AWARD NUMBER: W81XWH-19-1-0390

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

PRINCIPAL INVESTIGATOR: Edwin M Posadas, MD

CONTRACTING ORGANIZATION: Cedars-Sinai Medical Center, Los Angeles, CA

REPORT DATE: October 2021

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2021	Annual Report	01Sep2020-31Aug2021

4. TITLE AND SUBTITLE

A Nanotechnology Solution for Early Detection of Micrometastatic

Prostate Cancer After Radical Prostatectomy

5a. CONTRACT NUMBER
PC180192

5b. GRANT NUMBER
W81XWH-19-1-0390

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Edwin M Posadas, MD

E-Mail: Edwin.Posadas@csmc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Cedars-Sinai Medical Center
8700 Beverly Blvd
Los Angeles, CA 90048

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

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

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	18	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

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:

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

3. ACCOMPLISHMENTS:

-What were the major goals of the project?

- 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 1-8 with 100% completion.
- Major Task 2: To carry out calibration studies to assess the performance of RNA quantification for ddPCR™.
  Timeline: Month 7-9 with 90% completion.
- Major Task 3: To carry out calibration studies to examine the complete CTC-RNA assay.
  Timeline: Month 10-12 with 90% completion.
- Major Task 4: Case-control study to verify the association between CTC-RNA markers and mPCa.
  Timeline: Month 13-24 with 50% 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?

1) Major activities
   - Calibration studies for CTC capture efficiency.
   - Calibration study of RT-ddPCR for the 3 gene-panel.
   - Examination of the specificity of ddPCR primers and probes sets.
   - Reproducibility studies for the CTC-RNA assay
   - Case-control study to verify the association between CTC-RNA markers and mPCa.
   - Exploration of other RNA markers 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.

