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PRINCIPAL INVESTIGATOR: Rajan Kulkarni, MD, PhD

CONTRACTING ORGANIZATION: University of California, Los Angeles
Los Angeles, CA 90095

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Rajan Kulkarni, MD, PhD

E-Mail:rkulkarn@g.ucla.edu

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14. ABSTRACT

Prostate cancer is a leading cause of cancer-related mortality in men. Although therapies that inhibit androgen receptor (AR) activity are initially effective, patients ultimately succumb to metastatic castration resistant prostate cancer (mCRPC). The molecular events leading to this are incompletely understood. There is a need for minimally invasive means to obtain tumor information to overcome these knowledge gaps. Circulating tumor cells (CTCs) are cells that break away from either the primary tumor or metastatic sites. Moreover, serial CTC analyses performed in the context of treatment changes can characterize the mutational landscape of tumors as they evolve, particularly for novel agents such as MEK inhibitors. However, the profiles of sensitivity or resistance to MEK inhibition are unknown, particularly as the tumor may evolve in response to treatment. We have begun to obtain blood samples from patients on a trial of MEK inhibition in prostate cancer to identify molecular predictors of sensitivity/resistance. These studies will also help us better understand the molecular basis of sensitivity or resistance to MEK inhibitors, which will help to provide new scientific insights into the pathogenesis of advanced prostate cancer and provide ideas of novel approaches that can be used to treat such tumors.

15. SUBJECT TERMS
metastatic castration resistant prostate cancer (mCRPC), circulating tumor cells (CTCs), MEK inhibition, liquid biopsy, personalized medicine, secondary resistance

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1. Introduction:

Prostate cancer is a leading cause of cancer-related mortality in men. Although therapies that inhibit androgen receptor (AR) activity are initially effective, patients ultimately succumb to metastatic castration resistant prostate cancer (mCRPC). The molecular events leading to this are incompletely understood. There is a need for minimally invasive means to obtain tumor information to overcome these knowledge gaps. Circulating tumor cells (CTCs) are cells that break away from either the primary tumor or metastatic sites. However, genome and transcriptome wide analyses of CTCs have been hampered by low purity and prolonged capture time. My mentors and I have developed and tested a CTC capture technology, Vortex Chip, which allows rapid isolation of highly purified CTCs. The purity of the samples obtained through Vortex Chip are high enough to enable rapid exome or transcriptome analysis. Moreover, serial CTC analyses performed in the context of treatment changes can characterize the mutational landscape of tumors as they evolve. This is particularly important, as mentor Dr. Matthew Rettig has recently started a trial to treat select patients with refractory prostate cancer with MEK inhibitors (trametinib) as MAPK pathway activation appears to play a role in growth in certain cases. However, the profiles of sensitivity or resistance to MEK inhibition are unknown, particularly as the tumor may evolve in response to treatment. The purpose of this research is twofold: 1) characterize intra- and inter-patient heterogeneity of prostate cancer CTCs and 2) identify molecular predictors of sensitivity/resistance to the use of novel MAPK pathway therapies for mCRPC. These studies will also help us better understand the molecular basis of sensitivity or resistance to MEK inhibitors, which will help to provide new scientific insights into the pathogenesis of advanced prostate cancer and provide ideas of novel approaches that can be used to treat such tumors. They will also facilitate a more personalized approach to cancer treatment by enabling repeatable, minimally invasive, and longitudinal genetic analyses during the course of the treatment of prostate cancer.

2. Keywords:

metastatic castration resistant prostate cancer (mCRPC), circulating tumor cells (CTCs), MEK inhibition, liquid biopsy, personalized medicine, secondary resistance

3. Accomplishments:

Major Goals of the project (from SOW):

i. Training and educational development in prostate cancer research
ii. Use Vortex Chip to characterize molecular changes in circulating tumor cells (CTCs) of metastatic castration-resistant prostate cancer (mCRPC) patients
iii. Characterize molecular profiles that predict sensitivity or resistance to MEK inhibition

