

AWARD NUMBER: W81XWH-16-1-0541

TITLE: Fusion Genes Predict Prostate Cancer Recurrence

PRINCIPAL INVESTIGATOR: Dr. Jianhua Luo

CONTRACTING ORGANIZATION: University of Pittsburgh  
Pittsburgh, PA 15213

REPORT DATE: October 2018

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.

# REPORT DOCUMENTATION PAGE

Form Approved  
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.**

<b>1. REPORT DATE</b> October 2018		<b>2. REPORT TYPE</b> Annual		<b>3. DATES COVERED</b> 15 Sep 2017-14 Sep 2018	
<b>4. TITLE AND SUBTITLE</b> Fusion Genes Predict Prostate Cancer Recurrence				<b>5a. CONTRACT NUMBER</b>	
				<b>5b. GRANT NUMBER</b> W81XWH-16-1-0541	
				<b>5c. PROGRAM ELEMENT NUMBER</b>	
<b>6. AUTHOR(S)</b> Jianhua Luo  E-Mail: Luo@upmc.edu				<b>5d. PROJECT NUMBER</b>	
				<b>5e. TASK NUMBER</b>	
				<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  University of Pittsburgh, 3550 Terrace Street, Pittsburgh, PA15261				<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>	
				<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b>  Approved for Public Release; Distribution Unlimited					
<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> Most prostate are indolent, and may not require clinical intervention. Only a small fraction of cases progress to metastatic disease if not treated. Prediction of the clinical outcomes of prostate cancer remains a challenge. Recently, we discovered a panel of 8 fusion genes that occurred in aggressive prostate cancer. The presence of these fusion genes correlates 91% chance of recurrence of prostate cancer. In order to make the fusion gene test clinically ready as a predictor, we have modified to test into a semi-quantitative Taqman QRT-PCR. The tests were performed in CLIA certified lab. Four hundred and sixty samples of prostate cancers were collected. Prostate cancer cells were microdissected. Taqman QRT-PCRs were performed on these samples. Significant numbers of samples were found positive for some of these fusion genes. In addition, we have analyzed 150 samples from Stanford University and 155 samples from University of Wisconsin Madison. A training model has been constructed. We are in the process to apply the model to testing sets.					
<b>15. SUBJECT TERMS</b>					
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>  Unclassified	<b>18. NUMBER OF PAGES</b>	<b>19a. NAME OF RESPONSIBLE PERSON</b> USAMRMC
<b>a. REPORT</b>  Unclassified	<b>b. ABSTRACT</b>  Unclassified	<b>c. THIS PAGE</b>  Unclassified			<b>19b. TELEPHONE NUMBER (include area code)</b>

## Table of Contents

Page

<b>1. Introduction.....</b>	
<b>2. Keywords.....</b>	
<b>3. Accomplishments.....</b>	
<b>4. Impact.....</b>	
<b>5. Changes/Problems.....</b>	
<b>6. Products.....</b>	
<b>7. Participants &amp; Other Collaborating Organizations.....</b>	
<b>8. Special Reporting Requirements.....</b>	
<b>9. Appendices.....</b>	

**Major Task 1 :** We will conduct analysis of MAN2A1-FER, SLC45A2-AMACR, TRMT11-GRIK2, MTOR-TP53BP1, LRRC59-FLJ60017, CCNH-C5orf30, KDM4-AC011523.2, TMEM135-CCDC67 on 5106 prostate cancer samples collected from University of Pittsburgh, Stanford University and University of Wisconsin Madison. We will first establish prostate cancer recurrence model and short PSADT prediction models either by fusion gene status alone or in combination with nomogram based on the cohort from 600 radical prostatectomy samples from UPMC. This model will be locked in and tested on cohorts from University of Pittsburgh, Stanford University and University of Wisconsin. The prediction accuracy, sensitivity and specificity within each cohort will be evaluated.

**Subtask 1:** In the first 3 months of the funded period, we plan to establish this test in the CLIA certified laboratory at the University of Pittsburgh Medical Center. Fifty-six FFPE samples that were shown to be positive for at least one fusion transcripts in the matched frozen tissues. These FFPE samples had been tested in non CLIA certified laboratory, and achieved 98.9% sensitivity and 100% specificity. We will repeat the same tests on these samples in CLIA certified laboratories. All PCR products will be analyzed through Sanger’s sequencing to confirm the authenticity of the fusion products. In addition, all fusion minigene RNA templates will be serially diluted. TAQMAN QRT-PCR will be performed to evaluate the sensitivity of the test. Detection threshold will be obtained. Random selection of 600 prostate cancer samples with definitive clinical outcomes will be carried out in UPMC campus. TAQMAN QRT-PCR on  $\beta$ -actin will be used as RNA quality control. For sites 2 and 3, all relevant institutional review board exempt protocols will be secured and approved.

