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TITLE: Next-Generation Human Prostate Cancer Cocultures to Optimize Autologous Chimeric Antigen Receptor T-Cell Immunotherapy

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14. ABSTRACT
In this reporting period, we have optimized a methodology to process fresh primary human prostate cancer tissues obtained at standard-of-care prostatectomy and establish viable tissue slices that can be cultured for up to a week. Our preliminary studies indicate that the prostate cancer tissue slices can be maintained without a loss of cell viability or gross disruption of tissue architecture during this time period. Immunohistochemical studies showed maintenance of androgen receptor, prostate stem cell antigen, and Ki67 expression in these tissue slices. We have developed a multiparametric flow cytometry panel to examine changes in the tumor microenvironment of the tissue slices over time. We are now optimizing enzymatic digestion protocols to dissociate tissue slices to single cells to maximize cell recovery for these analyses. Once this technical optimization and characterization is complete, we intend to move forward with immunological co-culture studies using chimeric antigen receptor T cells and human primary prostate cancer tissue slices.

15. SUBJECT TERMS
Prostate cancer, tissue slice culture, chimeric antigen receptor, immunotherapy

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INTRODUCTION

Background: Cancer therapies that hold preliminary promise based on preclinical studies in cell culture or in small animal models often fail to be effective humans. This applies as well to immunotherapies where available systems may oversimplify human biology. Solid tumors including prostate cancer are composed of mixed cell populations in an evolving three-dimensional mass within which only small fractions of the numerous cellular and intercellular interactions are understood. Specific to immune dysregulation in cancer, tumor cells, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), T regulatory cells (Tregs), and defective antigen presenting cells (APCs) all play active roles in inducing immune suppression. The use of pre-clinical models that recapitulate this biological complexity is necessary to appropriately interrogate the efficacy and safety of candidate cellular therapies like chimeric antigen receptor (CAR) T cells. The purpose of this research is to establish human prostate cancer tissue slices and propagate them *ex vivo* to preserve their *in vivo* cell and tissue properties. We will then use these prostate cancer tissue slices to examine and optimize the activity of CAR T cells targeting prostate stem cell antigen (PSCA) in immunological co-cultures. This platform may enable the rapid interrogation of combination immunotherapy approaches with PSCA CAR T cell therapy to discover strategies that will enhance clinical antitumor efficacy.

KEYWORDS

Prostate cancer, tissue slice culture, chimeric antigen receptor, immunotherapy

ACCOMPLISHMENTS

To summarize the research accomplishments to date, the tasks described in the proposed Statement of Work are itemized here with a brief update for each task.

SA 1: Optimize human prostate cancer tissue slice cultures (TSCs) for use in immunological co-cultures (months 1-32)

Task 1: Confirm the preservation of viability and tissue architecture in short-term primary prostate cancer TSCs (months 1-7) Completed. We have obtained multiple fresh human primary prostatectomy specimens from the University of Washington Genitourinary Research Laboratory. From these tissues, ~350 um thick sections were sliced on a Krumdieck tissue slicer, placed on titanium well inserts in 6-well plates filled with media on a fixed-angle 3D platform rotator (**Fig. 1**). Importantly, this experimental setup allows for the tissue slices to have both air and liquid interfaces and the media is changed daily. We initially tested two media conditions (**Fig. 2A**) with modifications adapted from a published protocol of human prostate TSC [1]. Using

