally been aliquoted and transferred to our lab for SOMAscan proteomics analysis.

For Aims 2 and 3 (major task 4), we identified A2A study samples and transferred to our lab for SOMAscan analysis. We completed the SOMAscan run for the A2A samples, assessed quality control metrics, and created a covariate dataset for further analyses.

2) Specific Objectives

a. Obtain IRB approval.
b. Obtain HRPO approval.
c. Identify, select, and transfer plasma samples for endometriosis cases and matched controls from the NHSII study for proteomics measurement by SOMAscan.
d. Identify, select, and transfer plasma samples for endometriosis cases and matched controls from the A2A study for proteomics measurement by SOMAscan.
e. Run A2A samples on SOMAscan proteomics platform.

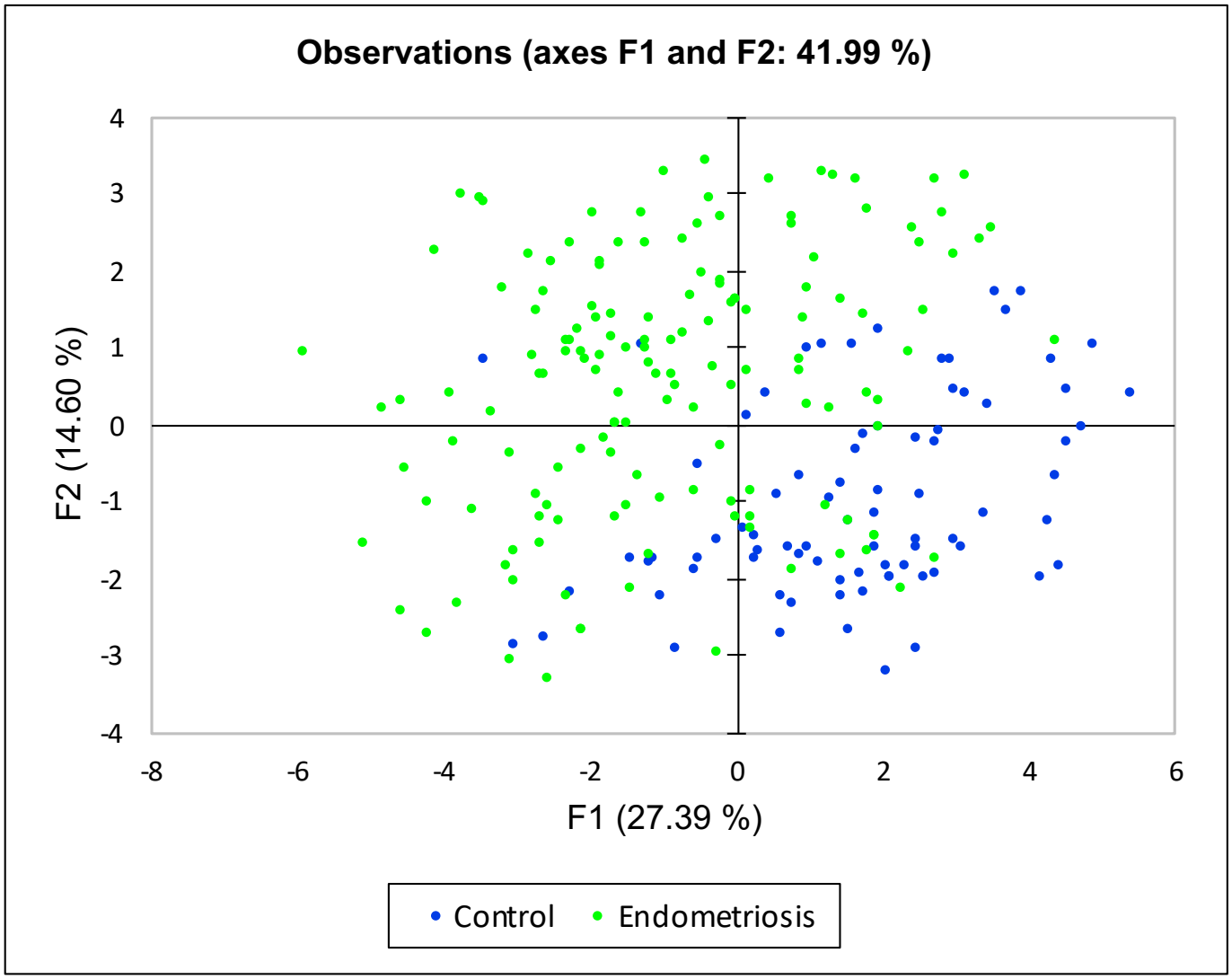
3) Significant results or key outcomes, including major findings, developments, or conclusions