**Progress:** We have procured the CLIA certified lab space in the beginning of the funded period. To accommodate the reality of formalin-fixed and paraffin-embedded tissues, we have designed a set of new primers and Taqman PCR probes for highly fragmented RNA species. These sets of primers and probes were subsequently tested and validated on synthetic mini-fusion genes of MAN2A1-FER, TRMT11-GRIK2, MTOR-TP53BP1, CCNH-C5orf30, KDM4-AC011523.2, SLC45A2-AMACR, TMEM135-CCDC67, and LRRC59-FLJ60017. The probe and primers for  $\beta$ -actin were also revised to accommodate a shorter RNA fragment. The analyses showed that these assays detect as low as 600-1000 molecules of these fusion transcripts. We then analyzed 56 FFPE samples whose frozen counterparts have been previous found to contain at least one fusion gene using these sets of probes and primers. All samples that were positive for these fusion genes were also positive in the new Taqman qRT-PCR assays. The positive match rate is 100%. All participating institutes, including University of Pittsburgh, Stanford University and University of Wisconsin Madison, had obtained the institutional approval for the exempt protocols.

**Subtask 2:** From month 4-9 of the first funded year, we will perform TAQMAN QRT-PCR and Sanger’s sequencing on a randomly selected cohort of 600 samples from phase 1 that have at least 5 years clinical follow-up. These tests will be performed in CLIA certified laboratory of University of Pittsburgh. The prediction models of PCa recurrence and PSADT mentioned will be developed based on this large number of samples. For sites 2 and 3, the first 300 prostate cancer cases from each site will be selected and evaluated for sufficient materials for the assay.

**Progress:** To create a training set, we performed Taqman qRT-PCR using the primers and probes as mentioned from above on 271 samples from University of Pittsburgh, 155 samples from University of Wisconsin Madison, and 150 samples from Stanford University. The results show surprisingly high positive rate of SLC45A2-AMACR in Stanford and Wisconsin cohort, reaching 96% and 92.6% respectively. Among these fusion genes, the lowest frequent

**Table 1 Positive rate of fusion in prostate cancers**

Cohort	MAN2A1/ FER	TRMT11/ GRIK2	MTOR/ TP53BP1	CCNH/ C5orf30	KDM4B/ AC011523.2	SLC45A2/ AMACR	TMEM135/ CCDC67	LRRC59/ FLJ60017
UPMC	13% (60)	25.8% (119)	2.8% (13)	33.4% (154)	0.4% (2)	50.1% (234)	1% (5)	3.4% (16)
Stanford	18% (9)	20% (10)	10% (5)	12% (6)	4% (2)	96% (48)	6% (3)	22% (11)
UWisc	19% (31)	12.9% (21)	4.3% (7)	76.7% (125)	9.2% (15)	92.6% (151)	0.6% (1)	26% (43)

**Table 2  
The cutoffs(and OR) of each fusion gene in each cohort**

Cohort	MAN2 A1/FER	MAN2A1/F ER-actin	TRMT11 /GRK2	MTOR/T P53BP1	CCNH/C 5orf30	KDM4/ACO 11523.2	SL45A2/ AMACR	TMEM135 /CCDC67
UPMC	32(26.3)	0 (25.9)	43(5.53)	42(inf)	39(0.12)	44(inf)	34(1.57)	47(1.54)
Wisconsin	35(14.8 1)	3(7.57)	42(inf)	40(23.6)	38(0.49)	40(1.5)	31(1.7)	N/A
Stanford	39(1.71)	0(0.34)	39(4.03)	39(inf)				

one is TMEM135-CCDC67: A total of 8 samples were found positive. In addition, high positive rate of CCNH-C5orf30 was also found in the prostate cohort from University of Wisconsin. In general, the rates of fusion gene positive samples are comparable among the 3 cohorts (table 1). Subsequent analyses showed that MAN2A1-FER (or normalized MANA1-FER), TRMT11-GRIK2, and mTOR-TP53BP1 gene fusions have the highest odd ratios for predicting the recurrence of prostate cancer for UPMC and University of Wisconsin cohorts (Table 2).

