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
PRINCIPAL INVESTIGATOR:	Dr. Kathleen Kelly
CONTRACTING ORGANIZATION:	The Geneva Foundation 917 Pacific Ave #600 Tacoma, WA 98402
REPORT DATE:	October 2020
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; Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2020	Annual Report	30 Sept 2019 - 29 Sept 2020

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 W81XWH-18-1-0767

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Kathleen Kelly

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. # 600
Tacoma, WA 98402

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)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Objective: Our goal is to guide the design of future clinical trials for aggressive prostate cancer and the optimum patient selection for those trials. 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.

Impact: This innovative proposal is designed to address a major limitation in our knowledge concerning the breadth of therapeutic vulnerabilities for advanced prostate cancer and the molecular properties associated with drug responsiveness. If successful, we expect that novel combinations comprised of clinically translatable agents could proceed directly to biomarker-driven phase II clinical trials, addressing the PCRP Overarching Challenge to develop effective treatments and address mechanisms of resistance for men with high-risk or metastatic prostate cancer, and the PCRP Focus Area of Therapy and Mechanisms of Resistance and Response. Indeed, the NIH Clinical Center is well-poised to conduct such a trial. In addition, the availability of an extensive drug response database will provide to the community a platform that can be further leveraged for preclinical studies, bioinformatics/statistical mining, and mechanistic analysis.

15. SUBJECT TERMS
Oncology, Cancer, Prostate Cancer

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	10	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

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.

2. KEYWORDS:

Prostate cancer, high throughput screening, organoids, patient-derived xenografts, effective treatment combination therapy

3. ACCOMPLISHMENTS:.

What were the major goals of the project?

Key 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.

What was accomplished under these goals?

1) **Major activities that are completed or ongoing:**
   - **a)** Complete the high throughput drug screens on organoids and on 2D and 3D LNCaP and RWPE models, b) organize and analyze drug screening data, c) summarize and generate graphical representations of data, d) perform in vitro analysis of potential drug combinations utilizing drug-model combinations selected from the high throughput screen, e) analyze RNAseq data obtained from organoids grown under the same conditions as used for the drug screen, f) perform preliminary correlation analyses for drug responsiveness and molecular markers, including PDX transcriptomic data, g) select initial PDX clinical trial therapeutics and models and initiate trial, h) 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 2:**
   a) Complete identification of single agent therapeutics with activity in advanced prostate cancer, b) perform in vitro assays for drug combinations, selected based upon the HTS data, including the standard of care drugs, enzalutamide and carboplatin, c) perform PDX clinical trials based upon in vitro drug responses to single agents and combinations, d) Begin PDX RNAseq and drug correlation analyses.

What opportunities for training and professional development did the project provide?
How were the results disseminated to communities of interest?
The Kelly lab and Corey lab teams meet monthly to discuss ongoing experiments and the interpretation of results. Drs. Corey and Kelly met at the PCF2019 annual meeting and were schedule to meet at the AACR Prostate 2020 meeting, which was cancelled due to the COVID pandemic. In addition, Dr. Kelly meets regularly with her team via one-on-one and group meetings.

How were the results disseminated to communities of interest?
Preliminary results were disseminated via an oral presentation by Dr. Kelly ("High-Throughout Drug Screening in a Clinically Representative Cohort of mCRPC Patient-Derived Organoids*"*) at the Annual Prostate Spore retreat, February 2020.

What do you plan to do during the next reporting period to accomplish the goals and objectives?
We will continue in-depth bioinformatics analyses of the HTS in combination with molecular markers (mutational and transcriptomic features) to establish relationships between drug responses and molecular phenotypes. We will continue to test promising drug combinations deduced from the HTS in conjunction with the statistical analyses, first in vitro and then in vivo. We will expand the promising, ongoing PDX clinical trials to establish sufficient statistical significance of the results. We will continue to test additional models and proceed to investigation of molecular correlates of the response.

4. IMPACT:

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

The HTS data will be widely used throughout the prostate cancer community. We anticipate publishing the data in 2021 following additional bioinformatics analyses. This is the first comprehensive drug screen coupled with molecular markers, allowing generalizations and in-depth correlative analyses. The data will be used by basic researchers investigating mechanisms of drug response as well as translational/clinical investigators designing clinical trials. In particular, we have identified treatments and combination treatments that were highly effective in vivo for neuroendocrine prostate cancer.

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
Our progress was somewhat slowed down due to the COVID situation. Investigations at NIH were suspended for approximately 4 months. However, we are adjusting to a new normal and starting additional bioinformatics, in vitro, and animal studies, albeit safety precautions limit work hours and continue to slow work to some degree. We do meet regularly via virtual meetings.
Experimentally, we have encountered toxicity of some of the treatments and the combinations used, so we are adjusting the doses of the agents for the subsequent studies. Otherwise, we are continuing as planned and described in SOW.

Actual or anticipated problems or delays and actions or plans to resolve them:
Please see above.

