Award Number: W81XWH-18-1-0576
PC171066

TITLE: A Novel Blood-based RNA Assay for Early Detection of Lethal Prostate Cancers

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REPORT DATE: OCTOBER 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel 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 2019	ANNUAL	15SEP2018 - 14SEP2019

4. TITLE AND SUBTITLE
A Novel Blood-based RNA Assay for Early Detection of Lethal Prostate Cancers

5a. CONTRACT NUMBER
W81XWH-18-1-0576

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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E-Mail:

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Cedars-Sinai Medical Center
LOS ANGELES, CA 90048

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Materiel 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: Our research collaborator, Dr. Michael Freeman (CSMC) has developed a new transcriptome-based subtyping: Prostate Cancer Classification System (PCS)1. PCS categorizes PCa into 3 subtypes which are related to survival, response to therapy and may even predict resistance to certain types of treatment. The limitation of these genomic assays, however, is the need for tissue biopsy. Given the risk and invasiveness of the procedure, a non-invasive assay with the reference to molecular features driving clinical behavior and outcomes would potentially address this unmet need in PCa. RNA-based molecular signatures can be detected in circulating tumor cells (CTCs), making this an opportune way to further use of these evolving genomic signatures. While promising, this process requires improvement and further development before it can be used in the clinic. Over the past decade, our team has pioneered the NanoVelcro CTC assay, in which capture agent-coated nanosubstrates are used to selectively enrich CTCs. Recently, we have introduced the ThermoResponsive (TR)-NanoVelcro CTC purification assay, which allows capture and release of CTCs with intact RNA. This allows for seamless coupling with NanoString nCounterÂ® platform2 to accurately quantify the expression of RNA transcripts. Using these tools, our goal is to develop CTC-based PCS panel that will measure the aggressiveness of PCa. This tool could be used not only for early detection, but also for continuous monitoring of disease in response to treatment as serial blood collection is safe and easy. This allows for timely detection of emerging drug resistance and progression that will be of particular benefit to those patients with advanced mCRPC and their treating physicians.**

15. SUBJECT TERMS

NONE LISTED

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	17	19b. TELEPHONE NUMBER *(include area code)*

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1. Introduction

Our research collaborator, Dr. Michael Freeman (CSMC) has developed a new transcriptome-based subtyping: Prostate Cancer Classification System (PCS)[1]. PCS categorizes PCa into 3 subtypes which are related to survival, response to therapy and may even predict resistance to certain types of treatment. The limitation of these genomic assays, however, is the need for tissue biopsy. Given the risk and invasiveness of the procedure, a non-invasive assay with the reference to molecular features driving clinical behavior and outcomes would potentially address this unmet need in PCa. RNA-based molecular signatures can be detected in circulating tumor cells (CTCs), making this an opportune way to further use of these evolving genomic signatures. While promising, this process requires improvement and further development before it can be used in the clinic. Over the past decade, our team has pioneered the NanoVelcro CTC assay, in which capture agent-coated nanosubstrates are used to selectively enrich CTCs. Recently, we have introduced the ThermoResponsive (TR)-NanoVelcro CTC purification assay, which allows capture and release of CTCs with intact RNA. This allows for seamless coupling with NanoString nCounter® platform[2] to accurately quantify the expression of RNA transcripts. Using these tools, our goal is to develop CTC-based PCS panel that will measure the aggressiveness of PCa. This tool could be used not only for early detection, but also for continuous monitoring of disease in response to treatment as serial blood collection is safe and easy. This allows for timely detection of emerging drug resistance and progression that will be of particular benefit to those patients with advanced mCRPC and their treating physicians.

2. Key Words

Metastatic, castration-resistant prostate cancer (mCRPC), NanoVelcro Assay, Prostate Cancer Classification System (PCS)

3. Accomplishments

- **What were the major goals of the project?**

Training-Specific Tasks:

Training and educational development in prostate cancer research.

Milestone(s) Achieved: Presentation of project data at a national meeting or preparation for publication.

- **Jan YJ** et al. A Circulating Tumor Cell RNA Assay for Dynamic Assessment of Androgen Receptor Signaling Inhibitors Sensitivity in Metastatic Castration-Resistant Prostate Cancer. Journal of Clinical Oncology. 2019;37(7_suppl):157-. doi: 10.1200/JCO.2019.37.7_suppl.157. GU Cancers Symposium 2019, San Francisco, CA. (poster presenter)
- **Jan YJ** et al. A Circulating Tumor Cell-RNA Assay for Assessment of Androgen Receptor Signaling Inhibitor Sensitivity in Metastatic Castration-Resistant Prostate Cancer. Theranostics. 2019;9(10):2812-26. doi: 10.7150/thno.34485.
- Teng PC, **Jan YJ**, Chen JF et al. Circulating tumor cells with small nuclear size associate with poor survival and clinical outcomes in advanced prostate cancer. Submitted to Journal of National Cancer Institute. (co-1st author, in submission)

Research-Specific Tasks

Specific Aim 1: Assess the feasibility of this CTC-PCS assay using artificial blood samples.

Major Task 1: Calibration studies of TR-NanoVelcro assay for CTC capture efficiency and release yield.

Milestone(s) Achieved Achieve temperature-dependent recovery (capture then release) of purified CTCs in 20 min, with >90% capture efficiency and >80% recovery yield.

