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TITLE: Fusion Genes Predict Prostate Cancer Recurrence

PRINCIPAL INVESTIGATOR: David F Jarrard

RECIPIENT: University of Wisconsin System
Madison, WI 53715

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This Impact Award between the University of Pittsburgh, Stanford University and University of Wisconsin at Madison seeks to provide definitive clinical validation of low copy number transcript fusions as prognostic markers in clinically localized prostate cancer. The Stanford site is charged with providing formalin fixed paraffin embedded samples for independent validation of markers discovered at University of Pittsburgh. To that end, we have shipped 300 samples for assay testing and have accumulated over 600 FFPE blocks of radical prostatectomy specimens with detailed clinical follow-up. The assay results suggest that there are some artifacts, and University of Pittsburgh will be addressing these issues. Next year we will increase the number of samples accumulated to get to our goals. We will be sending specimens blinded to the Pittsburgh site and include controls for assay performance. We have agreed to allow the Stanford and Wisconsin sites to receive data from Pittsburgh to perform correlation with clinical data and correlation with outcomes at these sites.

15. SUBJECT TERMS

None provided

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

The University of Pittsburgh site, University of Wisconsin site and Stanford site have previously published work demonstrating that, with very deep sequencing, we could identify gene fusions that predict with high sensitivity recurrence after radical prostatectomy (Am J Path **184:** 2857-2866, 2014). In this proposal, we seek to test whether the following fusions correlate with outcome: MAN2A1-FER, SLC45A2-AMACR, TRMT11-GRIK2, MTOR-TP53BP1, LRRC59-FLJ60017, CCNH-C5orf30, KDM4-AC011523.2, TMEM135-CCDC67. Furthermore, we propose to build a clinical model that includes some or all of the fusions to predict outcomes after surgery. The study design is to build a model on an initial set of samples from all institutions and validate on a second set of independent samples taken from the archival tissues available at each of the 3 sites.

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

Prostate cancer, prognosis, gene fusions, fusion transcripts

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.

1. *Provide samples to University of Pittsburgh for prognostic model construction (Phase 1).*
2. Accumulate additional samples at the University of Wisconsin site for definitive and independent validation (Phase 2)

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.

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.

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 institutions Pittsburgh, Stanford University and University of Wisconsin Madison, have 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. cancers.

Progress: We performed Taqman qRT- PCR using the primers and probes as mentioned from above on 460 samples from University of Pittsburgh, 163 samples from University of Wisconsin Madison, and 50 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 (see table 1). We are in the process to gather and update precise clinical outcome information of these prostate cancers to analyze the correlation between prostate cancer recurrence, death due to prostate cancer and response to treatment with the status of these fusion genes in the prostate cancer samples. Once the information is complete, we will build a training model to predict the clinical outcomes of prostate cancers.

To date Wisconsin has collected and annotated 300 samples and is on track to complete the total accrual of 1000 samples. Long term clinical data continues to be collected. Initial assay development is ongoing at Pittsburgh prior to validation of the model.

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. Both Drs. Brooks and Jarrard are working to select m inish total 1500 samples by the end of second year of funded period.

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.

Cedric Shi: This student undertook the initial work as technician on this project and has expanded his knowledge of pathology, histology, PCR techniques and prostate cancer clinical work during his work on this grant. He has left to pursue a PhD program at the University of Illinois.

Tyler Etheridge, BA a medical student taking a year to do research has joined the project. Work on this proposal has expanded his knowledge of pathology, histology, PCR techniques and prostate cancer clinical work. He will return to complete his MD degree this summer.

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 given continuing ongoing assay validation

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.

- Accumulate more samples. Send samples to Pittsburgh for assay development and validation
- Clinical scrutiny of assay performance

4. **IMPACT:** Describe 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).

Since we have sent blinded samples, we have been able to detect potential issues with assay fidelity and performance. We will continue to uphold high standards of evidence for assay performance.

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.

Nothing to report

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:

No changes

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.

Prognostic Model development has been delayed because standards of assay performance have not been met. We anticipate this will not change the outcomes significantly as ongoing sample collection continues at the prescribed pace.

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.

No changes

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

No changes

Significant changes in use of biohazards and/or select agents

No changes

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).*

Nothing to report

  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.*

None

- **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.

None

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

None

- **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.

None

- **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.*

None

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".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: David Jarrard, MD
Project Role: PI
Researcher Identifier (e.g. ORCID ID): jarrard@urology.wisc.edu
Nearest person month worked: 1.8
Contribution to Project: I have supervised the project at University of Wisconsin-Madison
Funding Support: This grant

Name: Tyler Etheridge, BS
Project Role: Medical student
Researcher Identifier (e.g. ORCID ID): tetheridge@wisc.edu
Nearest person month worked: 12 (July 2017- present)
Contribution to Project: Pulled original H & E slides, Confirmed pathology under supervision of Dr. Jarrard, Selected slides used for the project, H & E staining, Read and marked the slides for the area tissue to be cored, Generated cores, and clinical follow-up.
Funding Support: This grant

Name: Cedric Shi, BS
Project Role: Research specialist
Researcher Identifier (e.g. ORCID ID): zshi47@wisc.edu
Nearest person month worked: 12 (Sept 2016-Jun 2017)
Contribution to Project: Pulled original H & E slides, Confirmed pathology under supervision of Dr. Jarrard, Selected slides used for the project, H & E staining, Read and marked the slides for the area tissue to be cored, Generated cores, and clinical follow-up.
Funding Support: This grant

Name: Bing Yang, PhD
Project Role: Researcher
Researcher Identifier (e.g. ORCID ID): yangb@urology.wisc.edu
Nearest person month worked: 3.6
Contribution to Project: Organized the samples, Personnel management, Make sure all the protocols and procedures used for this project are correct, Shipped samples to U Pittsburgh
Funding Support: This grant

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.

Nothing to report

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: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.