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TITLE: A Nanotechnology Solution for Early Detection of Micrometastatic Prostate Cancer After Radical Prostatectomy

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The overall objective of this research proposal is to conduct the initial development of a rapid circulating tumor cell-based blood test that can identify men with micrometastatic disease in order to facilitate patient selection for salvage radiotherapy. Aim 1 of this study was do perform a technical validation study and Aim 2 to embark upon testing of banked clinical specimens then to test the new assay in the setting of the NRG-GU-006 clinical trial (salvage radiotherapy +/- apalutamide). In the first year of work we have begun a series of key technical validation studies while completing the sample collection from the now fully accrued NRG-GU-006 trial. Due to COVID-19 there were delays in progress but due to rapid clinical accrual and regearing of our studies, this effort is still on time.

15. SUBJECT TERMS
Prostate cancer, circulating tumor cells, nanotechnology, mRNA, expression profiling, biochemical relapse

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TABLE OF CONTENTS

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

Biochemical relapse (BCR) after prostatectomy (i.e. a rising PSA after surgery) is a delicate and important clinical finding. Several men with BCR are potentially still curable with salvage radiotherapy, while others have been harboring occult micro-metastatic disease outside of a standard salvage field and will continue to progress. We have proposed to conduct advanced development of a rapid blood test that identifies the molecular footprint of circulating tumor cells (versus relying upon morphologic review alone) focusing on the expression of key genes (PSA, PSMA, SCHLAP1). Aim 1 of this study was do perform a technical validation study and Aim 2 to embark upon testing of banked clinical specimens then to test the new assay in the setting of the NRG-GU-006 clinical trial (salvage radiotherapy +/- apalutamide).

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

Prostate cancer, circulating tumor cells (CTCs), nanotechnology, mRNA, expression profiling, biochemical relapse (BCR)

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.

- Major Task 1: Implement QA/QC protocols and carry out calibration studies for CTC capture efficiency and CTC recovery yield of the TR-NanoVelcro System.
  Timeline: Month 1-6 with 80% completion.
- Major Task 2: To carry out calibration studies to assess the performance of RNA quantification for ddPCR™.
  Timeline: Month 7-9 with 50% completion.
- Major Task 3: To carry out calibration studies to examine the complete CTC-RNA assay.
  Timeline: Month 10-12 with 50% completion.
- Major Task 4: Case-control study to verify the association between CTC-RNA markers and mPCa.
  Timeline: Month 13-24 with 30% completion.
- Major Task 5: Parallel testing of the CTC-RNA assay.
  Timeline: Month 13-24 with 30% completion.
- Major Task 6: Testing of the CTC-RNA assay using patients' samples from NRG-GU-006 trial.
  Timeline: Month 13-35 with 30% completion.

-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) Major activities
   - Implement QA/QC protocols of TR-NanoVelcro CTC purification system.
   - Preparation of artificial samples (LNCaP, MCF7, SkBr3, Huh7, A549, 22Rv1, and PC3).
   - Automated fluorescent microscope and image cytometry analysis for CTC counting.
   - Calibration studies for CTC capture efficiency.

- To carry out calibration studies to assess the performance of RNA quantification for with cDNA mixture of spiking different PCa cell lines into 5000 WBCs at densities of 400-500, 100-150, 40-50 and 1-5 cells per sample.
- Bioinformatic pipeline for panel development in addition to the originally proposed PSA, PSMA, and SCHLAP1.
- Sample collection from the NRG-GU006 trial.

2) Specific objectives
   - Technical validation using artificial and patient samples.
   - Path to implementation and initial clinical test of the CTC-RNA assay.
3) Significant results
   - Capture efficiency of artificial samples