**Table 3**

To establish a prediction model, we combined top 6 fusion genes that have prediction power to construct classification models to predict prostate cancer recurrence. As shown in table 3, all three models (Random Forest,

Model	Fusion genes only					2x2 table		
	Sensitivity	Specificity	Youden	Accuracy	AUC		Recurrent (n=107)	Non-Recurrent (N=164)
RF	0.80	0.81	0.61	0.81	0.862	Positive	TP=86	FP=31
	Top 6, cutoff=0.2					Negative	FN=21	TN=133
SVM	0.71	0.87	0.58	0.81	0.77	Positive	TP=76	FP=21
	Top 4, cutoff=0.2					Negative	FN=31	TN=143
LDA	0.71	0.88	0.59	0.81	0.85	Positive	TP=76	FP=20
	Top 6, cutoff=0.4					Negative	FN=31	TN=144

Support vector machine and Linear discriminant analysis) yielded very similar accuracy: 81%, even though the specificity and sensitivity may vary. When combined with gleason's score and TNM pathology staging, the accuracy improves to 84-86%.

When the same models were applied to the dataset from University of Wisconsin, the accuracy rate yielded 75-84%. Interestingly, when combined with Gleason's grade and pathology TNM staging, the accuracy rate improved to 88-90%. However, the same model fared worse in Stanford data set: 67-68% accuracy was found. Combination with fusion genes, Gleason's grade and pathology TNM staging improves the accuracy to 75%.

When all data were pooled, and 10 fold cross validation was performed. The accuracy of fusion gene prediction rate is 76-77%. When combined fusion genes, Gleason's grading and TNM staging, the accuracy is improved to 81-82% (table 4). In contrast, if prediction is only relied on Gleason's grade and TNM

**Table 4**

Model	Gleason and TNM stage + fusion genes					2x2 table		
	Sensitivity	Specificity	Youden	Accuracy	AUC		Recurrent (n=208)	Non-Recurrent (N=368)
RF	0.79	0.82	0.61	0.81	0.86	Positive	TP=164	FP=67
	top 5 fusion genes, cutoff=0.2					Negative	FN=44	TN=301
SVM	0.75	0.83	0.59	0.81	0.84	Positive	TP=157	FP=61
	Top 6 fusion genes, cutoff=0.2					Negative	FN=51	TN=307
LDA	0.77	0.85	0.61	0.82	0.87	Positive	TP=160	FP=57
	Top 5 fusion genes, cutoff=0.4					Negative	FN=48	TN=311

staging, the prediction accuracy is 74-76% across all three data set. As a result, we concluded that fusion contains independent prediction value and can assist in predicting the clinical outcomes of prostate cancer.

**Subtask 3:** From month 10 of the first year to the end of year 3 of the funded period, we will validate predictive models based on the fusion transcript panel and clinical and pathological parameters on independent datasets from the University of Pittsburgh, University of Wisconsin and Stanford University. The UPMC cohort includes up to 1900 well-annotated and -followed radical prostatectomy samples. University of Wisconsin will provide up to 1000 samples for the analysis. All samples will have at least 5 years of clinical follow-up at the end of year 3. The Stanford cohort will select up to 1500 samples from 3100 cases with a minimum of 5 years

of follow-up. Over 70% of these cases will have 7-15 years clinical follow-up. The model established in phase 2 and our preliminary results will be used to assess the risk of recurrence and short PSADT of each case. From month 10 to 36, TAQMAN QRT-PCR on selected genes will be performed on 100 random selected samples in sites 1, 2 and 3 simultaneously to assess the reproducibility of the assays. Each case will have 3 separate loci of prostate cancer. All loci will be tested for the heterogeneity of the prostate cancer in terms of fusion gene distribution.

**Progress:** Investigators in University of Pittsburgh, Stanford University and University of Wisconsin Madison, are selecting additional samples for validation of the prediction model. In particular, Dr. Nelson in University of Pittsburgh had already selected additional 250 samples for the purpose of a testing cohort. Dr. Brooks have submitted PCa 150 samples, while Dr. Jarrard submitted 166 samples. Both Drs. Brooks and Jarrard are working to select more samples for the testing set. We plan to finish total 1500 samples by the end of third year of funded period.