While IRB and HRPO approval took longer than expected and the Covid-19 pandemic shutdown after approval slowed the selection and transfer of samples and other lab work until late June 2020, we succeeded to run the first set of samples from the A2A study on the SOMAscan platform.

We identified and selected A2A plasma samples according to our inclusion criteria (superficial peritoneal cases who has surgery at baseline, completed the baseline and year 1 questionnaire, have no/little missing data for menstrual and acyclic pain, blood drawn within 90 days prior to surgery, baseline questionnaire completed within 6 months of blood draw, no more than 3 freeze/thaw cycles, and no hemolysis). Unfortunately, we were only able to identify 139 of the 150 samples planned that met these criteria. Therefore, we increased the number of controls included from 50 to 72 samples. In total our A2A sample set included 330 EDTA samples consisting of 89 paired (preoperative/postoperative) case samples, 50 single baseline case samples, 72 control samples, and 30 blinded quality control (QC) samples.

These 330 EDTA plasma samples from the A2A study were run on the manual version of SOMAscan Assay Kit 1.3K, Human Plasma (item 900-00011). The kit provided pooled plasma controls were run 5X on each SOMAscan plate as well as a no protein buffer control. 345 out of 350 samples passed the SomaLogic standard quality control and normalization criteria.
To further evaluate the quality of the SOMAscan data we performed a Spearman correlation analysis comparing all samples with all 1,305 proteins to each other. Typically, we expect to see a correlation between samples >0.9, indicating that the majority of proteins across all samples change very little. Indeed, we observed that the correlation between all samples was typically >0.91 with only a few samples between 0.86-0.9. This analysis further confirmed that there is no significant QC problem with the samples and data and that technically everything performed as expected. In addition, we observed excellent inter- and intra-plate CVs for blinded QC aliquots of <15% for well over 90% of the proteins.

Preliminary data analysis revealed that 155 proteins are significantly differentially expressed between 139 endometriosis cases and 72 controls with a p-value <0.01. Principal Component Analysis (PCA) using the top 20 proteins separates endometriosis cases (green) from controls (blue) quite well as seen in the figure below.



In initial evaluation of specific proteins enriched in endometriosis, there are several that we identified in our analyses that have been previously linked to endometriosis in the literature such as VEGF. In the next funding period, we will further explore the A2A SOMAscan data as well as running the 400 NHSII samples on SOMAscan.

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

The project provided opportunities for professional development for Dr. Naoko Sasamoto, early career investigator, to gain hands-on training in A2A sample selection for the proposed aims. In addition, the funding provided support for Dr. Sasamoto to publish a paper on CA125 and endometriosis symptoms in the A2A cohort.

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?
During the next reporting period we will perform the detailed bioinformatics, statistics, and systems biology analysis on the SOMAscan data from the A2A participants to identify plasma proteins associated with endometriosis that may inform candidate biomarkers for early diagnosis. Among other analyses we plan to define endometriosis subtypes and its pathophysiology. We will also run the 440 NHSII prediagnostic samples on SOMAscan.

IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
We have completed running blood samples for endometriosis cases and matched controls on the SOMAscan proteomics platform that measures 1,305 proteins in the circulation. Once we have analyzed the data, we anticipate identifying blood biomarkers that discriminate between endometriosis and controls as well as identifying different subtypes of endometriosis. Such a phenotyping of endometriosis will help with differential diagnosis and improved management of patients with endometriosis and may also identify new druggable therapeutic targets. We expect that our data by the end of the funding period will have a major impact on predicting who will develop endometriosis and on how best to monitor and manage endometriosis patients.