As

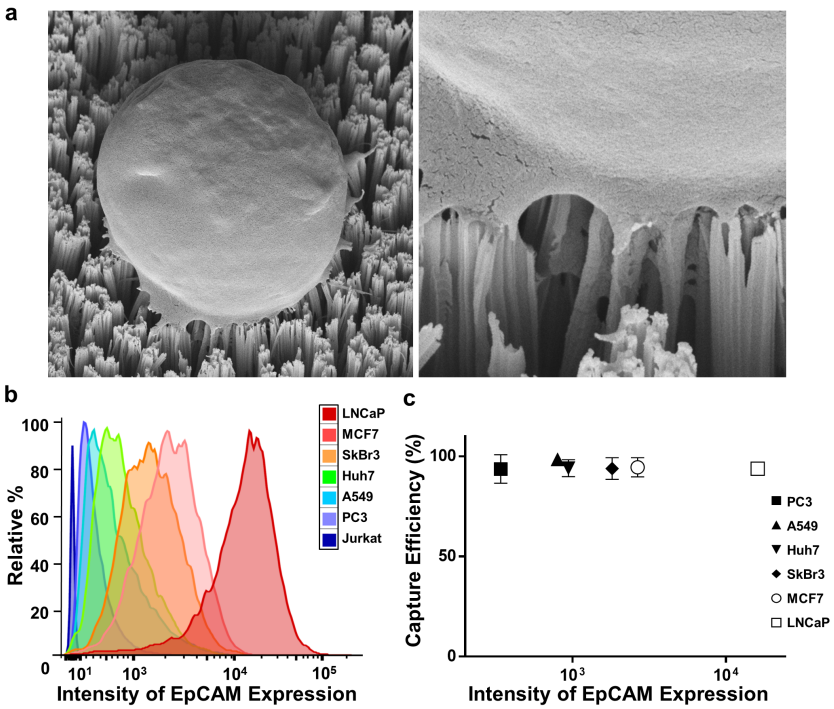

Figure1. (a) Scanning electron microscope (SEM) images of SkBr3 cells on nanosubstrates and magnification of interface between filopodia of cancer cell and nanowire. **(b)** Identify EpCAM expression level on cell membrane of various cancer cells. To detect EpCAM on different cancer cells, anti-EpCAM antibody and FITC conjugated secondary antibody were used to identify by flow cytometry. The rainbow color indicates the lower protein expression to higher expression from blue to red as shown in the graph. **(c)** The capture efficiency of various cancer cells relative to EpCAM expression is examined. 500 cells of various cancer types were mixed with 0.5 million Jurkat cells as background to load to the capture system.

opposed to these existing CTC detection technologies, the uniqueness of TR-NanoVelcro Chip is the use of NanoVelcro substrates – specifically, capture agent-coated nanosubstrates based on silicon, TiO_2, polymer nanowires[3], and other nanostructured materials – which allow for enhanced local topographic interactions between the nanosubstrates and nanoscale cellular surface components, resulting in vastly improved cell-capture affinity (**Fig. 1a**) compared to that observed for non-structured (i.e., flat) substrates. Capture agents (e.g., anti-EpCAM) are grafted onto the nanosubstrates to confer specificity for detecting CTCs of epithelial origin. The capture efficiency of various cancer cell lines showed no difference between each other. High capture efficiency was observed in all cell lines even with low EpCAM expression (**Fig. 1b**) because of enhanced contact area. Our pioneering work has attracted the attention of other researchers, who have started to explore the use of a wide spectrum of nanostructured substrates (with different capture agents) for specific applications in the field of rare-cell sorting. Continuous development of NanoVelcro CTC Assays has led to four generations of devices for different clinical utilities.

- Identification of CTCs using 3-color immunocytochemical (ICC) protocol

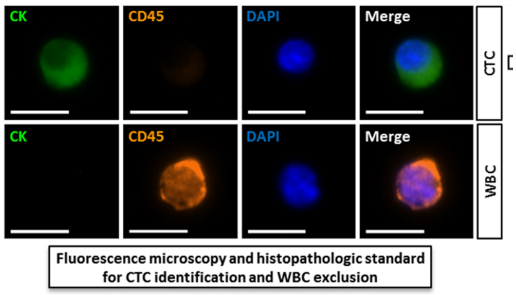

Figure 2. NanoVelcro CTC Chips for CTC enumeration and morphologic analysis. The NanoVelcro CTC Chips identify PCa CTCs using the 3-color ICC protocol (DAPI+/CK+/CD45-).

A 3-color ICC protocol using DAPI, anti-CD45, and anti-CK for identification of NanoVelcro-immobilized CTCs (DAPI+/CK+/CD45-, Fig. 2), we developed CTC enumeration method using NanoVelcro platforms.

- Technical validation of CTC-RNA assay.