- We reproduced the cell line study in PCa similar to the study described in our previous publication of the TR-NanoVelcro assay (which validated the performance using lung cancer cell line and patient samples).[3] We successfully captured C4-2B parental and treatment resistant lines with 90% of efficiency and recovered 80% of the captured cells.

Major Task 2: Calibration studies to assess the performance of RNA quantification for NanoString nCounter® platform.

Milestone(s) Achieved: Achieve a sensitivity of RNA detection down to the density of 1-5 cells/mL with an intra-class correlation coefficient (ICCC) greater than 0.9.

- We have developed a CTC-specific PCS1 panel through a rigorous bioinformatic process. Among the 3 PCS subtypes[1], PCS1 phenotype is likely to be independent of AR pathway and associated with the worst prognosis, visceral metastasis, and resistance to androgen receptor signaling inhibitor (ARSI). The performance of the TR-NanoVelcro chip as well as the CTC-PCS1 panel is well-validated PCa in cell lines (see Appendix A, Figure 1 & 2).

Major Task 3: Calibration studies to examine the complete CTC-PCS assay.

Milestone(s) Achieved: Achieve a sensitivity of RNA detection down to the density of 1-5 cells/mL with an intra-class correlation coefficient (ICCC) greater than 0.9.

- In cell line studies, the treatment resistant C4-2B cell lines had higher CTC-PCS1 Z scores compared to the parental lines (see Appendix A, Figure 3).

Specific Aim 2: Assess the performance of this CTC-PCS assay in annotated clinical blood samples.

Major Task 4: Assess the performance of this CTC-PCS assay in annotated clinical blood samples.

Milestone(s) Achieved: Complete the evaluation of the performance of this CTC-PCS assay. Work with collaborators to review, discuss and refine this assay if needed.

- In an exploratory clinical analysis, ARSI-resistant patients had significantly higher CTC-PCS1 Z scores comparted to ARSI-sensitive patients (see Appendix A, Figure 4 & 5).
- This work was published in *Theranostics* 2019.[4]

- **What was accomplished under these goals?**

See Appendix A.

- **What opportunities for training and professional development has the project provided?**

Training

- One-on-one work with mentor, Dr. Edwin Posadas, for clinical study design, execution, data collection, and interpretation
- One-on-one work with co-mentor, Dr. Hsian-Rong Tseng, for optimization of NanoVelcro CTC assay and development of subsequent approaches for CTC-based RNA measurement
- Monthly meeting with consultant, Dr. Leland Chung, for experimental design, data analysis and interpretation

- Quarterly meeting with consultant, Dr. Michael Freeman, for experimental design, data analysis and interpretation
- Attendance of Biostatistics and Bioinformatics Research Center Presentation at Cedars-Sinai Medical Center

Professional development

- Attendance of GU Cancers Symposium 2019

- **How were the results disseminated to communities of interest?**

Conference presentations:

- Teng PC, **Jan YJ** et al. A Circulating Tumor Cell Assay for Dynamic Assessment of Drug Sensitivity in Metastatic Castration-Resistant Prostate Cancer (Abstract 453). American Association for Cancer Research (AACR) Annual Meeting 2019, Atlanta, GA.
- Teng PC, **Jan YJ** et al. 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.
- **Jan YJ** el al. A Circulating Tumor Cell RNA Assay for Dynamic Assessment of Androgen Receptor Signaling Inhibitors Sensitivity in Metastatic Castration-Resistant Prostate Cancer. Journal of Clinical Oncology. 2019;37(7_suppl):157-. doi: 10.1200/JCO.2019.37.7_suppl.157. GU Cancers Symposium 2019, San Francisco, CA. (poster presenter)
- Teng PC, **Jan YJ** et al. 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.

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

Generally, the necessary experiment for the project is finished and we are going to do the final analysis of the raw data. Some additional experiment may be needed if the results are not as expected. We have submitted an abstract regarding this project to 2020 GU Cancers Symposium. We plan to submit another bioinformatics-oriented article this year which will demonstrate our unique and rigorous bioinformatics pipeline to dissect molecular signals from background WBCs.

4. Impact

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

Success in this endeavor will produce tool for measuring dynamic, biological alterations that point to the emerging resistance to therapy. Optimally, this blood test will detect those changes responsible for the failure of particular cancer therapies in patients that define the biology of lethal PCa while providing new potential therapeutic targets for future exploration. This insight may also help refine the timing of changes in therapy to optimize outcomes and minimize toxicity.

- **What was the impact on other disciplines?**

Combination of engineering and informatics will create a means for translating other emerging gene/protein expression based signatures into blood tests that could be useful to developing blood-based companion diagnostics for PCa/CRPC.

- **What was the impact on technology transfer?**

Nothing to report.

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

The ultimate goal of this research is to pave the way for developing the use of CTC as a putative biomarker for aggressive prostate cancer, which will allow oncologists to implement therapy that will alter the natural history of advanced prostate cancer.

5. Changes/Problems

- **Changes in approach and reasons for change**

Nothing to report.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report.