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."

CHANGES/PROBLEMS:
We do not have any significant changes to report. The project is following the approved SOW. The only problem we have encountered in the first year is the delay in getting the proposed experiments executed in the original timeframe due to the Covid-19 related shut down of all labs for almost four months as well as due to the delay in getting the HRPO approval which we obtained only on 4/3/20. However, since getting IRB and HRPO approval and after reopening of the labs we are quickly caught up on lost time. For Aim 1 we are back on the planned timeline and for Aim 2 we are a few months ahead of the timeline.

PRODUCTS:
Journal publications
Evaluation of CA125 in relation to pain symptoms among adolescents and young adult women with and without surgically-confirmed endometriosis.
Sasamoto N, DePari M, Vitonis AF, Laufer MR, Missmer SA, Shafrir AL, Terry KL.PLoS One. 2020 Aug 24;15(8):e0238043. doi: 10.1371/journal.pone.0238043. eCollection 2020.PMID: 32833998
Acknowledgement of federal support: yes

Other products
We have generated the SOMAscan proteomics data for the A2A study samples including endometriosis cases and matched controls.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name: Towia Libermann, PhD
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0002-4006-8179
Nearest person month worked: 2
Contribution to Project: Dr. Libermann oversees the study protocol, SOMAscan proteomics work, data analysis, interpretation of the data and manuscript writing.
Funding Support: Next section

Name: Long Ngo, PhD
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0002-8903-9352
Nearest person month worked: 1
Contribution to Project: Dr. Ngo is leading the data analysis and statistical analysis of the study, data interpretation and manuscript preparation.
Funding Support: Next Section

Name: Simon Dillon, PhD
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0002-4417-178X
Nearest person month worked: 4
Contribution to Project: Dr. Dillon oversees and performs all the proteomics experiments of the study. He is also contributing to data interpretation and manuscript writing.
Funding Support: NA

OTHER SUPPORT

LIBERMANN, TOWIA

Active Research Support

P30 CA006516 (Glimcher) 12/01/2011-11/30/2021 7% / 0.84 CM
NIH/NCI $45,468
Cancer Center Support Grant
Cancer Proteomics Core
Specific Aim: The Cancer proteomics Core will provide access to high sensitivity, high throughput, and high resolution proteomics for all Cancer Center members
The primary goal of the center is to promote collaborative interactions that will lead to new approaches to cancer prevention, diagnosis and treatment.
Program Officer: Joseph Woodill
Role: Core Leader

R01 AG 051568 (Marcantonio, Libermann) 01/15/2016 – 12/31/2020 17.3% / 2.07 CM
NIH/NIA $410,939
Title: Advancing the Understanding of Postoperative Delirium Mechanisms via Multi-Omics
Our goal is to define integrated multi-omics based plasma and CSF protein signatures and biomarkers for postoperative delirium.
Specific Aim 1. Define and validate a plasma protein signature for delirium
Specific Aim 2. Define lipid and metabolite delirium signatures, integrate with the protein signature, and validate this integrated signature across the SAGES cohort and in the independent HIPOR sample
Specific Aim 3. Define an integrated CSF-based protein, lipid, and metabolite signature for delirium
Program Officer: Molly Wagster
Role: PI

R21CA218960-1A1 (Libermann) 06/30/2018-07/31/2021 10% / 1.2 CM
NIH/NCI $150,000
Identification of Plasma- and Exosome-Based Protein Biomarkers for Early Detection of Pancreatic Cancer Using SOMAscan Technology

The goal is to define candidate protein biomarkers for pancreatic ductal adenocarcinoma using ultra-sensitive SOMAscan.
Specific Aims:
1) Define candidate plasma protein biomarkers for PDAC using ultra-sensitive SOMAscan
2) Define candidate EMV protein biomarkers for PDAC using ultra-sensitive SOMAscan

