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TITLE: Military Exposure-Related Pleural Mesothelioma: An Innovative Translational Approach to Inform Novel Molecular-Targeted Treatment Development

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

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Not

14. ABSTRACT
Malignant pleural mesothelioma (MPM) is a highly aggressive form of cancer that develops within the pleural lining of the lungs. Asbestos-related malignancies dropped precipitously in military/veteran populations upon the removal of asbestos from naval ship construction. However, older naval vessels and military facilities still containing asbestos were still in use decades later, resulting in thousands of veterans suffering asbestos exposure. Indeed, it is estimated that military veterans account for one third of all MPM patients. Despite this estimation, there is little data on the phenotype of military exposure and MPM pathogenesis. We recently defined the mutational landscape of MPM and have identified the most commonly mutated genes as BAP1, NF2, TP53, and SETD2, as well as other frequent mutations. We have also classified MPM into 4 distinct molecular clusters that provide new opportunities to identify MPM patients with better prognosis as well as to rationally divide tumors based on distinct molecular/biochemical driving mechanisms.

15. SUBJECT TERMS
Malignant pleural mesothelioma (MPM)

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1. **INTRODUCTION:**

Malignant pleural mesothelioma (MPM) is a highly aggressive form of cancer that develops within the pleural lining of the lungs. Asbestos-related malignancies dropped precipitously in military/veteran populations upon the removal of asbestos from naval ship construction. However, older naval vessels and military facilities still containing asbestos were still in use decades later, resulting in thousands of veterans suffering asbestos exposure. Indeed, it is estimated that military veterans account for one third of all MPM patients. Despite this estimation, there is little data on the phenotype of military exposure and MPM pathogenesis. We recently defined the mutational landscape of MPM and have identified the most commonly mutated genes as BAP1, NF2, TP53, and SETD2, as well as other frequent mutations. We have also classified MPM into 4 distinct molecular clusters that provide new opportunities to identify MPM patients with better prognosis as well as to rationally divide tumors based on distinct molecular/biochemical driving mechanisms. The objective of the study is to refine the classification of MPM into biologically and prognostically distinct sub-groups, relate these sub-groups to the military-exposed veterans and rationally design potential biomarker-selected targeted therapies for the military/veteran population for future human trials. This study aims to define and compare MPM tumors from military versus non-military cases for diagnosis and prognosis, using the type of mutations and cluster membership by RNA expression. This study also intends to identify potential novel therapies utilizing genetically-engineered mouse models (GEMMs) to interrogate MPM-specific tumorigenesis, invasion, and metastasis. Finally, this study plans to translate potential molecular targets into therapeutics using an *in-vivo* PDXs model. MPM tumors from civilian and military/veteran patients will be genotyped for the five most frequently mutated genes in MPM and will then be used to establish the distribution of mutations of all types in the 4 molecular cluster groups that have been classified. Frequently observed mutations or other genomic aberrations will be further interrogated using GEMMs to more completely understand MPM carcinogenesis and progression, as well as to identify potential targets for therapy. PDXs models will then be developed *in vivo* from the diagnostic/prognostic biomarkers that are identified in the civilian and military populations to focus pre-clinical therapeutics on the two extreme sub-types of MPM: 1 and 4.

2. **KEYWORDS:**

Malignant pleural mesothelioma (MPM)

genetically-engineered mouse models (GEMMs)

patient-derived xenograft (PDX)

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

The major goals established in the approved SOW are:

A. Establish Consortium Collaborative Infrastructure (Responsible PI, Harpole-Duke) – 95% complete
   i. Expected completion: 1-3 months
   ii. Actual completion: Pending UCSD ACURO approval
B. Perform RNA-seq analyses on the prospectively-collected, fresh-frozen MPM tumors (Responsible PI, Bueno-BWH) – 86% complete
   i. Expected completion: 3-9 months
   ii. Actual completion: Pending. 154 non-military cases of RNA have been sequenced and analysis has been completed. Sixty-two more cases are in the process of being sequenced this month (analysis pending). We expect completion will occur mid second year.
C. Investigate whether there are any genomic / genetic differences between civilian and veteran MPM tumors based on the consensus cluster expression and mutational genotyping (N=250 FFPE) (Responsible PI, Bueno-BWH)- 40% complete
   i. Expected completion: 6-18 months
   ii. Actual completion: We have compared 50 military to 154 non-military cases and found no differences in the distribution of cluster status. We have also performed all the NF2 FISH analysis to date. We expect to hit target completion outlined in the SOW.
D. Identification of Novel Therapies (Responsible PI, Harpole-Duke)- 0% complete
   i. Expected completion: 12-24 months
   ii. Actual completion: Pending

E. To translate potential molecular targets into therapeutics using an in-vivo PDX model (Responsible PI, Harpole-Duke) - 0% complete
    i. Expected completion: 24-36 months
    ii. Actual completion: Pending

What was accomplished under these goals?

A. Establish Consortium Collaborative Infrastructure (Responsible PI; Harpole, Duke)

Material transfer agreements have been completed for Duke, Brigham and Women's Hospital (BWH) and Cornell. Material and data can be shared between Duke, Cornell and BWH at this time. The MTA with UCSD is in process and will not affect moving forward with the proposed work. The study data dictionary has been completed and has been implemented into RedCAP. Duke and BWH finalized discussions regarding the addition of more variables and the definitions of these variables and their respective permissible values. Additional agreed upon variables were added to the project's central RedCAP database. The database has been developed, tested and is currently in production. The Duke dataset has been uploaded into RedCAP. The database has been opened to the study team at BWH and user rights have been established for them to begin uploading their dataset. Duke and the Brigham have obtained local IRB approval as well as HRPO approval under their respective awards. Duke, the Brigham, and UCSD have obtained local IACUC protocol approval from their respective institutions. Duke and the Brigham have also obtain ACURO approval under their respective awards. UCSD has submitted their protocol to ACURO and it is pending approval.

B. Perform RNA-seq analyses on the prospectively-collected, fresh-frozen MPM tumors (Responsible PI; Bueno, BWH)

Samples for analysis have been identified and RNA-seq assays have begun on the fresh-frozen MPM tumors under their approved HRPO. We compared and contrasted the distributions of gender, histology and the four transcriptional clusters between veteran and non-veteran patients within our cohort. We found that all veterans were male. For histology and the four transcriptional clusters we first compared the distributions for all categories using a chi-squared test. Next we combined all non-epithelioid samples into a non-epithelioid category for histology, and combined all non-cluster 1samples into a non-cluster 1 category. We then compared non-veteran and veteran distributions of theses implied histology and transcriptional cluster categories using both a chi-squared and a Fisher's exact test. We found no statistically significant differences in any of histology or transcriptional cluster distributions between the veteran and non-veteran populations. This work is still underpowered and we are continuing it in the new amplified size cohorts.

C. Investigate whether there are any genomic / genetic differences between civilian and veteran MPM tumors based on the consensus cluster expression and mutational genotyping (N=250 FFPE) (Responsible PI; Bueno, BWH)

Samples for analysis have been identified under Duke's HRPO. Samples are currently being cut and prepared. Forty-two (42) samples have been shipped to and received by BWH and are currently being prepared for analysis. Thirty-eight (38) additional samples are scheduled for shipment to BWH this week. Also, utilizing immunohistochemistry, 277 cases of mesothelioma have been analyzed for BAP1 and NF2 at BWH.

D. Identification of Novel Therapies – (Responsible PI; Harpole, Duke)

These studies have not begun. These will begin upon ACURO approval of UCSD's the animal use protocol. However, SOPs and preliminary work under UCSD's local IACUC are currently in development in preparation for approval so as not to delay starting this portion of the grant.

E. To translate potential molecular targets into therapeutics using an in-vivo PDX model. (Responsible PI; Harpole, Duke)

These studies are not anticipated to begin until year three of the grant.