- **Changes that had a significant impact on expenditures**

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

- **Jan YJ**, Yoon J, Chen J-F, Teng P-C, Yao N, Cheng S, Lozano A, Chu GCY, Chung H, Lu Y-T, Chen P-J, Wang JJ, Lee Y-T, Kim M, Zhu Y, Knudsen BS, Feng FY, Garraway IP, Gao AC, **Chung LWK**, **Freeman MR**, You S, **Tseng H-R**, **Posadas EM**. A Circulating Tumor Cell-RNA Assay for Assessment of Androgen Receptor Signaling Inhibitor Sensitivity in Metastatic Castration-Resistant Prostate Cancer. Theranostics. 2019;9(10):2812-26. doi: 10.7150/thno.34485.
- Chen P-J, Teng P-C, Zhu Y, **Jan YJ**, Smalley M, Afshar Y, Chen L-C, Pisarska MD, **Tseng H-R**. Noninvasive Prenatal Diagnostics: Recent Developments Using Circulating Fetal Nucleated Cells. Current Obstetrics and Gynecology Reports. 2019. doi: 10.1007/s13669-019-0254-x. (co-1st author)
- Teng P-C, **Jan YJ**, Chen J-F, Cook-Wiens G, Cheng S, Yao N, Chu GCY, Chen P-J, Zhu Y, Ho H, Huang J, Li K-C, **Chung LWK**, **Freeman MR**, Rogatko A, **Tseng H-R**, **Posadas EM**. Circulating tumor cells with small nuclear size associate with poor survival and clinical outcomes in advanced prostate cancer. Submitted to Journal of National Cancer Institute. (co-1st author, in submission)
- Dong J, **Jan YJ**, Cheng J, Zhang RY, Meng M, Smalley M, Chen PJ, Tang X, Tseng P, Bao L, Huang TY, Zhou D, Liu Y, Chai X, Zhang H, Zhou A, Agopian VA, **Posadas EM**, Shyue JJ, Jonas SJ, Weiss PS, Li M, Zheng G, Yu HH, Zhao M, **Tseng HR**, Zhu Y. Covalent chemistry on nanostructured substrates enables noninvasive quantification of

gene rearrangements in circulating tumor cells. Science Advances. 2019. doi: 10.1126/sciadv.aav9186.

- Dong J, Zhang RY, Sun N, Smalley M, Wu Z, Zhou A, Chou SJ, **Jan YJ**, Yang P, Bao L, Qi D, Tang X, Tseng P, Hua Y, Xu D, Kao R, Meng M, Zheng X, Liu Y, Vagner T, Chai X, Zhou D, Li M, Chiou SH, Zheng G, Di Vizio D, Agopian VG, **Posadas EM**, Jonas SJ, Ju SP, Weiss PS, Zhao M, **Tseng HR**, Zhu Y. Bio-inspired nanovilli chips for enhanced capture of tumor-derived extracellular vesicles: Toward non-invasive detection of gene alterations in non-small cell lung cancer. ACS applied materials & interfaces. 2019. doi: 10.1021/acsami.9b01406.

Conference presentations:

- Teng P-C, **Jan JY**, Yoon J, Chen J-F, Chen P-J, Yao N, Cheng S, Lozano A, **Freeman MR**, You S, **Tseng H-R**, **Posadas EM**. A Circulating Tumor Cell Assay for Dynamic Assessment of Drug Sensitivity in Metastatic Castration-Resistant Prostate Cancer (Abstract 453). American Association for Cancer Research (AACR) Annual Meeting 2019, Atlanta, GA.
- Teng P-C, **Jan YJ**, Yoon J, Chen P-J, Chen J-F, Yao N, Cheng S, Lozano A, **Freeman MR**, You S, **Tseng H-R**, **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.
- Chen P-J, **Jan YJ**, Teng P-C, Chen J-F, Cheng S, Yao N, Reis-Sobreiro M, Lozano A, Gomez A, **Freeman MR**, Tseng H-R, **Posadas EM**. A Noninvasive Prognostic Biomarker for Metastatic Castration-Resistant Prostate Cancer: Very small nuclear circulating tumor cells. Journal of Clinical Oncology. 2019;37(7_suppl):179-. doi: 10.1200/JCO.2019.37.7_suppl.179. GU Cancers Symposium 2019, San Francisco, CA.
- **Jan YJ**, Yoon J, Chen J-F, Chen P-J, **Teng P-C**, Yao N, Cheng S, Lozano A, **Freeman MR**, You S, **Tseng H-R**, **Posadas EM**. A Circulating Tumor Cell RNA Assay for Dynamic Assessment of Androgen Receptor Signaling Inhibitors Sensitivity in Metastatic Castration-Resistant Prostate Cancer. Journal of Clinical Oncology. 2019;37(7_suppl):157-. doi: 10.1200/JCO.2019.37.7_suppl.157. GU Cancers Symposium 2019, San Francisco, CA. (poster presenter)
- Pai-Chi Teng, **Yu Jen Jan**, Jie-Fu Chen, Galen Cook-Wiens, Shirley Cheng, Nu Yao, Amber Lozano, Gina C.Y. Chu, Pin-Jung Chen, Hao Ho, Yingying Yang, Jiaoti Huang, Ker-Chau Li, **Leland W.K. Chung**, Sungyong You, Yazhen Zhu, **Michael R. Freeman**, Andre Rogatko, Ju Dong Yang, **Hsian-Rong Tseng**, **Edwin M. Posadas**. 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.
- Pai-Chi Teng, **Yu Jen Jan**, Junhee Yoon, Jie-Fu Chen, Pin-Jung Chen, Minhyung Kim, Nu Yao, Shirley Cheng, Amber Lozano, **Michael R. Freeman**, Sungyong You, **Hsian-Rong Tseng**, **Edwin M. Posadas**. 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.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

In this research, we have developed the CTC-PCS1 Assay which can detect prostate cancer specific RNA signals in CTCs. This aggressive signature is correlated with treatment resistance (published in *Theranostics*. 2019;9(10):2812-26).

