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14. ABSTRACT Melanoma is a leading cause of cancer-related mortality amongst Americans, particularly when metastatic. Although there are several new molecularly-based treatments, not all patients may be candidates due to lack of mutant genotype, and resistance often develops. The molecular events that lead to resistance are not completely 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. However, the profiles of sensitivity or resistance to PD-1 inhibition are largely unknown, particularly as the tumor may evolve in response to treatment. These studies will also help us better understand the molecular basis of sensitivity or resistance to PD-1 inhibitors, which will help to provide new scientific insights into the pathogenesis of advanced melanoma 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 melanoma.					
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1. Introduction:

Melanoma is a leading cause of cancer-related mortality amongst Americans, particularly when metastatic. Although there are several new molecularly-based treatments, not all patients may be candidates due to lack of mutant genotype, and resistance often develops. The molecular events that lead to resistance are not completely 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. However, the profiles of sensitivity or resistance to PD-1 inhibition are largely 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 melanoma CTCs and 2) identify molecular predictors of sensitivity/resistance to the use of PD-1 immunotherapies for advanced melanoma. These studies will also help us better understand the molecular basis of sensitivity or resistance to PD-1 inhibitors, which will help to provide new scientific insights into the pathogenesis of advanced melanoma 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 melanoma.

2. Keywords:

malignant melanoma, immunotherapy, PD-1 inhibition, circulating tumor cells (CTCs), liquid biopsy, personalized medicine, secondary resistance

3. Accomplishments:

Major Goals of the project (from SOW):

- i. Training and educational development in melanoma/translational research and next generation sequencing
- ii. Characterize transcriptome changes in CTCs of advanced melanoma patients
- iii. Characterize molecular profiles that predict sensitivity or resistance to PD-1 therapy

What was accomplished under these goals?

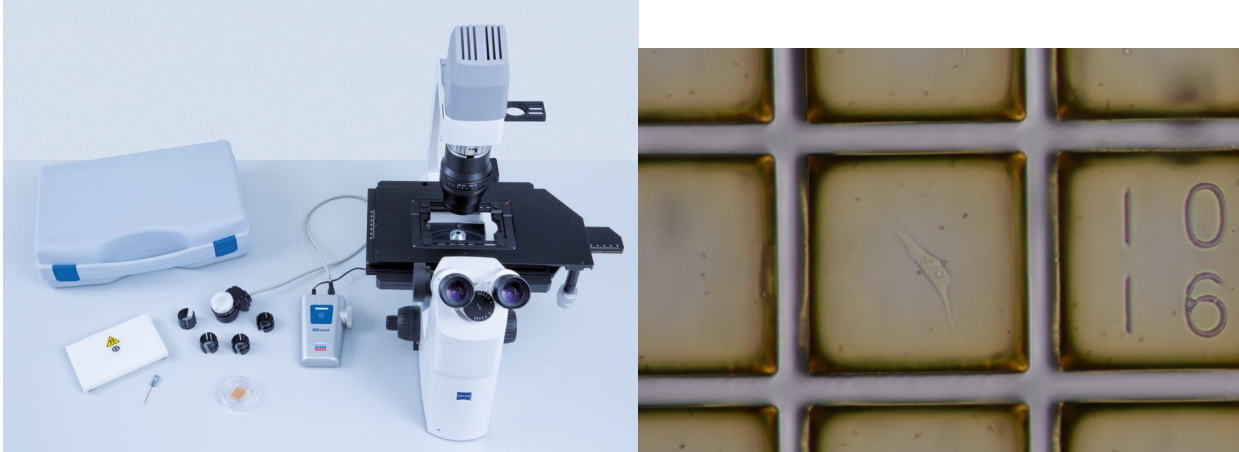
Training and educational development in melanoma/translational research and next generation sequencing

For goal 1 (training and educational development), 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 Melanoma Research Alliance meeting in 2018 (by invitation only) meeting in 2017 and plan to attend again in 2019.

Characterize transcriptome changes in CTCs of advanced melanoma patients

For this goal, we had both basic laboratory and translational approaches incorporated into the goals. For the basic laboratory approaches, I worked with Ramin Nazarian on developing and refining single cell isolation methods using Qiagen QIAScout apparatus. We have been able to successfully isolate individual melanoma cells known to be BRAF V600E positive (spiked into a whole blood sample) and can perform PCR/qPCR followed by sequencing and are able to isolate the mutant allele (see below).

