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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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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Geneva Foundation
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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

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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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 clinical trials with docetaxel and docetaxel combined with S63845
f) validate molecular markers of response (RB1, pATR, HNF1, BCLXL, and MCL1) using biochemical or protein markers

Specific objectives- year 4:

a) Expand the in vivo validation of docetaxel responsiveness and in combination with selected apoptotic inducing agents.
b) validate HNF1 as a mechanistic marker of docetaxel responsiveness
c) validate HNF1 as predictive biomarker using available clinical correlates

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. 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. In the next year, we will finish the validation for these findings with mechanistic studies and correlative analyses in clinical data sets. We anticipate finishing the preparation of a manuscript and submission before the end of fiscal 2023.

1) Major activities. The major activities during the past year were focused upon expanded validation of selected drugs, based upon the efficacy of responsiveness across multiple models, with respect to analyzing in vivo responses as well as in-depth analysis of correlated biomarkers defined by transcriptomic signatures and protein expression.
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. This is the first validation of such biomarkers across multiple, clinically relevant mCRPC models.
2) 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 Figures 1 and 2).
3) 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.

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. We also are exploring the use of circulating DNA that can be used to interpret predicted transcriptomic patterns. 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 meet 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 and at the NIH Prostate Spore annual meeting. 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?
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 the University of Minnesota Cancer Center and the NCI Prostate Spore 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.

During the no cost extension of this grant, we will add additional taxane and taxane/navitoclax PDX clinical trial data, complete mechanistic validation of HNF1 as a taxane nonresponse marker based upon genetic manipulation of expression in responder and nonresponder models, investigate the characteristics of HNF1 expression in patient populations, submit the work for publication and respond to requests for additional data validation relative to reviews of the initial submission.

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. We anticipate publishing the data in 2023. 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 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 has slowed the completion of work due to delays in receiving supplies as well as insufficient personnel at NIH, which have slowed obtaining data through technical core-supported work as well as animal studies that rely on specialized veterinary technical contributions. We are experiencing problems that are occurring throughout the scientific community. Work slowdowns are sporadic and outside of our control, but we do our best to make adjustments and perform the work to the best of our ability.

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 have decreased our use of supplies and core services.

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

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: *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

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. 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.*

Nothing to report

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 provided financial support for the salaries of Dr. Kelly, Dr. Senatorov, and Mr. Bowman.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

APPENDIX I: SUPPORTING FIGURES

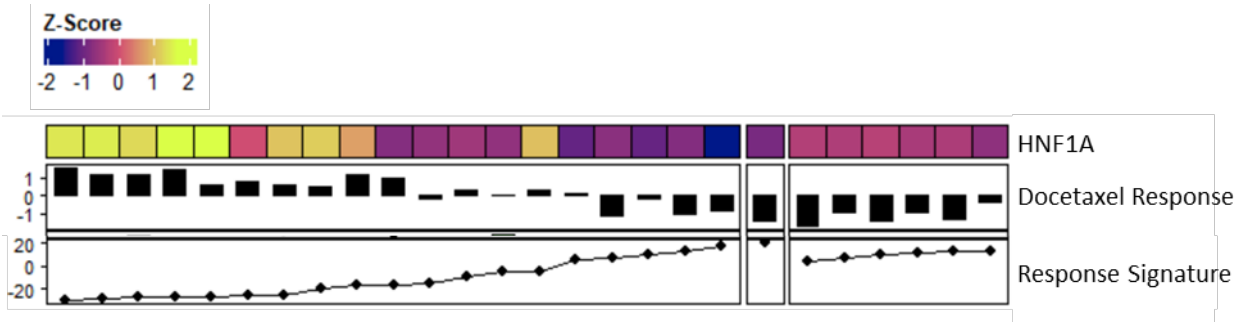

Figure 1: Composite summary showing HNF1A is almost exclusively expressed in docetaxel resistant models, while docetaxel response and HNF1A expression correlates strongly with Response Signature.

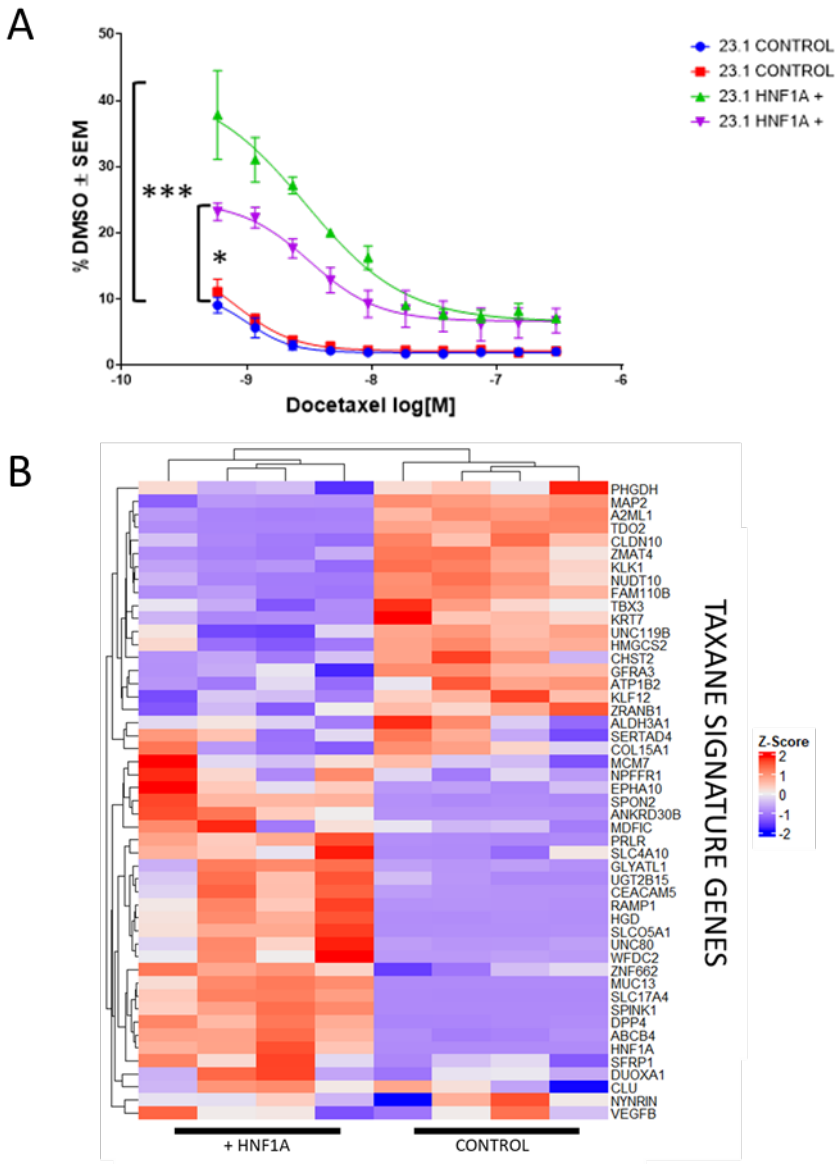

Figure 2: A) Drug response curves comparing 2 biological replicates of LuCaP 23.1 ectopically expressing HNF1A vs. control vector. *** denotes $p < 0.001$, * denotes $p < 0.05$. B) Transcriptomic heatmap comparing 4 biological HNF1A+ 23.1 replicates with their corresponding native controls showing directional change in nearly every gene present in the response signature upon expression of HNF1A confirming its transcriptional role in signature score.