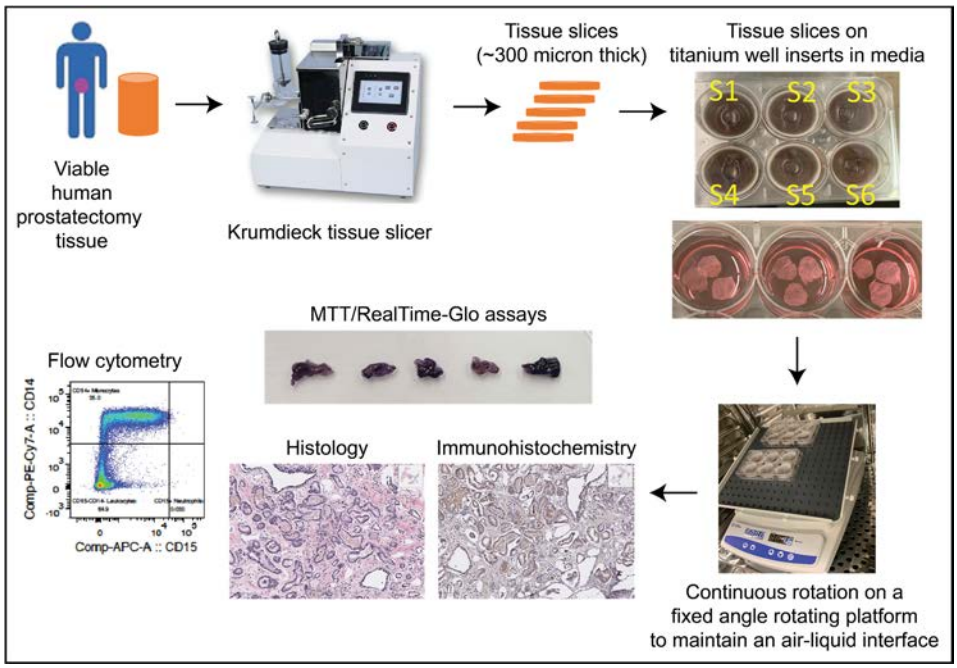

Fig. 1. Workflow to initiate and analyze human primary prostate cancer tissue slice cultures.

these conditions, we discovered that tissue slice viability by calorimetric MTT assay decreased significantly after 48 hours in both conditions (**Fig. 2B**). Ultimately, we tested a third media condition which more closely resembled the previously reported complete PFMR-4A media [1] but is based on commercial Advanced DMEM/F12 media (**Fig. 3A**). Upon conversion to this media, tissue slice viability was generally maintained or increased over the course of 120 hours by both MTT and by RealTime-Glo assays (**Fig. 3B**). We performed histologic and immunohistochemical analyses of the prostate tissue slices, comparing sections at day 0, 3, and 5 of TSC (**Fig. 3C**). Overall, we observed preservation of tissue architecture, evidence of cell proliferation based on Ki67 staining, retained androgen receptor (AR) expression, and p63 expression lining the basal layer of normal glands.

A

Media Condition 1	Media Condition 2
Advanced DMEM/F12	RPMI 1650
2 mM GlutaMAX	10% FBS
10 mM HEPES	2 mM GlutaMAX
1.25 mM N-acetylcysteine	1.134 g/L NaHCO3
B27 supplement	14.5 mM HEPES
5 ng/ml hEGF	20 mg/L L-cysteine
100 ng/ml hNoggin	
500 ng/ml hR-spondin 1	
10 ng/ml hFGF10	
5 ng/ml hFGF2	
1 uM prostaglandin E2	
10 mM nicotinamide	
10 uM SB202190	
500 nM A83-01	
10 uM Y027632	
50 nM R1881	

B

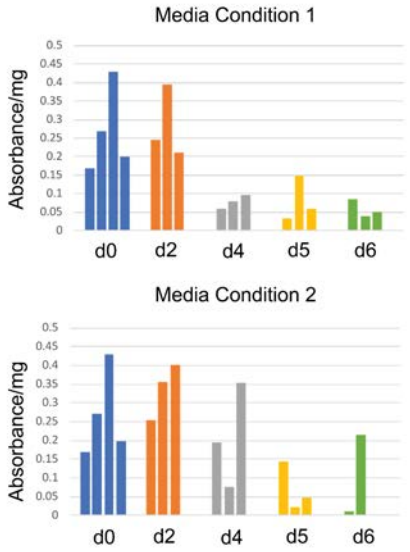

Fig. 2. Evaluation of media conditions and tissue slice viability over time. (**A**) Composition of media used for initial prostate cancer TSC experiments. (**B**) Tissue slice viability measured by MTT assay and shown as absorbance normalized to weight of each tissue slice over time for each media condition.