Program Officer: Matthew Young
Role: PI

P01 AG031720 (Inouye) 09/1/2018 – 08/31/2023 10% effort; 1.2 Cal
NIH/NIA $207,042
Title: Delirium, Dementia, and the Vulnerable Brain: An Integrative Approach
Project 2: The role of inflammation in the pathophysiology of delirium and its associated long-term cognitive decline
Our goal is to identify inflammatory mechanisms and related biomarkers in plasma and CSF among an elderly cohort of surgical patients with delirium and LTCD using SOMAscan and CyTOF
Specific Aims:
1) To use the state-of-the-art SOMAscan proteomics to identify new inflammatory proteins analyzing PREOP and POD2 plasma from 32 matched pairs from SAGES I with and without delirium, including 16 delirium cases with complicated delirium. We will also examine PREOP CSF from 32 matched pairs with and without delirium from SAGES II, including 16 delirium cases at high risk for complicated delirium.
2) To use state-of-the-art mass cytometry technology (CyTOF) to characterize circulating immune cells from the same 32 matched delirium-no delirium pairs of SAGES II participants from Aim 1.
3) To measure CRP and the inflammatory index in banked plasma (PREOP, POD2) and freshly collected plasma and CSF from 32 matched quartets of SAGES I participants.

Program Officer: Luci Roberts
Role: PI of Project 2 (Multiple PI)

NIH/NIA R01AG051568-03S1 (Libermann) 01/01/2019-12/31/2020 2% / 0.24 CM
NIH/NCI $89,465
Advancing the Understanding of Postoperative Delirium Mechanisms via Multi-Omics (Administrative Supplement)
To advance the ongoing work of the parent project by applying the latest cutting edge technologies for metabolite and lipid analysis to two previously conducted studies of older orthopedic patients.
Aim 1. To improve and optimize the metabolomics and lipidomics workflow for discovery and validation of delirium biomarkers in plasma from the SAGES study and CSF from the HIPOR study. H1A: Inclusion of isotope spike-ins and pooled QC samples will result in highly reproducible inter-assay and intra-assay consistency and dramatically improve the discovery of metabolite and lipid delirium biomarkers.
Aim 2. To develop an optimized bioinformatics analysis workflow for metabolomics and lipidomics. H2A: A new bioinformatics workflow for normalization and calibration based on the inclusion of isotope spike-ins and repeated pooled QC samples will lead to generation of the optimal metabolite and lipid delirium predictors.
Program Officer: Luci Roberts
Role: PI

PR181444 (Terry, Libermann) 09/01/2019-08/31/2022 15% / 1.8 CM
DOD $381,892
Using affinity based proteomics to identify diagnostic and prognostic plasma biomarkers for endometriosis
Our goal is to utilize data and specimens from the two population-based cohort studies – NHSII, a prospective cohort study with blood samples collected months to years before endometriosis surgical diagnosis, and A2A, a deeply phenotyped cohort of endometriosis patients, to identify non-invasive diagnostic and prognostic protein biomarkers for endometriosis.
Specific Aims:

Aim 1. In prospectively collected plasma samples obtained up to 6 years before diagnosis, identify proteins that differentiate women who will be diagnosed with endometriosis from controls. We hypothesize that:
a. Inflammation, immune, and angiogenesis related proteins will be altered and can be used to predict those with endometriosis years before diagnosis.
b. The association between protein markers and endometriosis diagnosis will differ by infertility status, age at diagnosis or BMI.
Aim 2. Determine whether proteins differ between endometriosis subtypes. We hypothesize that:
a. Inflammation, immune, and angiogenesis related proteins will perform better than CA125 alone to discriminate between endometriosis cases and controls overall and by subtype.
b. Secondarily, inflammatory, immune, and angiogenesis related proteins will be more dysregulated in women with red or clear lesions compared to women with black, blue or brown lesions.
Aim 3. In pre and post-operative samples, identify proteins and pathways that discriminate between those who continue to be impacted by the disease, characterized by continued chronic pain and poor quality of life, and those who improve after surgery. We hypothesize that:
a. Proteins indicative of inflammation, immune dysfunction, and angiogenesis will be elevated at baseline in those who have surgery resistant disease symptoms.
b. Compared to presurgical values, post-surgical inflammatory, immune, and angiogenic marker levels will increase or remain unchanged in those with surgery resistant disease symptoms.
Program Officer: Sherry M. Apperson
Role: PI

