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CONTRACTING ORGANIZATION: Cedars-Sinai Medical Center

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14. ABSTRACT

Metastatic, castration-resistant prostate cancer (mCRPC) may exhibit varied clinical courses. Some mCRPC patients will develop metastasis beyond the bone and lymph nodes, including the liver, lungs, and adrenal glands. These conditions are termed visceral metastases (VM). Patients with VM have significantly poorer overall survival than patients with non-VM, as their clinical course involves rapid deterioration from organ failure. Certain CRPC treatments have been shown to push the progression of the cancer to its more aggressive VM form. This highlights the importance of developing a means of detecting and predicting cancer progression to the viscera. Using the NanoVelcro Chip designed to capture circulating tumor cells (CTCs), we identified a morphologically unique subgroup of these cells, which we termed very-small-nuclear CTCs (vsnCTCs). We found that these vsnCTCs appear in patients with VM and begin emerging before the disease progresses to VM. Thus we hypothesize that vsnCTCs are associated with the development of VM and are biologically distinct from non-vsnCTCs, which lead to a different clinical course. We aim to analyze the association between vsnCTCs and VM, as these cells could play a key role in detection VM. Additionally, we aim to compare the gene expression of vsnCTCs and non-vsnCTCs to gain greater insight into the biology of VM.

15. SUBJECT TERMS
Prostate Cancer, biomarkers, circulating tumor cells.

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1. Introduction

In this proposed study, we hypothesized that very-small-nuclear circulating tumor cells (vsnCTCs, defined by CTCs with nuclear size < 8.54 μm) are associated with the development of visceral metastasis (VM) in metastatic castration-resistant prostate cancer (mCRPC) and harbor expression signatures distinct from other non-vsnCTCs on several oncogenic pathways. The hypothesis will be tested by a retrospective analysis for the association of vsnCTCs with VM and prognosis, and comparison of gene expression signatures of vsnCTCs.

2. Key Words

Metastatic, castration-resistant prostate cancer (mCRPC), visceral metastasis, very-small-nuclear circulating tumor cells (vsnCTCs), 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.

- **Teng P-C** 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. (Poster Presenter)
- **Teng P-C** 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. (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 P-C** et al. Very Small Nuclear Circulating Tumor Cells Are Associated with Poor Clinical Outcomes in Metastatic Castration-Resistant Prostate Cancer. Submitted to Annals of Oncology. (In Submission)

Research-Specific Tasks

Specific Aim 1: Retrospective analysis for the association between vsnCTCs and VM.

Major Task 1: CTC enumeration studies using NanoVelcro Chip on the specimens from the blood specimen/CTC bank.

Milestone(s) Achieved: identify at least 15 patients and their specimens for each of the following metastatic categories: no metastasis, osseous/lymph node metastasis only, visceral metastasis present.

Milestone(s) Achieved: Complete the CTC enumeration studies and match the clinical annotation for all the identified specimens.

- We have included 76 patients with mCRPC and available blood specimens from Dr. Posadas' (primary mentor) clinic and conducted CTC enumeration. These samples were well clinically annotated.

Major Task 2: Mathematical modeling of CTC nuclear size.

Milestone(s) Achieved: Complete the association analysis.

- Raw data of CTC enumeration has been sent to our prior collaborative mathematician for modeling (pending results).

Specific Aim 2: Comparing gene expression signatures of vsnCTCs and non-vsnCTCs.

Major Task 3: Optimize NanoVelcro-LCM platform for CTC isolation and downstream expressional analysis.

Milestone(s) Achieved: Identify vsnCTC-specific and/or VM-specific expression signatures.

- We have identified patients with presence of vsnCTC (i.e., vsnCTC+) have aggressive gene expression compared to patients without presence of vsnCTC (i.e., vsnCTC-).

