

AWARD NUMBER: W81XWH-18-1-0767

TITLE: High-Throughput Screen of Advanced Prostate Cancer Organoids and PDX Preclinical Trials to Identify Single and Combination Therapies Correlated with Genotype

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REPORT DATE: October 2019

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel
Command Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE Oct 2019		2. REPORT TYPE Annual Report		3. DATES COVERED 30 Sep 2018 – 29 Sep 2019	
4. TITLE AND SUBTITLE High-Throughput Screen of Advanced Prostate Cancer Organoids and PDX Preclinical Trials to Identify Single and Combination Therapies Correlated with Genotype				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-18-1-0767	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Kathleen Kelly, PhD E-Mail: kellyka@mail.nih.gov				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Geneva Foundation 917 Pacific Ave. Suite 600 Tacoma WA 98402 (253) 383-1398				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S) USAMRMC	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The goal of our proposal is to identify new highly effective agents and combinatorial therapies and identify biomarkers of responsive tumors for clinical trial patient selection. To achieve this goal, we have developed new methodology, and our research will provide validation of a platform that will allow fast, economic testing of new treatment strategies that provide highly translational results. We are proposing one of the first comprehensive screens with clinically relevant models of advanced prostate cancer. Our models, patient-derived xenografts, were established directly from patient tumors and grown in mice. These tumors were shown to look like and behave like tumors in the patients. However, because these models are growing in mice, they cannot readily be used for large scale studies. To address this shortcoming, we have recently optimized the conditions to take these tumors from mice and grow them in three dimensional cultures (organoids) in the laboratory. Moreover, in preliminary experiments we have achieved reproducible results screening drugs with these organoids in a robot-assisted drug screening facility. The use of advanced prostate patient-derived xenografts, the robust growth of prostate organoid cultures and the use of organoids in high-throughput screening approaches are all tremendous advances for the field.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

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1. Introduction:

Metastatic castration resistant prostate cancer (mCRPC), which develops in response to suppression of androgen receptor pathway signaling, is responsible for almost all prostate cancer-related deaths. The development of therapeutic approaches for advanced prostate cancers have centered upon androgen receptor (AR) signaling pathway inhibition (ARIs), sometimes followed by taxane or platinum chemotherapeutics. Thus, there are multiple agents for the same target, AR, but few agents for other key vulnerabilities. However, clinical and genomic characterization of mCRPC tumors have revealed substantial heterogeneity with respect to various drivers of disease progression and mechanisms of resistance. Outside of ARI based therapies, *BRCA1* and *BRCA2* deficiencies are the only approved genomic biomarkers for targeted therapies in CRPC. We seek to discover additional effective therapies for mCRPC and to identify phenotypic or genomic properties that guide their use. This project takes advantage of using a large collection of mCRPC patient derived xenografts (the LuCaP PDX cohort) that represent the genomic and phenotypic diversity of patient tumors in combination with newly developed organoid culture techniques that have enabled in vitro growth of the above PDX models. The purpose of the project is to establish novel efficacious drug responses, singly and in combination, and to identify associated molecular markers. The scope of the project encompasses a high throughput in vitro drug screen across about 40 organoid models using 110 drugs, selected in vivo PDX clinical trial validation, and computational analyses to identify molecular correlates of drug responses.

2. Keywords:

SPECIFIC AIM 1:

The first major goal/milestone of this project is to identify effective single therapeutic agents with a target date for completion in the first 18 months. The evaluation of therapeutic agents encompasses an initial in vitro high throughput screening phase, which is 90% completed. The first in vivo analyses are in progress.

SPECIFIC AIM 2:

The second milestone is the identification of novel effective combination therapies based upon knowledge gained from the first milestone. These novel compounds are analyzed in vitro and in vivo with established therapies (enzalutamide for adenocarcinoma and carboplatin for neuroendocrine prostate cancers) and with each other. The target date for completion is the end of the granting period; our progress till today represents about 15% of the anticipated analyses.