- Compare exome/transcriptome and proteome of tumor at time of initiation of MEK inhibitor treatment and on disease progression
- Determine molecular profile of primary versus secondary resistance to MEK inhibitors (trametinib)

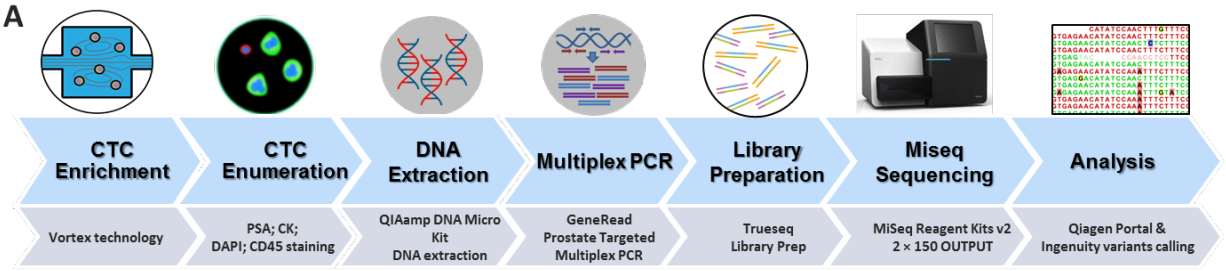
What was accomplished under these goals?

<u>Training and educational development in prostate cancer research</u>

For this goal (goal 1), I completed the UCLA CTSI Track 2 program in Translational Research and took courses in biostatistics, translational study design, and clinical trials methodologies. I plan to take courses in genomics and bioinformatics analysis in the coming year. I also was able to attend the annual Prostate Cancer Foundation (PCF) meeting in 2017 and the invitation only PCF Coffey-Holden Academy in June 2018 (see below).

Use Vortex Chip to characterize molecular changes in circulating tumor cells (CTCs) of metastatic castration-resistant prostate cancer (mCRPC) patients

For this goal (goal 2), we have enrolled five patients (of a total of 30 patients), and we are beginning analysis of the data from these CTCs. We are also working on development of the analysis workflow for these samples, using cell line cells to test these approaches (LNCaP prostate cancer cells). The current workflow methodology is outlined below, using a multiplex PCR approach:



In the coming year, we are also planning to collaborate with Hisham Mohammed at OHSU/Knight Cancer Institute on single cell RNA-sequencing of the CTCs; he is one of the world experts in single cell sequencing methods. We have not yet been able to access the archived library tissues as we are still obtaining IRB/HRPO approval for these studies, but this is in progress and we hope to have all those approvals shortly.

Characterize molecular profiles that predict sensitivity or resistance to MEK inhibition

For this goal (goal 3), Dr. Rettig's MEK-inhibitor clinical trial was finally opened. There were some IRB and institutional delays and so the first patients were not enrolled until March 2018; however, since March, ten patients have been enrolled (of a total projected enrollment of 40). All patients had an initial blood collection and most had a metastatic biopsy performed as well. Of these patients, 2 patients have since died before follow up could be performed, and an additional 4 patients have left the study. Of the remaining 4 patients, followup is planned every 12 weeks with serial blood monitoring. In future years, up to an additional 30 patients will be enrolled into the study. The blood, CTCs, and metastatic biopsies are now being processed for analysis of tumor whole exome and transcriptomes.

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

The PI, Rajan Kulkarni, has had several opportunities for training and professional development through the first year of the project. More detail is provided above; in summary, I have been able to attend the PCF Annual Meeting in 2017 in Washington DC and was invited to participate in the PCF annual Coffey-Holden

Academy in 2018; this academy is by invitation only and is an intimate setting to discuss timely research issues and questions related to prostate cancer. I was fortunate to attend in June 2018 and make several connections with potential future collaborators including Sumit Subudhi at MD Anderson Cancer Center and Julie Graff and Joshi Alumkal at OHSU in Portland, OR.

How were the results disseminated to communities of interest?

Nothing to report. However, we plan to present at regional/national conferences as we accumulate and analyze more data.

