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TITLE: Molecular Characterization of Very-Small-Nuclear Circulating Tumor Cells: A Putative Biomarker for Visceral Metastasis in Prostate Cancer

PRINCIPAL INVESTIGATOR: Pai-Chi Teng, M.D.

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

The prognostic value of circulating tumor cells (CTCs) in prostate cancer (PCa) has been validated in multiple studies.[1-3] Our team previously identified a subset of CTCs demarcated by nuclei less than 8.5 µm in size, vsnCTCs (Appendix

***Figure* 1**). These vsnCTCs associated with existing and developing lethal visceral metastasis (VM) in PCa.[4] Furthermore, additional studies now show that vsnCTCs are associated with poorer over survival (OS) and progression-free survival (PFS) in metastatic, castration-resistant PCa (mCRPC patients). These results have prompted us to characterize the underlying molecular alterations specific to vsnCTCs.

Our research collaborator, Dr. Michael Freeman (consultant, CSMC) has developed a new transcriptome-based subtyping: Prostate Cancer Classification System (PCS)[5]. PCS categorizes PCa into 3 subtypes (i.e., PCS1-3) 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 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 with intact RNA. This allows for seamless coupling with NanoString nCounter® platform[6] to accurately quantify the expression of RNA transcripts. Using these tools, we were able to develop CTC-based PCS panel that will measure the aggressiveness of PCa. This allows for timely detection of aggressive clinical behaviors including VM and emerging drug resistance 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), visceral metastasis (VM), 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. Circulating tumor cells with small nuclear size are associated with poor clinical outcomes in advanced prostate cancer. Submitted to *Cancer*. (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. Sixty-six of the 76 patients (87%) had detectable CTCs. vsnCTCs were more frequently seen in patients with presence of VM or those who developed VM during the clinical course.
- Fifty of the 76 patients had available blood specimens prior to a systemic therapy (i.e., pre-treatment samples). These patients were suitable for PFS analysis. Forty-three of the 50 patients (86%) with pre-treatment samples had detectable CTCs.

Major Task 2: Mathematical modeling of CTC nuclear size.

Milestone(s) Achieved: Complete the association analysis.

- We explored the relationship between CTC nuclear size and clinical outcomes with a different approach testing the hypothesis that smaller minimum CTC nuclear size (i.e., the smallest CTC nucleus among all the CTCs in each specimen) is associated with poorer prognosis. Patients without CTCs (N=10) were excluded. **Figure 2** depicts the relationship between OS and minimum CTC nuclear size using a p-spline plot (N=66). Generally, the hazard ratio (HR) of OS increased as the minimum CTC nuclear size decreased. This association was statistically significant in a range of 4.9 - 7.2 μm and 8.4 - 11.8 μm, as the 95% confidence interval (CI) in these ranges did not cross 1. This suggests that a minimum CTC nuclear size below 7.3 μm pointed toward poorer OS, whereas a minimum CTC nuclear size larger than 8.4 μm pointed toward better OS. The cutoff in our previous study (8.5 μm)[4] is very close to 8.4 μm, which means that our previously defined vsnCTCs have clinically meaningful value.
- Interestingly, the prognostic significance only holds true with minimum CTC nuclear size but not with the mean or median CTC nuclear size. A potential explanation for this observation is that clinical course is largely determined by the most biologically aggressive clones which were represented by the CTCs with smaller nuclear size. As a result, it was the CTCs with the smallest nucleus, rather than the overall CTC population, that best reflected the aggressiveness of the overall disease.

- The Kaplan-Meier analyses comparing vsnCTC status and clinical outcomes (OS/PFS) are shown in **Figure 3**. Patients with vsnCTCs (i.e., vsnCTC+) had significantly shorter OS and PFS compared to patients with no vsnCTC (i.e., vsnCTC-).
- This research work has been summarized in a manuscript entitle "Circulating tumor cells with small nuclear size are associated with poor clinical outcomes in advanced prostate cancer". (In submission)

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

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

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

- Among the 3 PCS[5] subtypes, 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 NanoVelcro chip as well as the CTC-PCS1 panel is well-validated in our previous publication.[7]
- We have identified vsnCTC+ patients have aggressive gene expression (i.e., CTC-PCS1 genes) compared to vsnCTC- patients (**Figure *4***).