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

The provisional patent application entitled "A Circulating Tumor Cell-RNA Assay for Assessment of Androgen Receptor Signaling Inhibitor Sensitivity in Metastatic Castration-Resistant Prostate Cancer" was filed in 2019 to cover the intellectual properties associated with the proposed CTC-RNA Assay and the related clinical applications. Drs. Jan (PI), Tseng (co-mentor), Posadas (co-mentor) and Freeman (consultant) are included as co-inventors.

- **Other Products**

Nothing to report.

7. Participants & Other Collaborating Organizations

- **What individuals have worked on the project?**

Name: *Yu Jen Jan, M.D.*
Project role: *PI*
Unchanged

Name: *Edwin M. Posadas, M.D.*
Project role: *Primary mentor*
Unchanged

Name: *Hsian-Rong Tseng, Ph.D.*
Project role: *Co-mentor*
Unchanged

Name: *Leland W.K. Chung, Ph.D.*
Project role: *Consultant*
Unchanged

Name: *Michael Freeman, Ph.D.*
Project role: *Consultant*
Unchanged

- **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

- Facilities

8. Appendices

Appendix A: Abstract and figures regarding this project.

A Circulating Tumor Cell-RNA Assay for Assessment of Androgen Receptor Signaling Inhibitor Sensitivity in Metastatic Castration-Resistant Prostate Cancer

Background: Our objective was to develop a circulating tumor cell (CTC)-RNA assay for characterizing clinically relevant RNA signatures for the assessment of androgen receptor signaling inhibitor (ARSI) sensitivity in metastatic castration-resistant prostate cancer (mCRPC) patients.

Methods: We developed the NanoVelcro CTC-RNA assay by combining the Thermoresponsive (TR)-NanoVelcro CTC purification system with the NanoString nCounter platform for cellular purification and RNA analysis. Based on the well-validated, tissue-based Prostate Cancer Classification System (PCS), we focus on the most aggressive and ARSI-resistant PCS subtype, i.e., PCS1, for CTC analysis. We applied a rigorous bioinformatic process to develop the CTC-PCS1 panel that consists of prostate cancer (PCa) CTC-specific RNA signature with minimal expression in background white blood cells (WBCs). We validated the NanoVelcro CTC-RNA assay and the CTC-PCS1 panel with well-characterized PCa cell lines to demonstrate the sensitivity and dynamic range of the assay, as well as the specificity of the PCS1 Z score (the likelihood estimate of the PCS1 subtype) for identifying PCS1 subtype and ARSI resistance. We then selected 31 blood samples from 23 PCa patients receiving ARSIs to test in our assay. The PCS1 Z scores of each sample were computed and compared with ARSI treatment sensitivity.

Results: The validation studies using PCa cell line samples showed that the NanoVelcro CTC-RNA assay can detect the RNA transcripts in the CTC-PCS1 panel with high sensitivity and linearity in the dynamic range of 5-100 cells. We also showed that the genes in CTC-PCS1 panel are highly expressed in PCa cell lines and lowly expressed in background WBCs. Using the artificial CTC samples simulating the blood sample conditions, we further demonstrated that the CTC-PCS1 panel is highly specific in identifying PCS1-like samples, and the high PCS1 Z score is associated with ARSI resistance samples. In patient bloods, ARSI-resistant samples (ARSI-R, n=14) had significantly higher PCS1 Z scores as compared with ARSI-sensitive samples (ARSI-S, n=17) (Rank-sum test, P=0.003). In the analysis of 8 patients who were initially sensitive to ARSI (ARSI-S) and later developed resistance (ARSI-R), we found that the PCS1 Z score increased from the time of ARSI-S to the time of ARSI-R (Pairwise T-test, P=0.016).

Conclusions: Using our new methodology, we developed a first-in-class CTC-RNA assay and demonstrated the feasibility of transforming clinically relevant tissue-based RNA profiling such as PCS into CTC tests. This approach allows for detecting RNA expression relevant to clinical drug resistance in a non-invasive fashion, which can facilitate patient-specific treatment selection and early detection of drug resistance, a goal in precision oncology.

