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14. ABSTRACT
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. 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. In the funding period, Two hundred seventy-one prostate cancer samples with clinical follow-up were collected from University of Pittsburgh Medical Center. In addition, 194 prostate cancer samples from University of Wisconsin, Madison and 108 prostate cancer samples from Stanford University were collected. Taqman QRT-PCRs were performed on these samples. Significant numbers of samples were found positive for some of these fusion genes. The expression of MAN2A1-FER, SLC45A2-AMACR, MTOR-TP53BP1 fusions are associated with prostate cancer recurrence in the UPMC cohort. Cross-validation showed that fusion gene model predicts up to 91% clinical outcomes of prostate cancer accurately. When cohorts of UPMC, Stanford and Wisconsin were combined, the accuracy is 74%. The combination of fusion with Gleason appeared to improve the overall accuracy from 77% (Gleason) to 92% (Gleason+fusion) in the UPMC cohort, and from 71% (Gleason) to 82% (Gleason+fusion) when all three cohorts are combined. When fusion combined with both pathology stage and Gleason, the accuracy was improved a little further: 93% accuracy in the UPMC cohort and 83% when all three cohorts are combined. In summary, fusion transcript prediction model may have a role in prostate cancer prognosis prediction and guiding the management of prostate cancer patients.

15. SUBJECT TERMS
Prostate Cancer, prognosis, gene fusions, fusion transcripts

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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 β-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.

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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 β-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 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 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	MAN2A1/FER	MAN2A1/FER-actin	TRMT11/GRK2	MTOR/TP53BP1	CCNH/C5Orf30	KDM4/AC011523.2	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(14.81)	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)				

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,

Table 3

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%.

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

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 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.

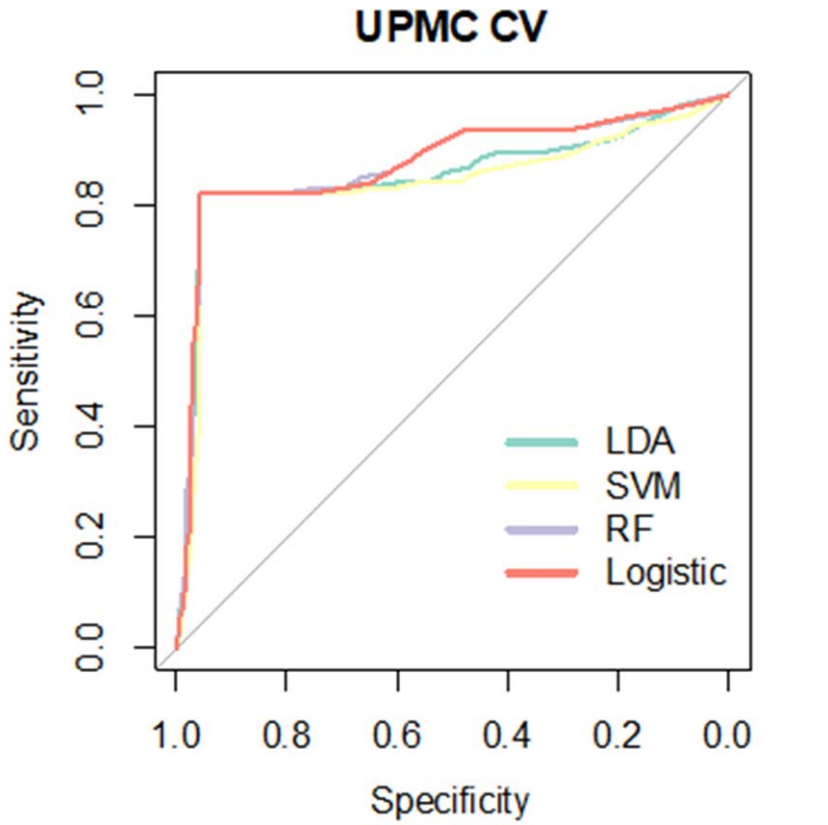

Figure 1. ROC analyses of fusion transcript model predicting prostate cancer recurrence of UPMC prostate cancer samples. The model utilized fusion transcripts only as prediction factor. LDA-Linear discriminant analysis; SVM-Support-vector machine; RF-Random forest.

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.

