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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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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 None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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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-0derived xenografts, effective treatment combination therapy

3. ACCOMPLISHMENTS:

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

Ongoing activities:

a) perform PDX clinical trials with selected therapeutic combinations (including volasertib, roniciclib, S63845, carboplatin, and combinations of the former with carboplatin- 7/13 models assayed in year 3; docetaxel and docetaxel combined with S63845)
b) validate molecular markers of response using biochemical or protein markers
c) test predictive molecular markers using the clinical response/circulating tumor cell RNAseq data generated in the PROPHECY clinical trial
d) coordinate and optimize collaborative goals

Specific objectives- year 3:

a) Validate in vivo those single and combination drug treatments selected from in vitro drug screening
b) identify predictive biomarkers of drug responses
c) validate predictive biomarkers using available clinical response data.

Significant results/key outcomes:
A number of novel conclusions have been discovered, which represent the first comprehensive analysis of responsiveness to multiple drug classes and the associated biomarkers for multiple CRPC and NEPC models. Underlying supporting data for some of the conclusions can be found in the attached appendix as indicated.

1. Chemotherapeutic agents, S/G2/M cell cycle checkpoint inhibitors, and apoptosis pathway targets demonstrate overlapping response profiles across models **(Fig. 1)**.
2. Drugs targeting a single signaling pathway (e.g. PI3K pathway) overlap in their response profiles, and demonstrate relatively rare activity as single agents across models **(Fig. 1,2)**.
3. *RB1* loss and replication stress initiated by distinct DNA repair mutations are biomarkers of drug responsiveness.
4. A transcriptomic-based predictive signature for docetaxel response has been generated. *RB1* loss dependent gene expression has been excluded to minimize selection based on neuroendocrine phenotypes/genotypes **(Fig. 3)**.
5. Volasertib treatment demonstrated significant anti-tumor efficacy in some of PDX models but the other monotherapies (roniciclib, S63845, carboplatin) had generally no to low efficacy B **(Fig. 4)**. However, the combinations tested exhibited significant anti-tumor activities in adenocarcinoma CRPC as well as in neuroendocrine PC. Signaling pathway markers were associated with in vivo responses classes **(Fig. 5)**
6. The combination of BCLXL and MCL1 inhibitors is highly synergistic in vitro. In vivo the combination is also highly effective, but tumor lysis syndrome limits utility.

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. The opportunities for trainees to present work has been limited due to COVID restrictions. However, postdoctoral fellows from Dr. Kelly's lab have presented data from this project at the Laboratory of Genitourinary Cancer Pathogenesis and the Data Sciences departmental seminar series as well as to the CCR Prostate Cancer PI Working Group. 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 presented by Dr. Kelly via institution-wide virtual seminars at Northwestern, Thomas Jefferson, and the FDA.

What do 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 complete the PDX clinical trials, complete protein marker evaluations, complete the analysis of PROPHECY trial data to determine the utility of the taxane response signature which was derived from this project, submit the work for publication and respond to requests for additional data validation relative to reviews of the initial submission.

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 2022. 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. If the molecular signature we have developed proves to be predictive in clinical trials, we anticipate further validation and development for clinical use.

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
Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them:
The COVID pandemic has slowed the completion of work due to delays in receiving supplies and mice 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. Similarly, at the University of Washington, there were delays due to receiving supplies as well as limitations on personnel being allowed in vivarium and present at the work site. 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
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:

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
Nothing to report.

Journal publications:
Nothing to report.

Books or other non-periodical, one-time publications:
Nothing to report.

Other publications, conference papers, and presentations:
Chung Lee Lectureship at Lurie Cancer Center, Northwestern University, Chicago

Sidney Kimmel Cancer Center seminar series, Thomas Jefferson University, Philadelphia
FDA Neuroendocrine Prostate Cancer Mini-symposium

Website(s) or other Internet site(s):
Nothing to report.

Technologies or techniques:
We have developed techniques for high throughput screening of organoids and for improved CRPC organoid growth. We have shared our protocols with several labs upon request.