- **What was accomplished under these goals?**
  - Identify the association between vsnCTCs and VM.
  - Identify the association between clinical outcomes (OS/FS) with vsnCTCs as well as CTC nuclear size.
  - Validate the clinically significant cutoff for vsnCTCs.
  - Transform the tissue-based PCS classifier into blood-based CTC-PCS assay using the NanoVelcro chip and the NanoString nCounter platform.
  - Validate CTC-PCS1 genes as aggressive molecular signatures.
  - Verify that vsnCTC+ patients had more expression of aggressive CTC-PCS1 genes.
- **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.
- **Teng P-C** 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**)
- **Teng P-C** 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**)
- **Teng P-C** et al. Prostate cancer CTC-RNA Assay: A new method for contemporary genomics and precision medicine via liquid biopsy. Journal of Clinical Oncology 38, 170-170, doi: 10.1200/JCO.2020.38.6_suppl.170 (2020). GU Cancers Symposium 2020, San Francisco, CA.
- Wang, J. J. et al. Association of very small nuclear circulating tumor cell (vsnCTC) with clinical outcomes in metastatic castration-resistant prostate cancer. Journal of Clinical Oncology 38, 168-168, doi: 10.1200/JCO.2020.38.6_suppl.168 (2020). GU Cancers Symposium 2020, San Francisco, CA.

- **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 “Circulating tumor cells with small nuclear size are associated with poor clinical outcomes in advanced 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?**

While bone metastases are frequent events in PCa, men with osseous involvement typically live for years. Men who develop VM are known to have significantly foreshortened survival[8] with median survivals of 21 vs 13 months. In autopsy series, VM are present in more than 45% of metastatic cases at the time of death.[9] The perception of the rarity of VM stems from infrequent monitoring of the viscera.[10] This underscores the need to alert treating physicians of clinical and biological events that point toward impending clinical crisis that may prompt more timely imaging and intervention. With advances in clinical genomics, it is recognized that suppression of AR signaling (the focus of many active and commonly utilized therapies) can result in a biological transformation that drives the emergence of a small cell/neuroendocrine phenotype which requires different forms of therapy from conventional PCa.[11] While optimal therapy for VM is not known, most clinicians will change therapy based upon this phenotype to include cytotoxics addressing the emergence of this neuroendocrine feature with agents such as platinum or other emerging strategies such as the antibody-drug conjugate rovalpituzumab tesirine (NCT02709889), other DNA-damage repair targeted therapies (e.g., PARP inhibitors), and immune-oncologics. Moreover, a considerable amount of effort is being exerted in clinical research to understand the optimal non-AR therapy for these patients with agents such as PARP inhibitors and immune checkpoint inhibitors. Thus, the need to better understand the details of this emerging biology is of paramount importance to field.

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 unmet need for PCa clinical care as hormonal treatments may 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 PCa. 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?**

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 assay (including enumeration and molecular profiling) 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.[12,13]

Based on the success with PCa, we also utilized this platform in other diseases including melanoma[14], hepatocellular carcinoma[15], lung cancer[12], pancreatic cancer[16] and noninvasive prenatal diagnostics[17].

- **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 PCa, which will allow oncologists to implement therapy that will alter the natural history of VM in PCa.

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)
- Ahn JC, **Teng P-C**, Chen P-J, Posadas EM, Tseng H-R, Lu S , Yang JD. Detection of circulating tumor cells and their implications as a novel biomarker for diagnosis, prognostication, and therapeutic monitoring in hepatocellular carcinoma. Hepatology, doi:10.1002/hep.31165 (2020).
- **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 are associated with poor clinical outcomes in advanced prostate cancer. Submitted to Cancer. (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)

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**)
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- 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**)
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- Wang JJ, **Teng P-C**, Jan YJ, Chen J-F, Cook-Wiens G, Yao N, Chu GC-Y, Chen P-J, Ho H, Yang Y, Lee Y-T, Huang J, Chung LWK, You S, Zhu Y, Freeman M, Rogatko A, Yang JD, Tseng H-R, Posadas EM. Association of very small nuclear circulating tumor cell (vsnCTC) with clinical outcomes in metastatic castration-resistant prostate cancer. Journal of Clinical Oncology 38, 168-168, doi: 10.1200/JCO.2020.38.6_suppl.168 (2020). GU Cancers Symposium 2020, San Francisco, CA.