Changes that had a significant impact on expenditures
The work slowdown is reflected in our use of less supplies than would be the case otherwise.

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:
Nothing to report.

Significant changes in use of biohazards and/or select agents:
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:

Preliminary results were disseminated via an oral presentation by Dr. Kelly ("High-Throughout Drug Screening in a Clinically Representative Cohort of mCRPC Patient-Derived Organoids ") at the Annual Prostate Spore retreat, February 2020.

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:

Name:	Craig Thomas, PhD
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	0000-0001-9386-9001
Nearest person month worked:	1
Contribution to Project:	Directed HTS infrastructure
Funding Support:	NIH (NCATS, NCI)

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:

QUAD CHARTS:
See attached

9. APPENDICES:

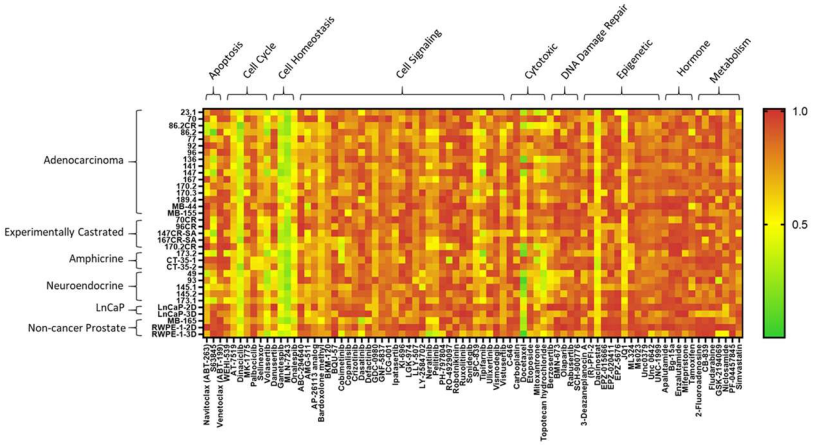


Figure 1: High-throughput drug screen results of compounds across multiple mechanistic classes tested across a cohort of phenotypically distinct prostate cancer patient-derived organoids. Data represented as normalized area under the curve (nAUC).

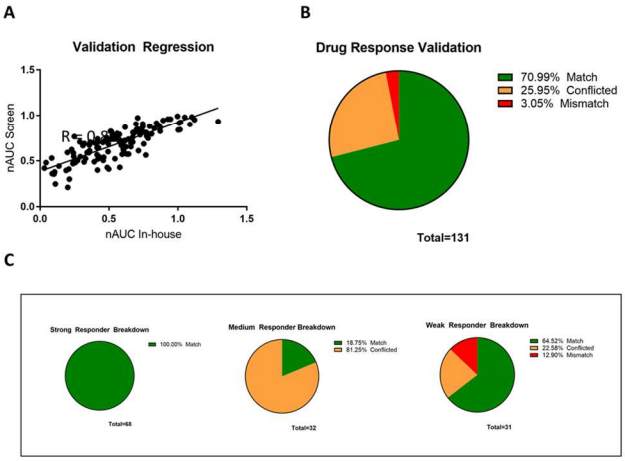


Figure 2: (A) Linear Regression of normalized Area Under Curve (nAUC) derived from the screen matched with the same model-drug pair tested as a biological replicate in-house. (B) Compound-specific validation using screen-wide nAUC average with 'Strong Responders' classified as organoid repsonses falling 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 perform as a Weak Responder in-house or vice versa. (C) For each Responder Class, percentages of compounds matching, conflicting, or mismatching.

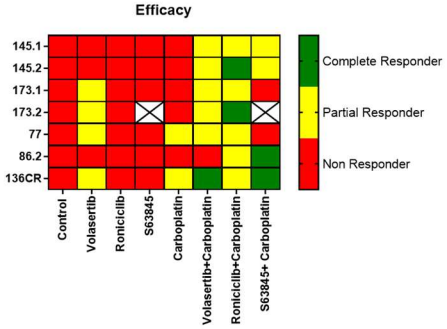


Figure 3: In-vivo response summaries to monotherapy or combination therapy with carboplatin

