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TITLE: Targeting Resistance in Colorectal Cancer with a Novel Lineage-Tracking Technology

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CONTRACTING ORGANIZATION: The University of Texas at Austin

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
This proposal addresses the FY17 PRCRP Topic Area of Colorectal Cancer and the Military Relevance Focus Area "Gaps in cancer prognosis, treatment and/or survivorship" that impact the health of military service members, veterans, and their beneficiaries. Treatment of colorectal cancer is complicated by the high degree of intratumoral heterogeneity. Recent studies have demonstrated the utility of DNA barcode libraries in monitoring heterogeneous cell populations. This is achieved by labeling each cell with a unique, heritable sequence; lineage abundance is tracked over time by sequencing the barcode ensemble. Changes in clonal dynamics after perturbations, such as treatment with a pharmacological agent, reveal variation in lineage survival or growth rate. This approach enables the simultaneous observation of many lineage trajectories to reveal high-resolution details of cancer population dynamics. With this tool, the ability to concurrently track clonal fitness dynamics and generate lineage-specific genomic and transcriptomic data over longitudinal studies provides new insight into the response to therapeutics.

15. SUBJECT TERMS
Colorectal carcinoma, tumor heterogeneity, chemotherapy, chemoresistance, clonal dynamics, lineage tracing

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1. INTRODUCTION:

This proposal addresses the FY17 PRCRP Topic Area of Colorectal Cancer and the Military Relevance Focus Area "Gaps in cancer prognosis, treatment and/or survivorship" that impact the health of military service members, veterans, and their beneficiaries. Treatment of colorectal cancer is complicated by the high degree of intratumoral heterogeneity. Recent studies have demonstrated the utility of DNA barcode libraries in monitoring heterogeneous cell populations. This is achieved by labeling each cell with a unique, heritable sequence; lineage abundance is tracked over time by sequencing the barcode ensemble. Changes in clonal dynamics after perturbations, such as treatment with a pharmacological agent, reveal variation in lineage survival or growth rate. This approach enables the simultaneous observation of many lineage trajectories to reveal high-resolution details of cancer population dynamics. With this tool, the ability to concurrently track clonal fitness dynamics and generate lineage-specific genomic and transcriptomic data over longitudinal studies provides new insight into the response to therapeutics.

2. KEYWORDS:

Colorectal carcinoma, tumor heterogeneity, chemotherapy, chemoresistance, clonal dynamics, lineage tracing

3. ACCOMPLISHMENTS:

What were the major goals of the project?
There are two major goals of the project, as described in the approved SOW.

Aim 1: To utilize a novel cell lineage tracking system BAAR to measure cell lineage dynamics and isolate specific lineages of interest in cultures of patient-derived colorectal carcinoma.

Aim 2: To demonstrate the utility of BAAR for the identification of tumor subpopulations that vary in chemosensitivity and resistance.

Specific Aim 1: To utilize a novel cell lineage tracking system *BAAR* to measure cell lineage dynamics and isolate specific lineages of interest in cultures of patient-derived colorectal carcinoma cells.	**Timeline**
Major Task 1: To validate the BAASE lineage tracking and isolation in patient-derived colorectal carcinoma cells (mutant KRAS)	
Subtask 1: Local IRB review has been completed (March 2018) and all documents have been submitted for HRPO review and approval. No work will commence on colorectal cells until HRPO review and approval is complete.	1-3

