

AD _____

Award Number: W81XWH-12-1-0032

TITLE: "DNA Damage and Genetic Instability as Harbingers of Prostate Cancer"

PRINCIPAL INVESTIGATOR: Dr. Amit Algotar

CONTRACTING ORGANIZATION: The University of Arizona
Tucson, AZ 85719-4824

REPORT DATE: June 2013

TYPE OF REPORT: Final

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.

REPORT DOCUMENTATION PAGE		<i>Form Approved</i> OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.			
1. REPORT DATE June 2013	2. REPORT TYPE Final	3. DATES COVERED 15 January 2012 – 14 May 2013	
4. TITLE AND SUBTITLE “DNA Damage and Genetic Instability as Harbingers of Prostate Cancer”		5a. CONTRACT NUMBER	
		5b. GRANT NUMBER W81XWH-12-1-0032	
		5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Dr. Amit Algotar E-Mail: algotar@email.arizona.edu		5d. PROJECT NUMBER	
		5e. TASK NUMBER	
		5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Arizona Tucson, AZ 85719-4824		8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSOR/MONITOR'S ACRONYM(S)	
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			
13. SUPPLEMENTARY NOTES			
14. ABSTRACT Current diagnostic modalities are inadequate to reliably differentiate between aggressive and indolent forms of prostate cancer (PCa). A significant number of men receive potentially unnecessary treatment along with associated morbidities. Identification of novel risk factors will allow for more reliable early diagnosis of PCa. Defective sensors of DNA damage and the resulting genetic instability may be involved in the early development of PCa. The objective of this project is to determine if DNA damage and genetic instability are harbingers of PCa using prostate biopsy samples from men at high risk for this disease. I am happy to report that this project has been completed six months ahead of time and below are its final results.			

15. SUBJECT TERMS Prostate cancer Prostate specific antigen ERG PTEN DNA damage					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U	UU	12	USAMRMC
					19b. TELEPHONE NUMBER <i>(include area code)</i>

Table of Contents

	<u>Page</u>
Introduction.....	5
Body.....	5
Key Research Accomplishments.....	9
Reportable Outcomes.....	10
Conclusion.....	11

INTRODUCTION:

Current diagnostic modalities are inadequate to reliably differentiate between aggressive and indolent forms of prostate cancer (PCa). A significant number of men receive potentially unnecessary treatment along with associated morbidities. Identification of novel risk factors will allow for more reliable early diagnosis of PCa. Defective sensors of DNA damage and the resulting genetic instability likely are involved in the early development of PCa. The objective of this project is to determine if DNA damage and genetic instability are harbingers of PCa using prostate biopsy samples from men at high risk for this disease. Expression of H2AX and RAD51 will be used as markers of DNA damage and repair whereas ERG and PTEN expression will be used as indicators for genetic instability. Formalin-fixed, paraffin-embedded prostate tissue biopsy slides from 150 men enrolled in a chemoprevention trial conducted at the Arizona Cancer Center. This trial was known as the negative biopsy trial (NBT) and the its primary aim was determine if selenium supplementation can reduce incidence of prostate cancer as compared to placebo. Primary analysis of this trial indicated no statistically significant effect of selenium supplementation on prostate cancer incidence. As a result, all the data, biological samples and biopsy tissue that were collected during the conduct of this trial could be used for subsequent research projects. The slides collected during NBT and identified for the current project will be processed for expression of markers of DNA damage and genetic instability using immunohistochemistry and fluorescence in-situ hybridization.

Body:

Tasks completed during the course of second project year.

Block 1: Months 1-6, January 2013 to June 2013.