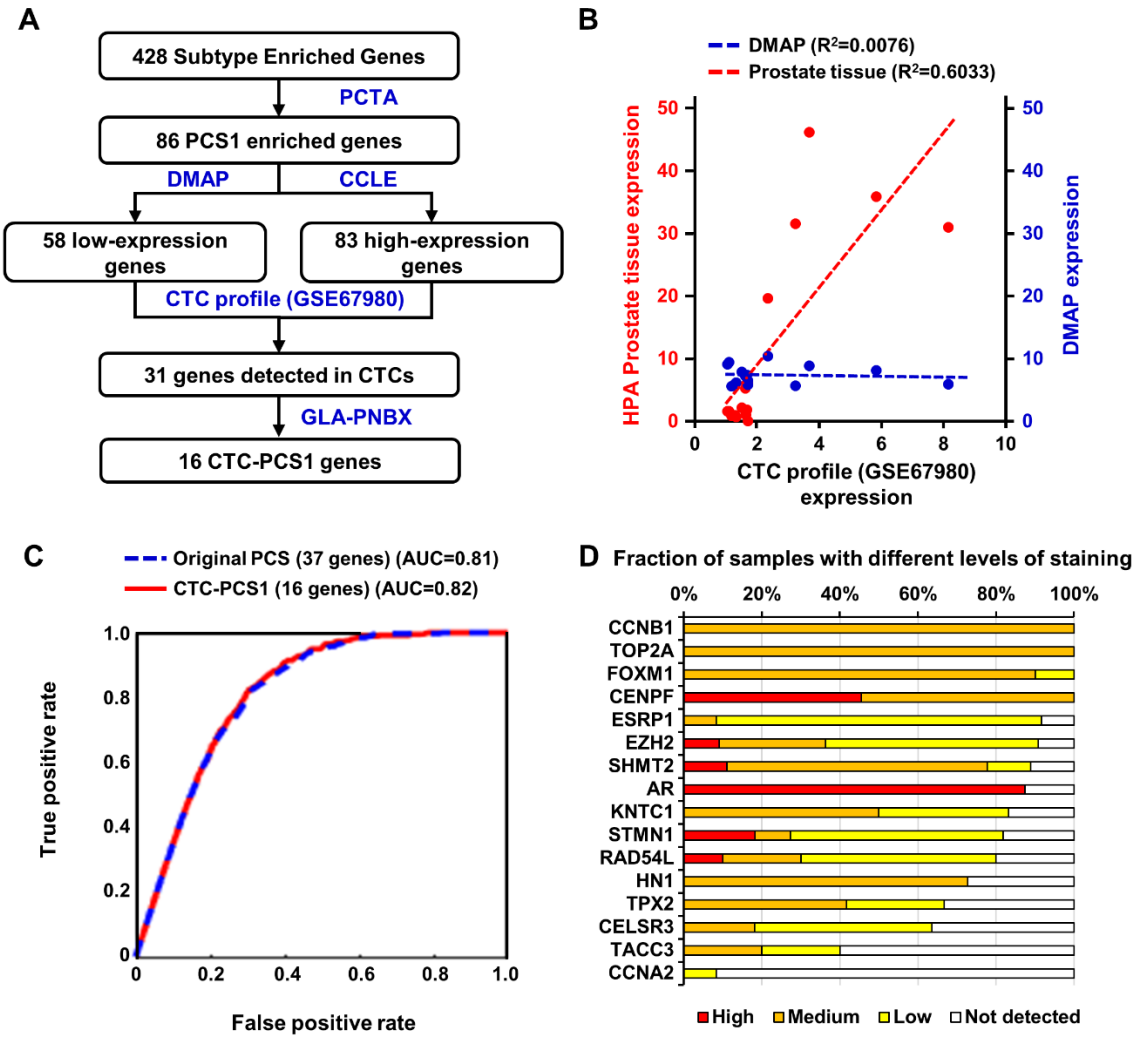

Figure 1. Selection and performance of the CTC-PCS1 gene panel. (A) Schematic flow of the selection of 16 CTC-PCS1 genes. **(B)** Scatter plot and regression lines shows expression of the 16 CTC-PCS1 genes in comparisons of CTCs (GSE67980) versus HPA prostate tissue, and CTCs (GSE67980) versus DMAP immune cells. Red dots and red dotted line indicate expressions in CTCs and prostate tissue, and blue dots and blue dotted line indicate expressions of CTCs and immune cells. **(C)** ROC curves of classifiers using the 16 CTC-PCS1 genes and original 37 PCS gene panel shows comparable level of performance of both classifiers. Red line indicates performance of 16 CTC-PCS1 genes and blue dotted line indicates performance of original 37 PCS gene panel for identification of PCS1 subtype. (D) Stacked bar graph depicts human PCa tissue staining of 16 CTC-PCS1 gene products in HPA database (https://www.proteinatlas.org/).

