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TITLE: Genomics of Early Lung Cancer Among Military Personnel (GELCAMP)

PRINCIPAL INVESTIGATOR: Robert Browning, MD FACP, FCCP

CONTRACTING ORGANIZATION: Walter Reed National Military Medical Center, Bethesda, MD

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6. AUTHOR(S)
Robert Browning, MD FACP, FCCP
E-Mail: robert.f.browning2.civ@mail.mil

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Walter Reed National Military Medical Center
8901 Wisconsin Avenue
Bethesda, MD 20889

Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814

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14. ABSTRACT
Lung cancer is a leading cause of death worldwide, and improvements in morbidity and mortality hinge on continued investigation into the biology of cancer. Whole genome sequencing can uncover cancer-associated mutations that can meaningfully impact clinical care and decision-making. In this study, blood, bronchial epithelial cells and tumor tissue (as applicable) will be sequenced from 50 cases with confirmed cancer and 50 non-cancer controls. The resulting profiles will be analyzed for genetic markers that that may enable earlier diagnosis, guide therapy decisions and improve patient outcomes.

15. SUBJECT TERMS
Lung cancer, whole genome

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- **INTRODUCTION:**

  Currently several biomarkers are being validated on the DECAMP specimen, but none of these use the whole genome sequencing (WGS) technology. WGS offers a novel view of mutational changes in early lung cancer. We are testing for genomic biomarkers for the detection of early lung cancer using whole genome sequencing of DNA specimens from blood, endobronchial brushings and lung tumor specimens (for those with lung cancer). While genomic testing of lung cancer has been performed in other studies, testing other sites within the lung as well as peripheral blood for these biomarkers has not been attempted. The DEAMP cohort is unique for several reasons. The DECAMP cohort is the only cohort that specifically enrolls military personnel and offers the potential to identify mutations that might be linked to military specific exposures. It is also one of the most comprehensive and detailed cohorts associated with bronchoscopic brushings and tissue matched with CT imaging, clinical history in a longitudinal cohort.

- **KEYWORDS:**
  Cancer
  Lung
  Biomarkers
  Genomic
  Genome
  Tumor
  DNA
  Whole Genome Sequencing
  WGS

- **ACCOMPLISHMENTS:**
  - **What were the major goals of the project?**
    - Aim 1: Evaluate DNA copy number alterations and somatic mutations in the airway and tumor as well as germline polymorphisms that can distinguish benign vs. malignant nodules**.**
    - Aim 2: Characterize DNA copy number alterations and somatic mutations in the airway and tumor that associate with prognosis among those with lung cancer.

  - **What was accomplished under these goals?**
    - Discussed COVID-19 phase re-opening of laboratory operations with Boston University and USU to ship GELCAMP pilot samples
    - Examined alternatives with Boston University to conduct GELCAMP pilot samples due to COVID delays with USU lab operations
    - Successfully completed GELCAMP pilot DNA extraction from subject tumor slides yielding more than adequate DNA for sequencing.
    - Teleconference with Boston University and USU to discuss results of pilot sample of DNA extraction at Boston Laboratory and details for sequencing at USU.
      - DNA isolation metrics were generated from the pilot study at BU successfully isolating RNA and DNA from formalin fixed paraffin embedded (FFPE) tumor samples.

        - Consensus to add additional samples to provide more confidence in the methodology for isolation
    - Successfully isolated RNA and DNA from additional FFPE/slides tumor samples and from 2 fresh frozen tissue samples.
        - In effort to potentially preserve the frozen samples to be utilized across additional technologies the frozen sample was embedded into optimal cutting tissue compound (OCT), maintaining the sample to remain frozen during the processing. Samples were then sectioned into 10 (20 micron) sections that are subject to lysis and stored at -80 conditions. Utilizing the Qiagen AllPrep DNA/RNA Kit, RNA and DNA were simultaneously isolated from 2 OCT -tumor sample. Following an initial processing we were able to isolate an abundant amount of both RNA (avg. 5,000ng) and DNA (avg.10,000 ng), where the quality was variable (RIN 5-2.7) and DNA quality was excellent (DIN 8).