Progress: After the collection of the results of all the qRT-PCR analyses for the prostate cancer samples from UPMC, we performed a leave-one-out cross-validation to examine whether fusion transcripts are predictive of prostate cancer recurrence. As shown in figure 1, 90.4% accuracy was achieved based the detection of 4 fusion transcripts (MAN2A1-FER, SLC45A2-AMACR,

mTOR-TP53BP1 and LRRC59-FLJ60017 in the cancer samples. The sensitivity is 82% and the specificity 95%. When all fusion transcripts were included, the accuracy only slightly improved to 91%. Among the fusion transcripts, MAN2A1-FER is most associated with prostate cancer recurrence, with a p-value <0.000001. Survival analysis showed that patients with positive recurrence prediction had only 15% PSA-free survival 5 years after the radical prostatectomy (figure 2), while patients with negative prediction had over 90% PSA-free survival. When the same leave-one-out approach was applied to Gleason grading prediction, it generates 77% accuracy of prediction with 57% sensitivity and 91% specificity. When fusion transcripts were considered along with Gleason grade, the accuracy improves to 92% with sensitivity of 89% and specificity of 93%. When fusion transcripts, Gleason grade and pathology stage are combined as a model for prediction, the accuracy improves to 94% with sensitivity and specificity 94.4% and 93.9%, respectively. When the analyses includes the cohorts from Stanford University (108 samples) and University of Wisconsin (194 samples), the same model prediction yielded an accuracy of 74% with sensitivity of 41% and specificity of 94%, very similar to the Gleason grade prediction (75% accuracy with sensitivity of 47% and specificity of 92%). When fusion transcripts were combined with Gleason grade, the accuracy improved to 77%. The highest accuracy (83%) was achieved when fusion transcripts, Gleason grade and pathology stage were combined as a model (figure 3). The PSA-free survival analysis on the combined prediction model showed that prostate cancer patients with positive prediction had less than 20% PSA-free survival in 5 years, while patients with negative prediction had PSA-free 5-year survival rate more than 85% (figure 4).

When cohort from Wisconsin was independently analyzed, the fusion gene predicted 84% recurrence accurately, comparable with 85% Gleason prediction rate. When Gleason grade was combined with fusion gene model, it improved to 86%. In contrast, Stanford cohort showed that fusion transcript model was less accurate, generating only 65% accuracy in predicting prostate cancer recurrence, comparing with 75%

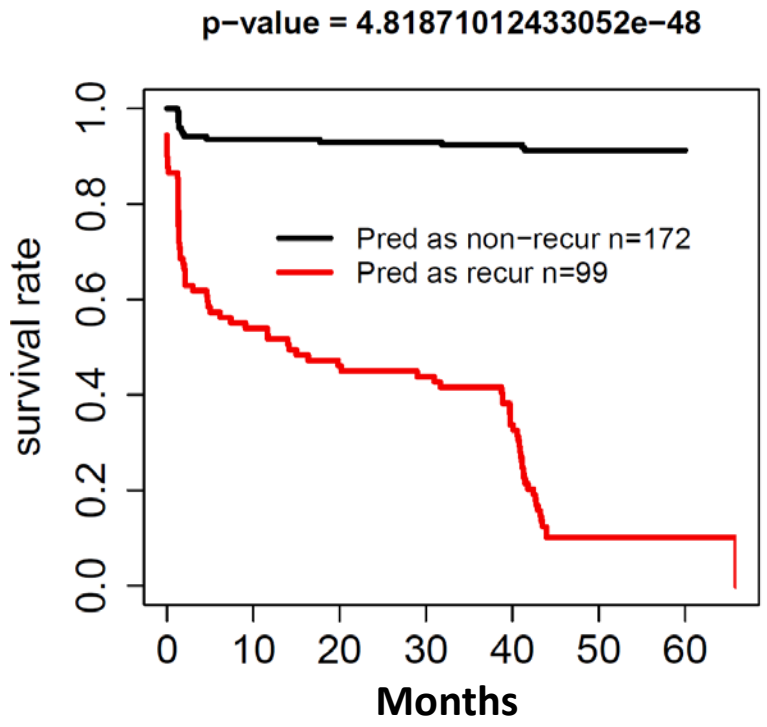

Figure 2. Kaplan-Mier analysis of PSA-free survival of prostate cancer based on fusion prediction model on prostate cancer samples from UPMC cohort. The prediction was based on the results from Random Forest method.