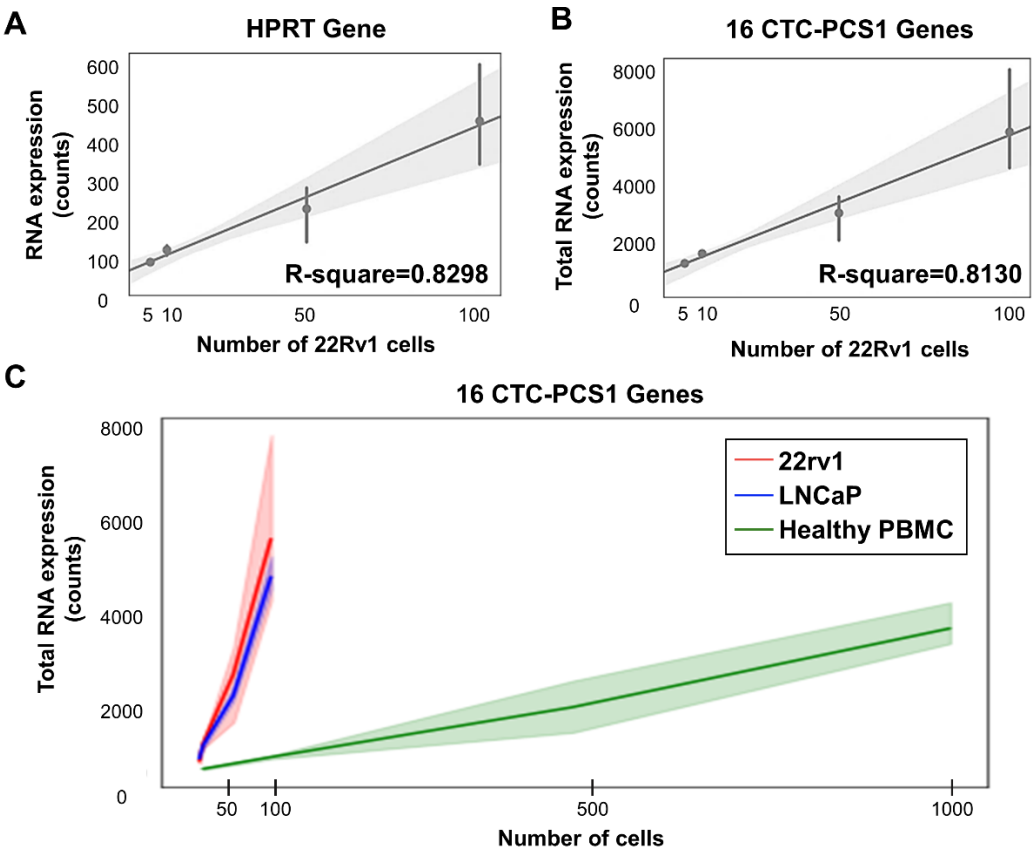

Figure 2. Analytical validation studies of the NanoVelcro CTC-RNA assay and CTC-PCS1 panel. (A) The HPRT RNA expression of PCa cell line 22Rv1 in different cell numbers measured by the NanoVelcro CTC-RNA assay. **(B)** NanoVelcro CTC-RNA assay quantification of the total CTC-PCS1 panel (16 genes) RNA expression of PCa cell line 22Rv1 in different cell numbers. **(C)** The total CTC-PCS1 panel (16 genes) RNA expression directly quantified by NanoString nCounter platform using 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.