1R03AG061582-01 (Vasunilashorn) 02/15/2019-11/30/2020 3% / 0.36 CM
NIH/NIA $50,000
Genetic and Neuroinflammatory Mechanisms of Delirium and Alzheimer's Disease and Related Dementias
The goal of this proposal is to test the hypothesis that neuroinflammation is a shared pathophysiological pathway linking delirium and Alzheimer's disease and related dementias (ADRD), and that these relationships differ by genetic predisposition. Delirium and ADRD have strong epidemiological associations: ADRD has long been recognized as a risk factor for delirium, and recently delirium has been implicated as a risk factor for incident ADRD. Although this points to a clear link between delirium and ADRD, the shared pathophysiology underlying these relationships remains largely unknown.
Specific Aims:
Aim 1: Determine whether neuroinflammation (YKL-40) is associated with delirium and ADRD.
H1A: Plasma and CSF levels of YKL-40 will be at least moderately correlated (r=0.30; HiPOR).
H1B: Delirium will be associated with higher CSF (HiPOR) and plasma (HiPOR, SAGES) levels of YKL-40.
H1C: In SAGES, incident ADRD will be associated with higher plasma levels of YKL-40.
Aim 2. Evaluate whether the associations of neuroinflammation (YKL-40) with delirium and ADRD, vary between those with and without genetic risk modifiers (APOE ε4, COMT Val/Val) in SAGES.
H2A: The risk of delirium and ADRD associated with high plasma YKL-40 will be greater in APOE ε4 carriers
than in APOE non-ε4 carriers.
H2B: The risk of delirium and ADRD associated with high plasma YKL-40 will be lower in patients with the COMT Val/Val genotype than in patients with one or more COMT Met alleles.
Program Officer: Luci Roberts
Role: Co-Investigator

R21 AR074016-01 (Jimenez) 07/01/2019-05/31/2021 5% / 0.6 CM
NIH/NIAMS $58,994
Aptamer Proteomic Analysis of Systemic Sclerosis Serum Exosome
The discovery of objective non-invasive biomarkers that may allow to diagnose Systemic Sclerosisassociated interstitial lung disease (SSc-ILD) at an early stage and to identify patients with Raynaud's Phenomenon evolving into SSc would be highly valuable for prompt initiation of SSc-disease- modifying therapy. Our goal is to utilize novel Aptamer-Based Proteomics for the analysis of the protein cargo of Serum Exosomes isolated

from patients with recent-onset diffuse SSc with and without SSc-ILD and from patients with Primary Raynaud's Phenomenon and patients with Raynaud's Phenomenon with positive serum antinuclear autoantibodies (ANA).
Specific Aims:
Specific Aim 1: Identify employing aptamer-based proteomics differentially expressed proteins in serum exosomes from patients with recent onset SSc-ILD compared with exosomes from SSc patients without SSc-ILD.
Specific Aim 2: Identify employing aptamer-based proteomics differentially expressed proteins in serum exosomes from patients with Primary Raynaud's Phenomenon compared with exosomes from patients with Raynaud's Phenomenon at high risk of evolving into SSc.
Specific Aim 3: Examine whether the Calt differentially increased proteins identified in Aims 1 and 2 induce in vitro a profibrotic phenotype in normal human lung fibroblasts or endothelial to mesenchymal transition (EndMT) in normal human lung microvascular endothelial cells.
Program Officer: Heiyoung Park
Role: Co-Investigator

R21 CA238651-01 (Zhang) 12/01/2019 -11/30/2021 3% / 0.36 Cal
NIH/NCI $13,735
Proteomic Study of Non-virus Related Hepatocellular Carcinoma Risk
The goals are to employ SOMAscan to develop plasma-based protein biomarkers for early detection of non-virus hepatocellular cancer
Specific Aims:
1) Determine plasma protein biomarkers for early detection of non-virus related HCC using SOMAscan.
2) Verify the SOMAscan identified protein biomarkers in an independent study.