The

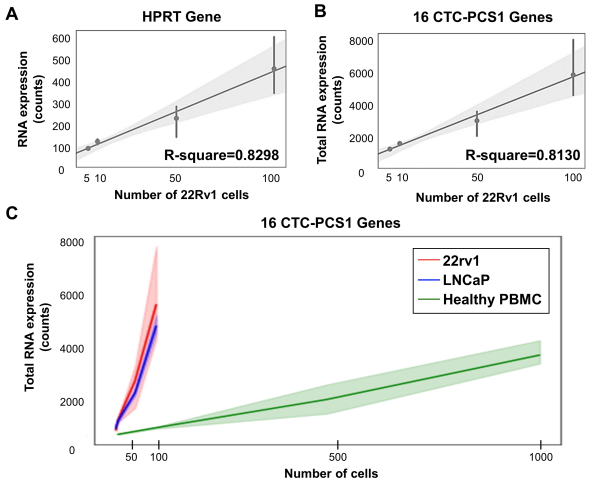

Figure 3. Analytical validation studies of the NanoVelcro CTC-RNA assay and CTC-PCS1 panel. (A) The HPRT RNA expression of PCa cell line 22Rv1 in different cell numbers measured by the NanoVelcro CTC-RNA assay (R-square= 0.8298). **(B)** NanoVelcro CTC-RNA assay quantification of the total CTC-PCS1 panel (16 genes) RNA expression of PCa cell line 22Rv1 in different cell numbers (R-square= 0.8130). **(C)** The total CTC-PCS1 panel (16 genes) RNA expression of PCa cell lines 22Rv1, LNCaP and healthy donor PBMCs in different cell numbers. Slopes of the curve- 22Rv1: 47 counts/cell, LNCaP: 44 counts/cell, healthy donor PBMC: 3 counts/cell.

RNA expression background in which CTCs exist is mostly contributed by WBCs, which is significantly different background than that of tumor tissue biopsies. As such, a bioinformatic process of refining tissue-based RNA panels for use with CTC analysis is also required. In brief, genes highly expressed in PCa cell lines are included and genes highly expressed in WBCs need to be excluded. We use CTC-PCS1 panel as a proof of concept. The Prostate Cancer Classification System (PCS)[19] is one of Dr. Sungyong You's achievements for improving prediction of prognosis and treatment sensitivity using datasets specific to gene expression in PCa/mCRPC[19]. PCS categorizes PCa into 3 subtypes, i.e., PCS1-3. Among them, PCS1 is associated with the worst prognosis, shortest time to metastasis, and highest risk of ARSI resistance. In comparison to other classification methods, PCS can identify tumors that will progress to lethal disease even in tissues with a low Gleason score[19]. To determine the sensitivity and dynamic range of the NanoVelcro CTC-RNA assay for quantification of the CTC-PCS1 signature, we first tested the assay with a PCa cell line, i.e., 22Rv1 using different cell numbers (n = 5, 10, 50, and 100 cells) that mimicked the CTC numbers present in 2-mL clinical blood samples. We demonstrated that the NanoVelcro CTC-RNA assay can detect RNA transcripts of a housekeeping gene (i.e., HPRT) and the 16 genes in CTC-PCS1 panel with high sensitivity and linearity in the dynamic range of 5-100 cells (**Figure 3A, 3B**). We then demonstrated that the CTC-PCS1 panel is capable of detecting PCa CTC-derived PCS1 signatures in the presence of WBC background by quantifying CTC-PCS1 RNA expression using three sets of RNA

samples extracted from 2 PCa cell lines (i.e., 22Rv1 and LNCaP), and healthy donor PBMCs with cell numbers mimicked the CTC and WBC numbers (i.e., 5-100 PCa cells and 50-1000 WBCs) in the CTC samples purified by the TR-NanoVelcro system[21] from 2-mL of patient blood. We demonstrated that the RNA counts of the CTC-PCS1 panel genes in PCa cells were significantly higher than the RNA counts in WBCs in the given cell number range (**Figure 3C**). This further validated the bioinformatic process of developing the CTC-PCS1 panel.

- Panel expansion to assess tumor burden (TB) and metastatic potential (MP) in addition to the originally proposed PSA, PSMA, and SCHLAP1 markers.

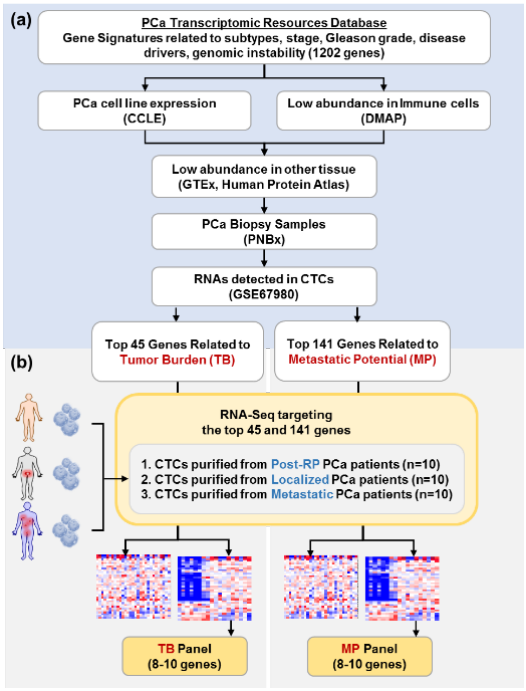

Figure 4. An integrated data analysis framework for selection of the PCa-CTC specific TB panel and MP panel. This diagram depicts the detailed steps of our integrated data analysis framework, consisting of two stages: **a)** 45 and 141 candidate genes related to tumor burden and metastatic potential respectively were selected from the PCa Transcriptomic Resources Database by a subtractive approach based on the integrated analysis of the cancer, immune cells and multi-tissue transcriptome profiles. **b)** Targeted RNA sequencing of PCa CTCs purified from post-RP PCa patients, localized PCa patients and metastatic PCa patients will be performed to validate these top 186 candidate genes. 8 to 10 genes that can best assess tumor burden and metastatic potential will be selected respectively as the mRNA markers of TB and MP panels.