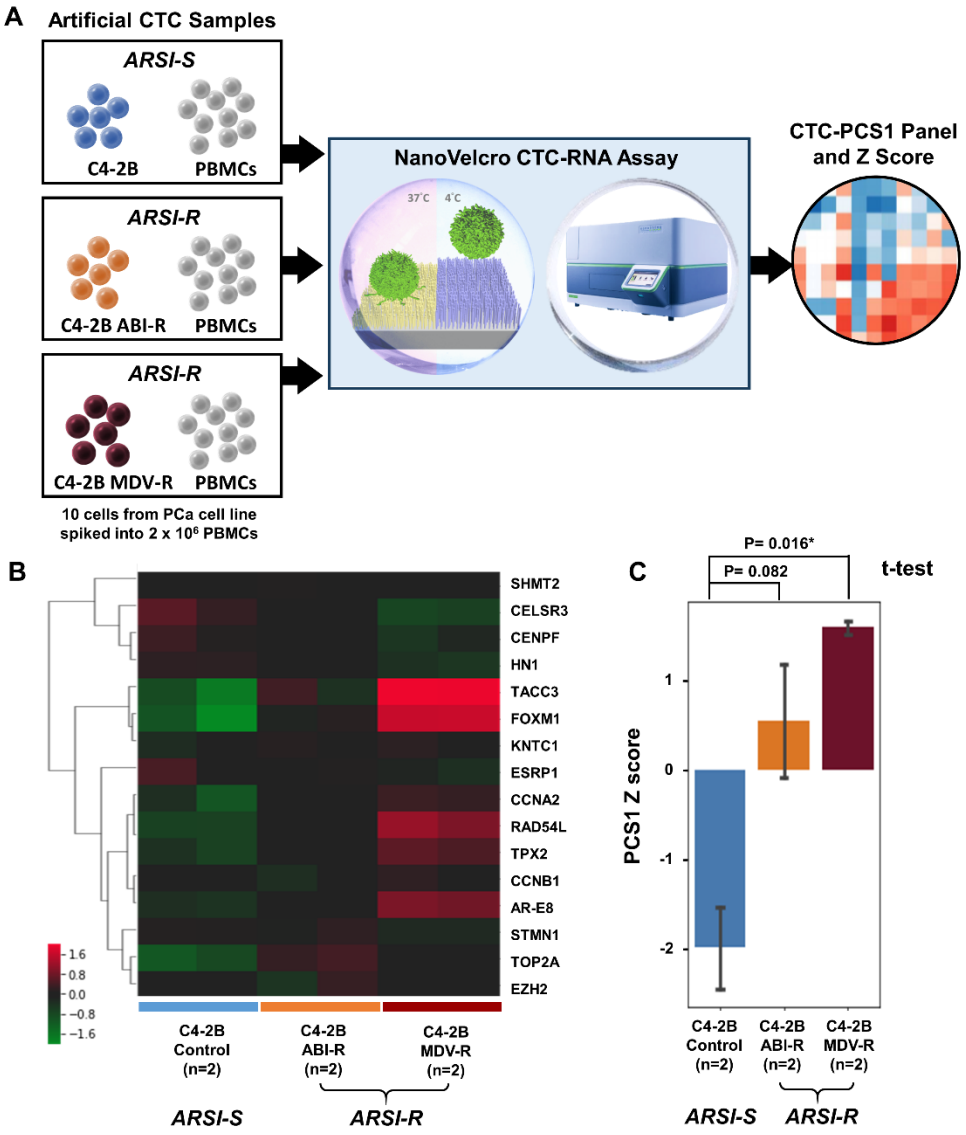

Figure 3. Cell line study of CTC-PCS1 panel for distinguishing ARSI sensitivity (A) Study workflow of the NanoVelcro CTC-RNA assay and CTC-PCS1 panel for profiling artificial blood samples of different ARSI sensitivities. Artificial CTC were prepared by spiking 10 cells of C4-2B treatment naïve (control, ARSI-S), C4-2B ABI-R (abiraterone-resistant, ARSI-R), and C4-2B MDV-R (enzalutamide-resistant, ARSI-R) into 2 x 106 healthy donor PBMCs respectively to simulate actual CTC blood samples. These artificial samples were then subjected to the NanoVelcro CTC-RNA assay, generating CTC-PCS1 RNA expression and Z scores. **(B)** Normalized and hierarchical clustered heatmap of PCS1 RNA expression in C4-2B treatment naïve (control, ARSI-S), C4-2B ABI-R (abiraterone-resistant, ARSI-R) and C4-2B MDV-R (enzalutamide-resistant, ARSI-R) artificial blood samples. (2 sets of 10 C4-2B control cells spiked in 2 x 106 healthy donor PBMCs, 2 sets 10 C4-2B ABI-R cells spiked in 2 x 106 healthy donor PBMCs and 2 sets 10 C4-2B MDV-R cells spiked in 2 x 106 healthy donor PBMCs). **(C)** PCS1 Z score comparison of C4-2B control artificial blood samples comparing to C4-2B ABI-R artificial blood samples and C4-2B MDV-R artificial blood samples. (T-test, P=0.082 and 0.016* respectively)