Subtask 2: 3 CRC cells lines will be obtained from Drs. Eckhardt and Van Den Berg at the Developmental Therapeutics Lab and maintained as organo-cultures. These organo-cultures will be labeled with reference barcode libraries.	3-6
Subtask 3: Recall plasmids for the reference barcode sets will be constructed and confirmed by Sanger sequencing.	1-2
Subtask 4: Transfection parameters will be optimized for Recall plasmid delivery.	3-6
Subtask 5: Activation of a GFP reporter gene by the Recall plasmid will be measured by flow cytometry. The percent of GFP+ cells will be compared to the known composition of the reference populations to measure the false positive/false negative rates of lineage activation.	6-12
Milestone(s) Achieved: To use a low diversity reference population of barcodes to establish the parameters for barcode-driven reporter activation and low false positive/false negatives rates in primary CRC.	
Major Task 2: Label CRC cell with high diversity barcode library	
Subtask 1: An additional set of 3 cell lines will be obtained from the Developmental Therapeutics Lab and maintained as organo-cultures.	4-12
Subtask 2: All 6 cell lines from MT1 and MT2 ST1 will be labeled with a high diversity BAASE barcode library, using the parameter conditions optimized in Major Task 1.	9-12
Subtask 3: Activation of Recall plasmid GFP expression by flow cytometry. Transfection conditions, dCas9- VPR ratios, and timing of isolation from Major Task 1 will be confirmed in these 6 cell lines.	9-12
Milestone(s) Achieved: To confirm the experimental conditions for barcode-driven reporter activation and low false positive/false negative rates in six high diversity populations of barcode labeled primary CRC.	

Specific Aim 2: To demonstrate the utility of *BAAR* for the identification of tumor subpopulations that vary in chemoresponsiveness and resistance.		
Major Task 1: To isolate lineages resistant to irinotecan, oxaliplatin and 5-FU		

Subtask 1: The 3 cell lines in MT1 will be used to establish baseline dose response curves. IC_{50} will be measured for CRC organo-cultures treated with irinotecan, oxaliplatin and 5-FU alone and in combination.	3-6	Brock
Subtask 2: Barcode labeled organo-cultures of all 6 cell lines will be treated with irinotecan, oxaliplatin and 5-FU, alone and in combination. A control population will be monitored without any drug treatment (vehicle only).	12-15	Brock
Subtask 3: For each of 6 cell populations, barcode ensembles will be quantified by NGS and between 2 and 3 high abundance resistant lineages will be identified.	12-18	Brock
Subtask 4: Recall plasmids will be constructed to isolate high abundance resistant lineages.	15-18	Brock
Subtask 5: Resistant lineages will be isolated by GFP activation and FACS	15-18	Brock
Milestone Achieved: Here we will identify and isolate the top 2-3 cell lineages which proliferated despite drug treatment. These will be identified for all 6 tumor cell populations.		
Major Task 2: To characterize resistant lineages by RNASeq and determine their sensitivity to other therapies.		
Subtask 1: Transcriptomes of isolated resistant lineages will be measured by RNASeq to identify altered signaling pathways that may be targeted by other therapies. The CTEP database will be examined for matching drug targets.	18-24	
Subtask 2: Isolated lineages will be subcultured and LD_{50} will be measured to the other drugs and combinations in this proposal (irinotecan, oxaliplatin and 5-FU).	18-24	
Milestone(s) Achieved: We expect to demonstrate feasibility of the BAASE system to identify and isolate specific cells of interest in the population response to chemotherapy and the onset of resistance.	24	

What was accomplished under these goals?

In Major Task 1, we have completed Sub-tasks 1-3 for one CRC cell line. Due to delays in HRPO review (approved July 2019), we have so far been able to perform these studies with one CRC cell population. Having now established these proof-of-principle studies for one cell line, we are currently expanding to include additional cell populations. Organoid cultures were labeled with a barcode library and cells positive for the transduced barcodes were identified by mCherry expression and collected by FACS to instantiate the labeled cell population. In this step, we optimized the transduction conditions in order to achieve an MOI of 0.074. This is critical because an MOI greater than 0.1 would increase the likelihood of an individual cell receiving two different labels (by Poisson statistics), requiring more complicated informatics analysis to deconvolve the correct barcodes.

We tested the efficiency of various transfection protocols for these cells, including spinoculation and nucleofection, and multiple liposome-based delivery reagents. We were able to achieve 28% activation of GFP in organoid CRC cells using liposome-based transfection

In next project period we will be focused on applying these tools— by treating barcoded patient-specific cell populations with cytotoxic and targeted therapeutic agents, measuring changes in clonal survival, and characterizing the resistant subpopulations. In addition, we will be able to expand the established methodology from one patient cell population to the additional cell lines described in the SOW. In our Statement of Work these tasks correspond to Major Task 1, Subtasks 3-5 and Major Task 2 in Specific Aim 1 and the studies described in Specific Aim 2.