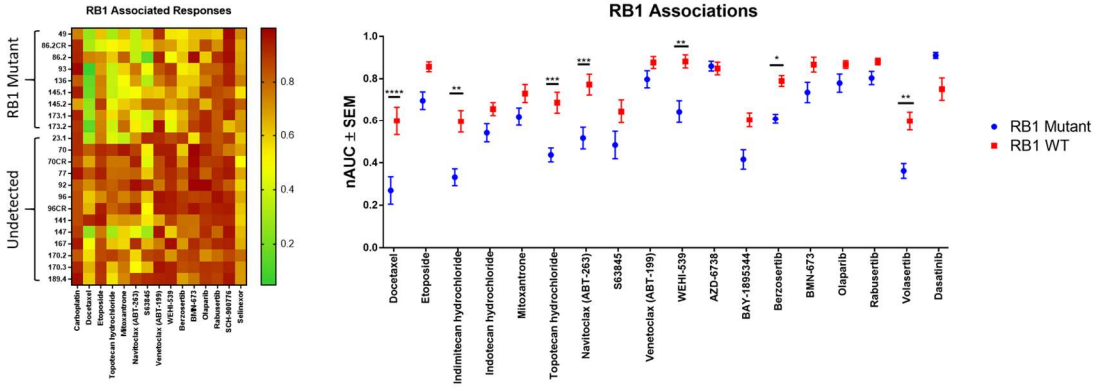


Figure 4: RB1 status segregates models in terms of drug efficacy for a variety of compounds spanning cytotoxic, apoptosis, DNA damage repair, and cell cycle regulatory mechanisms. **** denotes $p < 0.001$, *** denotes $p < 0.005$, ** denotes $p < 0.01$, * denotes $p < 0.05$

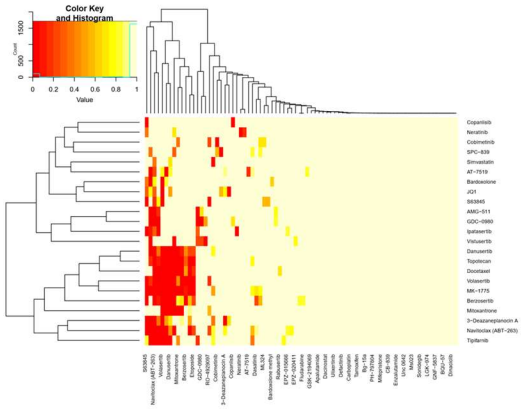


Figure 5: Heatmap representing p-values showing statistically co-enriched positive responses across screened models based on individual compounds compared to other compounds in the screen to suggest options for combination therapy. Agents targeting mitotic and replicative processes, apoptosis, cell cycle regulation, and DNA damage repair have significant efficacy overlap.

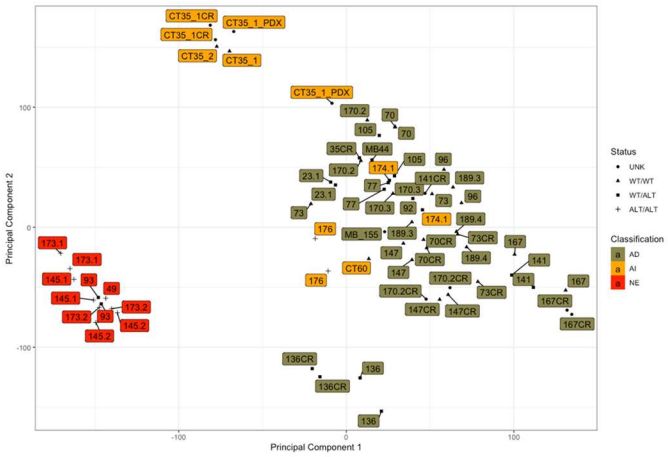
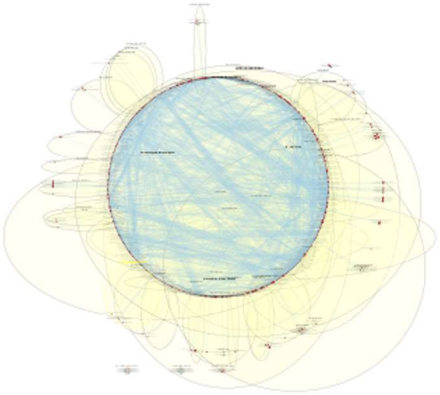


Figure 6: Principal components analysis plot of organoid RNA sequencing data. Individual models are labelled with colors by their histological classifications; adenocarcinoma (AD), amphicrine (AI), and neuroendocrine (NE). Mutational status of RB and TP53 is encoded using shape with status recorded as unknown at both loci (UNK), or wildtype (WT) or alternative (ALT) at both loci for RB and TP53 respectively.



Cluster	Nodes
wnt noncanonical	25
ifn interferon	16
met interacts interleukin	14
presentation folding disease	11
ichiba sengupta nasopharyngeal	9
apoptotic programmed bh3	8
vart pre notch	7
metabolism enzymes	6
chk1 chk2 cds1	6
dorn adenovirus infection	6
cd8 tcr 5pathway	5
environmental stress kyng	5
foxo acetylation classii	5
g1e regulatore nunoda	5
kokkinakis methionine deprivation	5
large mori immature	5
masri aromatase inhibitors	5
mclachlan dental caries	5
negative pi3k akt	5
capped intron mrna	4
cards korkola tumor	4
cytotoxicity dap12 nkcells	4
linoleic acid derivatives	4
mcbryan end bud	4
relapse bone smid	4
tnf signaling nfkb	4
yap1 taz stimulated	4

Figure 7: Circos plot showing how differentially expressed genes between docetaxel responders and non-responders cluster into signaling nodes of known biological processes providing an overview of the biological difference between responsive and nonresponsive CRPC