MAJOR TASK 1: Preliminary in vitro screens have been completed.

MAJOR TASK 2: In vivo combination screens with carboplatin are in progress.

SPECIFIC AIM 3:

The milestone is the identification of predictive biomarkers in silico and their validation in vivo. This work spans the granting period and initially results from analyzing in vitro therapeutic responses relative to molecular (transcriptomic and genomic) markers.

MAJOR TASK 1: RNA has been collected as biological duplicates of organoid cultures from 40 models. Samples were submitted for sequencing August 2 2019 and the results are anticipated to be returned any day.

MAJOR TASK 2: Initial platform tools have been built and used for these analyses, and a portion of the molecular data has been collected.

Key Research Accomplishments

1) Major activities that are completed or ongoing:

- a) Import, verify, and characterize the organoid growth characteristics of LuCaP PDX models, b) perform high throughput drug screens on organoids, c) determine the range of accuracy for high throughput drug screens, d) consult with Palantir platform software engineers to establish drug response pipeline, build additional tools for analysis and visualization as well as biomarker correlations, e) organize and analyze drug screening data, f) summarize and generate graphical representations of data, g) collect organoids for RNAseq analysis, h) perform preliminary correlation analyses for drug responsiveness and PDX transcriptomic data, i) obtain ACURO approval, j) select initial PDX clinical trial therapeutics and models and initiate trial, k) coordinate and optimize collaborative goals (participate in monthly conference calls between Kelly and Corey laboratories and meet twice per year in person).

2) Specific objectives- year 1:

- a) Identify single agent therapeutics with activity in advanced prostate cancer, b) select initial therapeutics and models from in vitro analysis and begin testing single agent in vivo responses, c) initiate in vitro assays combining enzalutamide or carboplatin with single agent screens, d) obtain RNAseq data from organoids to begin PDX RNAseq and drug correlation analyses.

3) Significant outcomes:

- a) The major accomplishment in this reporting period has been the screening of 35 CRPC organoid models (>45 high throughput screens) against more than 110 drugs. Importantly, as this is one of the first comprehensive organoid screens, the screening has been validated using independent biological replicates in both robotic high throughput and lab bench formats (Fig. 1-appendix). High throughput screening has been carried out by plating organoids in a 3D matrix in 384 well plates, using 10 serial dilutions for each therapeutic, seven day incubations with 2 media changes, and duplicate plates for averaging. The analysis of the data has been accomplished using a customized program built on the Palantir data base platform. The ability to analyze a large cohort of PDX-based organoids has required significant infrastructure support to import, grow, verify, and characterize the organoid growth characteristics of a large number of PDX models. One adjustment we have made to optimize the amount of data collected has been to modify our therapeutics library after the first 20 screens, eliminating therapeutics that showed no evidence of activity (about 20 drugs) and replacing these with other drugs of interest. Several conclusions can be drawn from the HTS data. We have analyzed the data using various different response parameters.
- b) A relatively stringent response criteria ($IC_{50} < 1\mu M$ and Max response <20%) showed that approximately one-third of the drugs (~40) were active against at least one model. This data identified potentially specific drugs and drug classes with the most promise for advanced prostate cancer.
- c) About 20 drugs demonstrated a distributed range of responses across models. These drugs are most promising for exhibiting specificity relative to genotypic and phenotypic markers, and we are concentrating the majority of our efforts for single agent responses with these drugs. Normalized area under curve (nAUC) is used as a more definite numerical characterization for drug response as it allows for statistical regression with genomic and transcriptomic expression data in search of biomarkers.

- d) In considering response phenotypes relative to phenotypic classes, apoptosis modulators and signaling inhibitors demonstrated the most heterogeneity (Fig. 2-appendix).