- Significant results calibration studies for CTC capture efficiency

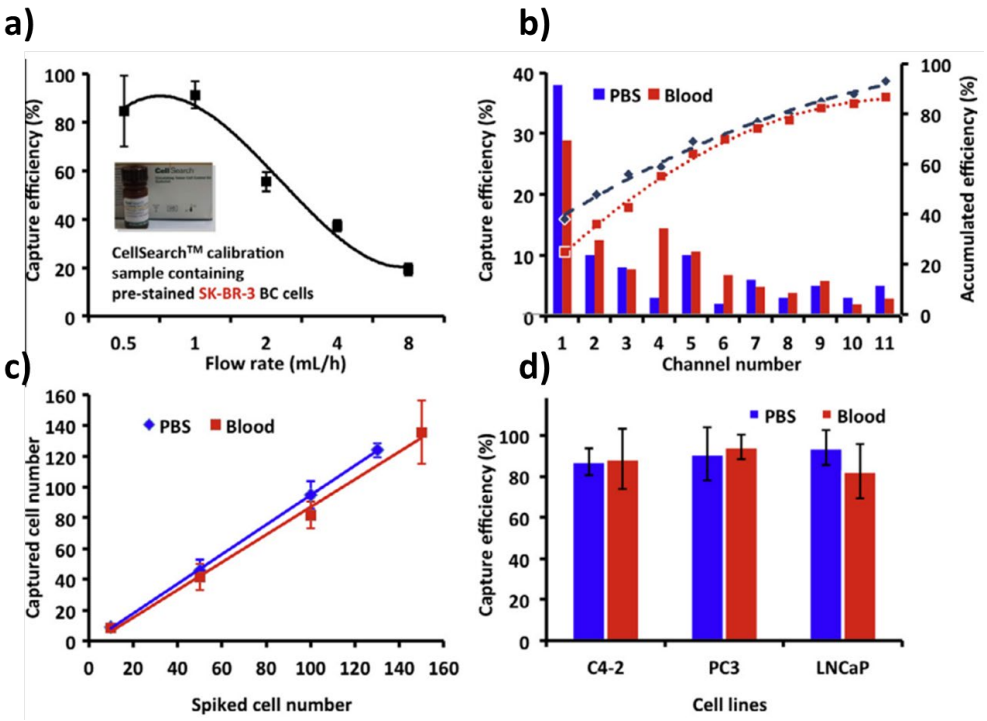

Figure 1. Optimization of the capture efficiency. a) Cell-capture efficiency of TR-NanoVelcro CTC Chip at flow rates of 0.5, 1, 2, 4, and 8 mL/h. Error bars show standard deviations (n = 3–4). CellSearch™ calibration samples containing 100 pre-stained EpCAM-positive SK-BR-3 breast cancer cells were spiked into PBS as a model system. b) The cell distribution and accumulative cell-capture efficiency in a TR-NanoVelcro CTC Chip were assessed in PBS and normal blood. c) Cell-capture efficiency at different cell numbers ranging from 10 to 150 cells mL-1 in two different types of samples: whole blood and PBS buffer. d) The capture efficiency observed for different prostate cancer cell lines in PBS and blood.

- We spiked 0.3 mL of the CellSearch™ calibration sample, containing 100 pre-stained EpCAM-positive SK-BR-3 breast cancer cells, into 0.7 mL of PBS as a model system. After labeling with biotinylated anti-EpCAM, samples were pumped through the TR-NanoVelcro CTC chips at different flow rates (Figure 1a). The results showed that the capture efficiency was optimized at the rate of 0.5 to 1 mL/h. We chose a flow rate of 0.5mL/h for experiments to decrease shearing force in order to preserve potentially fragile CTCs. The cell distribution and cumulative cell-capture efficiency were assessed along the length of the total channel. Samples utilized included cancer cells spiked into PBS and cells spiked into healthy donor blood. These tests showed that over 60% of the captured cells were located in the first 4 channels, with the majority captured in the first 1 to 2 channels. This finding was consistent across the PBS and donor blood model systems (Figure 1b). The capture efficiencies were also constant across different numbers of cells in PBS and blood (Figure 1c). Finally, we tested different prostate cancer cell lines on the NanoVelcro Chips. Three commonly utilized prostate cancer lines, LNCaP, C4-2, and PC3, showed a similar capture efficiency of 80–95% in both PBS and blood (Figure 1d).

- Calibration study of RT-ddPCR for the 3 gene-panel. We have conducted the calibration study for the RT-ddPCR for the proposed 3 gene panel (**Figure 2a/b**). *PSA, PSMA, SchLAP1* were quantified by ddPCR with standardized cDNA mixtures of PCa cell lines equivalent to different cell numbers. All three genes demonstrated excellent linear regression with R^2 >0.98.

