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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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14. ABSTRACT Despite the advances in our knowledge of prostate cancer (PC) biology and the widespread use of new FDA approved drugs (e.g. abiraterone acetate (AA), enzalutamide (ENZA), cabazitaxel, Radium 223, and Sipuleucel T), approximately 27,000 men will still die this year of advanced PC ¹ . <u>Essentially no patients with metastatic PC are cured of their disease.</u> Thou, substantial research efforts continue to be directed toward identifying new targets and therapeutics for metastatic castration resistant PC (mCRPC), <u>it is challenging to develop new effective therapies for mCRPC.</u> 1) There are multiple agents for the same target but no agents for other key factors. 2) Majority of new trials evaluate single agents and not combinations despite clear evidence in other malignancies of superior efficacy of combinations. 3) There are limited numbers of patients with mCRPC enrolling in clinical studies, a fact that impedes the breadth of drugs tested and the pace of evaluation. 4) There is a growing body of evidence demonstrating that mCRPC exhibits substantial inter-individual heterogeneity with respect to underlying molecular drivers. Only a few trials are designed using precision medicine paradigms that select patients based on molecular predictors of response and consequently the future <i>optimal deployment</i> of a therapeutic is hindered. We will perform high throughput screen using PC organoids to identify active combinatorial therapies and validate the outcome in vivo in patient derived xenografts clinical trial.					
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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.

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 scheduled to meet at the AACR Prostate 2020 meeting, which was cancelled due to the COVID pandemic. In addition, Dr. Kelly and Dr. Corey meet regularly with their teams 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-Throughput 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?

In year 3 we will carry on as planned and depicted in SOW. 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 and UW 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-Throughput 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. SPECIAL REPORTING REQUIREMENTS:

QUAD CHARTS:

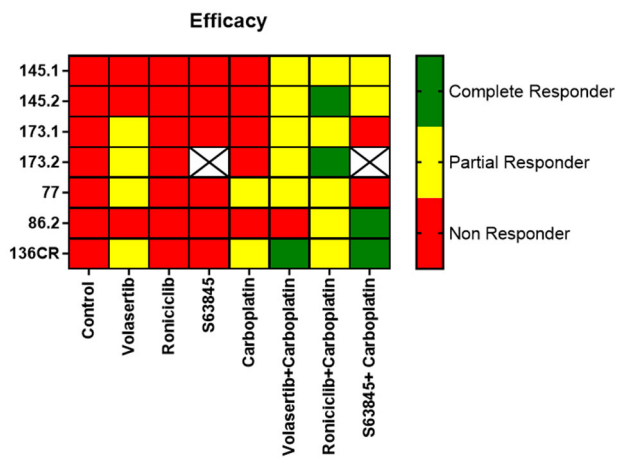


Figure 3: In-vivo response summaries to monotherapy or combination therapy with carboplatin