Program Officer: Mukesh Verma
Role: Co-I

Recently Completed

1R01AT006464-01 (Benson) 12/01/10-08/31/16 9.5% / 1.14 CM
NIH $120,120
Quantification of outcome measures for mind-body interventions (Genomics, Libermann)
The goal of this grant is to identify and compare genomic determinants of responsiveness to relaxation response and yoga in healthy volunteers.
Specific Aims:
Aim 1. Isolate RNA and DNA from PBMCs and compare PBMC gene expression and methylation profiles in patients at baseline with matched patients without disease using Affymetrix whole genome GeneChips and whole genome Tiling Arrays and in depth bioinformatics analysis.
Aim 2. Examine changes in PBMC gene expression and methylation profiles in patients before and after treatment with mind-body intervention.
Aim 3. Examine gene expression and methylation profiles in patients before treatment in relation to the short- and long-term outcomes.
Program Officer: John R. Glowa
Role: Subcontract PI

Grant (Denninger) 07/01/2013-07/31/2016 23% / 2.76 CM
Adolph Coors Foundation $126,082
Impact of a relaxation response mind-body intervention on the immune-system cancer multiple myeloma, and its precursors
Our goal is to determine whether relaxation response impacts on gene expression, psychological and biological factors in patients with precursors of multiple myeloma.
Aim 1. Explore the biological, clinical, and psychological impact of the relaxation response intervention on

MGUS and SMM patients.
Aim 2. Build on our previous work to identify additional immune-related genes and their genomic regulators that are central to multiple myeloma and which, when modulated by the relaxation response, may delay the progression of MGUS and SMM to multiple myeloma.
Program Officer: John Jackson
Role: Subcontract PI

P01-AG031720-01A1 (Inouye) 04/01/10-03/31/16 5% / 0.6 CM
NIH/NIA $187,442
Project 2 (Marcantonio)- Interdisciplinary Study of Mechanisms and Long-Term Outcomes of Delirium
The goal of this grant is to identify and validate biomarkers for delirium.
Aim 1. To create a biorepository of genetic material and plasma that will be a resource for future biomarker discovery studies. We will perform Apo-E genotyping for all participants to be used as a covariable in analyses examining long term cognitive decline.
Aim 2. To describe a cytokine "signature" for delirium using the Luminex multiplex analyzer system and validate this cytokine signature in both the same population and an independent population of 100 surgical participants (50 with delirium, 50 matched controls) using ELISA methods
Aim 3. Using 50 surgical participants with delirium, their matched controls, and the longitudinal design described above, we will use quantitative mass spectrometry proteomics to describe a plasma protein "signature" for delirium. We will perform two validations: first, using proteomics in an independent population of 100 surgical participants, and then using ELISA methods in the combined population of 200 participants.
Aim 4. To examine the relationship of the cytokine and protein signatures for delirium with cognitive and functional outcomes up to 18 to 42 months after non-cardiac surgery.
Program Officer: Molly Wagster
Role: Co-Investigator

R21 TR001753 (Kokkotou) 11/01/2016-10/31/2018 5% / 0.6 CM
NIH/NCATS $200,000
Pre-clinical evaluation of a neutrophil elastase inhibitor for the treatment of inflammatory bowel disease
The goal of this project is to test the hypothesis that AZD9668, an oral inhibitor of human neutrophil elastase (NE) developed for the treatment of lung diseases, is an effective therapeutic option for patients with inflammatory bowel disease (IBD).
Specific Aims:
1) Demonstrate drug efficacy in mouse models of experimental colitis.
2) Proof-of-principle drug testing using mucosal explants from patients with IBD.
Program Officer: Bobbie Ann Austin Role: Co-Investigator

