Award Number: W81XWH-07-1-0144

TITLE: Detection of Prostate Cancer Progression by Serum DNA Integrity

PRINCIPAL INVESTIGATOR: Dave S.B. Hoon, Ph.D.

CONTRACTING ORGANIZATION: John Wayne Cancer Institute
Santa Monica, CA 90404

REPORT DATE: April 2008

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.
Detection of Prostate Cancer Progression by Serum DNA Integrity

Dave S.B. Hoon, Ph.D.

E-Mail: hoond@jwci.org

John Wayne Cancer Institute
Santa Monica, CA 90404

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

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The main objective/hypothesis for this proposal is that additional diagnostic and prognostic insight can be obtained through analysis of tumor-related DNA integrity and methylation in patient serum. Additionally, DNA biomarkers will be assessed for their usefulness in predicting early disease recurrence and progression in patients during PCa treatment. The scope of the studies is to optimize and validate Alu, LINE1 and uLINE1 assays for assessment of prostate cancer (PCa) patient serum targeted for clinical utility. For the first year, we have put in place specimen procurement and processing protocols and optimized assays for Alu qRT and LINE1 qRT. The uLINE1 AQAMA (absolute quantitative allele methylation assay) assay is also near optimal. We have utilized prostate cell lines and tumor specimens for the initial assay optimization and validation. In the coming year, more PCA patients will be accrued as well as further accrual of serum at defined treatment intervals. Assays for the markers will be carried out once we have obtained statistically meaningful numbers of serum sets of PCa patients in treatment. We are also continuing our efforts in discovering additional clinically relevant DNA biomarkers.

15. SUBJECT TERMS
Circulating DNA, serum, prostate cancer, methylation, PCR

16. SECURITY CLASSIFICATION OF:

17. LIMITATION OF ABSTRACT
UU

18. NUMBER OF PAGES
12

19. NAME OF RESPONSIBLE PERSON
USAMRMC

19B. TELEPHONE NUMBER (include area code)
# Table of Contents

- **Introduction** .................................................. 4  
- **Body** .................................................................. 5  
- **Key Research Accomplishments** .............................. 7  
- **Reportable Outcomes** ............................................ 8  
- **Conclusions** ...................................................... 9  
- **References** .......................................................... 10  
- **List of Personnel** .................................................. 11  
- **Appendices** ......................................................... 12
INTRODUCTION

The main hypothesis of the proposal is that the detection of serum circulating tumor-related DNA marker(s) are surrogate genetic indicators of primary prostate cancer (PCa) tumor status and can be used to facilitate diagnosis, prognosis, predict treatment response, and aid in the surveillance of early disease recurrence. In the program years, our efforts will specifically focus on 1) Assessment of the Alu DNA integrity marker in PCa patients’ serum using a quantitative direct assay to determine its clinical utility; 2) Assessment of LINE1 DNA integrity marker and uLINE1 marker in PCa patients’ serum using quantitative assays to determine their clinical utility; and 3) Determine the combined clinical utility of the three circulating serum tumor-related DNA markers in monitoring patients’ response to treatment. The long-term goal is to validate the clinical utility of these markers.
In this first year of the proposal period, we began accruing PCa patients and normal donor serum for the study with an IRB approved protocol. As outlined in Task 1, Samples are being collected, coded, logged into a database, processed for serum and quality assured. Task 1 will be ongoing until we reach our proposed sample numbers.

Optimization of Alu and LINE1 assays are currently underway. The use of PCa cell lines facilitated the progress of the task. By using paraffin-embedded (PE) PCa tissue samples as clinical samples, we were able to further validate our assays. We have demonstrated proficiency in DNA extraction from paraffin-embedded tissues which were first micro-dissected using LCM. Because of the multifocal nature of the tumor, micro-dissection is necessary to isolate the cancer cells for validation of these markers as tumor markers.

We have devoted major efforts in developing the uLINE1 AQAMA assay. The protocol involved designing and testing specific primer sequences for methylated (LINE 1) and unmethylated (uLINE1) LINE1. In a pilot study, 18 prostate cancer PE tissues with matched adjacent normal tissues were assessed with the assay. Although the specimen numbers are limited, we observed that tumors tend to show higher uLINE1 index compared with normal tissue. Additionally, unifocal cancer showed significantly high U index compared with multifocal cancer (p=0.0067) (Figure A). Tumor U index is significantly correlated with prostate volume (p=0.0191) (Figure B).

In patient serum samples, we have worked on optimizing the assay. We were able to use samples from a small patient population and normal donors to establish the sensitivity and specificity of the LINE1 assay (Figure C).
So far, with the limited samples we have tested, we were able to confirm LINE1 is highly methylated in normal male donor serum and unmethylated in prostate cancer patients.

We plan to continue our efforts and optimize these assays to begin assessing patients serum in the coming year. We will accrue patients with BPH and prostatitis as well as age matched normal male donors. Refer to the grant tasks itemization and list for our future direction.
KEY RESEARCH ACCOMPLISHMENTS

1. PCa patient accrual for serum.
3. Database constructed and utilized to keep track of samples.
4. Clinical data recorded and collected in the database.
5. Blood is processed for serum; DNA is extracted and quantified
6. LINE1 methylation biomarkers in serum are detected.
7. LINE1 methylation biomarkers assessed and optimized for detection in serum.
8. LINE1 methylation biomarkers optimized for specificity and sensitivity.
REPORTABLE OUTCOMES

No reportable outcomes have occurred during this time period.
CONCLUSIONS

The planned studies are being conducted according to the approved schedule as delineated in the protocol.
REFERENCES

None.
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dave S.B. Hoon, MSc, PhD</td>
<td>John Wayne Cancer Institute at Saint John’s Health Center</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>Frederick R. Singer, MD</td>
<td>John Wayne Cancer Institute at Saint John’s Health Center</td>
<td>Co-Investigator</td>
</tr>
<tr>
<td>David Elashoff, PhD</td>
<td>University of California, Los Angeles</td>
<td>Co-Investigator</td>
</tr>
<tr>
<td>Ryuichi Mizuno, MD</td>
<td>John Wayne Cancer Institute at Saint John’s Health Center</td>
<td>Research Fellow</td>
</tr>
<tr>
<td>Tung Nguyen</td>
<td>John Wayne Cancer Institute at Saint John’s Health Center</td>
<td>Research Associate</td>
</tr>
</tbody>
</table>
APPENDICES

None.