4. Changes/Problems

We plan to make a technical change that does not impact the goals or scope of the project but simply involves an improvement to the proposed procedure. We originally proposed to analyze HTS data in the continuous presence of enzalutamide for five adenocarcinoma models and in the presence of carboplatin for five neuroendocrine/double negative models. Our initial analysis of one adenocarcinoma and two neuroendocrine models in the proposed format showed no IC₅₀ or MaxR shifts. Although there may be technical reasons in the performance of the high throughput screening assay (such as increased variability) that contribute to this result, it is also likely that other parameters such as the length of the assay may need to be individualized for specific therapeutics. HTS assays are not practical when individualized parameters must be considered. We believe that the most reliable results will be obtained by focusing on active single agents combined with the above appropriate standard of care treatments performed “by hand” with the incorporation of various assay variables such as length of assay and order of therapeutic addition.

5. Conclusions

In year 01 we have accomplished all tasks proposed for this period and in years 02-03, we will continue the experiments as proposed with the exception of the alteration described above.

6. Publications

- a) Abstract for the Annual Prostate Cancer Foundation Meeting: “Organoid culture screen for combination therapies to target aggressive variant prostate cancer,” David VanderWeele, Keith Jansson, Ilya Senatorov, Remi Adelaiye-Ogala, Yen Thi Minh Nguyen, Adlai Grayson, Aian Neil Alilin, Supreet Agarwal, Michael Beshiri, Craig Thomas, Kathleen Kelly