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

Graduate students supported by this grant participate in ongoing training and professional development activities. Within the department, trainees participate in weekly lab group meetings to share technical skills and gain practical experience in critical evaluation of the literature, oral communication and experiment design.

At the department level, students participate in two biweekly seminar series—one on Cancer Biology and one focused on Computational Oncology. A student-led troubleshooting session provides an additional setting for peer mentoring in computational work and statistical analyses. This session includes research scientists and postdocs, as well as graduate students, and is highly interactive.

At the college/university level, trainees have participated in two short courses through The University of Texas at Austin Genomics and Sequencing Analysis Core and the Texas Advanced Computing Center to improve technical skills in bioinformatics and computational biology. In combination with foundational course work, these classes have enabled students to gain new skills in informatics; this has proved useful for analysis of the NGS data in this project.

How were the results disseminated to communities of interest?

The PI has presented seminars locally at the Livestrong Cancer Center, Dell Medical School, to educate clinicians and researchers about the potential utility of the tools developed in this work.

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

In Fall 2019 and Winter 2020, we were able to complete the labeling of 2 patient-specific cell populations (Major Task 2, Subtask1-2) and confirm the accuracy of recall (Aim 1, Major Task 2, Subtask 3). We performed dose response studies to determine the IC50 for these cells (Aim 2, Subtask 1-3) which lays the groundwork for all further Aim 2 studies.

We were of course impacted by the COVID-19 shutdown beginning in March 2020 but are now working to label one additional patient-specific population and will focus on the remaining Aim 2 studies with chemotherapy-treated populations for the next year.

4. IMPACT:

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

This project has accomplished the important step of demonstrating that this barcoding technology can be utilized in patient-derived tumor cells. This is a key step, as our earlier work has used only established, immortalized cell lines which have been maintained in laboratories for many years and thus have some biological differences from cancer cells that are treated in the clinic. This proof-of-technology has opened up feasibility for other collaborators to also use this system in patient-derived tumor cells and we will continue to make these tools available for wide sharing.

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?

Nothing to report.

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

In year 1, we experienced a significant delay in HRPO review of our documents (supporting documents uploaded via eBRAP Aug 2018, supporting documents submitted by email Dec 27, 2018; HRPO clarification requested by email May 20, 2019; final decision received by email July 15, 2019). HRPO agreed with the previous recommendation of our local institutional review board and this research was deemed exempt.

We experienced COVID-19 related delays in spring 2020 but now have all lab members working back in the wet lab and making progress towards the aims of this project.

Changes that had a significant impact on expenditures

Spending was reduced in spring to summer 2020 due to the pandemic.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Significant changes in use of biohazards and/or select agents

Nothing to report

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **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:	Amy Brock, Ph.D.
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	amybrock (eRA Commons)
Nearest person month worked:	1
Contribution to Project:	Dr. Brock oversees all aspects of the project, coordinates and plans studies, contributes to data analysis and interpretation and communication.
Funding Support:	n/a
Name:	Carla Van Den Berg, PharmD
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	vandenbc (eRA Commons)
Nearest person month worked:	1
Contribution to Project:	Dr. Van Den Berg…
Funding Support:	n/a
Name:	Gail Eckhardt, MD
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	Eckhardt.Gail (eRA Commons)
Nearest person month worked:	1
Contribution to Project:	Dr. Eckhardt…
Funding Support:	n/a
Name:	Daylin Morgan
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Mr. Morgan constructed the barcode libraries, established the plasmids for the study, optimized transfection conditions of PDO cells and performed dose response studies.
Funding Support:	n/a
Name:	Russell Durrett
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Mr. Durrett assisted in design and construction of barcode libraries and performed analysis of library diversity.
Funding Support:	n/a

Name:	Eric Brenner
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Mr. Brenner assisted in design and construction of barcode libraries and performed analysis of library diversity
Funding Support:	n/a

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A

9. APPENDICES: None