We also examined whether these conditions may differentially support the growth of normal prostate or prostate cancer tissues. Two regions of a single human prostatectomy sample, one enriched in normal tissue and the other in prostate cancer as determined by a pathologist, were subjected to TSC. Based on RealTime-Glo assays, we did not identify a difference in the viability of the tissue slices for up to six days (**Fig. 4**), after which a decline was evident. The tissue slices from regions of cancer demonstrated a higher overall level of luminescence in the RealTime-Glo assays compared to those from normal areas. Additionally, we appreciated variability in signal between tissue slices within the groups which is likely a consequence of inherent differences in cellularity and tissue hypoxia and necrosis related to prostatectomy.

Task 2: *Profile the effects of TSC on immune cell subsets in the tumor microenvironment of human prostate cancer (months 1-5)* In progress. In collaboration with the Immune Monitoring Shared Resource at Fred Hutch, we have generated an antibody panel for multiparametric flow cytometry to quantify human B and T lymphocytes, monocyte, neutrophil, and macrophage populations. In preliminary work, we have validated the performance of this panel on healthy donor peripheral blood mononuclear cells and dissociated human prostate tissue (**Fig. 5**) with the use of fluorescence minus one (FMO) controls (not shown). We are currently optimizing the enzymatic digestion of prostate cancer tissue slices using collagenase type II and dispase to maximize cell recovery for flow cytometry analysis. Due to restrictions related to COVID-19, the availability of viable primary prostate cancer tissues for research purposes through the University of Washington Genitourinary Cancer Research Laboratory was restricted between the months of March and September 2020. We have since resumed these studies and anticipate completion in the next three months.

Task 3: *Adapt prostate cancer TSC to metastatic prostate cancer tissues (months 10-32)* Not yet started. Due to the COVID-19 pandemic, the University of Washington Tissue Acquisition Necropsy Program was inactive and research autopsies on deceased patients with metastatic prostate cancer have not been performed since March 2020.