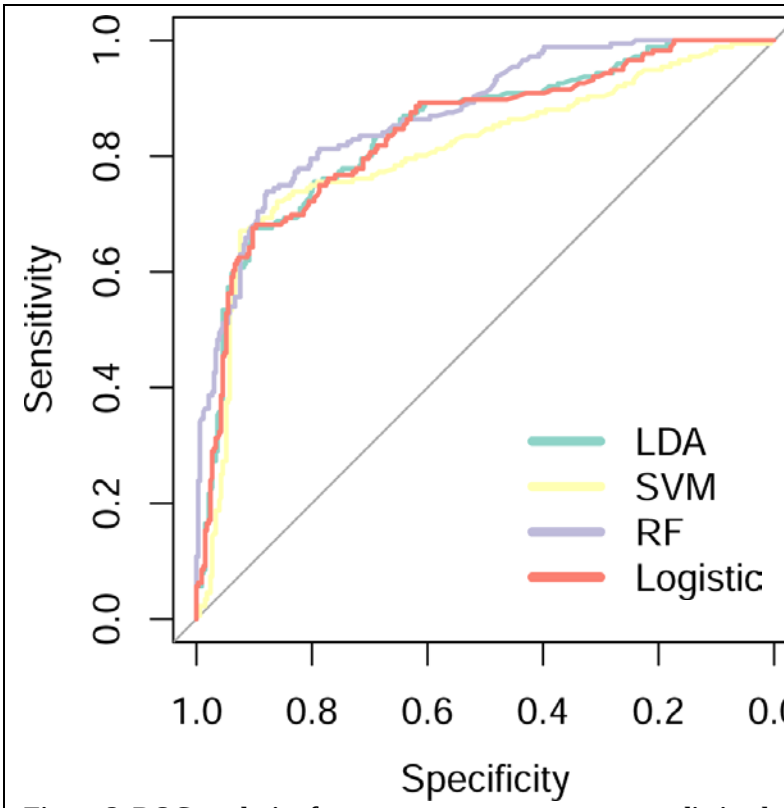

Figure 3. ROC analysis of prostate cancer recurrence prediction based on prostate cancer samples of combined cohorts of UPMC, Stanford and University of Wisconsin. The model utilized fusion transcripts, Gleason grade and pathology stage as prediction factors. LDA-Linear discriminant analysis; SVM-Support-vector machine; RF-Random forest.

accuracy by Gleason grade. However, when fusion model was combined with Gleason grade, the model prediction improved to 81% accuracy. When we combined cohorts from Wisconsin and Stanford into one validation cohort, the fusion prediction model predicts 72% recurrence accurately, slightly lower than Gleason grade prediction (73%). Combining Gleason with fusion transcripts improves the correct prediction rate to 75%. Fusion transcript also improve on Gleason plus pathology stage model (78% over 76%).

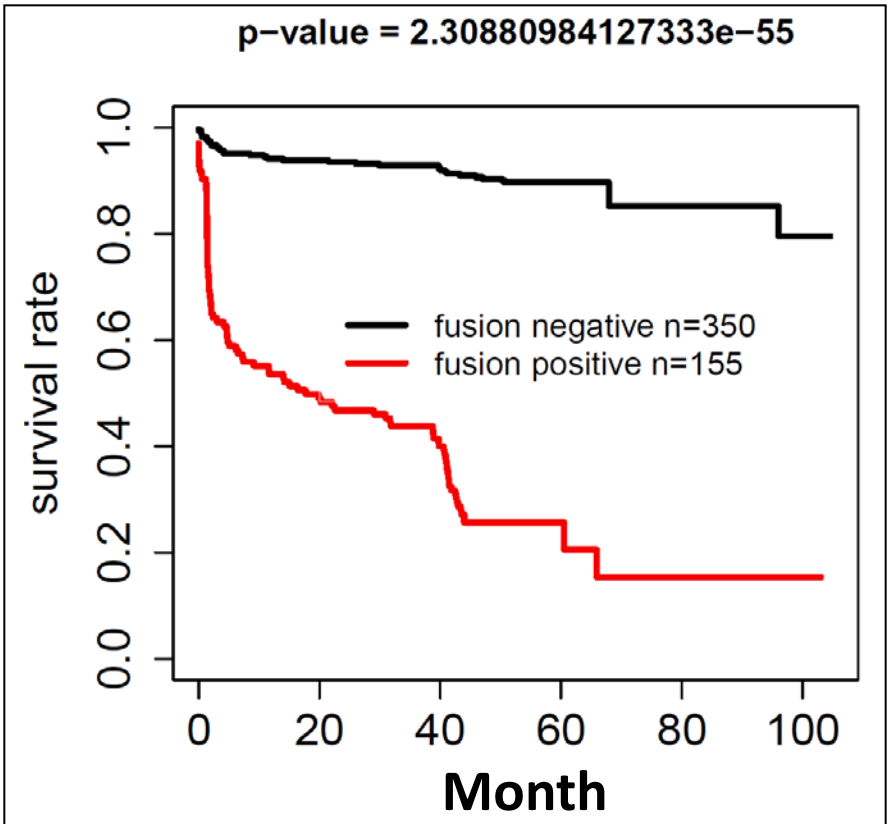

Figure 4. Kaplan-Mier analysis of PSA-free survival of prostate cancer based on fusion prediction model on prostate cancer samples from UPMC, Stanford and University of Wisconsin cohort. The prediction was based on the results from Random Forest method using fusion transcripts, Gleason grade and pathology stage as prediction factors.