Task 1: Gaining clinical knowledge regarding prostate cancer

- In order to gain in-depth clinical knowledge regarding prostate cancer, Dr. Algotar attended the urology oncology clinic with Dr. Mitchell Sokoloff, division head for Urology at The University of Arizona Cancer Center. Dr. Sokoloff's practice is exclusively focused on prostate cancer. Here Dr. Algotar was able to learn about clinical aspects of prostate cancer such as history taking, physical examination, appropriate diagnostic modalities, their advantages and disadvantages and their optimum utility. He also learned appropriate biopsy and surgery techniques as well as associated risk factors and limitations of each. Attending pre and post diagnosis patient counseling sessions allowed Dr. Algotar to understand the perspective of patients and their spouse to this disease and the effect clinical interventions and treatment modalities have on not only the physical aspects of the patients and their spouses but also their mental and psychological well-being, thus providing him with a well-rounded insight and understanding regarding this deadly disease which is of tremendous public health important especially in elderly US population.

Task 2: Training in laboratory techniques needed for this project

- Optimization of immunohistochemistry procedures and protocols for staining the study slides for two markers (H2AX and RAD51), was carried out by Dr. Algotar under the supervision of experienced laboratory manager Mary Krutzsch. Antibody for H2AX was obtained from Cell Signaling Technologies while the antibody for RAD51 was ordered from Abcam chemicals. A total of 300 slides were processed by hand, 150 for H2AX and 150 for RAD51.
- Result interpretation for H2AX was carried out with an experience genitourinary pathologist (Dr. Raymond Nagle) using a continuous scale. Result interpretation for RAD51 was carried out using Spectrum Version 10.2.2.2315, a quantifiable image analysis software from Aperio Technologies.

Task 3: Data analysis and Results:

- Data cleaning, quality control and statistical analyses were carried out by the project PI (Dr. Algotar) using Stata12 statistical software (StataCorp, College Station, TX).
- For H2AX, the pathologist (Dr. Nagle) scored slides using a continuous scale. After finding a representative gland, he estimate what percent of luminal cells were stained 1+, 2+, 3+ or completely negative (scored as zero). Total score for each case was calculated as follows. If a subject was scored as having 20% luminal cells to be 1+, 10% to be 2+ and 5% to be 3+, the subject's total H2AXscore would be $= (20*1)+(10*2)+(5*3) = 55$. Figure 1 demonstrates positive (blue arrow) and negative staining (red arrow) for H2AX in prostate biopsy tissue. Results of logistic regression indicate that H2AX score was not statistically significantly associated with diagnosis of prostate cancer ($p=0.696$) however, it was statistically significantly associated with serum prostate specific antigen levels ($p = 0.02$).

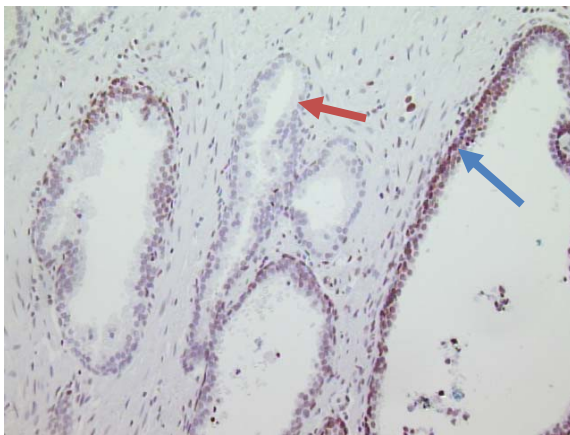


Figure 1: Prostate glandular tissue showing positive (blue arrow) and negative staining (red arrow) for H2AX

- For RAD51, proportion of percent positive nuclei were determined using above mentioned digital analysis software. After adjusting for age, race and baseline PSA, results of logistic regression indicate that RAD51 expression was statistically significantly associated with diagnosis of prostate cancer ($p < 0.001$). RAD51 expression was also a strong predictor of subsequent diagnosis of prostate cancer. Models comparing data currently available to urologist (model X1) and models including RAD51 data (model X2 = X1 + RAD51 expression data) showed that including RAD51 data statistically significantly increased model prediction for subsequent diagnosis of prostate cancer. Area under the curve for model X1 was 0.7324 whereas the area under the curve for model X2 was 0.8424 and the difference between these two areas was statistically significant, $p = 0.0081$ (Figure 3).