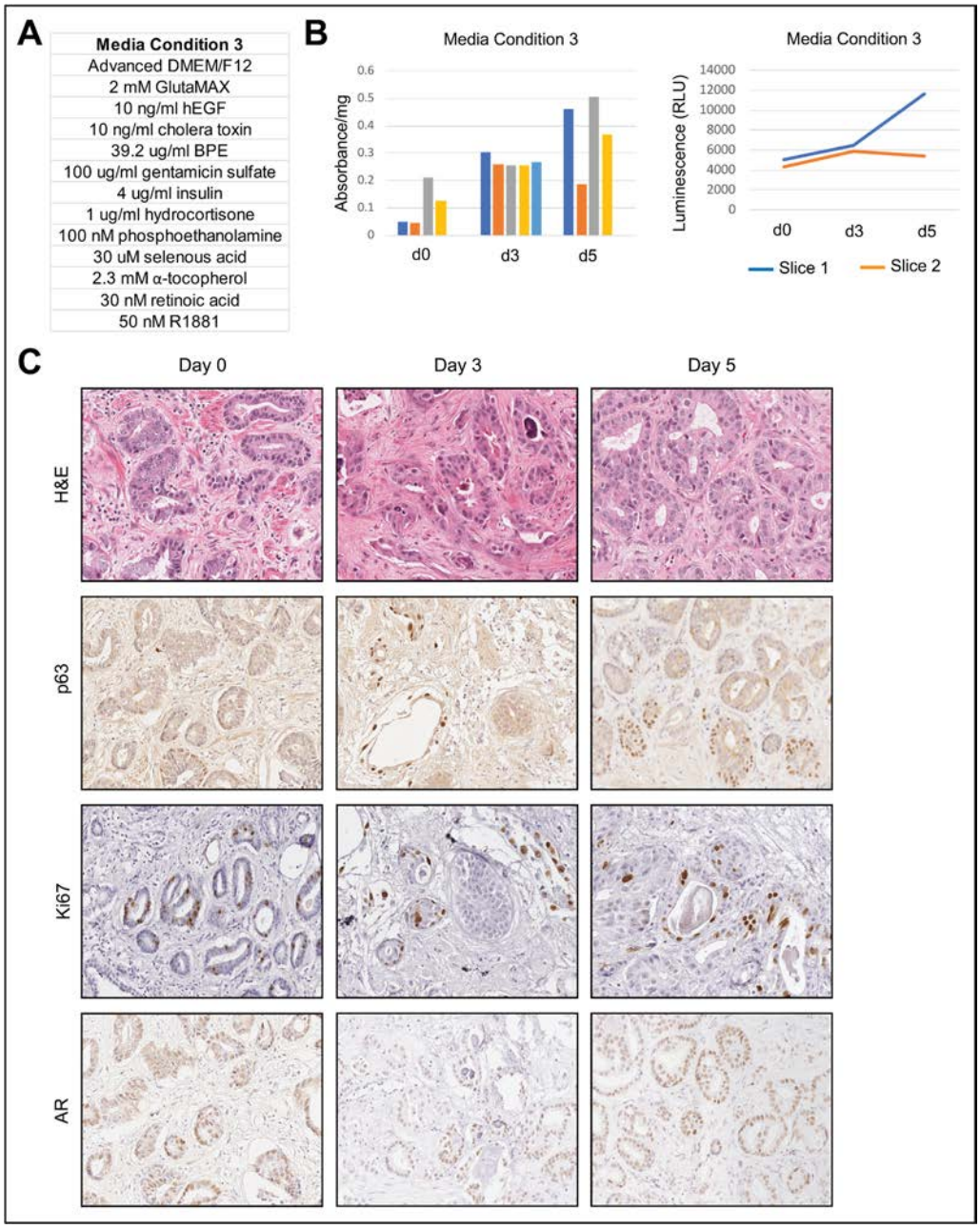

Fig. 3. Identification of a media condition that maintains prostate cancer tissue slice viability and cellular architecture. (**A**) Composition of media optimized for prostate cancer TSC. (**B**) *Left*, tissue slice viability measured by MTT assay and shown as absorbance normalized to weight of each tissue slice over time. *Right*, tissue slice viability measured by RealTime-Glo assay and shown as relative light units (RLU) over time. (**C**) Representative photomicrographs of sections from prostate cancer tissue slices stained with H&E or immunohistochemically stained with antibodies against p63, Ki67, and AR on days 0, 3, and 5 of TSC.

SA 2: Characterize the antitumor activity of autologous PSCA-BBζ CAR T cells in matched human prostate cancer tissue slice co-cultures (months 8-36)

Task 1: Establish co-cultures of primary prostate cancer and autologous PSCA- BBζ CAR T cells (months 8-23) Not yet started.

Task 2: Determine the antitumor effects of PSCA-BBζ CAR T cells in human prostate cancer TSCs (months 9-24) Not yet started.

Task 3: Evaluate the infiltration of PSCA-BBζ CAR T cells in human prostate cancer TSCs (months 9-36) Not yet started.

SA 3: Examine the effects of altering immune modulatory signals on PSCA-BBζ CAR T cell activity (months 18-36)

Task 1: *Screen immunotherapies combined with PSCA-BBζ CAR T cells in prostate cancer TSCs to identify agents that enhance anti-tumor activity (months 18-30)* Not yet started.

Task 2: *Validate combination immunotherapy strategies with mouse PSCA-CARs in immunocompetent mouse models of prostate cancer (months 24-36)* Not yet started.

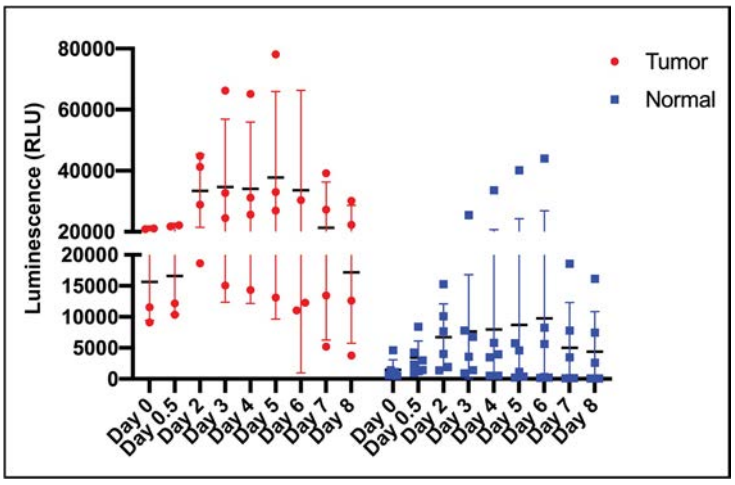

Fig. 4. Comparison of the viability of tissue slices from benign or cancer-enriched regions of primary human prostate tissue. Tissue slice viability measured by RealTime-Glo assay and shown as relative light units (RLU) over time. Individual values from each replicate tissue slice, the mean, and standard deviation are shown.