- **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
- Attendance of AACR Annual Meeting 2019
- Attendance of ASCO Annual Meeting 2019

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

Conference presentations:

- **Teng P-C** 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. (Poster Presenter)
- **Teng P-C** 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. (Poster Presenter)

- Chen P-J et al. 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 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.
- **Pai-Chi Teng** et al. 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. (Poster Presenter)
- **Pai-Chi Teng** 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. (Poster Presenter)

- **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 2 abstracts regarding this project to 2020 GU Cancers Symposium. A manuscript entitled “Very Small Nuclear Circulating Tumor Cells Are Associated with Poor Clinical Outcomes in Metastatic Castration-Resistant Prostate Cancer” which focuses on the direct correlation between vsnCTCs and poor prognosis in patients with mCRPC is in submission.

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. We will also associate the aggressive gene expression with presence of vsnCTC and poor prognosis or poor treatment response.

4. Impact

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

Through our research, we aim to confirm the association of vsnCTCs with VM and poor prognosis. Moreover, we intend to characterize these cells and identify the mechanism underlying VM disease progression. The overarching goal of our research inquiry is to develop of a new assay for predicting VM. This is an important an unmet need for prostate cancer clinical care as hormonal treatments drive more patients’ conditions towards VM. By identifying men early in their transition to this more aggressive, VM-disposed disease, oncologists can implement therapy that will alter the natural history of VM in prostate cancer. Our studies will elucidate the biological differences between VM and non-VM prostate cancer, as revealed through CTC and tissue analysis, and lead to refined therapeutic strategies. Our work will lead to significant progress toward a putative biomarker for aggressive prostate cancer.

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

Nothing to report.

- **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 VM in 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. Very Small Nuclear Circulating Tumor Cells Are Associated with Poor Clinical Outcomes in Metastatic Castration-Resistant Prostate Cancer. Submitted to Journal of Clinical Oncology Precision Oncology. (In Submission)
- Huang Y-W, Huai K, Chen P-J, **Teng P-C**, Chou S, Sun N, Wu Z, Qi D, Jan YJ, Zhu Y, Posadas EM, Tseng H-R. A New Generation of NanoVelcro System for Enumeration of Circulating Tumor Cells with Different EpCAM Levels. Submitted to Lab on a Chip. (In Submission)

- Ahn JC, **Teng P-C**, Chen P-J, Posadas EM, Tseng H-R, Lu S , Yang JD. Circulating tumor cells in Hepatocellular Carcinoma. Submitted to World Journal of Gastroenterology. (In Submission)

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. (Poster Presenter)
- **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. (Poster Presenter)
- 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.
- **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. (Poster Presenter)
- **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. (Poster Presenter)

- **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 presence of vsnCTC (see Appendix A) and treatment resistance (published in *Theranostics*. 2019;9(10):2812-26).

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

Nothing to report.

- **Other Products**

Nothing to report.

7. Participants & Other Collaborating Organizations

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

Name: *Pai-Chi Teng, 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?**

Jie-Fu Chen, MD, the submitting PI of this research, has left the position of postdoctoral fellow at Cedars-Sinai Medical Center in June 2017, due to personal career plan. The proposed work will be continued by his successor, Pai-Chi Teng, MD, starting from January 2019. The proposed research has not changed and will be carried out according to the abovementioned plan.

- **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)