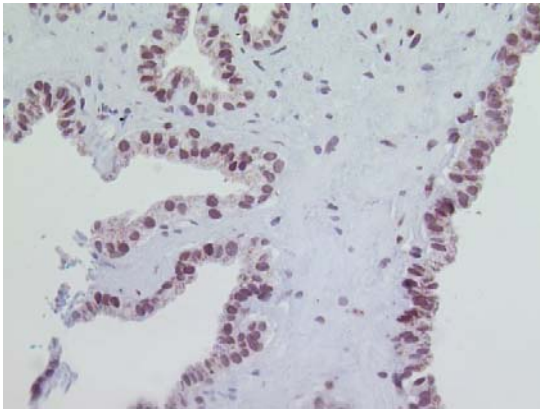


Figure 2: Brown nuclear and cytoplasmic staining indicates positive staining for RAD51

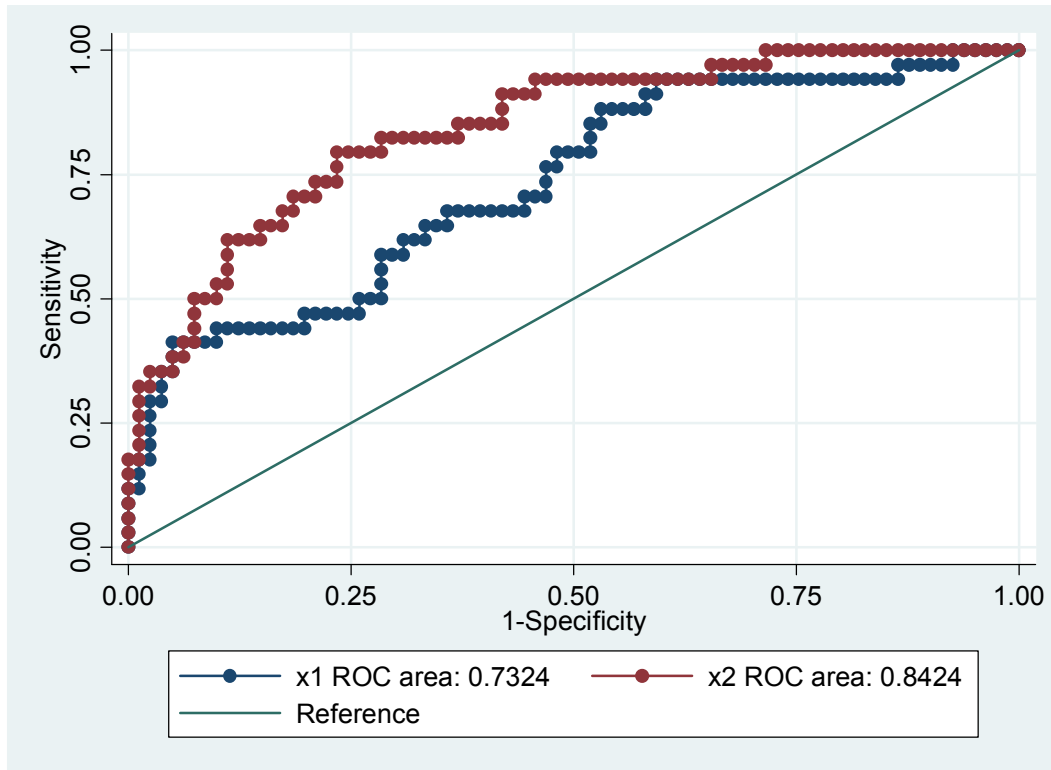


Figure 3: ROC curves comparing models with (X2) and without RAD51 (X1) data.