While the original proposal focused on digital RT-PCR of PSA, PSMA, and SCHLAP1 as a primary focus, we have gained the capacity to conduct other rapid sequencing approaches that we will explore in parallel to the directed RT-PCR to further optimize the performance of the assay. It is known that as cancers progress and metastasize, increasing numbers of tumor cells are shed into the blood stream[18]. These cellular and clinical events are driven by alternations in changes in molecular pathways that govern growth and metastasis[20]. Our proposed PCa CTC-RNA assay will directly characterize these alterations by measuring two parameters – tumor burden (TB) and metastatic potential (MP) respectively through molecular profiling of the enriched CTCs in addition to the originally proposed PSA, PSMA, and SCHLAP1 markers.

As we illustrated in **Figure 4a**, we have identified PCa gene signatures relevant to subtypes, stages, Gleason grade, disease driver gene networks, and genomic instability in the PCa Transcriptomic Resources Database (**Figure 4**). These genes were selected by various approaches. To this end, we have performed a non-negative matrix factorization based unsupervised clustering[22] for selecting stage and grade-specific genes, integrated hypothesis testing[23] of primary versus metastatic tumors for metastasis specific genes, correlation analysis with tumor mutational burden and survival outcomes, master regulator analysis to select driver genes, and literature survey for PCa subtype-specific genes[19,24], resulting in a total of 474 genes. To establish a highly sensitive and specific TB and MP panel for the CTC test, genes in the panels should have high expression in PCa CTCs and low/absent expression in other tissues and immune cells to reduce background signals. We applied a subtractive approach by integrating independent transcriptome profiles that are relevant to PCa gene expression and various tissues and immune cells. We then assessed whether the genes in the PCa gene signatures are highly expressed in PCa cell lines from the Cancer Cell Line Encyclopedia (CCLE)[25] by comparing PCa cell lines with other cancer cell lines. Further evaluation of these gene expression levels in various tissues and immune cells using Genotype-Tissue Expression (GTEx)[26], Human Protein Atlas (HPA)[27], and Differentiation Map (DMAP)[28] data, enabled us to exclude genes with similar levels of expression in immune cells and other tissues, leaving a panel of genes that would be highly specific for genes expressed in PCa. Finally, we selected the top 186

genes previously detected in PCa CTCs using CTC RNA sequencing (RNA-seq) data (GSE67980)[29], allowing for high sensitivity in profiling PCa CTC signatures.

- Sample collection from our biobank. We have identified 20 metastatic and 20 localized patients from the Cedars-Sinai IRB protocol # 33050, 42197, and 51931.
- Sample collection from the NRG-GU006 trial. As noted in the original application, through our participation in the NRG network, our laboratory group has been receiving specimens from NRG-GU-006: A Phase II, Double-Blinded, Placebo Controlled Randomized Trial of Salvage Radiotherapy With or Without Enhanced Anti-androgen Therapy With Apalutamide in Recurrent Prostate Cancer (BALANCE). This is an international study of salvage radiotherapy that is being conducted through NRG Oncology- an NCI supported cooperative group. This trial had a proposed sample size of 311 and completed accrual in March 2020. We have received specimens from 238 unique patients on this study and await maturation of clinical outcomes.

-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.

