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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
PRINCIPAL INVESTIGATOR:	Dr. Kathleen Kelly
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PREPARED FOR:	U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012

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The Geneva Foundation
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Tacoma, WA 98402

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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

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Page

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

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:** *Provide a brief list of keywords (limit to 20 words).*

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

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

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.

Major activities completed or ongoing:
Completed activities:
a) analyze drug model responses to determine correlated responses among drugs as well as correlated model responses
b) analyze genomic markers of response
c) analyze transcriptomic markers of response
d) perform PDX clinical trials with selected therapeutic combinations (including volasertib, roniciclib,

S63845, carboplatin, and combinations of the former with carboplatin)
e) perform PDX clinical trials with docetaxel and docetaxel combined with apoptosis inhibitors (S3845 and navitoclax)
f) validate molecular markers of response (RB1, pATR, HNF1, BCLXL, and MCL1) using biochemical or protein markers
g) Expand the in vivo validation of docetaxel responsiveness and in combination with selected apoptotic inducing agents
h) validate HNF1 as a mechanistic marker of docetaxel responsiveness
i) validate HNF1 as predictive biomarker using available clinical correlates
j) Prepare a publication describing the high throughput screen results and the in-depth analysis of taxane responses.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Summary: We have successfully completed and validated for the first time an extensive high throughput drug screen with multiple models representative of patient samples and in conjunction with the analysis of transcriptomic and protein biomarkers. This data will be extremely valuable to both the clinical and the basic/translational community, and we have presented this data in various public forums and shared the data with multiple investigators for their use.
We have determined the molecular features of mCRPC models that respond to various replication stress targeted drugs and G2/M cell cycle inhibitors. Overall, we have identified responder and nonresponder models to approximately 20 drugs. Two drugs not previously recognized for their activity in CRPC are the MCL-1 inhibitor S3845 and the farnesyltransferase inhibitor, tipifarnib. The distribution of responses over ~30 models will be available to the prostate cancer community.
Importantly, we have also analyzed the response to docetaxel, a highly clinically relevant drug for CRPC, and determined response and nonresponder biomarker classes as well as a class of drugs (apoptosis inducers) that appear to synergize efficaciously. For the first time, we have identified the transcription factor, HNF1, as a biomarker of taxane nonresponsiveness. Because this finding has major implications for improving personalized medicine treatment for men with CRPC, we chose to pursue validation and in-depth analysis of this finding. In the final year, we were able extend our initial observation by experimental validation with mechanistic studies and correlative analyses in clinical data sets. We have prepared and submitted a manuscript describing these results.

1) Major activities. The major activities during the past year were focused upon expanded validation of taxane responses, based upon the efficacy of responsiveness across multiple models, with respect to analyzing in vivo responses, analysis of correlated biomarkers defined by transcriptomic signatures and protein expression, and investigations into the mechanism of HNF1-mediated taxane nonresponsiveness.

2) Specific objectives. The specific objectives were to validate the results from the high throughput drug screen at the level of preclinical data collection that could be translated to phase 1 clinical trials in the form of predictive biomarkers and efficacious drugs or drug combinations.

Key conclusions/outcomes.

1) As described previously, we have established the response to a cluster of drugs, enriched for activity in the G2/M phases of the cell cycle, across multiple models that include both NEPC and Adenocarcinomas. Importantly, these models can be identified by direct or indirect loss of *RB1* activity, which is correlated with various biomarkers that we have validated in the models. These biomarkers include an RB1 transcriptomic signature score as well as pATR and/or SLFN11 levels (see appended manuscript and As described previously, we have established the response to a cluster of drugs, enriched for activity in the G2/M phases of the cell cycle, across multiple models that include both NEPC and Adenocarcinomas. Importantly, these models can be identified by direct or indirect loss of *RB1* activity, which is correlated with various biomarkers that we have validated in the models. These biomarkers include an RB1 transcriptomic signature score as well as pATR and/or SLFN11 levels (see appended manuscript and Agarwal, S., L. Fang, K. McGowen, J. Yin, J. Bowman, A. T. Ku, A. N. Alilin, E. Corey, M. Roudier, L. True, R. Dumpit, I. Coleman, J. Lee, P. S. Nelson, B.J. Capaldo, I. S. Senatorov, A. G. Sowalsky, E. M. Hurt and K. Kelly (2023).“Tumor-derived biomarkers predict efficacy of B7-H3 antibody-drug conjuate treatment in metastatic prostate cancer models.” Journal of Clinical Investigation 133(22):e162148. https://doi.org/10.1172/JCI162148. Federal support acknowledged). This is the first validation of such biomarkers across multiple, clinically relevant mCRPC models.

2) We have identified previously unappreciated activity for S3845, an MCL-1 inhibitor, and Tipifarnib, a farnesyltransferase inhibitor, as drugs that lead to prostate cancer cell death across multiple CRPC models (see appended manuscript).

3) We have made the important discovery that docetaxel nonresponsiveness in mCRPC adenocarcinoma is highly correlated with HNF1 expression and with an HNF1-driven transcriptomic signature (see appended manuscript). We have validated this finding in PDX clinical trials and in experimentally manipulated models. We have also determined that one mechanistic explanation of this finding is the transcriptional induction of *BIRC3* by HNF1, which leads to an inhibition/blunting of mitotic apoptosis induced by docetaxel. Inhibition of BIRC3 with the drug LC161 converts taxane nonresponsive models to responders.