- **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?**
    - Continue routine teleconferences between Boston University and investigators at USU and WRNMMC to coordinate study activities and establish the optimal method for RNA and concomitant DNA isolation
    - Assess DNA quality from the additional FFPE and frozen tissue samples.
    - Review and discuss DNA quality metrics
    - Ship GELCAMP pilot samples to USU for test extraction and assure DNA quality is sufficient
    - Based on pilot samples, finalize with USU Investigators the number of slides needed in order to have enough material to isolate DNA and RNA for whole genome sequencing
    - Coordinate with Boston University to generate a master list with GELCAMP identifiers to be shared with USU and Brown University
    - Finalize existing DECAMP shipping SOP for GELCAMP requirements.
    - Ship rest of samples to USU along with the master list to perform extraction on the rest of the selected DECAMP tumor tissue slides and for whole genome sequencing.
    - Coordinate with Brown University to collect clinical data from the selected DECAMP samples, have data files relabeled and sent to USU for linkage.
    - Modify existing USU array processing SOP for GELCAMP protocol.
    - Coordinate with technicians at USU to have samples relabeled appropriately using the master list.
    - Modify and finalize data tracking / sharing mechanism between WRNMMC and USU

- Coordinate with Brown University to perform interim and final analysis of the linked clinical and whole genome sequencing data
- Continue operational teams and routine teleconferences to discuss study progress and timelines between Brown University, Boston University and investigators at USU and WRNMMC
- Prepare and submit manuscript to peer review journal.

- **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.

- **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**
    - *COVID19 Research Priorities:* A pilot of tissue slides was selected for shipment to USU for extraction. Originally, it was planned that USU would perform DNA and RNA extraction using these slides to determine the amount of material that could be extracted from each slide. Unfortunately, the research efforts and sample shipments were put on hold as the USU Genome Center was assigned to COVID-19 efforts. Non COVID research was then allowed to restart at Walter Reed and USU a couple of months later but the priority for research time and efforts was primarily COVID focused research particularly for the USU DOD genomics lab. To avoid more delays, the team decided to have Boston University conduct the pilot samples. DNA isolation metrics were generated from the pilot study that was conducted to identify the best means of isolation of RNA and DNA from formalin fixed paraffin embedded (FFPE) tumor samples. The metrics which demonstrated promising results, were presented and discuss with the partners. There was a consensus to include additional FFPE samples as well as samples from fresh frozen tissue to further establish the ideal method for isolation for the rest of the samples. Unfortunately, this step was further delayed due to BU not having all the samples residing in house. At the time, BU was in the process of transitioning its biorepository from their current pathology core lab but issues with the sub-contract as well as shipping delays due to COVID, delayed this transition. Despite the progress with the pilot study, we submitted a request for a second no cost extension due to significant delays impacting this study with HRPO/IRB approval, biorepository issues, and COVID closures.

- **Changes that had a significant impact on expenditures**
  - Delays in protocol approval and COVID closures have delayed collaborators contributions,
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - Nothing to Report.

- **PRODUCTS:** Nothing to Report.

- **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
  - **What individuals have worked on the project?** Individuals supporting the project for the past year in excess of 160 hours are:
    - Dr. Robert Browning, PI
    - Luis Rojas, Research Coordinator
    - Folashade Akani, Research Assistant

  - **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?**
    - Uniform Services University, Bethesda MD
      - Facilities: The American Genome Center
      - Collaboration: Dr. Clifton Dalgard
    - Brown University, Providence, RI
      - Collaboration: Dr. Fenghai Duan (no contributions thus far due to protocol delay)
    - Boston University, Boston, MA

- **SPECIAL REPORTING REQUIREMENTS**
  - COLLABORATIVE AWARDS: Nothing to Report

- **APPENDICES:**
  - Quad Chart