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

Nothing to Report

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? We expect to receive

ACURO approval for UCSD within the next month

A. Sixty-two cases are in the process of RNA-seq analysis and we anticipate completion of this aim by the middle of year 2. This will include unsupervised analysis to identify potential novel, distinct MPM subgroups that could be added to our 4 consensus clusters as well as the validation analysis of our MPM consensus clusters in an independently-collected cohort of MPM tumors.
B. With continued submission and analysis (rt-PCR and genotyping) of our sample set, we will continue to investigate whether there are any genomic / genetic differences between civilian and veteran MPM tumors based on the consensus cluster expression and mutational genotyping and address the validation of the consensus clusters.
C. Upon approval of UCSD's ACURO protocol, animal studies will begin for the purpose of identification of genetic drivers that result in neoplasia from genes identified in consensus clusters 1 and 4.

4. **IMPACT:**

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

Nothing to Report. However, this comprehensive proposal for molecular characterization of mesothelioma whose goal is identification of novel targeted therapies specifically matched with genetically-identified subsets of tumors study, seeks to identify genetic markers specific to military-related MPM. Thus, these findings will be relevant to thousands of military veterans who were exposed to asbestos. The identification of these markers could lead to earlier/enhanced diagnosis and treatment strategies for veterans afflicted with this deadly disease, and improve patient survival.

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

Significant changes in use or care of human subjects: No changes have been made regarding the use or care of human subjects

Significant changes in use or care of vertebrate animals: No changes have been made regarding the use or care of vertebrate animals

Significant changes in use of biohazards and/or select agents: No changes have been made regarding the use of biohazards and/or select agents.

6. **PRODUCTS:**

Publications, conference papers, and presentations:

Nothing to Report.

Journal publications:

None at this time

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:

- **A.** Collaborative mesothelioma clinical RedCAP database
- **B.** Collaborative Biospecimen collection of mesothelioma tumor samples in FFPE – samples are continuing to be added as the project progresses
- **C.** Collaborative data repository of RNA-seq analyses on mesothelioma tumor samples – data is continuing to be added as analyses are completed

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

What individuals have worked on the project?

Name:	David Harpole, MD (Duke) – no change
Name:	Mary-Beth Joshi, MPH (Duke) – no change
Name:	Tam How (Duke)
Project Role:	Research Analyst
Nearest person month worked:	1
Contribution to Project:	manages the IACUC and ACURO submission and will perform PDX work
Funding Support:	DoD W81XWH-17-1-0372

Name:	Soraya Voight, MD (Duke)
Project Role:	Surgery Resident
Nearest person month worked:	2
Contribution to Project:	compiles and enters clinical outcomes data for data set, works with pathology to identify appropriate FFPE samples for submission for analysis
Funding Support:	Departmental

Name:	Karla Ballman, PhD (Cornell) – no change

Name:	Mark Onaitis, MD (UCSD) – no change

Name:	Priyanka Chaudhary, PhD (UCSD)
Project Role:	Postdoc
Nearest person month worked:	1
Contribution to Project:	managing IACUC and ACURO submission, Designing and performing experiments
Funding Support:	DoD W81XWH-17-1-0372

Name:	Guangfang Wang (UCSD)
Project Role:	Mouse-technician
Nearest person month worked:	1
Contribution to Project:	Managing breeding colonies
Funding Support:	DoD W81XWH-17-1-0372

Name:	Raphael Bueno (BWH) – no change

Name:	Nhien Dao (BWH)
Project Role:	Technician
Nearest person month worked:	2
Contribution to Project:	Performing technical effort in preparing samples for analysis
Funding Support:	DoD W81XWH-17-1-0372

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

Nothing to Report.

What other organizations were involved as partners?

Duke and Brigham and Women's are partnering PIs on this study.

8. **SPECIAL REPORTING REQUIREMENTS**

COLLABORATIVE AWARDS: Independent reports will be submitted by BOTH the Initiating PI and the Collaborating/Partnering PI.

9. **APPENDICES:** Not applicable