Key Research Accomplishments:

- Optimization of media conditions that maintain the viability, tissue architecture, and epithelial differentiation markers of human primary prostate cancer tissue slices in TSC for up to five days.

- TSC conditions appear suited for both benign and cancerous human prostate tissue.

- Development of an antibody panel for multiparametric flow cytometry for impending quantification of immune cell subsets from dissociated human primary prostate cancer tissue slices.

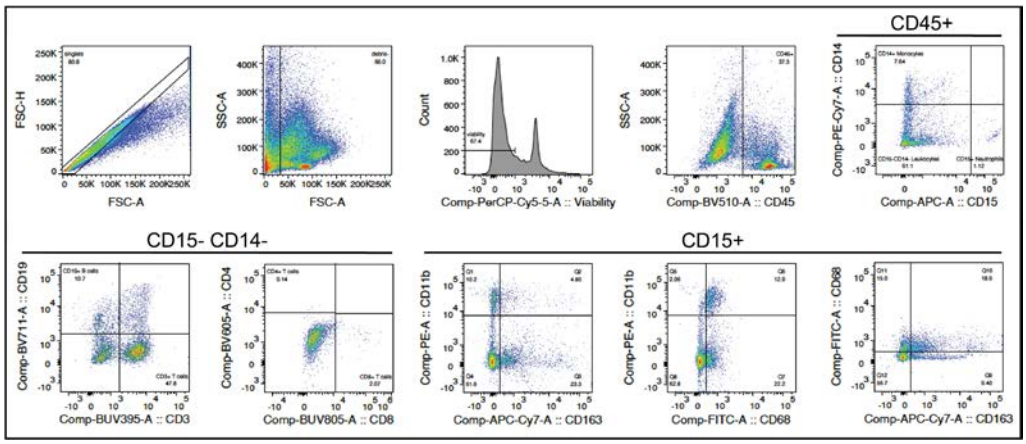

Fig. 5. Multiparametric flow cytometry to quantify immune cell subsets. Example of the use of an antibody panel for multiparametric flow cytometry of dissociated human primary prostate cancer tissue.

Opportunities for Training and Professional Development:

This reporting period included my second year as an independent investigator in the Prostate Cancer Program in the Human Biology Division at Fred Hutch. My laboratory consists of three post-doctoral fellows, a graduate student, two technicians, and a staff scientist that I oversee and mentor. I continue to meet with my junior faculty mentoring committee consisting of Drs. Pete Nelson, Valeri Vasioukhin, and Bob Eisenman.

My professional development includes multiple areas of service to the institution as a member of the Cancer Consortium Scientific Review Committee, the Faculty Diversity and Inclusion Committee, and the Proteomics Faculty Advisory Committee. I am also actively involved with the Prostate Cancer Foundation and the Pacific Northwest Prostate Cancer SPORE. I continue in my role as the co-leader of the Prostate Cancer Foundation Young Investigator Microenvironment and Immunology Working Group. In the past year, I received the Eula and Donald S. Coffey Innovative Research Award and Young Investigator Award from the Society for Basic Urologic Research as well as a Young Physician-Scientist Award from the American Society for Clinical Investigation. My new associations with these organizations have further expanded my professional network.

I am a co-investigator on the UCLA SPORE in Prostate Cancer Project 3 with Drs. Owen Witte, Stephen Forman, and Saul Priceman. Dr. Priceman and I remain in constant communication through monthly teleconference calls to discuss the progress of our shared research projects related to CAR T cell therapy for prostate cancer through the UCLA SPORE and Department of Defense Prostate Cancer Research Program.

Dissemination of Results:

The findings of our work have been shared locally with the Fred Hutch Prostate Cancer Program. We will plan to share additional results in the next six months through the Genitourinary Oncology Seminar Series sponsored by the Pacific Northwest Prostate Cancer SPORE. As the data mature, we also anticipate disseminating the results at national meetings including the Prostate Cancer Foundation Scientific Retreat and Society for Immunotherapy of Cancer Annual Meeting.