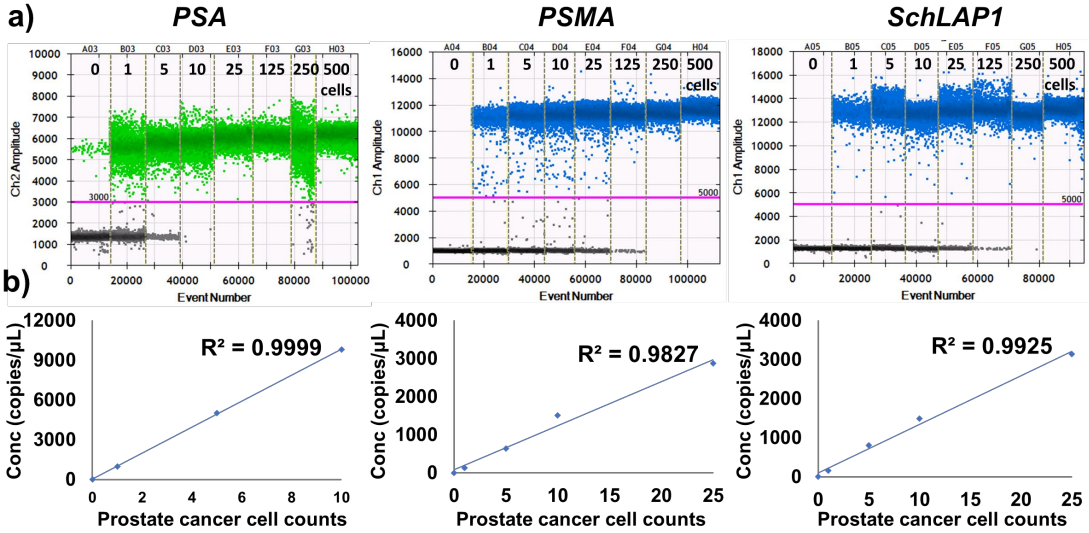

Figure 2. Calibration study of **a)** RT-ddPCR for PCa-specific RNA markers of PSA, PSMA, SchLAP1. **b)** Linear regression among all the RNA markers with R^2 >0.98.

- Confirm the specificity of the primers and probes of the proposed 3 gene panel using the CTC-RNA assay

- The specificity of the primers and probes for the gene panel - *PSA/PSMA/SchLaP1* was determined by quantifying the copy number detected in 5000 WBCs from male healthy donor, which is the background WBC counts on the TR-NanoVelcro chip. . HPRT is a housekeeping gene showing constant expression between prostate cancer cell lines and background WBCs. The results show the designed ddPCR primers and probes can detect abundant expression of these genes (*PSA, PSMA, SchLaP1*) in prostate cancer cell lines with absent detection in WBCs from male healthy donors. This confirms the specificity of the ddPCR primers probes sets for detecting the interested 3 genes under WBC background (Figure 3).

- Case-control study to verify the association between CTC-RNA markers and mPCa.
  - To carry out case-control study to verify the association between CTC-RNA markers and mPCa, banked PBMC samples from 18 localized PCa patients and 23 metastatic PCa patients were tested by the CTC-RNA assay. With batch calibration and normalization with housekeeping gene RPL13A, the expression of PCa markers is shown in the heatmap without clustering and centering (Figure 4a). The expression of PCa markers *PSMA* and *SChLAP1* was computed as a mPCa index. Boxplot (Figure 4b) shows the mPCa index are higher in metastatic PCa patients than those in localized PCa patients, but it doesn't reach statistical significance (Mann-Whitney test, P=0.33). To improve the separation, more samples are being tested to increase the samples size. Other marker candidates more correlated with mPCa are also in exploration.

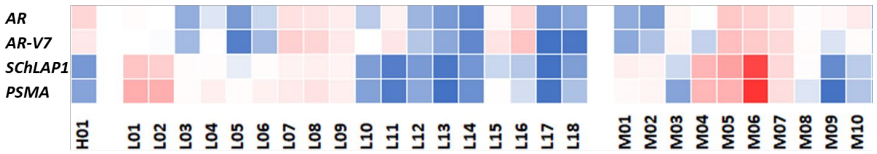

Figure 4. RNA expression in purified CTCs from localized and metastatic PCa patients donor, 18 localized and 23 metastatic PCa patients. **b)** mPCa index computed from 18 locali P=0.33).

- Exploration of other RNA markers in addition to the originally proposed *PSA, PSMA* and *SChLAP1*.
  - To explore other RNA markers that may have better distinguishing performance than the proposed *PSA, PSMA* and *SChLAP1*, we sought to expand the panel by exploring other existing transcriptomic signatures in PCa.
  - PAM50 is a transcriptome-based classifiers initially developed from breast cancer and is the basis for the commercially available Prosigna test.(Wallden B, Storhoff J, Nielsen T, et al. Development and verification of the PAM50-based Prosigna breast cancer gene signature assay. *BMC Med Genomics*. 2015;8:54.) This classifier and concept were then applied to PCa, in which the molecular subtyping by luminal and basal status is prognostic for clinical outcomes and may be associated with response to postoperative androgen deprivation therapy[1].