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

In the coming year, I plan to continue my training plan as detailed in the SOW and to attend relevant courses in bioinformatics and genomics. I also plan to attend and hopefully present data at relevant prostate cancer conferences, including the 2018 PCF meeting and potentially the next AACR and/or ASCO meetings in 2019. For the second goal, I am commencing a collaboration with Dr. Hisham Mohammed at OHSU on single cell analysis of CTCs; he is a leading expert in single cell RNA sequencing methods and analysis and he will help me to streamline and optimize methodologies for analysis of Vortex isolated CTCs. I also plan to continue to enroll patients as described. For the third goal, Dr. Rettig's MEK trial is continuing to accumulate patients, and I will continue to process and analyze the blood and tissue samples from these patients. So far, the treatment has been well tolerated but several patients have died (not from this treatment but due to debility from metastatic prostate cancer) or have otherwise left the study. We will continue to monitor even those living patients who have left the study as is possible. We will also begin to analyze the metastatic tissue biopsies to help elucidate markers that may predict response, non-response, or secondary resistance to MEK inhibition.

4. Impact:

What was the impact on the development of the principle discipline of the project?

We have shown that MEK inhibition can be utilized in cases of advanced prostate cancer and that we are starting analysis to determine mechanisms of sensitivity or resistance to this novel agent. Data are limited as this is the first year (of 4) for the project, but we are hopeful that the trends described will continue and that we will continue to have an impact within prostate cancer treatment.

What was the impact on other disciplines?

Nothing to report at this time.

What was the impact on technology transfer?

Nothing to report at this time.

What was the impact on society beyond science and technology?

MEK inhibition may be an additional treatment option for advanced/metastatic prostate cancer, based on our initial results. Further analysis and monitoring will be

necessary in order to confirm the findings and to identify the subset of patients who will most likely benefit from this treatment in the future.

5. Changes/Problems:

Changes in approach

Nothing to report

Problems and delays and actions/plans to resolve them

There was a small delay in starting accruals of patients due to IRB and HRPO review, though these are almost complete now and patients are being enrolled and analyzed as described. There is one protocol still pending IRB/HRPO review and we hope it will be approved shortly.

Changes that had a significant impact on expenditures

The delay in starting patient accruals has meant that spending in the first year has been a bit delayed, though we are now starting to analyze data samples and will start performing the sequencing and data analysis as described in the SOW. In addition, UCLA has switched to a new financial system (UCPath) which delayed spending on the grant (particularly for PI salary, which ended up being covered by the department and the UCLA CTSI in the first year); this has now been implemented and full spending for PI salary should commence shortly. In addition, I have had a bit of difficulty in finding additional appropriate staff for the study (to function as research assistant), though I have identified someone who will hopefully be hired shortly.

Significant changes in use or care of human subjects

Nothing to report.

6. Products:

Journal publications: Nothing to report
Books or other non-periodical publications: Nothing to report
Other publications, conference papers, presentations: Nothing to report
Websites: Nothing to report
Technologies or techniques: Nothing to report yet
Inventions/patent applications: Nothing to report
Other products: Nothing to report

7. Participants and Other Collaborating Organizations

Name: Rajan Kulkarni
Project Role: PI
Researcher Identifier (ORCID): 0000-0002-2191-4085
Nearest person month worked: 6
Contribution to Project: Dr. Kulkarni has served as PI for the project and has helped coordinate all aspects including sample collection and analysis. In addition, he has completed career development activities as described above.
Funding Support: UCLA departmental funds, UCLA CTSI

Name: Ramin Nazarian
Role: Project Scientist
Researcher identifier: N/A
Nearest person month worked: 3
Contribution to Project: Dr. Nazarian has helped with analysis of the tumor and blood/CTC specimens and is helping to develop novel analysis methods for single cells (including CTCs).
Funding Support: UCLA Departmental Funds

Has there been a change in the active other support of the PI or other key personnel since last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report. I am initiating collaborations with Drs. Hisham Mohammed and Julie Graff and Joshi Alumkal at OHSU and we will likely have data from this collaboration in the next progress report.

8. Special Reporting Requirements: Not applicable

9. Appendices: Not applicable