- **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 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:	*Jie-Fu Chen, M.D.*
Project role:	*Previous PI*
Changed	*Grant transferred to Dr. Teng in 2019*

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, left the position of postdoctoral fellow at Cedars-Sinai Medical Center in June 2017, due to personal career plan. The proposed work was continued by his successor, Pai-Chi Teng, MD, starting from January 2019. The proposed research has not changed and was 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. Appendix

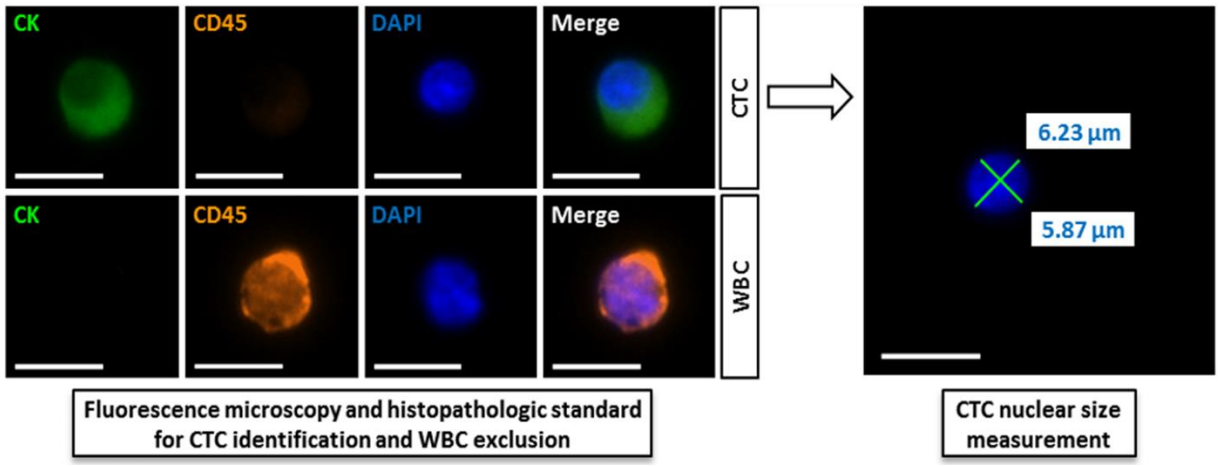

Figure 1. CTC candidates were captured using NanoVelcro assay[18] and characterized by DAPI+/CK+/CD45- events. Each candidate underwent subsequent review from a cytopathologist verifying that morphology of the candidate cell was of consistent with an epithelial origin. For all CTCs, the nuclear size was calculated using previously published methods[4]. Nuclear size was defined as the square root of the product of the long axis and the short axis. In this study, the nuclear size of every CTC from every patient was documented.