Association analysis showed that MAN2A1-FER, TRMT11-GRIK2 and SLC45A2-AMACR are associated with higher pre-operational PSA, with p-value 0.0025, 0,016, and 0.008, respectively. mTOR-TP53BP1 and TRMT11-GRIK2 are associated with higher Gleason's grade (p=0.002 for mTOR-TP53BP1 and 0.0002 for TRMT11-GRIK2). mTOR-TP53BP1 is associated with larger tumor volume (p=0.049) and more advanced stage of cancer (p=0.007). Samples with lymph node metastasis tends to have mTOR-TP53BP1 and SLC45A2-AMACR present in the primary cancer samples (p=0.008 for mTOR-TP53BP1 and p=0.044 for SLC45A2-AMACR).

Overall, fusion transcripts were found widely present in prostate cancer samples, and were associated with prostate cancer recurrence and other pathological features of the cancer. Even though there were significant inter-cohort variations in terms of predictability of prostate cancer clinical outcomes, fusion transcript detection improves on the current clinical means in predicting the cancer outcomes, and thus, can be utilized as an important tool in predicting cancer outcomes in patient management. In addition, repeat testing of fusion transcript in 20 organ donor prostates and 14 blood samples from healthy individuals yielded negative results. However, we have found fusion transcripts present in benign tissues adjacent to prostate cancer, suggesting potential field-effect of the prostate cancer genome.

Additional progress on fusion gene analysis supported by this grant:

Fusion transcripts are present in human prostate cancer and other cancer cell lines

In our previous studies, we have characterized eight fusion genes identified in aggressive prostate cancer samples. Additional analyses showed that one of the fusion genes called MAN2A1-FER is frequently present in 5 other types of human malignancies. To expand our analyses of other fusion genes in the panel, we analyzed TRMT11-GRIK2, MTOR-TP53BP1, CCNH-C5orf30, KDM4-AC011523.2, TMEM135-CCDC67, LRRC59-FLJ60017 in 20 cancer cell lines from six different human

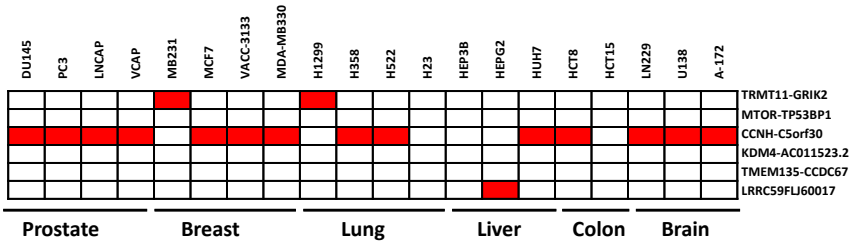

Figure 5. Fusion transcripts are present in human cancer cell lines. Representative cancer cell lines from lung, liver, prostate, breast, colon and brain were examined for the presence of fusion transcript TRMT11-GRIK2, CCNH-C5orf30, mTOR-TP53BP1, TMEM135-CCDC67, KDM4-AC011523.2 and LRRC59-FLJ60017 using quantitative TaqMan RT-PCR. Red denotes positive for the fusion transcript, while blank negative for the fusion transcript. All positive fusion samples were verified by Sanger sequencing.

malignancies (figure 5). TRMT11-GRIK2 fusion transcript was identified in breast cancer cell line MD-MB231 and lung cancer cell line H1299, while CCNH-C5orf30 was positive in 14 of 20 cancer cell lines, including all prostate cancer cell lines tested (PC3, DU145, LNCaP and VCaP), 3 of 4 breast cancer cell lines (MCF7, VACC-3133 and MDA-MB330), 2 of 4 lung cancer cell line (H358 and H522), 1 of 3 liver cancer cell lines (HepG2), 1 of 2 colon cancer cell lines (HCT8) and 3 of 3 GBM cell lines (LN229, U138 and A-172). Also, KDM4-AC011523.2 and LRRC59-FLJ60017 were present in liver cancer cell line HepG2. These results suggest that these fusion genes are not specific for prostate cancer. They may be present in the primary cancer samples of a variety of human malignancies.