7. Inventions, Patents and Licenses

None

8. References

None

9. Appendix

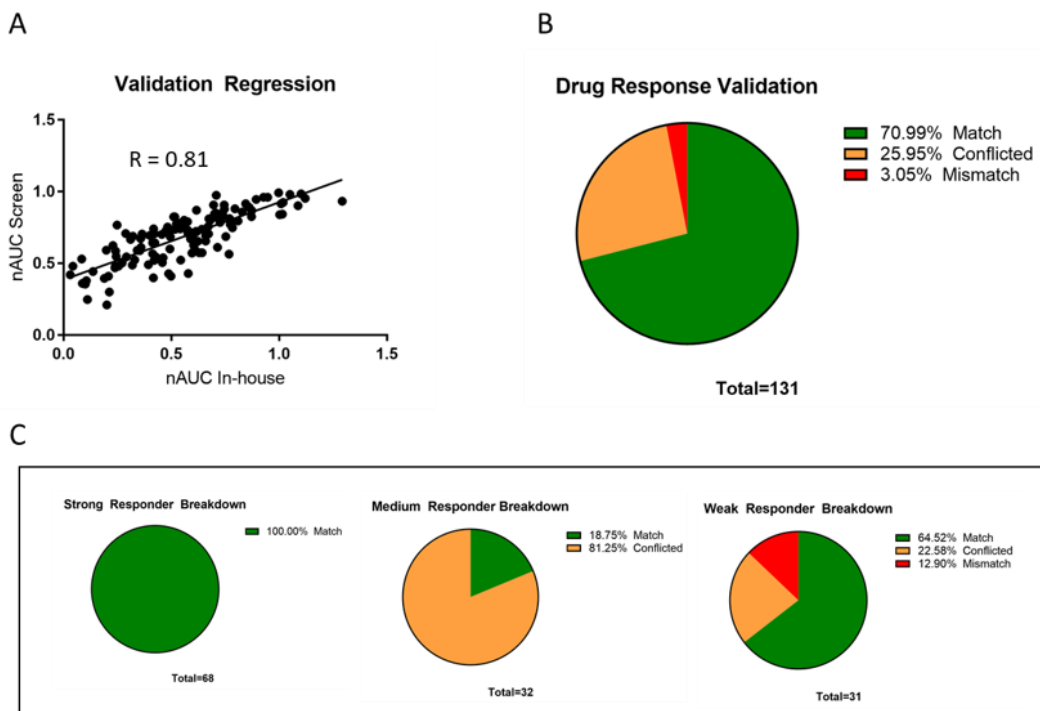


Figure 1. (A) Linear Regression of normalized Area Under Curve (nAUC) derived from the screen matched with the same model-drug pair tested as a biological replicate in-house. (B) Compound-specific validation using screen-wide nAUC average with 'Strong Responders' classified as organoid responses falling in the top 33%, 'Weak Responders' as the bottom 33%, and all others as 'Medium Responders'. 'Match' conditions require preserved response class from screen to in-house, 'Conflicted' are Medium Responders in the screen which classified as Weak or Strong in-house, and 'Mismatch' requires a screened Strong Responder to come up as Weak Responder in-house, or vice versa. (C) For each Responder Class, percentages of compounds matching, conflicting, or mis-matching.

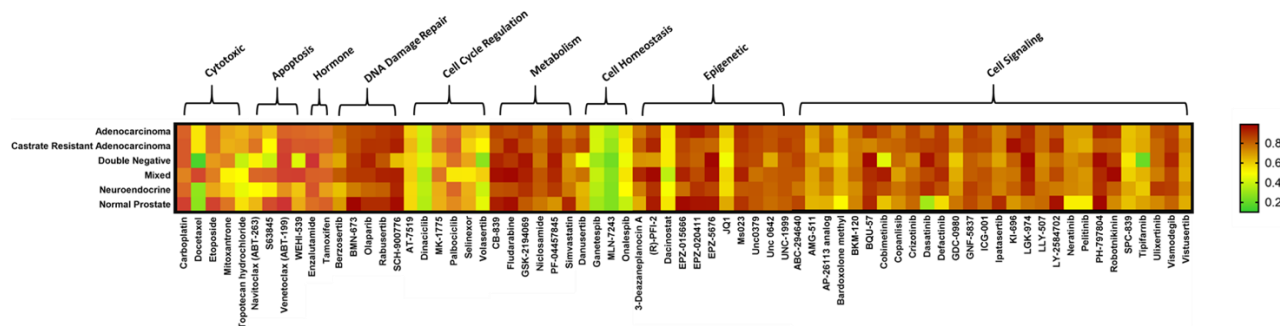


Figure 2. Heatmap representing averaged normalized Area Under Curve (nAUC) as a numerical measure of drug efficacy for each compound against phenotypically distinct model cohorts of CRPC: Adenocarcinoma (n = 18), Castrate-Resistant Adenocarcinoma (n = 5), Double Negative (n = 1), Mixed (n = 1), Neuroendocrine (n = 6), Normal Prostate (n = 1).

PC170244: High-Throughput Screen of Advanced Prostate Cancer Organoids and PDX Preclinical Trials to Identify Single and Combination Therapies Correlated with Genotype



PI: Kathleen Kelly, PhD., NCI, Maryland

Budget: \$765,046.00

Topic Area: Prostate Cancer Research Program

Mechanism: W81XWH-17-PCRP-IDA

Research Area(s): Prostate Cancer

Award Status: 9/30/2018 through 9/29/2021

Study Goals:

1. Our goal is to guide the design of future clinical trials for aggressive prostate cancer and the optimum patient selection for those trials.
2. Our objectives are
 1. To establish pre-clinically validated efficacious drugs and drug combinations together with predictive molecular correlates when possible, and
 2. Analyze and provide to the prostate cancer research community a large data set encompassing CRPC drug responsiveness for genotypically and phenotypically characterized patient-derived samples

Specific Aims:

- **Aim 1: Identify agents among a comprehensive, actionable drug library with high anti-tumor suppressive activity using PC organoids and patient-derived xenografts**
- **Aim 2: Determine efficacy of combinatorial treatment strategies of selected agents**
- **Aim 3: Integrate and analyze organoid/PDX molecular characteristics against response to therapeutic regimens and identify molecular determinants of response and candidate predictive biomarkers**

Publications: None to date

Patents: None to date

Funding Obtained: \$765,046.00