Task 4: Modified aim 1:

- Primary hypothesis of aim 1 of this grant was that ERG-PTEN expression patterns in prostate biopsy will be predictive of subsequent diagnosis of prostate cancer. However, as per the data analysis presented in the previous annual report this hypothesis did not hold true. As a result we presented and obtained approval for a modified hypothesis which states that ERG-PTEN expression patterns in prostate biopsy will be associated with prostate cancer aggressiveness. For this modified hypothesis, slides were pulled from subjects who had participated in the Watchful Waiting (WW) study. The WW was a Phase 2 chemoprevention trial carried out to determine the effect of selenium supplementation on prostate cancer progression. Patient clinical data and biopsy samples were collected as part of the WW and were IRB approved for future research. All subjects in this trial had a biopsy confirmed diagnosis of prostate cancer. Slides from these biopsies were pulled for staining for ERG and PTEN using established protocols and were carried out under the supervision an experienced genitourinary pathologist, Dr. Raymond Nagle. PSA velocity, rate of prostate specific change over time, a commonly used clinical marker of prostate cancer aggressiveness was used as a marker of prostate cancer aggressiveness for this study. Results indicate that ERG and PTEN expression is independently associated with lower PSA velocity ($p = 0.02$ and 0.037 respectively). Joint effect of ERG and PTEN expression on PSA velocity was also analyzed using logistic regression. As compared to the wild type (ERG+/PTEN-), ERG-/PTEN- and ERG-/PTEN+ subjects demonstrated increased risk for high PSA velocity. Odds ratios

(95% confidence intervals) are: 4.05 (0.68, 24.1) and 1.71 (0.12, 23.1) respectively. These models were adjusted for age, race and Gleason score. This is the first study to report on an association between ERG-PTEN expression and PSA velocity, an important clinical marker of aggressive prostate cancer. Results of this study are counterintuitive to the current understanding of ERG-PTEN mechanism in prostate carcinogenesis indicating presence of yet undiscovered molecular pathways. Hence further research is needed to confirm these findings and delineate these pathways to determine clinical utility of ERG and PTEN expression in prostate cancer.

Task 5: Meetings and conferences

- Attended and presented poster at American Association for Cancer Research annual meeting at Washington, DC (April 6-10, 2013).
- Attended weekly mentoring meetings with primary and secondary mentors
- Attended six monthly mentoring committee meetings with mentoring committee
- Attended weekly meetings and seminars: Prostate Invasion and Metastasis Group, Prostate Cancer Translational Research meetings, Urology Grand Rounds, Cancer Prevention and Control Seminar, and Cancer Biology Seminar.
- Attended monthly meetings and seminars: Medical Oncology Genitourinary Conference, Frontiers in Medical Research and Hematology-Oncology Conference
- Attended quarterly Post-doctoral training workshop

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

- H2AX expression in prostate biopsy tissue does not predict subsequent diagnosis of prostate cancer
- H2AX expression in prostate biopsy tissue is associated with lower PSA.
- RAD51 expression in prostate biopsy tissue does predict subsequent diagnosis of prostate cancer
- ERG-PTEN expression profile of prostate biopsy tissue maybe associated with PSA velocity, an important clinical indicator of aggressive prostate cancer.
- Improved understanding of the principal investigator regarding the clinical aspects of prostate cancer as well as the patient and spouse perspectives regarding prostate cancer.
- Findings from this project increase our understanding regarding the role of markers associated with DNA damage (H2AX and RAD51) and genetic instability (ERG and

PTEN) in diagnosis of prostate cancer and prostate aggressiveness and opens avenues for future research.

FUTURE PROJECTS:

- What effect does ERG-PTEN expression have on markers of DNA damage and repair?
- Are markers associated with DNA damage and repair associated with prostate cancer aggressiveness?
- Do markers associated with genetic instability and DNA damage predict response to treatment?