Fusion transcripts are present in 7 other types of human malignancies:

To investigate whether any of the above-mentioned fusion genes has a role in human cancers, Quantitative TaqMan qRT-PCRs using primers and probe specific for each fusion gene were performed on primary human cancer samples representing seven different types of human malignancies. Our results showed that TRMT11-GRIK2 is present in all seven types of human malignancies (figure 6), including breast cancer (41/60, 68.33%), colon cancer (25/60, 41.7%), esophageal adenocarcinoma (9/34, 26.5%), hepatocellular carcinoma (9/70, 12.9%), ovarian adenocarcinoma (28/61, 45.9%), glioblastoma multiforme (26/150, 17.3%) and non-small cell lung cancer (39/141, 27.7%). CCNH-C5orf30 transcript was also detected in 7 different types of human cancers with relatively high frequencies: 85% (51/60) breast cancer, 43% (26/60) colon cancer, 50.8% (31/61) ovarian cancer, 67.6% (23/34) esophageal adenocarcinoma, 41.8% non-small cell lung cancer (59/141), 37% (26/70) liver cancer and 53% (80/150) glioblastoma multiforme. mTOR-TP53BP1, on the other hand, was detected in five different types of human malignancies with significantly lower frequencies: breast cancer (10/60, 16.7%), colon cancer (4/60, 6.7%), ovarian adenocarcinoma (4/61, 6.6%), glioblastoma multiforme (7/150, 4.7%) and lung cancer (8/141, 5.7%). LRRC59-FLJ60017 was found present in four different types of human cancers: esophageal adenocarcinoma (3/34, 8.8%), ovarian adenocarcinoma (4/61, 6.6%), glioblastoma multiforme (16/150, 10.7%), and non-small cell lung cancer (33/141, 23.4%). Only two glioblastoma multiforme and one lung cancer samples were found positive for TMEM135-CCDC67. KDM4-AC011523.2 fusion was only found in 3 breast cancer and 3 lung cancer samples.

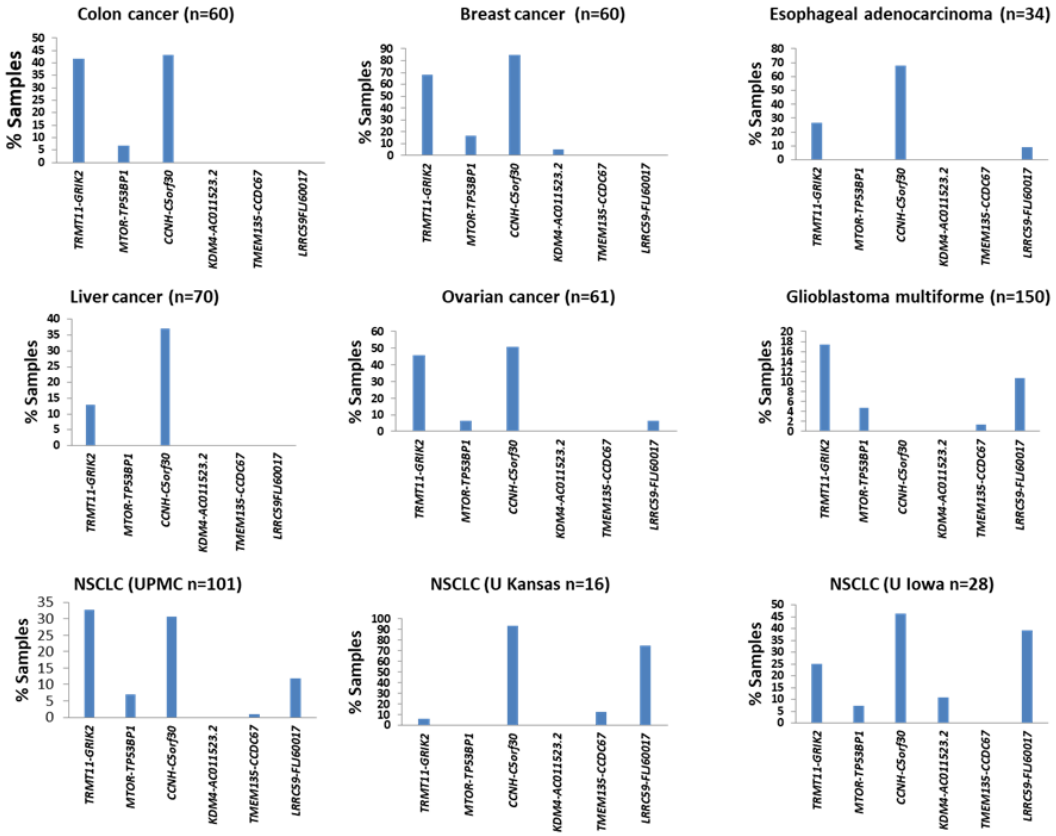

Figure 6. Frequency of gene fusion in primary human malignant samples. Real-time TaqMan qRT-PCRs were performed on formalin-fixed paraffin-embedded samples of breast cancer, colon cancer, esophageal adenocarcinoma, ovarian cancer, hepatocellular carcinoma, non-small cell lung cancer and glioblastoma multiforme. The numbers of samples of each cohort are indicated. Sanger sequencing was performed on 20% samples that were found positive for the fusion genes.