4) Docetaxel responsiveness is correlated with responsiveness to BCLXL inhibitors, suggesting that those models that are responsive to docetaxel have ongoing apoptotic stress, inhibited at least in part by BCLXL. These data suggest that taxanes in combination with BCLXL inhibitors or other appropriate drugs that target apoptosis downstream of BCLXL may convert some models of taxane nonresponders to responders. Our PDX clinical trial results demonstrated no synergism with S3845 and docetaxel and that Navitoclax was toxic, most likely as a result of thrombocytopenia, a known off-target effect of the drug.

Goals not met: It has been challenging to obtain clinical patient response data linked to transcriptomic data. This is largely due to the fact that those data sets that contain useful

transcriptomic data (such as SU2C cohorts) do not have sufficiently detailed clinical follow-up data (they were not originally designed for that purpose). We have analyzed various protein biomarkers that may be useful in post-hoc analysis of clinical trials if pathological biopsy material is available. Similarly, it has not been possible to directly validate our taxane finding with existing clinical trials due to the lack of molecular data associated with prospective clinical trials, which were performed ~25 years ago. We continue to interact vigorously with the clinical community to identify opportunities to validate our preclinical findings. This challenge does not preclude the value of our finding to the clinical community as our data suggests appropriate biomarkers to include in prospective trial design.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The Kelly lab and Corey lab teams met monthly to discuss ongoing experiments and the interpretation of results. The opportunities for trainees to present work has been somewhat limited due to ongoing COVID restrictions. However, postdoctoral fellows from Dr. Kelly's lab have presented data from this project at the Laboratory of Genitourinary Cancer Pathogenesis Department seminar series, the ACCR Prostate Cancer Meeting (March 2023) and at the Prostate Cancer Foundation Annual Meeting (October 2022). In addition, Dr. Kelly meets regularly with her team via one-on-one and group meetings. Trainees also participated in a variety of workshops and training sessions related to computational analyses.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Result have been disseminated by widely attended seminars at Fred Hutchison Cancer Institute presented as part of the Northwest Prostate Cancer Spore seminar series, the AACR Prostate Cancer meeting, and the Prostate Cancer Foundation Annual meeting. The data has also been shared by investigators who request information

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Nothing to report

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The HTS data will be widely used throughout the prostate cancer community. This data currently is under consideration for publication. This is the first comprehensive drug screen coupled with molecular markers for CRPC, 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 a new biomarker (HNF1) that potentially can be used to make treatment decisions for docetaxel, a commonly used drug for CRPC. This data contributes to clinical trial design for prospective biomarkers.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The COVID pandemic slowed the completion of work due to delays in receiving supplies as well as insufficient personnel at NIH, which slowed obtaining data through technical core-supported work as well as animal studies that rely on specialized veterinary technical contributions. We experienced problems that occurred throughout the scientific community. Work slowdowns were sporadic and outside of our control, but we did our best to make adjustments and perform the work to the best of our ability. The final year of the project did not run into problems or delays.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

The work slowdowns decreased our use of supplies and core services, necessitating the extension of the granting period. The final 1.5 years of the project proceeded expeditiously with respect to experimental plans, dissemination of data, and training opportunities for fellows.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year*

(international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

*High Throughput Organoid Drug Screening Reveals Drug Sensitivity and Resistance Profiles for Phenotypically Distinct Models of Prostate Cancer

Ilya S. Senatorov[1], Joel Bowman[1], Keith H. Jansson[1], Aian Neil Alilin[1], Brian Capaldo[1],Ross Lake[1], Morgan Riba[1], Yasmine C. Abbey[1], Crystal Mcknight[2], Xiaohu Zhang[2], Sonam Raj, Holly Nguyen[3], Craig J. Thomas[2] , Eva Corey[3], Kathleen Kelly[1]. "Castrate Resistant Prostate Cancer Response to Taxane is Determined by an HNF1-dependent Apoptosis Resistance Circuit." ***Cancer Cell*** *(Submitted January 2024).*Federal support acknowledged.

- *A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.*

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Our work demonstrated the utility of CRPC organoid models for high throughput purposes. The use of organoids greatly expands the availability of in vitro tractable CRPC models.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Kathleen Kelly PhD	No change
Eva Corey PhD	No change

Additional personnel not funded by the DOD:

1)

Name:	Ilya Senatorov
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	0000-0003-0460-5296
Nearest person month worked:	12
Contribution to Project:	Dr. Senatorov led the analysis of taxane responses and has performed validation experimentation.
Funding Support:	CCR/ NCI IRP fellowship

2)

Name:	Joel Bowman
Project Role:	Postbaccalaureate fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Mr. Bowman performed computational analysis for the project.
Funding Support:	CCR/NCI IRP fellowship

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

The Federal government (CCR, NCI, NIH) provided financial support for the salaries of Dr. Kelly, Dr. Senatorov, and Mr. Bowman.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: ***N/A***

QUAD CHARTS: N/A

9. APPENDICES:

APPENDIX I: SUPPORTING FIGURES

Please see the attached submitted manuscript.