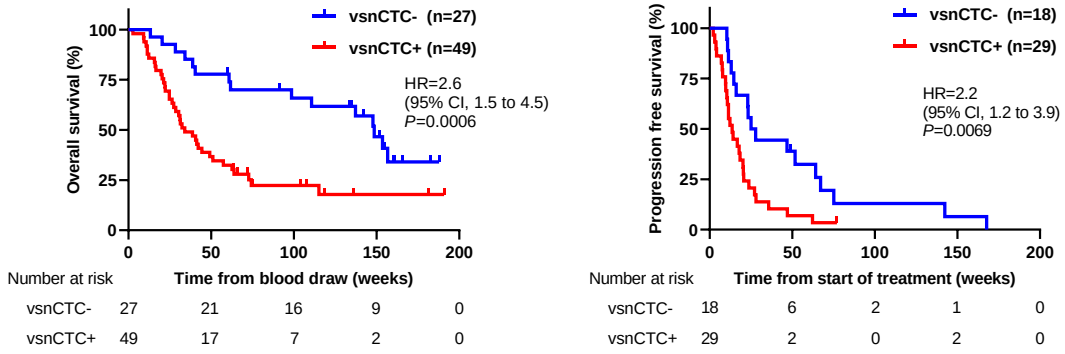
Partner's contribution to the project

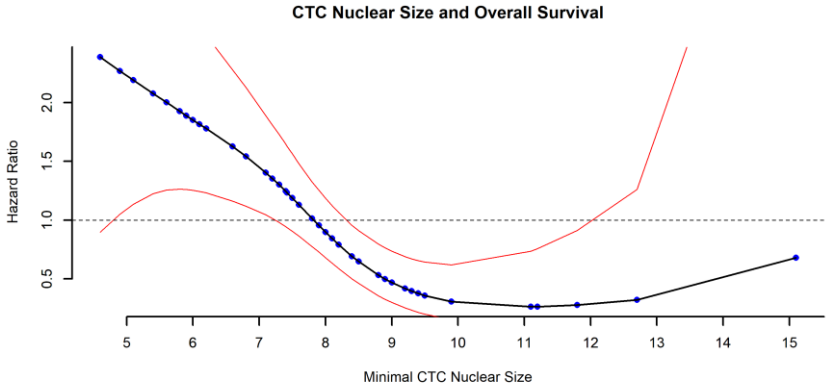
- Facilities

8. Appendices

Appendix A: abstracts and figures for pending publications regarding this project.

Very-Small-Nuclear Circulating Tumor Cell (vsnCTC) is Associated with Poor Clinical Outcomes in Metastatic Castration-Resistant Prostate Cancer





Background: Circulating tumor cells (CTCs) have arisen as contemporary noninvasive prognostic biomarkers for prostate cancer (PC). Previously, a subgroup of PC CTCs, with particularly small nuclei (<8.5 μm), were found to be correlated with the presence of visceral metastases. This subgroup was named very-small-nuclear CTCs (vsnCTCs). We proposed vsnCTCs as a putative biomarker of a lethal subtype in metastatic castration resistant PC (mCRPC).

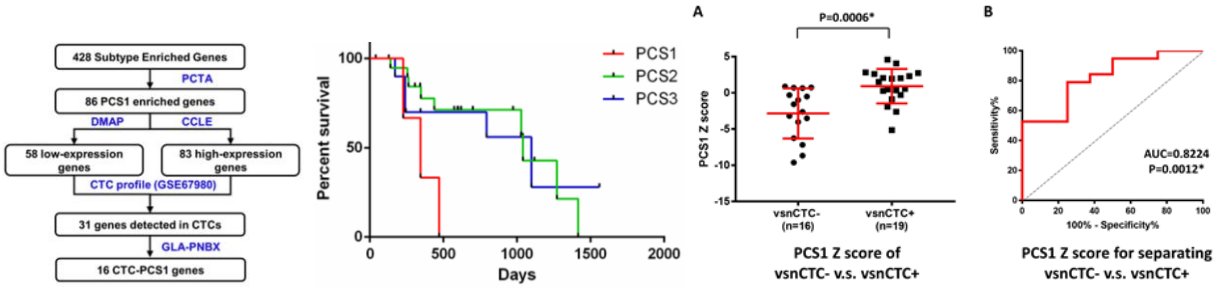
Methods: In this study, 76 patients with mCRPC were recruited for overall survival (OS) analysis. Of the 76 patients, 47 had available pre-treatment blood specimens prior to the initiation of androgen receptor signaling inhibitor (ARSI, e.g. abiraterone and enzalutamide) or taxane therapy. Using the NanoVelcro CTC Assay, CTCs were captured and subjected to immunofluorescence staining. CTCs were identified as DAPI+/CK+/CD45- with a round or oval nucleus. Additionally, CTC nuclear size was measured and defined as the square root of the product of the long axis and the short axis. Kaplan-Meier analysis and Cox proportional hazards model were conducted.