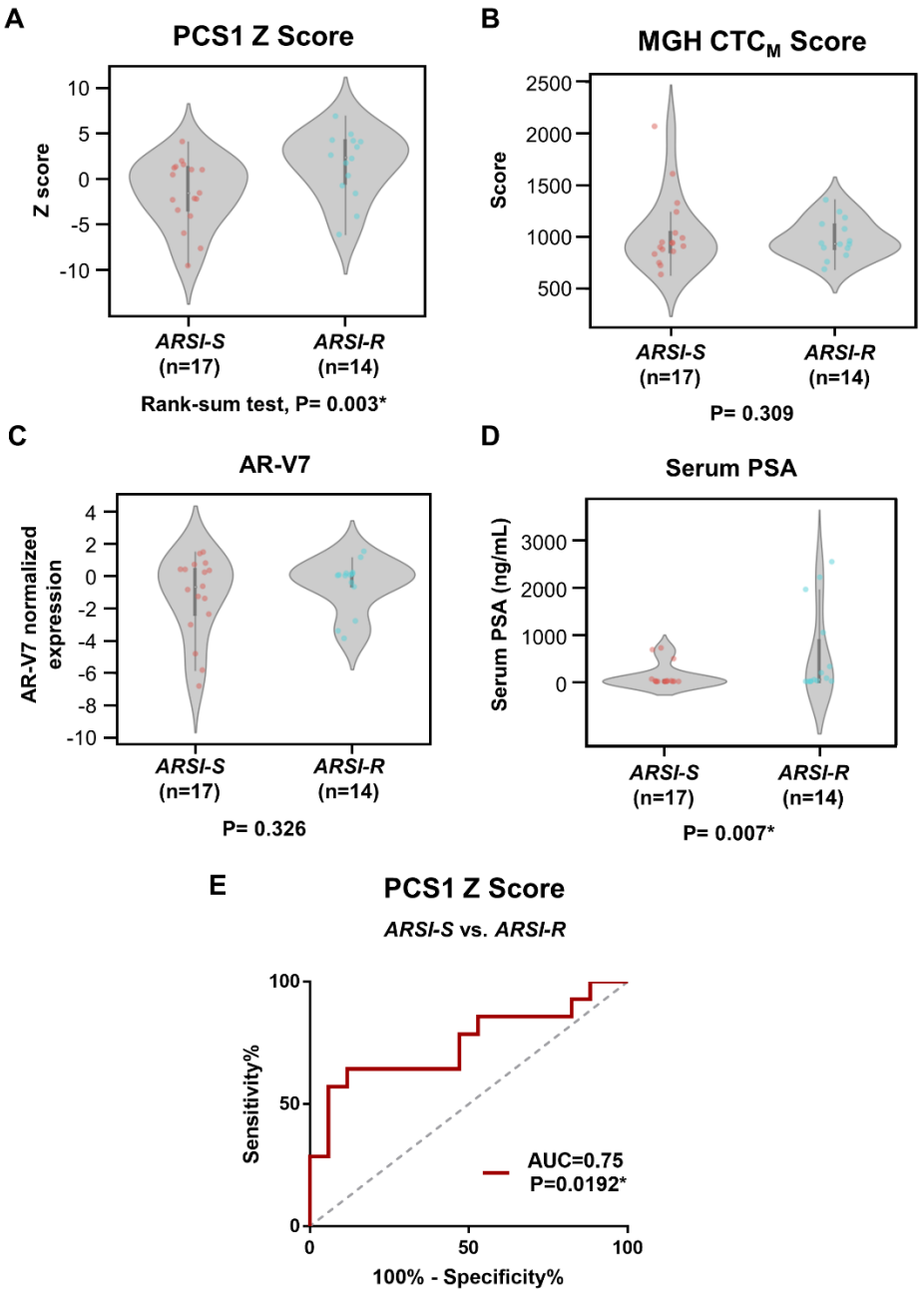

Figure 4. Analysis of gene scores between ARSI-S and ARSI-R samples. Comparison of **(A)** PCS1 Z score and **(B)** MGH CTCM score among 31 mCRPC samples, with 17 samples from ARSI sensitive state (ARSI-S), and 14 samples from ARSI resistant state (ARSI-R). PCS1 Z score is statistically significant higher in resistant patients (Rank-sum test, P=0.003*). No statistically significant trend was found in MGH CTCM score between the 2 groups (P=0.309). Similar tests performed using **(C)** AR-V7 expression and **(D)** serum PSA level are also shown. Serum PSA level exhibits statistically significant higher value in the resistant patients (P=0.007*). **(E)** Receiving Operating Characteristics (ROC) curve analysis of PCS1 Z score separating ARSI-S and ARSI-R patients. ROC curve exhibits Area Under Curve (AUC)= 0.75, P=0.0192*.

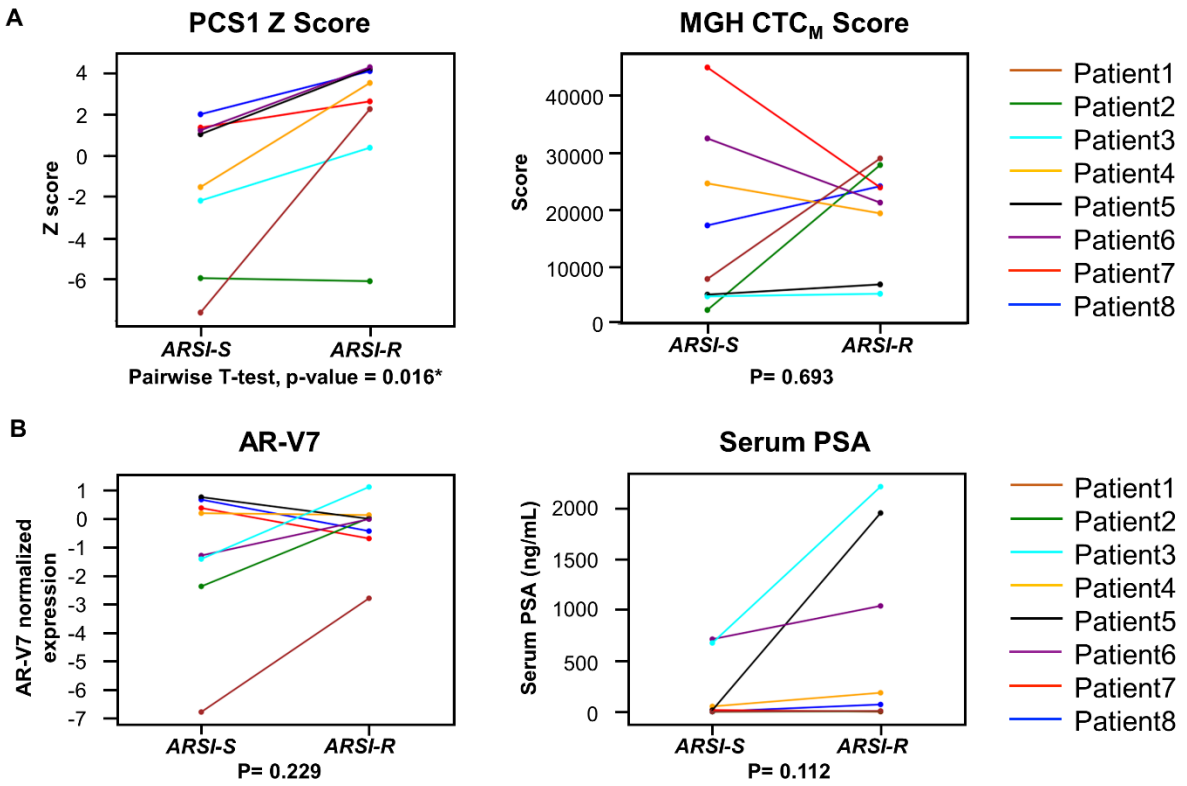

Figure 5. Analysis of gene signature score changes in continuous samples from individual patients. (A) Line plot depicts changes of PCS1 Z score for each patient from ARSI sensitive to resistant. Individual patients are displayed with different colors. Pairwise t-tests were conducted and PCS1 Z score showed a statistically significant increase from ARSI sensitive to resistant (P = 0.016*). No statistically significant trend of the MGH CTCM score was observed between the 2 timepoints (P = 0.693). **(B)** Same analysis was done with AR-V7 expression and serum PSA level. No statistically significant trend was observed in pairwise t-tests between the 2 timepoints (P = 0.229 and 0.112, respectively).

References

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2 Geiss, G. K. *et al.* Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nat Biotechnol* **26**, 317-325, doi:10.1038/nbt1385 (2008).

3 Ke, Z. *et al.* Programming thermoresponsiveness of NanoVelcro substrates enables effective purification of circulating tumor cells in lung cancer patients. *ACS Nano* **9**, 62-70, doi:10.1021/nn5056282 (2015).

4 Jan, Y. J. *et al.* A Circulating Tumor Cell-RNA Assay for Assessment of Androgen Receptor Signaling Inhibitor Sensitivity in Metastatic Castration-Resistant Prostate Cancer. *Theranostics* **9**, 2812-2826, doi:10.7150/thno.34485 (2019).