$$Nuclear\ size\ (\mu m) = \sqrt{Long\ Axis\ \times Short\ Axis}$$

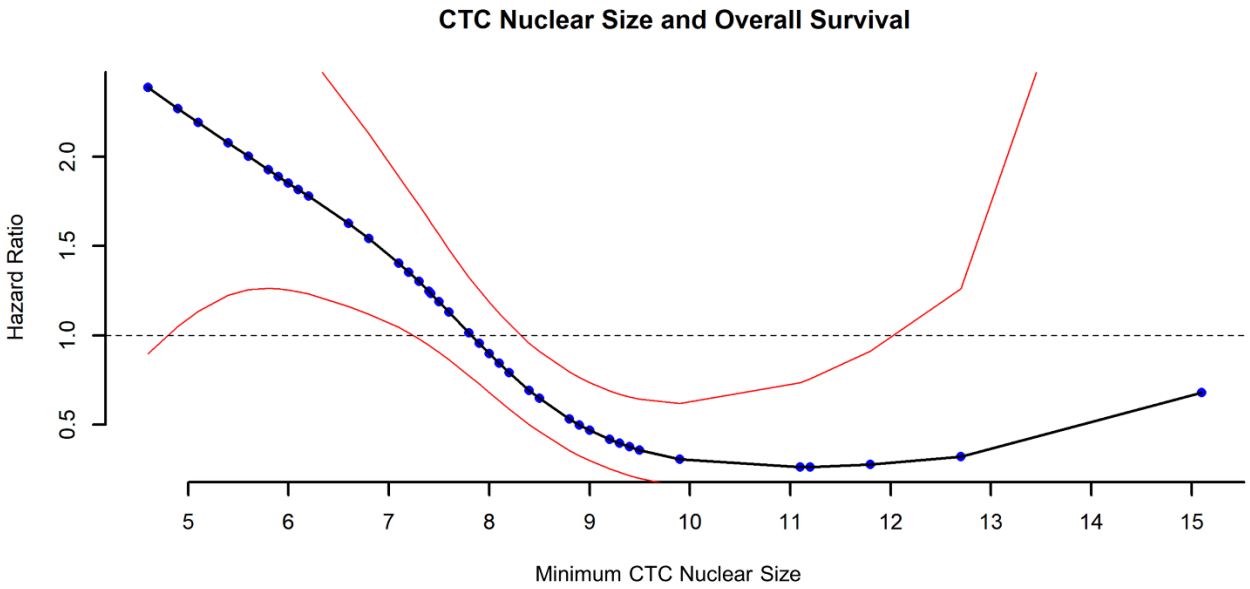

Figure 2. P-spline curve comparing minimum CTC nuclear size and hazard ratio for overall survival (N=66). As the minimum CTC nuclear size decreases, the hazard ratio (HR, univariate Cox regression model) of OS increases. Blue dots: HR of the corresponding minimum CTC nuclear size (i.e., the smallest CTC nuclear size in each specimen). Red lines: 95% confidence interval.

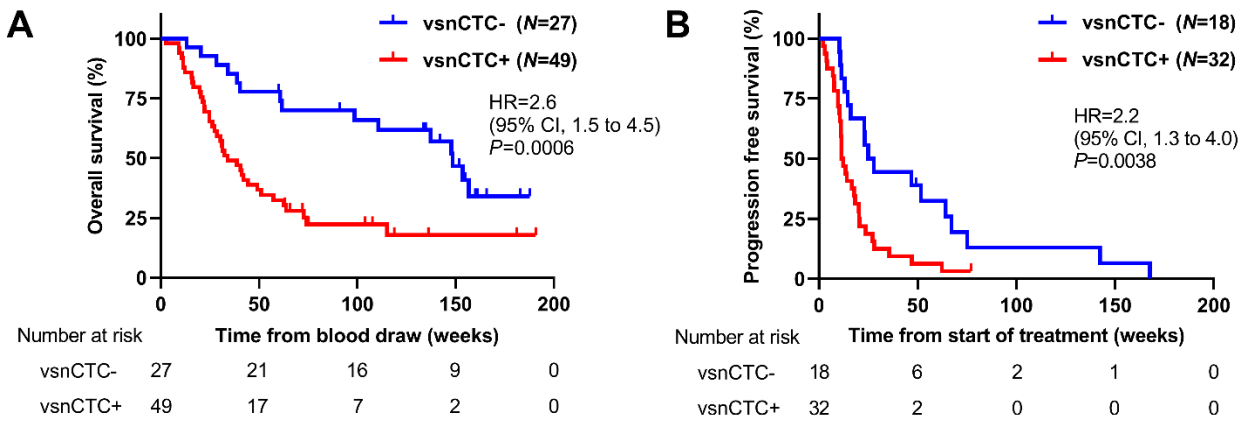

Figure 3. Kaplan-Meier analyses. (A) OS for all mCRPC patients (N=76) with available blood specimens. **(B)** PFS for patients with available blood specimens prior to ARSI, taxane, or other therapy (N=50).

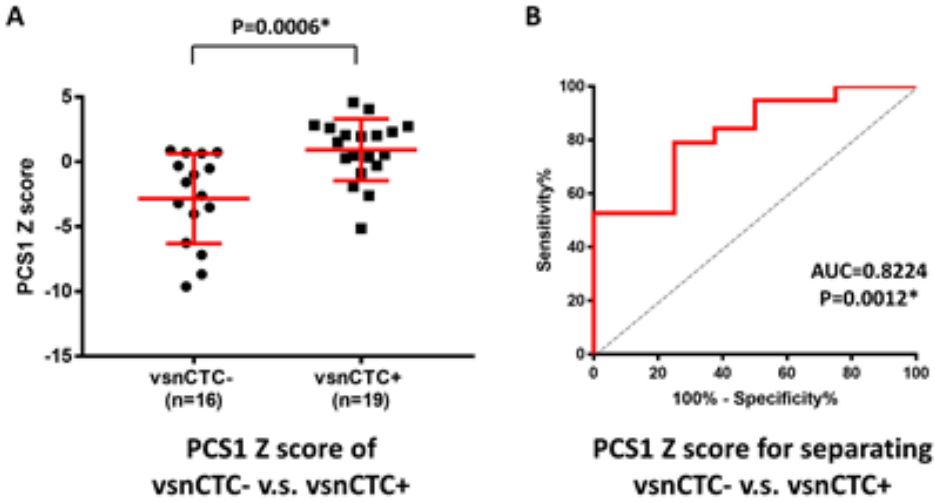

Figure 4. PCS1 analysis of PCa patients by vsnCTC category. (a) Comparing PCS1 Z score in vsnCTC- (n=16) patients and vsnCTC+ (n=19). vsnCTC+ patients had higher Z score (P=0.0006). (**b**) Area under curve (AUC) of PCS1 Z score separating vsnCTC- and vsnCTC+ patients is 0.82 (P=0.0012).