Interestingly, ductal type of breast cancers positive for TRMT11-GRIK2 was associated with less likely to develop local lymph node metastasis (46.7% versus 93.3%, p=0.014). Liver cancer positive for TRMT11-GRIK2 was also associated with a higher rate of overall survival (41.7% versus 6.9%, p=0.006). KDM4-AC011523.2 was only detected in lobular type breast cancer and adenocarcinoma of the lung. Patients with lobular breast cancers positive for mTOR-TP53BP1 were also less

likely to have lymph node metastasis (0% versus 40%, p=0.017). CCNH-C5orf30 was more frequent in adenocarcinoma of the lung cancer versus squamous type (67.7% versus 34.8%, p=0.001), and colon cancer with advanced stages at the time of diagnosis (52.3% versus 18.8%, p=0.037).

Fusion transcripts are present in metastatic lymph nodes:

To investigate whether fusion genes are also present in the metastatic lesion of human cancers, breast cancer, colon cancer and ovarian cancer samples with matched lymph node metastasis were analyzed (figure 7). Twenty-six of 30 metastatic breast cancers in the lymph nodes were positive for TRMT11-GRIK2, including seven metastatic cancers whose matched primary breast cancers were negative for the fusion. For colon cancers, however, the status of TRMT11-GRIK2 between primary cancer samples and lymph node metastases was matched by 78.5% (11/14): Eleven lymph node metastases were exactly matched with the status of the primary colon cancer samples, while two samples of lymph node metastases were found negative for TRMT11-GRIK2 fusion. One lymph node metastasis was found positive for the fusion gene while the matched primary sample was negative (figure 3). For ovarian adenocarcinoma, nine metastatic lesions were found to contain TRMT11-GRIK2 fusion gene, matching all primary samples. However, four lymph node metastases contained no TRMT11-GRIK2 while the matched primary cancer samples were positive. One lymph node metastasis gained the fusion of TRMT11-GRIK2 over the primary cancer sample. For CCNH-C5orf30, the matching rate of primary breast cancer with lymph node metastases was 62%, while the matching rates for ovarian cancer and colon cancer with their corresponding lymph node metastases were 72% and 73%, respectively. For mTOR-TP53BP1, two of 3 lymph node metastases retained the fusion in both colon and ovarian cancers. Two of 2 lymph node metastases in ovarian cancer retained LRRC59-FLJ60017 fusion. These results suggest significant heterogeneity of the cancer samples. However, most fusion genes were retained in metastatic lesions.