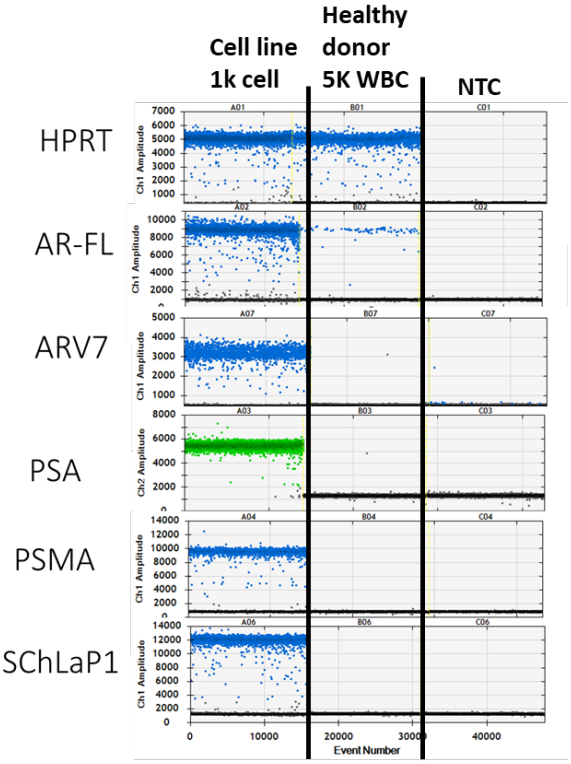

Figure 3. Validation of the specificity of the primers and probes sets. The specificity of the 3 gene panel to PCa was determined by ddPCR quantification of the genomic product extracted from 5,000 male healthy donor WBCs in comparison to those in PCa cell lines. Zero copy number of the 3 genes was detected in 5,000 WBCs. HPRT: housekeeping gene. NTC: no template control. AR-FL: Androgen receptor full length.

- The Prostate Cancer Classification System (PCS)[2] 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[2]. 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 androgen receptor signaling inhibitor resistance. In comparison with PAM50, the PCS system exhibits greater separation in multiple clinical outcomes and provides better separation of prostate luminal and basal characteristics[3]. This result was published on *Prostate Cancer Prostatic Dis* this year.
- To translate these tissue-based transcriptomic signatures to CTC-based signatures, a bioinformatics process was performed to filter out the background signals from WBCs. With an integrated data analysis framework using PCa and WBC datasets from the Prostate Cancer Transcriptome Atlas (PCTA, www.thepcta.org), PCa CTC RNA-seq (GSE67980)[4], Cancer