Inventions, patent applications, and/or licenses:
Nothing to report.

Other Products:
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

Name:	Kathleen Kelly- NO CHANGE
Project Role:	
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	

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Project Role:	
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	

Name:	Craig Thomas- NO CHANGE
Project Role:	
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	

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.

8. APPENDICES:

APPENDIX I: SUPPORTING FIGURES

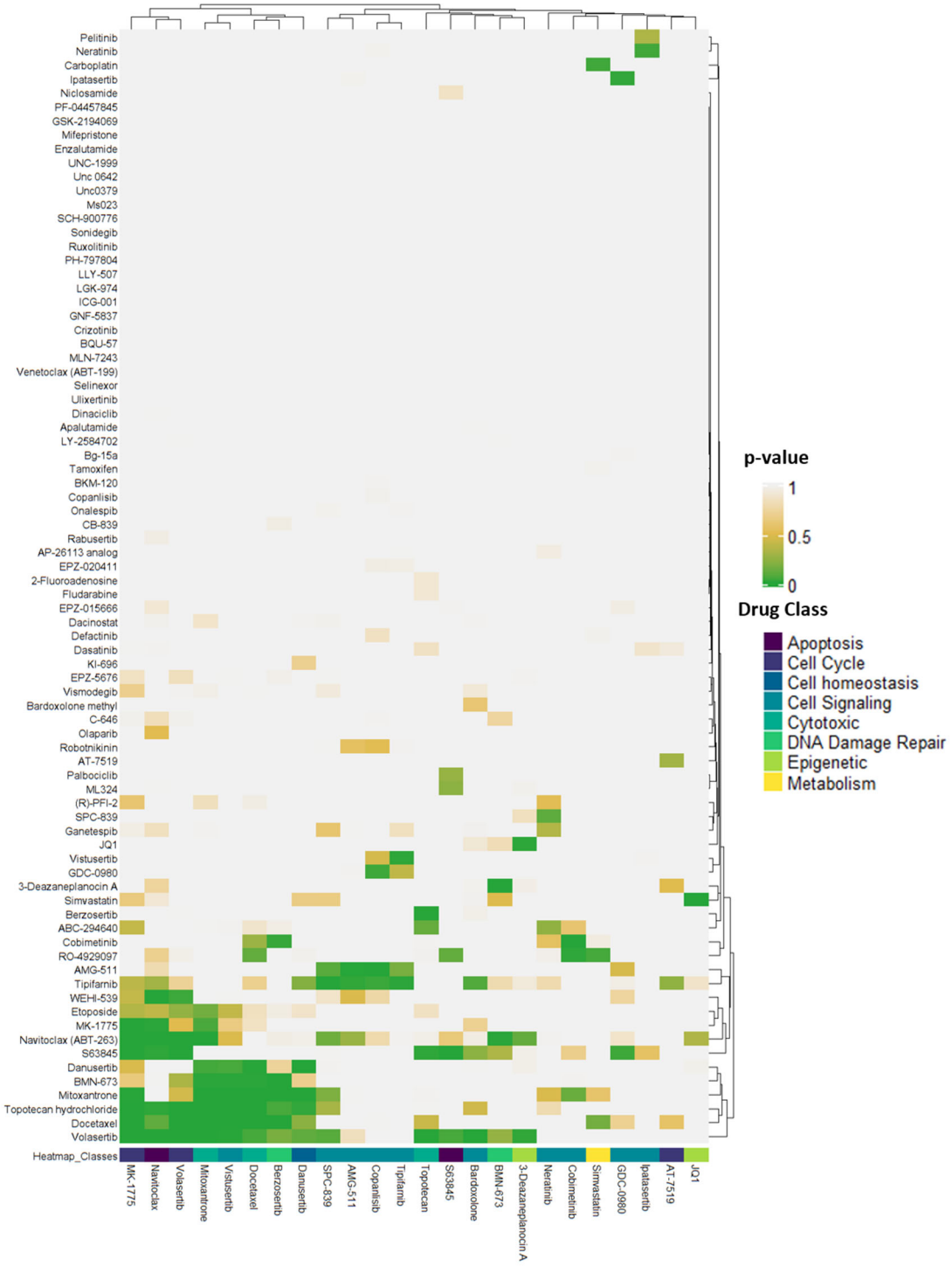