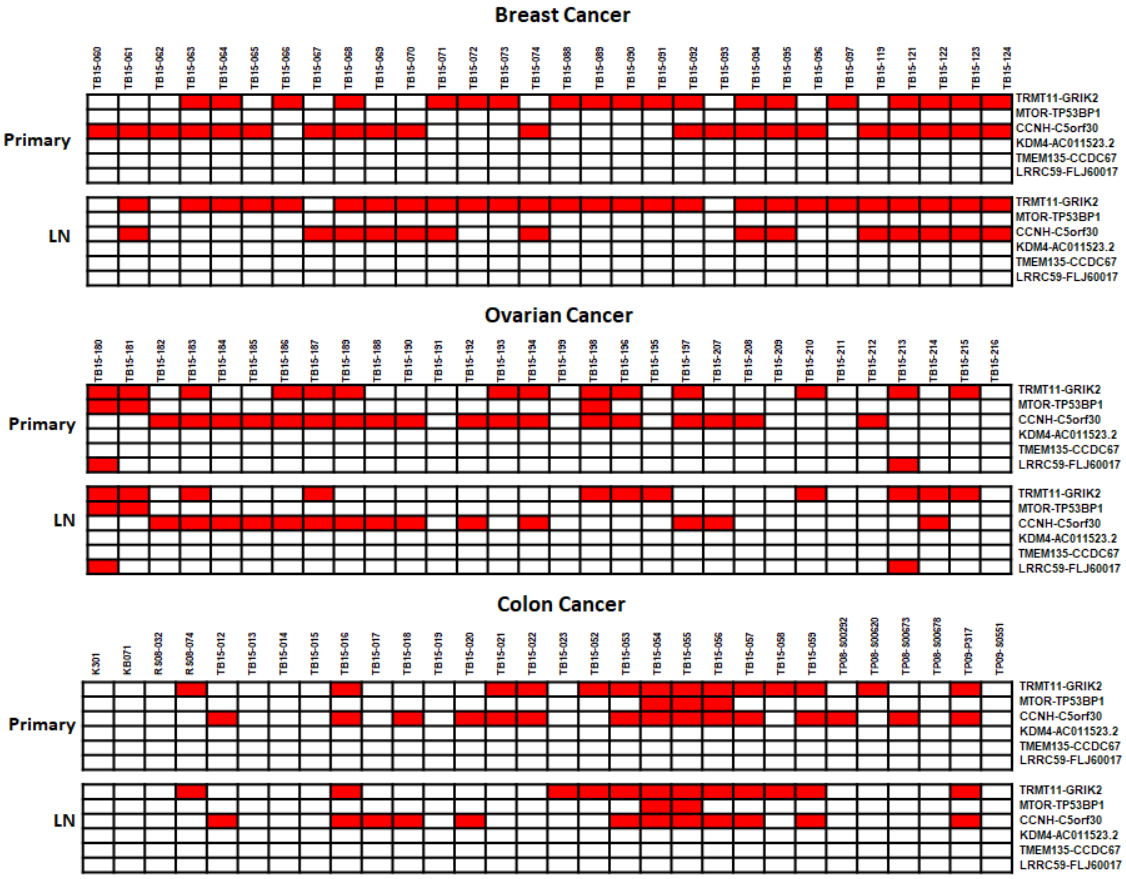

Figure 7. Fusion transcript in metastatic lymph node samples. Real-time TaqMan qRT-PCRs were performed on formalin-fixed paraffin-embedded samples of breast cancer (top), ovarian cancer (middle) and colon cancers (bottom) and their corresponding lymph node metastases. Both primary (top) and matched lymph node (bottom) cancer samples are shown. Red indicates positive detection of the fusion transcript, while blank indicates negative detection of the fusion transcript. Sanger sequencing was performed on 20% samples that were found positive for the fusion genes.

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Cancer specific fusion genes are the results of chromosome rearrangement in the cancer genomes. The detection of fusion transcripts in cancer cells may reflect the progression of human cancer. Previously, we have identified a panel of 8 fusion genes in prostate cancer. The presence of these fusion transcripts correlated with the aggressive behavior of prostate cancer. In this proposed study, we will conduct large scale analysis to evaluate whether the detection of these fusion transcripts is predictable for poor clinical outcomes.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Fusion gene, RNA, Taqman RT-PCR, in situ hybridization, RNA, prostate cancer, cancer relapse, chromosome

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

We will conduct analysis of MAN2A1-FER, SLC45A2-AMACR, TRMT11-GRIK2, MTOR-TP53BP1, LRRC59-FLJ60017, CCNH-C5orf30, KDM4-AC011523.2, TMEM135-CCDC67 on over 1000 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.

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. Detection threshold will be obtained.

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.

3) TAQMAN QRT-PCR analysis for the fusion genes will be carried out at the CLIA certified lab at the University of Pittsburgh using approximately 200 samples provided by Drs. Brooks and Jarrard. In addition, validation of selected prostate cancer samples on specific fusion genes using FISH will be performed. Statistical analyses will be performed to evaluate whether the fusion gene status is predicative for the clinical outcomes of prostate cancers.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1. We have reaffirmed the detection of fusion transcripts in prostate cancer samples.
2. Using sensitive detection methods, we have found that fusion genes are widely present in prostate cancer samples.
3. We have established a preliminary training model for the prediction of the clinical outcomes of prostate cancer.
4. We have found the fusion genes identified in prostate cancer are also present in other human malignancies.
5. We have found that the fusion transcripts are present in the serum samples of human cancers in cell-free RNA form.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Nothing to report

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

MAN2A1-FER is the first tyrosine kinase fusion genes found to play critical roles in prostate cancer, it opens a new door for the treatment of prostate cancer using tyrosine kinase inhibitors. In addition, we developed a novel approach to treat human cancers that are positive for fusion gene by inserting a suicide gene into the chromosomal breakpoint of a fusion gene in the cancer genome. This could be a new way to treat prostate cancers that are refractory to other modes of the cancer treatment.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Fusion transcripts mentioned in the proposed study was also present in ovarian cancer, breast cancer, colon cancer, lung cancer, liver cancer, GBM and esophageal adenocarcinoma, and may play roles in the developments of those cancers.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to report

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. Yan-Ping Yu, Allan Tsung, Silvia Liu, Michael Nalesnik, David Geller, George Michalopoulos and **Jian-Hua Luo** (2019). Detection of fusion transcripts in the serum samples of patients with hepatocellular carcinoma. Oncotarget 10, 3352-3360.