Results: Patients with vsnCTC (i.e., vsnCTC+) had a significantly shortened OS compared with patients without vsnCTC (i.e., vsnCTC-). The median OS was 34 (vsnCTC+, n=49) vs. 149 (vsnCTC-, n=27) weeks (log-rank HR=2.6 with 95% CI 1.5 to 4.5, p=0.0006). Progression free survival (PFS) analysis was performed for the 47 patients with pre-treatment blood samples. The

median PFS was 14 (vsnCTC+, n=29) vs. 26 (vsnCTC-, n=18) weeks (log-rank HR=2.2 with 95% CI 1.2 to 3.9, p=0.0069). We also found that the hazard ratio of overall survival increased significantly as the CTC nuclear size decreased using the p spline plot.

Conclusions: Our study showed that nuclear size reduction has importance in CTCs in a fashion similar to its utility in tissue. This study points toward the importance of the vsnCTC in patients with mCRPC, as vsnCTC+ patients represented a group at risk for faster clinical progression who are at the highest risk for morality. We posit that the vsnCTC represents a new hallmark of an aggressive subtype of mCRPC. This has potential importance in optimizing therapeutic choices.

Preclinical Development of a Circulating Tumor Cell Based RNA-Classifier to Optimize the Treatment Selection in Patients with Metastatic Castration-Resistant Prostate Cancer



Rationale: Our objective is to develop a circulating tumor cell (CTC)-RNA Assay for characterizing clinically relevant RNA signatures for the treatment selection of androgen receptor signaling inhibitor (ARSI) in patients with metastatic castration-resistant prostate cancer (mCRPC).

Methods: We developed the CTC-RNA Assay by combining the Thermoresponsive (TR)-NanoVelcro system with the NanoString nCounter platform for CTC purification and RNA analysis, respectively. Based on the well-validated, tissue-based Prostate Cancer Classification System (PCS, developed by our collaborators, Dr. Michael Freeman and Dr. Sungyong You at Cedars-Sinai Medical Center) which categorizes prostate cancer (PC) into 3 subtypes (PCS1-3), a CTC-PCS panel was developed using a rigorous bioinformatic process. Among them, PCS1 subtype is correlated with the worst prognosis. We applied the weighted Z-score method and nearest centroid classification method to calculate gene expression and to assign PCS subtype.

Results: We retrospectively enrolled 34 patients with mCRPC who were beginning therapy with ARSI (abiraterone, enzalutamide or apalutamide). Pre-treatment blood samples were subjected to the CTC-RNA Assay. Each patient's PCS subtype was assigned. The median overall survival for PCS1 (n=3), PCS2 (n=20) and PCS3 (n=11) was 49, 149 and 157 weeks, respectively. The p-value (log-rank test) was 0.0132 for PCS1 vs. PCS2, and 0.0847 for PCS1 vs. PCS3. Besides, we observed that vsnCTC+ patients had significantly higher PCS1 Z score compared with vsnCTC- patients (AUC=0.82).

Conclusion: In the original PCS panel, PCS1 correlates with the most clinically aggressive cases and points toward the lowest sensitivity to AR inhibition. Our blood-borne PCS classifier can categorize PC into 3 subtypes, which are related to aggressiveness of PC, including prediction of treatment response. Through early identification of high-risk biology and timely adjustment of therapeutic strategies, physicians could potentially prevent disease progression to lethal PC, thus improving clinical outcomes and reducing mortality.