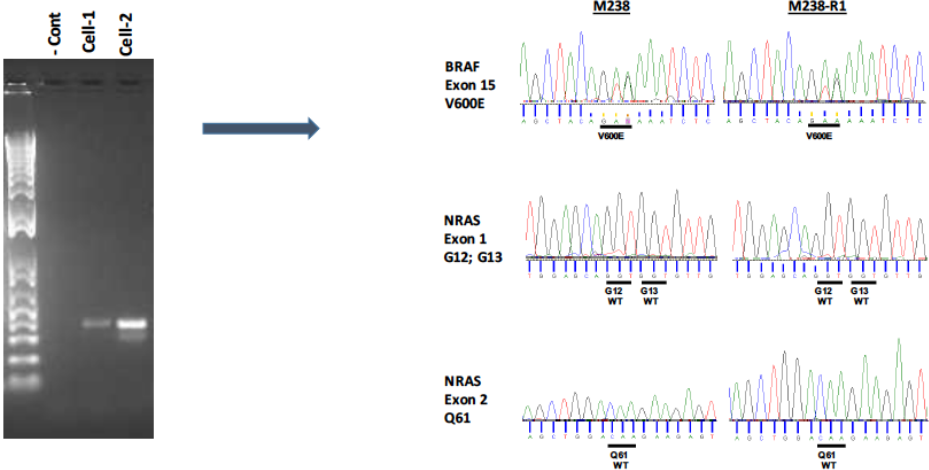


(Left) Qiagen Scout apparatus

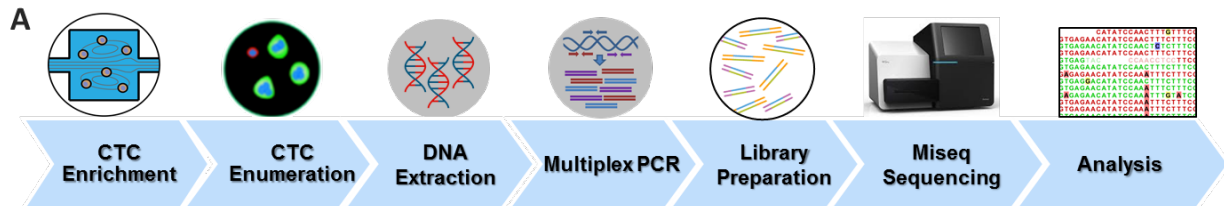
(Right) Single melanoma cell in a well

Detection of BRAF V600E point mutation in a single live melanoma cell isolated by magnetic micraft

Optimization of PCR amplification followed by sequencing of BRAF V600E mutation from a single live melanoma cell



We are now working to develop the necessary nucleic acid amplification strategies to ensure the most uniform coverage for targeted exome sequencing (see figure for analysis strategy; CTC enrichment involves using Vortex chip for enrichment followed by picking of single cells with the Qiagen QIAScout system as described). We are starting to accrue patients for the translational portion, see goal 3 below for further details.



Characterize molecular profiles that predict sensitivity or resistance to PD-1 therapy

For goal 3, there had unfortunately been some delays in receiving IRB/HRPO approvals for accruing patients for goals 2 and 3 (which were finally available in April 2018). In addition, I had to deal with family medical issues and emergencies from March until July of 2018, which are fortunately resolved. That being said, we have enrolled the first couple of patients for goal 2 and we are now beginning analysis of 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 (A375 melanoma cells). 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.

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. I have been able to attend the Melanoma Research Alliance (MRA) annual meeting (this meeting is by invitation only) in February 2018 and was invited to participate in the Young Investigator program at the MRA meeting. I have been invited again to the MRA annual meeting, scheduled for February 2019, and I plan to attend.

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 melanoma conferences, including the 2019 MRA meeting and potentially the next AACR and/or ASCO meetings in 2019. For the other goals, 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 CTCs. I also plan to continue to enroll patients as described.

4. Impact:

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

The methodologies that we are developing for single cell isolation and nucleic acid amplification using the Qiagen Scout apparatus have applicability for many fields of science; the ability to quickly and inexpensively isolate single cells is of great interest for improving our understanding of tumor heterogeneity and heterogeneity of other biological processes.

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?

Nothing to report at this time.

5. Changes/Problems:

Changes in approach

Nothing to report

Problems and delays and actions/plans to resolve them

There was a delay in starting accruals of patients due to IRB and HRPO review, followed by personal family medical emergencies. However, we now have IRB/HRPO approval and we are starting to enroll patients for analysis as described.

Changes that had a significant impact on expenditures

I switched mentors on this grant to Dr. Matthew Rettig in early 2018; this required approval by the contracting officer and an amended contract was issued to UCLA. UCLA was not able to set up spending on the account until the amended contract was issued and processed by the internal grants office, which finally occurred in late May. No funds could be spent until the grant was set up. In addition, the delay in starting patient accruals has meant that spending in the first year has been a bit delayed beyond May, 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 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: 3

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: 2

Contribution to Project: Dr. Nazarian has helped with analysis of the 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 Sancy Leachman 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