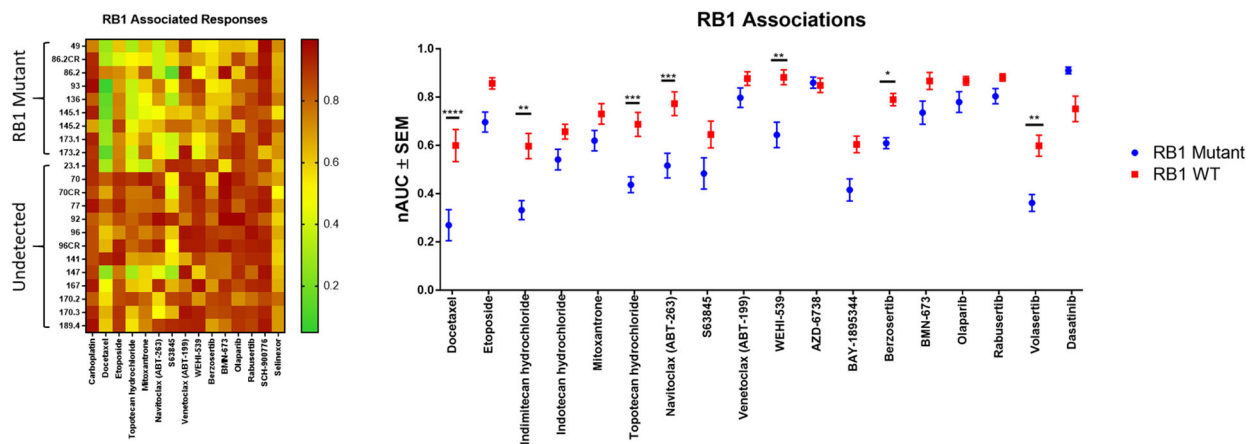


Figure 4: RB1 status segregates models in terms of drug efficacy for a variety of compounds spanning cytotoxic, apoptosis, DNA damage repair, and cell cycle regulatory mechanisms. **** denotes $p < 0.001$, *** denotes $p < 0.005$, ** denotes $p < 0.01$, * denotes $p < 0.05$

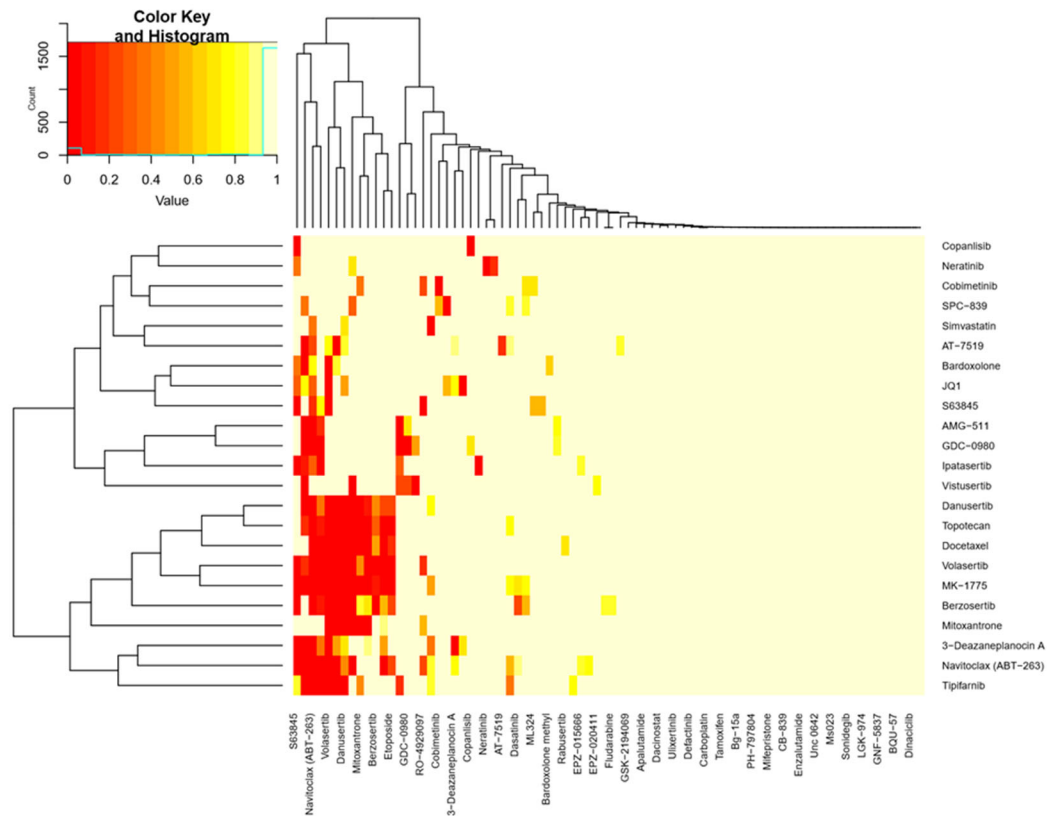


Figure 5: Heatmap representing p-values showing statistically co-enriched positive responses across screened models based on individual compounds compared to other compounds in the screen to suggest options for combination therapy. Agents targeting mitotic and replicative processes, apoptosis, cell cycle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