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

- Manuscripts:
 - o Singh P., **Algotar A.M.**, Bracamonte E. R. Prostate small cell carcinoma: diagnosis and management. *Journal of Cancer Therapy*. 2013 May; 4(3): 804-810.
 - o Nagle R.B., **Algotar A.M.**, Cortez C.C., Smith K., Jones C., Sathyanarayana U.G., Yun S., Riley J., Nagy D., Dittamore R., Dalkin B., Brosh L., Pestano G. ERG Overexpression and PTEN Status Predict Capsular Penetration in Prostate Carcinoma. *Prostate*. 2013 May 7. doi: 10.1002/pros.22675. [Epub ahead of print]
 - o **Algotar A.M.**, Cress A.E., Nagle R.B., Sokoloff M. H., Singh P., Ahmann F. R., Hsu C.H, Abril E., Drinkwitz D., Stratton S. P. ERG-PTEN expression profile of prostate biopsy tissue is associated with PSA velocity. (Under review).
 - o **Algotar A.M.**, Cress A.E., Nagle R.B., Hsu C.H, Kruttsch M., Drinkwitz D., Lodhia N., Bermudez Y., Stratton S. P. DNA damage in prostate biopsy specimen is associated with lower PSA. (Under review).
 - o **Algotar A.M.**, Cress A.E., Nagle R.B., Hsu C.H, Kruttsch M., Drinkwitz D., Lodhia N., Stratton S. P. Markers associated with DNA repair can be as predictors for diagnosing prostate cancer. (Under review).
- Posters:
 - o **Algotar A. M.**, Hsu C.H., Stratton S.P. Does selenium supplementation increase serum glucose levels in men at high risk for prostate cancer? American Association for Cancer Research Annual Meeting 2013 (April 6-10, 2013), Washington D.C. Poster number 3615.
 - o **Algotar A. M.**, Sokoloff M.H., Singh P. Hsu C.H., Stratton S.P. Negative association of smoking with PSA and PSA velocity. American Society for Clinical Oncology Annual Meeting. Chicago, IL. May 31-June 4, 2013. Abstract number e16060.

- Book chapter:
 - o **Algotar A.M.**, Stratton M.S., Cress A.E. A new paradigm for prostate cancer prevention studies. Fundamentals of cancer prevention. Third edition. Springer publications. 2013.
- Patents:
 - o **Algotar A. M.**, Cress A. E., Stratton S.P., Nagle R.B. Utility of DNA damage markers in prostate cancer diagnosis. (Pending)
 - o Pestano G., Nagle R.B., Cortez C., Vanpatten C., **Algotar A.M.** Expression of ETS related gene (ERG) & phosphatase & tensin homolog (PTEN) correlates with prostate cancer capsular penetration. (Pending)
- Grants applied:

Sponsor	Project Title
The University of Arizona Cancer Center Support Grants Pilot Projects	Pilot study of MRI scans to assess progression of disease in patients with prostate cancer
The University of Arizona Cancer Center Support Grants Pilot Projects	Pilot study to assess feasibility of Sequential Whole Genome sequencing in patients with prostate cancer progressing on different lines of treatment
The University of Arizona Faculty Small Grants Program	PSA isoform kinetics as predictors of prostate cancer
The University of Arizona Faculty Small Grants Program	Genomic profiling of castrate refractory prostate cancer

CONCLUSION:

Aim of this project is to determine if DNA damage and genetic instability are harbingers of prostate cancer using prostate biopsy samples from men at high risk for this disease. I am happy to report the completion of this project six months earlier than the project deadline. Results indicate that the genetic instability may not be associated with subsequent diagnosis of prostate cancer however, it does demonstrate association with prostate cancer aggressiveness. Marker associated with DNA repair demonstrates association with prostate cancer diagnosis and improves the predictive ability regarding subsequent diagnosis of prostate cancer.

This project has allowed the principal investigator to not only develop a strong understanding regarding the methods and techniques involved with basic and translational research in prostate

cancer but has also strengthened the PI's knowledge regarding the clinical aspects of prostate cancer building a very unique knowledge and skill set. Results of this project not only improve our understanding regarding the role of markers associated with DNA damage (H2AX and RAD51) and genetic instability (ERG and PTEN) in diagnosis of prostate cancer and prostate aggressiveness but also open avenues for future research such as those outlined in the future projects section of this document.