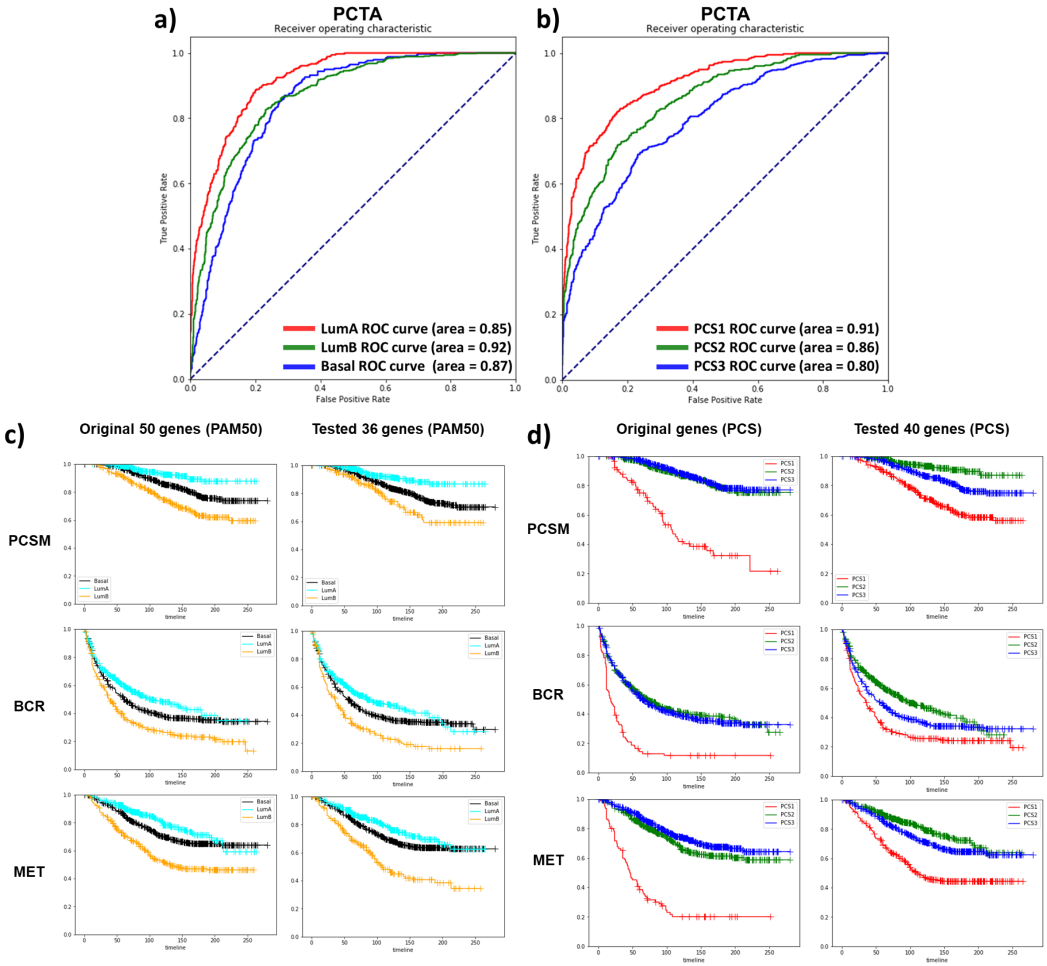

Figure 5. Comparison of CTC-PAM50/PCS and their original PAM50/PCS classifier in the PCTA database. a) ROC curve and the AUC of the CTC-PAM50 panel for distinguish the original tissue subtyping by the PAM50 panel. **b)** ROC curve and the AUC of the CTC-PCS panel for distinguish the original tissue subtyping by the PCS panel. **c)** Prognostic performance of original PCS and CTC-PCS panel in the GenomeDx GRID database. **d)** Prognostic performance of original PAM50 and CTC-PAM50 panel in the GenomeDx GRID database. PCSM: prostate cancer specific mortality; BCR: biochemical relapse; MET: metastasis free survival.

Cell Line Encyclopedia (CCLE)[5], and Differentiation Map (DMAP)[6], 36 genes from PAM50 and 40 genes from PCS were shown to be highly expressed in PCa CTCs with low expression in WBCs and were selected for the CTC-RNA assay.

- The discriminatory performance of CTC-PCS/PAM50 panel is compared with the original tissue-based PCS/PAM50 classifier in the public database PCTA. The area under curve (AUC) is 0.91, 0.86 and 0.80 for PCS1, PCS2 and PCS3, respectively (Figure 5a). The AUC

is 0.85, 0.92 and 0.87 for Luminal A, Luminal B and Basal, respectively (Figure 5b). The prognostic performance of these two CTC panels were validated in the GenomeDx GRID databases (Figure 5c/d).

- Banked PBMC samples of 17 localized PCa patients and 24 metastatic PCa patients from Urologic Oncology Program Blood Biospecimens Bank (UOPBBB, CSMC IRB#Pro00042197) were tested with the CTC-RNA assay. Nanostring nCounter platform was used for the downstream RNA quantification of the 40 CTC-PCS genes and 36 CTC-PAM50 genes.
- With batch calibration and normalization with housekeeping gene RPL13A, the results are shown in the heatmap (Figure 6). Rows are centered; unit variance scaling is applied to rows. Both rows and columns are clustered using correlation distance and complete linkage. PCa patients with metastatic diseases were clustered together in this heatmap. Further tests and analysis will be carried out to identify markers for detection of metastatic PCa.
-