2. Yan-Ping Yu, Peng Liu, Joel Nelson, Ronald L. Hamilton, Rohit Bhargava, George Michalopoulos, Qi Chen, Jun Zhang, Deqin Ma, Arjun Pennathur, Michael Nalesnik, George Tseng and **Jian-Hua Luo** (2019). Identification of recurrent fusion genes across multiple cancer types. Scientific Reports 9:1074. https://doi.org/10.1038/s41598-019-38550-6.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

J Luo, G Michalopoulos, Y Yu. Identification of recurrent fusion genes across multiple cancer types. The FASEB Journal 33 (1_supplement), 802.32-802.32.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	*James D. Brooks*
Project Role:	*Professor*
Nearest person month worked:	*0.12 calendar months since last reporting.* *0.84 calendar months for past year.*
Contribution to Project:	*I have supervised the project at Stanford*
Funding Support:	*Non-sponsored (designated, endowment, etc). Sponsored (DoD, NIH).*
Name:	*Rosalie Nolley*
Project Role:	*Pathology Technician*
Researcher Identifier (e.g. ORCID ID):	*none*
Nearest person month worked:	*2.16 calendar months* *3.36 calendar months for past year.*
Contribution to Project:	*Pulled H & E slides. Cored samples. Shipped to U Pittsburgh. Confirmed pathology under supervision of Dr. Brooks*
Funding Support:	*Non-sponsored (endowment). Sponsored (DoD, NIH).*
Name:	*Kieu My Huynh*
Project Role:	*Research Technician*
Researcher Identifier (e.g. ORCID ID):	*None*
Nearest person month worked:	*0.48 calendar months* *1.32 calendar months for past year.*
Contribution to Project:	*Pulled H & E slides with Mrs. Nolley.*
Funding Support:	*Non-sponsored (endowment). Sponsored (DoD, NIH).*
Name:	*Michelle Ferrari*
Project Role:	*Research Nurse Manager*
Researcher Identifier (e.g. ORCID ID):	*None*
Nearest person month worked:	*0.72 calendar months* *2.16 calendar months for past year.*
Contribution to Project:	*Pulled clinical data on all patients. Constructed clinical databases. Communicated blinded clinical data to Dr. Brooks who sends blinded data to Pittsburgh.*
Funding Support:	*Non-sponsored (endowment, gift, etc). Sponsored (DoD, NIH, PAVIR/VA).*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Title: Pathomic Predictors of Prostate Cancer Progression
1R01CA24989901
Effort: 0.60 calendar
Supporting Agency: NIH/NCI
Grants Officer: Romy Reis (romy.mondesir@nih.gov)
9609 Medical Center Dr.
Rockville, MD 20850
Performance Period: 04/16/2020-03/31/2025
Funding Amount:
Project Goals: This project proposes to build a rule-based classifier using domain knowledge for both pathological and clinical staging of prostate cancer to improve our previous work. We will build supervised and unsupervised classifiers for staging using a training set, tune the classifiers using a validation set, and assess the performance of the classifiers using a test set
Specific Aims: Aim 1: Profile the morphologies, cell types, environments, and molecular phenotypes of 234 prostate cancer tumors from patients with known favorable or adverse outcomes (median 9 years follow-up) using the Cell DIVE hyperplexed immunofluorescence platform. Aim 2: Classify the molecular and morphologic subtypes that jointly define the heterogeneity landscape of aggressive and benign prostate cancers at the single-cell level in tumor and surrounding stroma. Aim 3: Investigate the role of spatial and environmental factors in aggressive and benign prostate cancers using the hyperplexed molecular pathology data. Aim 4: Identify prognostic features and validate these features in a multi-institutional, ethnically diverse cohort of 1250 prostate cancer patients with known favorable and adverse outcomes.
PI: Parag Mallick
Role: Co-Investigator
Overlap: None.

Title: BMP5 cells and signaling in BPH pathogenesis
1R01DK123232 - 01A1
Effort: 0.24 calendar
Supporting Agency: NIH
Grants Officer: Pamela Love (lovepa@mail.nih.gov)
6701 Democracy Blvd.
Bethesda, MD 20817
National Institute of Diabetes, Digestive and Kidney Diseases
Performance Period: 09/01/2020-05/31/2023
Funding Amount:
Project Goals: The goal of this grant is to determine the role of BMP5 in benign prostatic hyperplasia using in vitro and transcriptomic approaches.
PI: Jonathan Pollack
Role: Multi Principal Investigator
Overlap: None.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: N/A

9. APPENDICES: *N/A*