1. Teng PC, Jan YJ, Yoon J, Chen PJ, Chen JF, Yao N, Cheng S, Lozano A, Freeman M, You S, Tseng HR, Posadas EM. A circulating tumor cell specific RNA assay for assessment of androgen receptor signaling inhibitor sensitivity in metastatic castration-resistant prostate cancer. Journal of Clinical Oncology. 2019;37(15_suppl):5059-. doi: 10.1200/JCO.2019.37.15_suppl.5059. American Society of Clinical Oncology (ASCO) Annual Meeting 2019, Chicago, IL. (Poster presentation)
2. Teng PC, Jan YJ, Chen JF, Cook-Wiens G, Cheng S, Yao N, Lozano A, Chu GCY, Chen PJ, Ho H, Yang Y, Huang K, Li KC, Chung LWK, You S, Zhu Y, Freeman MR, Rogatko A, Yang JD, Tseng HR, Posadas EM. Very-Small-Nuclear Circulating Tumor Cells: Nuclear Size Reduction is Associated with Poor Clinical Outcomes in Metastatic Castration-Resistant Prostate Cancer. 2019 NCI Alliance of Nanotechnology in Cancer Principal Investigator Meeting, Rockville, MD. (Poster presentation)
3. Teng PC, Jan YJ, Yoon Junhee, Chen JF, Chen PJ, Kim M, Yao N, Cheng S, Lozano A, Freeman MR, You S, Tseng HR, Posadas EM. Preclinical Development of a Circulating Tumor Cell Based RNA-Classifier to Optimize the Treatment Selection in Patients with Metastatic Castration-Resistant Prostate Cancer. 2019 NCI Alliance of Nanotechnology in Cancer Principal Investigator Meeting, Rockville, MD. (Poster presentation)

4. Wang JJ, Teng PC, Jan YJ, Chen JF, Cook-Wiens G, Yao N, Chu GCY, Chen PJ, Ho H, Yang Y, Lee YT, Huang J, Chung LWK, You S, Zhu Y, Freeman M, Rogatko A, Yang JD, Tseng HR, Posadas EM. Association of very small nuclear circulating tumor cell (vsnCTC) with clinical outcomes in metastatic castration-resistant prostate cancer. Journal of Clinical Oncology. 2020;38(6_suppl):168-. doi: 10.1200/JCO.2020.38.6_suppl.168. Genitourinary Cancers Symposium 2020, San Francisco, CA. (Poster presentation)
5. Teng PC, Jan YJ, Chen JF, Kim M, Yao N, Garraway I, Chu GCY, Chen PJ, Wang JJ, Lee YT, Zhu Y, Chung LWK, Feng FY, Freeman M, You S, Tseng HR, Posadas EM. Prostate cancer CTC-RNA Assay: A new method for contemporary genomics and precision medicine via liquid biopsy. Journal of Clinical Oncology. 2020;38(6_suppl):170-. doi: 10.1200/JCO.2020.38.6_suppl.170. Genitourinary Cancers Symposium 2020, San Francisco, CA. (Poster presentation)
6. Teng PC, Kim M, Jan YJ, Chen JF, Yao N, Chu GC, Chen PJ, Wang JJ, Lee YT, Zhu Y, Chung LWK, Feng FY, Freeman MR, You S, Tseng HR, Posadas EM. Gene expression of circulating tumor cells is predictive of treatment response in patients with advanced prostate cancer. American Association for Cancer Research (AACR) Annual Meeting 2020 (Poster presentation).
7. Wang JJ, Teng PC, Jan YJ, Chen JF, Cook-Wiens G, Yao N, Chu GC, Chen PJ, Yang Y, Yeo YH, Lee YT, Chung LWK, You S, Zhu Y, Freeman MR, Rogatko A, Yang JD, Tseng HR, Posadas EM. Nuclear size of circulating tumor cells is associated with prognosis in metastatic, castration-resistant prostate cancer. American Association for Cancer Research (AACR) Annual Meeting 2020 (Poster presentation).
8. Teng PC, Jan YJ, Kim M, Chen JF, Yoon J, Wang JJ, Chen PJ, Yao N, Lee YT, Lozano A, Gadilov R, Freeman M, You S, Tseng HR, Posadas EM. Development of a circulating tumor cell-based RNA classifier for patients with castration-resistant prostate cancer: CTC-PCS/PAM50. American Society of Clinical Oncology (ASCO) Annual Meeting 2020 (Virtual meeting).
9. Wang JJ, Teng PC, Jan YJ, Chen JF, Cook-Wiens G, Yao N, Chu GCY, Chen PJ, Ho H, Yang Y, Lee YT, Huang J, Chung LWK, You S, Zhu Y, Freeman M, Rogatko A, Yang JD, Tseng HR, Posadas EM. Association of very small nuclear circulating tumor cell (vsnCTC) with clinical outcomes in metastatic castration-resistant prostate cancer. American Society of Clinical Oncology (ASCO) Annual Meeting 2020 (Virtual meeting).

-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.