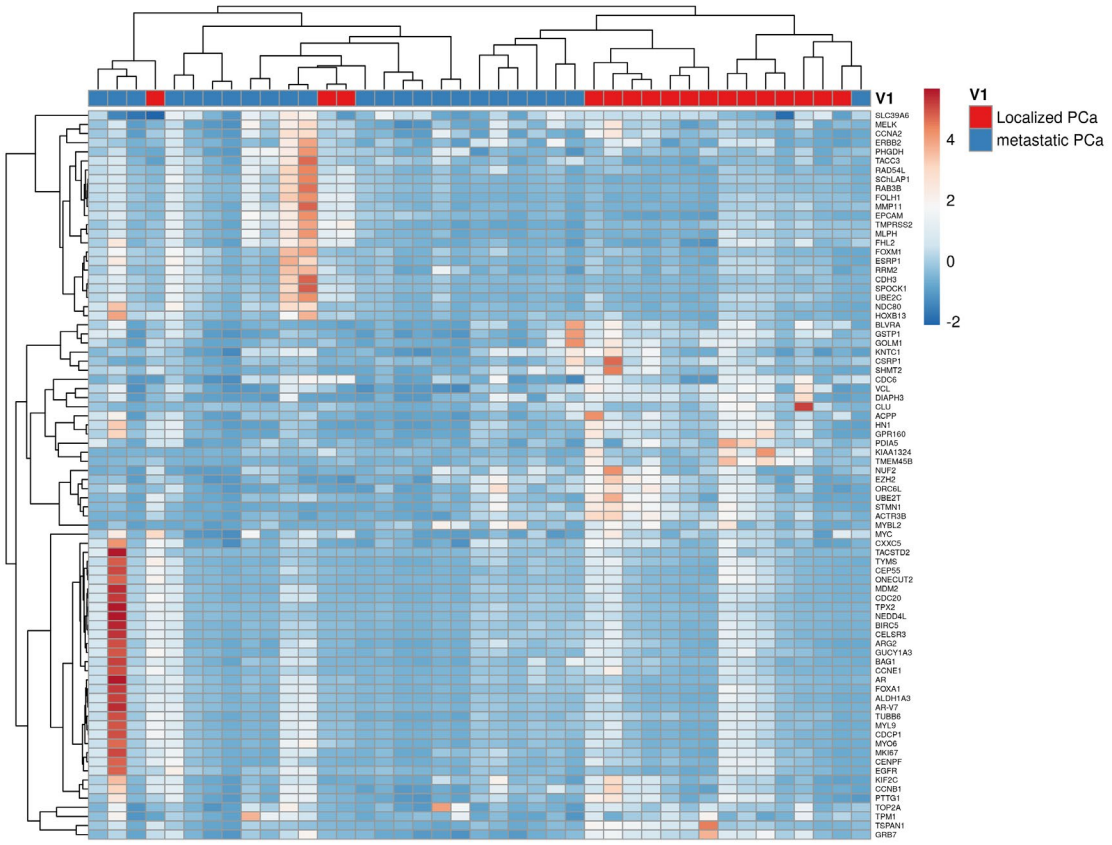

Figure 6. Heatmap depicting the expressions of CTC-PAM50 and CTC-PCS panel in purified CTCs from 17 localized and 24 metastatic PCa patients. Both rows and columns are clustered using correlation distance and complete linkage.

- Sample collection from our biobank. In preparation for the retrospective validation, 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?
Nothing to Report

-How were the results disseminated to communities of interest?

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).
10. Yoon J, Kim M, Posadas EM, Freedland SJ, Liu Y, Davicioni E, Den RB, Trock BJ, Karnes RJ, Klein EA, Freeman MR, You S. A comparative study of PCS and PAM50 prostate cancer classification schemes.

Prostate Cancer Prostatic Dis. 2021 Sep;24(3):733-742. doi: 10.1038/s41391-021-00325-4. Epub 2021 Feb 2. PMID: 33531653; PMCID: PMC8326303.
11. Wang JJ, Cavassani KA, Teng PC, Chen JF, Jan YJ, Chu GC, Lee YT, Gao A, Di Vizio D, Chung LW, You **S**, Zhu Y, Freeman M, Rogatko A, Yang JD, Tseng H-R, Posadas EM. Nuclear size of circulating tumor cells in advanced prostate cancer to reveal a potential biomarker for clinical outcomes and androgen receptor indifference. Journal of Clinical Oncology 2021 39:6_suppl, 167-167. Genitourinary Cancers Symposium 2021, San Francisco, CA. (Poster presentation)

-What do you plan to do during the next reporting period to accomplish the goals?