Plan for the Next Reporting Period:

In the next reporting period, we will complete the quantification of immune cell populations and characterize how they are maintained in human prostate cancer tissue slices over a period of five days in culture. Once this has been determined, we will begin to establish co-cultures of the human prostate cancer tissue slices with PSCA CAR T cells to examine cytotoxic activity. In addition, when the University of Washington Tissue Acquisition Necropsy Program begins research autopsies again, we will obtain metastatic prostate cancer tissues from which we will examine the ability to maintain tissue slice cultures using workflows and methodologies established during this reporting period.

IMPACT

Advanced prostate cancer is characterized by its immunologically “cold” and hostile tumor microenvironment which is a major barrier to the success of immunotherapies including cellular adoptive therapies. Our goal is to develop a human preclinical system that recapitulates this barrier and is sufficiently flexible to enable rapid hypothesis testing in order to advance therapeutic strategies to improve the effectiveness of CAR T cell therapy for prostate cancer. In the past year, we have systematically acquired the technical expertise and skill to viably propagate human prostate cancer tissue slices over a period of five days in culture. This represents an incredibly specialized technique of which only a handful of laboratories are capable of successfully employing. There are still unknown variables that we will address, namely whether the tumor immune microenvironment is preserved during this period of brief *ex vivo* culture. We expect to further optimize this system, identify combinatorial immunotherapies that enhance the antitumor efficacy of CAR T cells, and validate our findings in animal models. The major impact of this foundational work will be to advance new immunotherapy approaches in the future to reduce suffering and extend life in men with lethal, metastatic prostate cancer.

CHANGES/PROBLEMS

The project was impacted significantly by the COVID-19 pandemic in multiple ways. First, the research is dependent on human prostate cancer specimens obtained from patients undergoing prostatectomy or who have volunteered for research autopsies at death. The number of available research tissues from primary prostate cancer samples was reduced by diminished staffing during the COVID-19 pandemic. The University of Washington Tissue Acquisition Necropsy Program was placed on hold and no research autopsies have been performed since March 2020. In addition, my research laboratory was limited to minimal maintenance activities between the months of March and June 2020 due to physical distancing restrictions at the Fred Hutchinson Cancer Research Center.

No changes to the project are anticipated at this juncture.

PRODUCTS

None.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	*John K. Lee*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-6570-2180
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Lee has performed data analysis and overseen the conduct of the study.*
Funding Support:	

Name:	*Shan Li*
Project Role:	*Post-Doctoral Research Fellow*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*4*
Contribution to Project:	*Dr. Li has established short-term prostate cancer tissue slice cultures, developed a multiparametric flow cytometry panel to examine immune cell subsets, and performed cell viability assays, histology, and immunohistochemistry.*
Funding Support:	

Name:	*Tiffany Pariva*
Project Role:	*Research Technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2*
Contribution to Project:	*Ms. Pariva has assisted with tissue processing, cell viability assays, and histology.*
Funding Support:	

Name:	*Saul Priceman*
Project Role:	*Subaward PD/PI (City of Hope)*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Priceman has been involved in the overall experimental design and review of data related to the human prostate cancer tissue slice culture optimization.*
Funding Support:	

Name:	*Colm Morrissey*
Project Role:	*Subaward PD/PI (University of Washington)*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Morrissey has been involved in biospecimen acquisition, processing, characterization, maintenance of the clinical database, and distribution of specimens.*
Funding Support:	

Name:	*Lawrence True*
Project Role:	*Co-Investigator (University of Washington)*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. True, as a surgical pathologist, has been critical to prostate cancer tissue acquisition and the annotation of benign and cancerous regions of prostate tissue.*
Funding Support:	

Name:	*Lori Kollath*
Project Role:	*Research Sientist (University of Washington)*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Ms. Kollath has managed the biospecimen repository and has overseen all biospecimen collections, processing, and distribution.*
Funding Support:	

SPECIAL REPORTING REQUIREMENTS

Nothing to Report.

REFERENCES

1. Maund, S.L., R. Nolley, and D.M. Peehl, *Optimization and comprehensive characterization of a faithful tissue culture model of the benign and malignant human prostate.* Lab Invest, 2014. **94**(2): p. 208-21.

APPENDICES

None.