We have strengthened our interactions with Dr. Sungyong You, an expert in prostate cancer computation biology, we have been revising our approach to molecular characterization of CTCs using the CTC-RNA assay. While the original proposal focused on digital RT-PCR of PSA, PSMA, and SCHLAP1 as a primary focus, we have gained the capacity to conduct other rapid sequencing approaches that we will explore in parallel to the directed RT-PCR to further optimize the performance of the assay. Via our integrated data analysis framework using a large collection of PCa transcriptomic data consisting of >4,000 patients' profiles, we will select two optimal panels (i.e., TB and MP) of PCa CTC-specific mRNA markers that can assess Tumor Burden and Metastatic Potential, respectively. The panels and RT-ddPCR will be validated together using PCa cell lines. The top 186 genes will be further selected and validated by targeted sequencing of PCa CTCs purified from 10 post-RP PCa patients, 10 localized PCa patients and 10 metastatic PCa patients using the PCa CTC-RNA assay. The top 8-10 genes that can best assess TB and MP status will be selected respectively as the mRNA markers of TB and MP panels in addition to the originally proposed PSA, PSMA, and SCHLAP1 markers. Then we will validate these genes using banked samples from our biobank and the patient samples collected from the NRG-GU006 trial.

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).

Post-RP BCR PCa patients. PCa is the most common solid-organ malignancy and second leading cause of cancer death in American men[7]. Among the 190,000 of PCa cases diagnosed annually, about 92% of patients are diagnosed with localized cancers, which are commonly treated by radical prostatectomy (RP). After RP, approximately 35% of patients will experience biochemical recurrence (BCR)[8,9], clinically manifested as a rising serum prostate-specific antigen (PSA) concentration. For post-RP BCR patients without radiographic evidence of distant metastases, the mainstay of treatment is salvage radiotherapy (SRT) to the local prostate bed and the surrounding tissue, potentially salvaging the surgical failure and offering possibilities for cure[10].

Clinical unmet need: optimizing BCR management by detecting distant micrometastases in post-RP BCR PCa patients. Although SRT provides an opportunity for cure in post-RP BCR PCa patients, >50% of patients treated with SRT will experience disease progression[11-13]. Furthermore, patients receiving SRT often suffer from radiation toxicity, including long-term urinary incontinence and impotence[14]. The failure of SRT typically results from the presence of disease in the form of distant micrometastases outside the radiotherapy field. In this case, tumor cells will remain untreated by radiation[15]. Patients with distant micrometastases are best served by treating them as metastatic patients focusing on timely initiation of systemic therapy without the complications related to SRT. Current clinical imaging modalities (e.g., bone scan, CT, MRI, and/or PET) are helpful at times, but currently suffer from limited sensitivity and spatial resolution in detecting distant micrometastases[16,17]. As such, there is an urgent and unmet need to develop a diagnostic solution that will enable detection of distant micrometastases in post-RP BCR patients to personalize and optimize the use of SRT for better outcome.

PCa CTC-RNA assay as a diagnostic solution to detect distant micrometastases. It is known that as cancers progress and metastasize, increasing numbers of tumor cells are shed into the blood stream[18]. These cellular and clinical events are driven by alternations in changes in molecular pathways that govern growth and metastasis[20]. Our proposed PCa CTC-RNA assay will directly characterize these alterations through molecular profiling of the enriched CTCs. Applying this assay in post-RP BCR PCa patients, we are able to address the clinical unmet need to detect distant micrometastases, thereby improve treatment selection and clinical outcome.

Others. As the proposed assay is a combination of two existing technologies: the TR-NanoVelcro Assay and ddPCR™. The TR-NanoVelcro assay has been successfully deployed at test sites and ddPCR™ is now widely available. Thus, the merged assay can be deployed immediately following after our technical validation and QA/QC development. Expansion of FDA clearance of NanoVelcro platform to include the TR-NanoVelcro system which will allow for wider dissemination of this technology.

-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.

There are many platforms for enrichments or purifications of CTCs, which belong to the field of engineering. However, the subsequent studies of clinical applications are few. Our clinical validation of the CTC-RNA assay can provide positive feedback to the platform development. Indeed, our group has developed newer generations of NanoVelcro Chips which can purify CTCs with higher purity and throughput.[1,2]

Based on the success with PCa, we also utilized this platform in other diseases including melanoma[3], hepatocellular carcinoma[4], lung cancer[1], pancreatic cancer[5] and noninvasive prenatal diagnostics[6].

-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.*

The successful development of the proposed CTC-RNA assay is rapidly translatable, enabling a sensitive and biologically relevant CTC-based assay for optimizing the selection of salvage radiotherapy (SRT) candidates by identifying those who have micrometastases and will experience more harm than benefit from SRT. Such an approach will improve costs of care and, most important, quality of life for men dealing with BCR. Furthermore, Thermoresponsive (TR)-NanoVelcro Chips are expected to enable purification of CTCs from other solid tumors by targeting the corresponding surface markers, paving the way for the realization of a CTC-based RNA assay for cancer detection.