The initial case-control study to assess the performance of the 3 gene panel in distinguishing localized from metastatic PCa patients failed to reach adequate statistical significance. To improve this, more samples will be included to increase the sample size.
Meanwhile, 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 expanded our exploration to other PCa transcriptomic classifiers including PAM50 and PCS in parallel to the directed RT-PCR to further optimize the performance of the assay. Although bioinformatics process has been done to improve the specificity of these panel to PCa CTCs when being used in a liquid biopsy setting, further investigation into the normalization methods to filter out the WBC background will be carried out to optimize the output signals. On the basis of the initial tests with CTC-PAM50 and CTC-PCS panels, further tests and analysis will be done to identify appropriate markers for better detection of metastatic PCa.
Then we will validate these genes using banked samples from our biobank and the patient samples collected from the NRG-GU006 trial.

4. IMPACT:

-What was the impact on the development of the principal discipline(s) of the project?

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[19]. 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?

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.[20,21]

Based on the success with PCa, we also utilized this platform in other diseases including melanoma[22], hepatocellular carcinoma[23], lung cancer[20], pancreatic cancer[24] and noninvasive prenatal diagnostics[25].

-What was the impact on technology transfer?

Nothing to Report

-What was the impact on society beyond science and technology?

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:

-Changes in approach and reasons for change

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 genomic quantification 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

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
Nothing to Report

-Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
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:

- **Publications, conference papers, and presentations**

  Journal publications.
  Nothing to Report

  Books or other non-periodical, one-time publications.
  Nothing to Report

  Other publications, conference papers and presentations.

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).
10. Yoon J, Kim M, Posadas EM, Freedland SJ, Liu Y, Davicioni E, Den RB, Trock BJ, Karnes RJ, Klein EA, Freeman MR, You S. A comparative study of PCS and PAM50 prostate cancer classification schemes. Prostate Cancer Prostatic Dis. 2021 Sep;24(3):733-742. doi: 10.1038/s41391-021-00325-4. Epub 2021 Feb 2. PMID: 33531653; PMCID: PMC8326303.
11. Wang JJ, Cavassani KA, Teng PC, Chen JF, Jan YJ, Chu GC, Lee YT, Gao A, Di Vizio D, Chung LW, You **S**, Zhu Y, Freeman M, Rogatko A, Yang JD, Tseng H-R, Posadas EM. Nuclear size of circulating tumor cells in advanced prostate cancer to reveal a potential biomarker for clinical outcomes and androgen receptor indifference. Journal of Clinical Oncology 2021 39:6_suppl, 167-167. Genitourinary Cancers Symposium 2021, San Francisco, CA. (Poster presentation)

- **Website(s) or other Internet site(s)**
  Nothing to Report

- **Technologies or techniques**
  Nothing to Report

- **Inventions, patent applications, and/or licenses**

  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**
  Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

-What individuals have worked on the project?

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

No change

Name:	Jasmine Wang
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. Pai-chi Teng, has left for his residency training. Dr. Wang took over Dr. Teng'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:	Arthur Salonga Jr.
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. Gomez has left for her career plan. Mr. Salonga took over Ms. Gomez role for this project.

Name:	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.

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?

Nothing to Report

-What other organizations were involved as partners?