9. References

1 Danila, D. C. *et al.* Circulating tumor cell number and prognosis in progressive castration-resistant prostate cancer. *Clin Cancer Res* **13**, 7053-7058, doi:10.1158/1078-0432.CCR-07-1506 (2007).

2 Goldkorn, A. *et al.* Circulating tumor cell counts are prognostic of overall survival in SWOG S0421: a phase III trial of docetaxel with or without atrasentan for metastatic castration-resistant prostate cancer. *J Clin Oncol* **32**, 1136-1142, doi:10.1200/JCO.2013.51.7417 (2014).

3 Scher, H. I. *et al.* Circulating tumor cell biomarker panel as an individual-level surrogate for survival in metastatic castration-resistant prostate cancer. *J Clin Oncol* **33**, 1348-1355, doi:10.1200/JCO.2014.55.3487 (2015).

4 Chen, J. F. *et al.* Subclassification of prostate cancer circulating tumor cells by nuclear size reveals very small nuclear circulating tumor cells in patients with visceral metastases. *Cancer* **121**, 3240-3251, doi:10.1002/cncr.29455 (2015).

5 You, S. *et al.* Integrated Classification of Prostate Cancer Reveals a Novel Luminal Subtype with Poor Outcome. *Cancer Res* **76**, 4948-4958, doi:10.1158/0008-5472.CAN-16-0902 (2016).

6 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).

7 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).

8 Halabi, S. *et al.* Meta-Analysis Evaluating the Impact of Site of Metastasis on Overall Survival in Men With Castration-Resistant Prostate Cancer. *J Clin Oncol* **34**, 1652-1659, doi:10.1200/JCO.2015.65.7270 (2016).

9 Bubendorf, L. *et al.* Metastatic patterns of prostate cancer: An autopsy study of 1,589 patients. *Human Pathology* **31**, 578-583, doi:10.1053/hp.2000.6698 (2000).

10 Whitney, C. A. *et al.* In Men with Castration-Resistant Prostate Cancer, Visceral Metastases Predict Shorter Overall Survival: What Predicts Visceral Metastases? Results from the SEARCH Database. *Eur Urol Focus* **3**, 480-486, doi:10.1016/j.euf.2016.08.007 (2017).

11 Aggarwal, R. *et al.* Clinical and Genomic Characterization of Treatment-Emergent Small-Cell Neuroendocrine Prostate Cancer: A Multi-institutional Prospective Study. *J Clin Oncol* **36**, 2492-2503, doi:10.1200/JCO.2017.77.6880 (2018).

12 Dong, J. *et al.* Covalent chemistry on nanostructured substrates enables noninvasive quantification of gene rearrangements in circulating tumor cells. *Sci Adv* **5**, eaav9186, doi:10.1126/sciadv.aav9186 (2019).

13 Shen, M. Y. *et al.* Glycan Stimulation Enables Purification of Prostate Cancer Circulating Tumor Cells on PEDOT NanoVelcro Chips for RNA Biomarker Detection. *Adv Healthc Mater* **7**, doi:10.1002/adhm.201700701 (2018).

14 Hou, S. *et al.* Polymer nanofiber-embedded microchips for detection, isolation, and molecular analysis of single circulating melanoma cells. *Angew Chem Int Ed Engl* **52**, 3379-3383, doi:10.1002/anie.201208452 (2013).

15 Court, C. M. *et al.* A novel multimarker assay for the phenotypic profiling of circulating tumor cells in hepatocellular carcinoma. *Liver Transpl* **24**, 946-960, doi:10.1002/lt.25062 (2018).

16 Court, C. M. *et al.* Circulating Tumor Cells Predict Occult Metastatic Disease and Prognosis in Pancreatic Cancer. *Ann Surg Oncol* **25**, 1000-1008, doi:10.1245/s10434-017-6290-8 (2018).

17 Hou, S. *et al.* Imprinted NanoVelcro Microchips for Isolation and Characterization of Circulating Fetal Trophoblasts: Toward Noninvasive Prenatal Diagnostics. *ACS Nano* **11**, 8167-8177, doi:10.1021/acsnano.7b03073 (2017).

18 Jan, Y. J. *et al.* NanoVelcro rare-cell assays for detection and characterization of circulating tumor cells. *Adv Drug Deliv Rev* **125**, 78-93, doi:10.1016/j.addr.2018.03.006 (2018).