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:*

-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.

Expansion of the CTC-RNA assay: As a result of this project, we have strengthened our interactions with Dr. Sungyong You, an expert in prostate cancer computation biology, we have been revising our approach to molecular characterization of CTCs using the CTC-RNA assay. While the original proposal focused on digital RT-PCR of PSA, PSMA, and SCHLAP1 as a primary focus, we have gained the capacity to conduct other rapid sequencing approaches that we will explore in parallel to the directed RT-PCR to further optimize the performance of the assay.

-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.

COVID-19 related delays: During the up-ramping phase of our studies, both Cedars-Sinai and UCLA experienced a laboratory shut down which has negatively impacted the timelines of our proposed work. Both the Posadas and Tseng laboratories have re-opened at this point as of mid-July with staggered work hours. During this period, we have been able to continue planning and computational work, but the development of the artificial samples was complicated by the need to retrofit all equipment in the laboratory to minimize

aerosolization risks to laboratory personnel and to engage in campus required safety training to minimize potential transmission of respiratory pathogens.

-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.

Nothing to Report

-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).*

  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.*

1. Teng PC, Jan YJ, Yoon J, Chen PJ, Chen JF, Yao N, Cheng S, Lozano A, Freeman M, You S, Tseng HR, Posadas EM. A circulating tumor cell specific RNA assay for assessment of androgen receptor signaling inhibitor sensitivity in metastatic castration-resistant prostate cancer. Journal of Clinical Oncology. 2019;37(15_suppl):5059-. doi: 10.1200/JCO.2019.37.15_suppl.5059. American Society of Clinical Oncology (ASCO) Annual Meeting 2019, Chicago, IL.
2. Wang JJ, Teng PC, Jan YJ, Chen JF, Cook-Wiens G, Yao N, Chu GCY, Chen PJ, Ho H, Yang Y, Lee YT, Huang J, Chung LWK, You S, Zhu Y, Freeman M, Rogatko A, Yang JD, Tseng HR, Posadas EM. Association of very small nuclear circulating tumor cell (vsnCTC) with clinical outcomes in metastatic castration-resistant prostate cancer. Journal of Clinical Oncology. 2020;38(6_suppl):168-. doi: 10.1200/JCO.2020.38.6_suppl.168. Genitourinary Cancers Symposium 2020, San Francisco, CA.
3. Teng PC, Jan YJ, Chen JF, Kim M, Yao N, Garraway I, Chu GCY, Chen PJ, Wang JJ, Lee YT, Zhu Y, Chung LWK, Feng FY, Freeman M, You S, Tseng HR, Posadas EM. Prostate cancer CTC-RNA Assay: A new method for contemporary genomics and precision medicine via liquid biopsy. Journal of Clinical Oncology. 2020;38(6_suppl):170-. doi: 4.1200/JCO.2020.38.6_suppl.170. Genitourinary Cancers Symposium 2020, San Francisco, CA.
5. Teng PC, Kim M, Jan YJ, Chen JF, Yao N, Chu GC, Chen PJ, Wang JJ, Lee YT, Zhu Y, Chung LWK, Feng FY, Freeman MR, You S, Tseng HR, Posadas EM. Gene expression of circulating tumor cells is predictive of treatment response in patients with advanced prostate cancer. American Association for Cancer Research (AACR) Annual Meeting 2020.
6. Wang JJ, Teng PC, Jan YJ, Chen JF, Cook-Wiens G, Yao N, Chu GC, Chen PJ, Yang Y, Yeo YH, Lee YT, Chung LWK, You S, Zhu Y, Freeman MR, Rogatko A, Yang JD, Tseng HR, Posadas EM. Nuclear size of circulating tumor cells is associated with prognosis in metastatic, castration-resistant prostate cancer. American Association for Cancer Research (AACR) Annual Meeting 2020.
7. Teng PC, Jan YJ, Kim M, Chen JF, Yoon J, Wang JJ, Chen PJ, Yao N, Lee YT, Lozano A, Gadilov R, Freeman M, You S, Tseng HR, Posadas EM. Development of a circulating tumor cell-based RNA classifier for patients with castration-resistant prostate cancer: CTC-PCS/PAM50. American Society of Clinical Oncology (ASCO) Annual Meeting 2020.
8. Wang JJ, Teng PC, Jan YJ, Chen JF, Cook-Wiens G, Yao N, Chu GCY, Chen PJ, Ho H, Yang Y, Lee YT, Huang J, Chung LWK, You S, Zhu Y, Freeman M, Rogatko A, Yang JD, Tseng HR, Posadas EM. Association of very small nuclear circulating tumor cell (vsnCTC) with clinical outcomes in metastatic castration-resistant prostate cancer. American Society of Clinical Oncology (ASCO) Annual Meeting 2020.