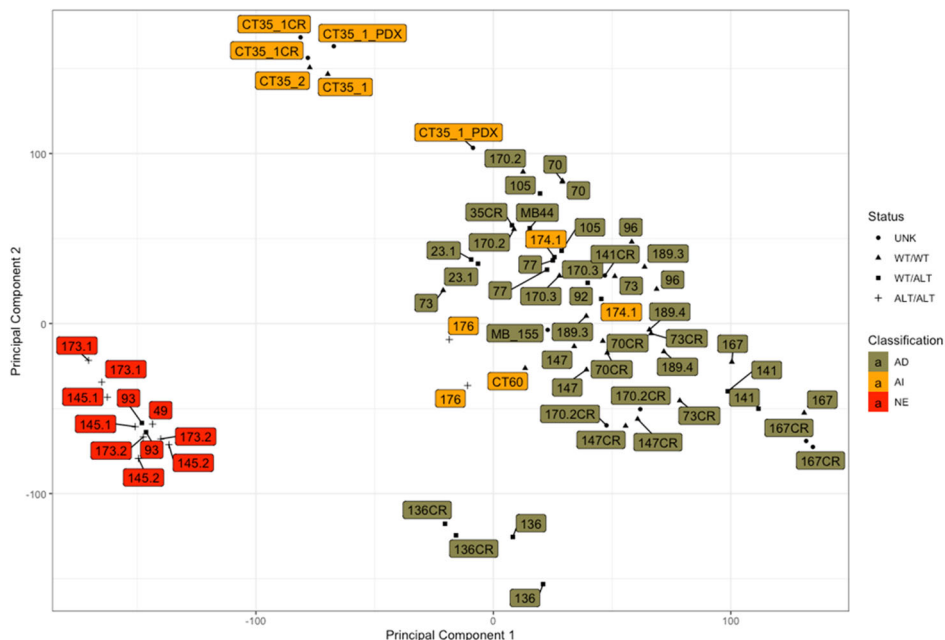


Figure 6: Principal components analysis plot of organoid RNA sequencing data. Individual models are labelled with colors by their histological classifications; adenocarcinoma (AD), amphotericin (AI), and neuroendocrine (NE). Mutational status of RB and TP53 is encoded using shape with status recorded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

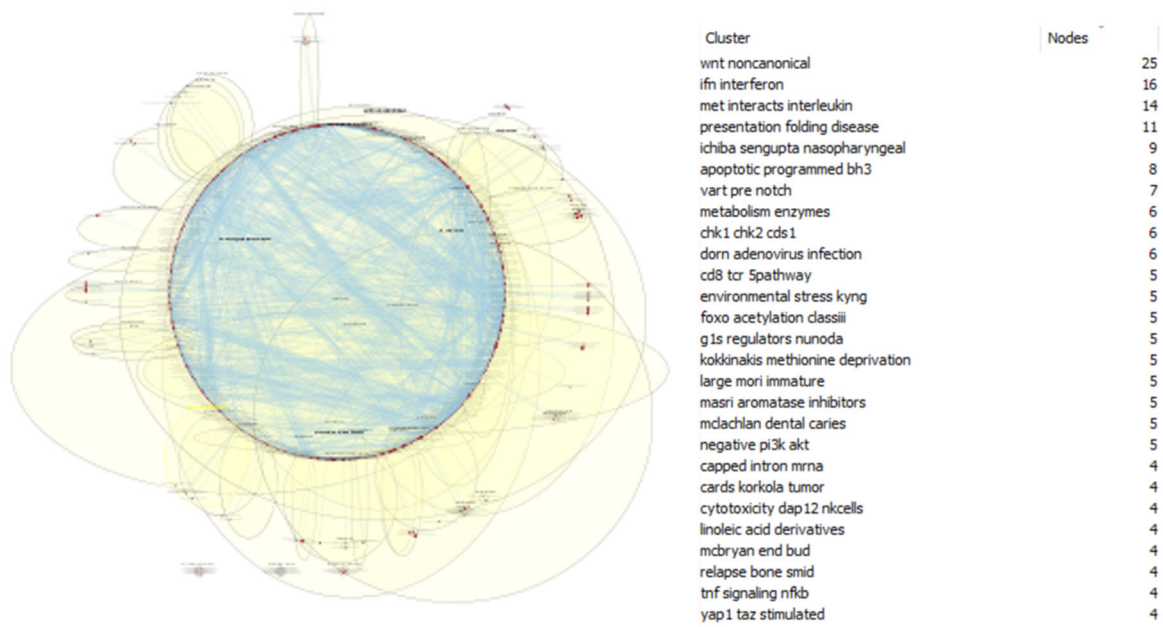


Figure 7: Circos plot showing how differentially expressed genes between docetaxel responders and non-responders cluster into signaling nodes of known biological processes providing an overview of the biological difference between responsive and nonresponsive CRPC