1R21 CA187843-01 (Libermann) 06/12/2015-05/31/2017 15% / 1.8 CM
NIH $150,000
Role of Axl in docetaxel resistance in prostate cancer
Our goal is to determine the relevance of Axl in resistance to docetaxel in metastatic castration-resistant prostate cancer.
Specific Aim 1. Determine the relevance of Axl expression/activity in docetaxel- and cabazitaxel-resistant prostate cancer cells
Specific Aim 2. Preclinical *in vivo* assessment of the relevance of Axl inhibition in docetaxel-resistant PCa xenografts to prevent, delay or overcome docetaxel resistance
Specific Aim 3. Determine the precise molecular mechanisms and signaling pathways involved in Axl regulation of docetaxel resistance
Program Officer: Suzanne Forry;
Role: PI

Onyx Pharmaceuticals (Bhasin) 12/10/2013 – 12/09/2018 1% / 0.12 CM
$53,723
Genomic and Psychosocial Effects of the Relaxation Response Resiliency Program (3RP) on Patients with MGUS and Smoldering Multiple Myeloma
Our goal is to determine whether relaxation response impacts on gene expression and NF-κB activity in bone marrow-derived multiple myeloma precursor cells in patients with precursors of multiple myeloma.
Specific Aims:
Aim 1. Determine the effects of relaxation response on bone marrow-derived multiple myeloma precursor cells in patients with precursors of multiple myeloma
A) Define transcriptome alterations induced by the relxation response therapy
B) Evaluate the effect of relaxation response therapy on NF-κB activity
Program Officer: Mary Joyce
Role: Co-Investigator

R01 DK 104346 (Martin) 09/18/2014 – 08/31/2018 13.5% / 1.62 CM
NIH/NIDDK $427,625
Title: Impact of fatty acid imbalance in intestinal health and disease in prematurity
This project will test our hypothesis through nutrigenomic strategies that early exposure to altered levels of critical fatty acids primes the premature infant's host responses and subsequent intestinal responses to the introduction of oral feedings resulting in an increased risk of this devastating intestinal inflammatory disease.
Specific Aim 1. Evaluate the priming effect of altered fatty acids induced by parenteral provision of lipid emulsions in preterm piglet model
Specific Aim 1. Directly evaluate the developmental intestinal adaptations of varying enteral fatty acids in *Murine* pup model
Program Officer: Padma Maruvada
Role: Co-Investigator

R21/33 AG057955 (Fong) 09/30/2017-05/31/2019 2% / 0.24 Cal
NIH/NIA $62,391
Blood-based Biomarkers of Neuronal Injury and Alzheimer's Disease: Predictors of Delirium and Long-term Cognitive Decline, and Potential Shared Pathophysiology
The goal is to define neuronal injury and AD miRNA biomarkers predictive of delirium and long-term cognitive decline in exosomes from plasma and CSF.
Specific Aims:
1) To identify candidate blood-based biomarkers (tau, NfL, and selected exosomal miRs associated with AD-related proteins) predictive of postoperative delirium or long-term cognitive decline (LTCD) – baseline, POD 2, discharge,
2) To investigate if the relationship of candidate biomarkers with delirium or LTCD is stronger among patients with mild cognitive impairment (MCI)
3) Candidate biomarkers identified in the R21 phase (to be associated with delirium) will be confirmed in a new prospective cohort of patients scheduled to undergo elective total hip or knee replacement under spinal anesthesia (n=125).
4) To determine if the relationship between candidate biomarkers, and delirium or LTCD is stronger in patients with preclinical AD. CSF tau and Aβ

Program Officer: Luci Roberts 301-496-9350 roberlu@mail.nih.gov
Role: PI

No Grant Number (Lai) 5/1/2017-4/30/19 12 % / 1.44 CM
Gilead $293,486
Biomarker Development Using Somascan Technology in NASH: Substudy for evaluation of the Gilead GS 384-1497 study of GS 4997 and simtuzamab in NASH patients