- **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.

  UCLA Technology Development Group filed the first patent application entitled "Click Chemistry-Mediated Rare-Cell Sorting in Microfluidic Devices" (UCLA # 2018-441) to cover the IPs associated with the Click Chips and the related research and clinical applications.
  The second provisional patent application entitled "A Circulating Tumor Cell-RNA Assay for Assessment of Androgen Receptor Signaling Inhibitor Sensitivity in Metastatic Castration-Resistant Prostate Cancer" (UCLA # 2019-740) was to cover the IPs associated with the PCa CTC-based RNA profiling technology.

- **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:	Edwin Posadas (no change)
Project Role:	Contact-PI
No change	

Name:	Pai-Chi Teng
Project Role:	Postdoc
Research Identifier (ORCID):	0000-0002-1872-466X

Nearest person month worked: 1.2
Contribution to project: Dr. Jan led the research team in Posadas Lab to perform and analyze the CTC-based RNA markers for clinical blood specimens at CSMC in cooperation with UCLA. He arranged and presented all findings to Drs. Posadas, Tseng and other team members as part of the monthly CTC-group meetings.
Funding Support: DoD/PCRP EIRA, PC151088
NIH/NCI, 1R01, CA218356-02

The previous postdoc, Dr. Yu Jen Jan, has left for his residency training. Dr. Teng took over Dr. Jan's work for this project.

Name: Nu Yao / Kai-Han Tu
Project Role: Lab Technician
Research Identifier: n/a
Nearest person month worked: 1.2
Contribution to project: Mr. Tu worked with Dr. Teng and Ms. Gomez to optimize the CTC-based RNA assay. He was responsible for operating the assay for the clinical blood specimens at CSMC.
Funding Support: NIH/NCI, 1R01, CA218356-02

Ms. Yao left in August 2020 for her career plan. Mr. Tu took over Ms. Yao's role for this project.

Name: Amber Lozano / Amy Gomez
Project Role: Lab Technician
Research Identifier: n/a
Nearest person month worked: 1.2
Contribution to project: Ms. Gomez's works involved picking up, processing and banking the clinical samples. She also helped Mr. Tu with isolation and molecular testing of CTCs from clinical samples using the proposed CTC-based RNA assay.
Funding Support: n/a

Ms. Lozano has left for her career plan. Ms. Gomez took over Ms. Lozano role for this project.

Name: Catherine Carroll / Zijing Chen
Project Role: Data Coordinator
Research Identifier: n/a
Nearest person month worked: 1.2
Contribution to project: Ms. Chen helped to provide clinical data annotation support for this project. She oversaw entry of clinical data into the existing database and work with Dr. Posadas to adapt the database to the needs of the research team. The clinical data from the NRG-GU-006 trial were also organized by Ms. Chen.

Ms. Carroll has left for her career plan. Ms. Chen took over Ms. Carroll role for this project.

Name: Hsian-Rong Tseng

Project Role: PI
No change

Name: Tom Lee
Project Role: Collaborator
No change

-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.*

Organization Name: University of California, Los Angeles (UCLA)
Location of Organization: 500 Westwood Plz, California NanoSystems Institute (CNSI)
Partner's contribution to the project
- Financial support
- In-kind support
- Facilities
- Collaboration
- Personnel exchanges

8. SPECIAL REPORTING REQUIREMENTS

- **NONE**

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.*

Literature Cited:

1. Dong J, Jan YJ, Cheng J, et al. Covalent chemistry on nanostructured substrates enables noninvasive quantification of gene rearrangements in circulating tumor cells. *Sci Adv.* 2019;5(7):eaav9186.
2. Shen MY, Chen JF, Luo CH, et al. Glycan Stimulation Enables Purification of Prostate Cancer Circulating Tumor Cells on PEDOT NanoVelcro Chips for RNA Biomarker Detection. *Adv Healthc Mater.* 2018;7(3).
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4. Court CM, Hou S, Winograd P, et al. A novel multimarker assay for the phenotypic profiling of circulating tumor cells in hepatocellular carcinoma. *Liver Transpl.* 2018;24(7):946-960.
5. Court CM, Ankeny JS, Sho S, et al. Circulating Tumor Cells Predict Occult Metastatic Disease and Prognosis in Pancreatic Cancer. *Ann Surg Oncol.* 2018;25(4):1000-1008.
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16. Erdi YE. Limits of Tumor Detectability in Nuclear Medicine and PET. *Mol Imaging Radionucl Ther.* 2012;21(1):23-28.
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