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:

Literature Cited:

1. Zhao SG, Chang SL, Erho N, et al. Associations of Luminal and Basal Subtyping of Prostate Cancer With Prognosis and Response to Androgen Deprivation Therapy. *JAMA Oncol.* 2017;3(12):1663-1672.
2. You S, Knudsen BS, Erho N, et al. Integrated Classification of Prostate Cancer Reveals a Novel Luminal Subtype with Poor Outcome. *Cancer Res.* 2016;76(17):4948-4958.
3. Yoon J, Kim M, Posadas EM, et al. A comparative study of PCS and PAM50 prostate cancer classification schemes. *Prostate Cancer Prostatic Dis.* 2021;24(3):733-742.
4. Miyamoto DT, Zheng Y, Wittner BS, et al. RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. *Science.* 2015;349(6254):1351-1356.
5. Barretina J, Caponigro G, Stransky N, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature.* 2012;483(7391):603-607.
6. Novershtern N, Subramanian A, Lawton LN, et al. Densely interconnected transcriptional circuits control cell states in human hematopoiesis. *Cell.* 2011;144(2):296-309.
7. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin.* 2020;70(1):7-30.
8. Freedland SJ, Humphreys EB, Mangold LA, et al. Risk of prostate cancer-specific mortality following biochemical recurrence after radical prostatectomy. *JAMA.* 2005;294(4):433-439.
9. Roehl KA, Han M, Ramos CG, Antenor JA, Catalona WJ. Cancer progression and survival rates following anatomical radical retropubic prostatectomy in 3,478 consecutive patients: long-term results. *J Urol.* 2004;172(3):910-914.
10. Pisansky TM, Thompson IM, Valicenti RK, D'Amico AV, Selvarajah S. Adjuvant and Salvage Radiotherapy after Prostatectomy: ASTRO/AUA Guideline Amendment 2018-2019. *J Urol.* 2019;202(3):533-538.
11. Goenka A, Magsanoc JM, Pei X, et al. Long-term outcomes after high-dose postprostatectomy salvage radiation treatment. *Int J Radiat Oncol Biol Phys.* 2012;84(1):112-118.
12. Stephenson AJ, Scardino PT, Kattan MW, et al. Predicting the outcome of salvage radiation therapy for recurrent prostate cancer after radical prostatectomy. *J Clin Oncol.* 2007;25(15):2035-2041.
13. Katz MS, Zelefsky MJ, Venkatraman ES, Fuks Z, Hummer A, Leibel SA. Predictors of biochemical outcome with salvage conformal radiotherapy after radical prostatectomy for prostate cancer. *J Clin Oncol.* 2003;21(3):483-489.
14. Thompson IM, Valicenti RK, Albertsen P, et al. Adjuvant and salvage radiotherapy after prostatectomy: AUA/ASTRO Guideline. *J Urol.* 2013;190(2):441-449.
15. Jackson WC, Suresh K, Tumati V, et al. Impact of Biochemical Failure After Salvage Radiation Therapy on Prostate Cancer-specific Mortality: Competition Between Age and Time to Biochemical Failure. *Eur Urol Oncol.* 2018;1(4):276-282.
16. Erdi YE. Limits of Tumor Detectability in Nuclear Medicine and PET. *Mol Imaging Radionucl Ther.* 2012;21(1):23-28.
17. Zhou Z, Qutaish M, Han Z, et al. MRI detection of breast cancer micrometastases with a fibronectin-targeting contrast agent. *Nat Commun.* 2015;6:7984.
18. Krebs MG, Metcalf RL, Carter L, Brady G, Blackhall FH, Dive C. Molecular analysis of circulating tumour cells-biology and biomarkers. *Nat Rev Clin Oncol.* 2014;11(3):129-144.

19. Shtivelman E, Beer TM, Evans CP. Molecular pathways and targets in prostate cancer. *Oncotarget.* 2014;5(17):7217-7259.
20. 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.
21. 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).
22. Hou S, Zhao L, Shen Q, et al. Polymer nanofiber-embedded microchips for detection, isolation, and molecular analysis of single circulating melanoma cells. *Angew Chem Int Ed Engl.* 2013;52(12):3379-3383.
23. 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.
24. 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.
25. Hou S, Chen JF, Song M, et al. Imprinted NanoVelcro Microchips for Isolation and Characterization of Circulating Fetal Trophoblasts: Toward Noninvasive Prenatal Diagnostics. *ACS Nano.* 2017;11(8):8167-8177.