The goal is to characterize and validate serum biomarkers that (i) accurately distinguish between NASH and NAFL simple steatosis, (ii) detect presence or absence of advanced fibrosis, (Ill) are causally involved in NASH pathogenesis, and (iv) predict as well as monitor response to therapy.
Specific Aims:
1) Validate our serum protein signatures for NAFL, NASH, and advanced fibrosis using SOMAscan for diagnostic testing. There are currently no accurate noninvasive tests to determine whether a patient has NASH or simple steatosis. Serological tests to determine risk of NAS >4 would markedly simplify screening for clinical trials and reduce the high rate of screen failure on liver biopsy. MR and ultrasound based fibrosis imaging and serum fibrosis tests are less than optimal. Again a novel strategy for fibrosis staging would be valuable as a clinical trial screening test
2) Test the diagnostic accuracy of the SOMAscan-derived serum protein signatures to detect change in disease activity in the GS-US-384-1497 trial on patients enrolled in the treatment phase of the trial
3) Identify baseline SOMAscan serum protein signatures that predict response to treatment
Program Officer: Mani Subramanian
Role: PI (Multiple PI)

GW150202 (Kokkotou) 09/01/2016-08/31/2019 5% / 0.6 CM
Grant ID No. W81XWH-11-1-0351 $167,419
DoD – New Investigator Award
GWI: Molecular Analysis of Disease Endophenotypes and Response to Acupuncture Treatment
The goals are to employ an unbiased proteomics screening with the following goals: a) to develop diagnostic biomarkers for GWI, and possibly biomarkers for specific disease clusters; b) to identify signatures of biological changes overtime and in proportion to acupuncture dosage; c) to establish markers of increasing susceptibility to the acupuncture's healing effects.
Specific Aims:
1) To identify GWI-specific biomarker signatures by comparing the plasma proteome of GWI patients against matched healthy individuals and two different disease controls
2) To facilitate personalized acupuncture treatment delivery based on biomarkers
3) To understand the biological mechanisms of acupuncture healing.
Program Officer: Kristy B. Lidie
Role: Co-Investigator

Grant No. NA (Libermann) 3/16/2018-06/30/19 1% / 0.12 CM
Hitachi $71,870
Retrospective study on bladder cancer recurrence
The goal is to determine whether specific mRNA biomarkers in extracellular vesicles isolated from urine can predict bladder cancer recurrence.
Specific Aims:
1. Measure previously identified urinary EV mRNA markers in retrospectively collected urine samples from bladder cancer patients and remission control groups.
2. Determine diagnostic performances (AUC, sensitivity, specificity) of urinary EV mRNA markers and cytology for recurrent bladder cancer detection.
3. Compare clinical concordance of urinary EV mRNA markers with cytology and cystoscopy.
Program Officer: Wakako Matsumoto
Role: PI

R21 LM012759-01A1 (Otu) 08/01/2018-07/31/2020 10% / 1.2 CM
NIH/NLM $14,586 (subcontract only)
Identification and characterization of interaction atlases in humans
The goal is to create an interaction atlas for cancer in humans.
Specific Aims:
1) Learn the interaction atlas in humans.
2) Network analysis of PDAC in comparison to other gastrointestinal cancers.

Program Officer: Jane Ye
Role: Co-Investigator

20161002 (Inouye) 2/1/2017-7/31/2020 1% / .12 CM
ADDF $94,666 (subcontract only)
Exploring the Role of Inflammation in Long-Term Cognitive Decline Following Surgery and Delirium
Goal: To follow a cohort of patients undergoing total hip and knee replacement in order to identify biomarkers for delirium and cognitive decline after surgery.
Specific Aims:

1) To develop a predictive model for long-term cognitive decline following delirium utilizing existing SAGES data. This model will help us to identify high risk patients for a clinical trial.
2) To assess the correlation of plasma, CSF, and [11C]-PBR28 PET-imaging biomarkers of inflammation in patients undergoing major orthopedic surgery under spinal anesthesia.

Program Officer: Dan Teng
Role: Co-I

Overlap
There is no scientific or effort overlap with any of the PI's Active Awards.