Figure 1: Heatmap showing positive drug-drug relationships demonstrating sensitivity and resistance overlap among multiple mechanistic drug classes. DNA inducing and cytotoxic agents, apoptosis modulators, and cell cycle inhibitors demonstrate co-efficacy with significant p-values < 0.05 showing potential for combination therapies and revealing a likely subset of vulnerability within prostate cancer that can be targeted by multiple mechanisms.

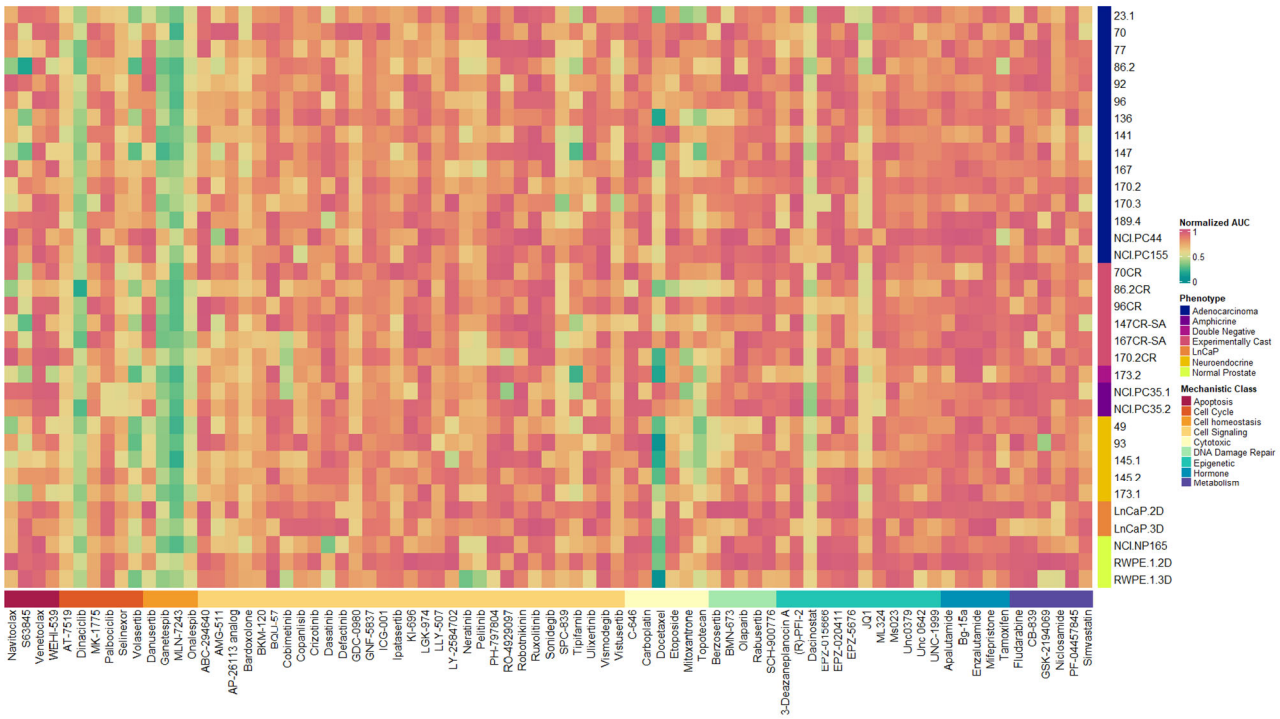

Figure 2: Heatmap of normalized area under curve (nAUC) as a single continuous variable summarizing response across 10 doses of every compound tested against every organoid. Organoids are annotated by phenotype and drugs are annotated by mechanistic class.

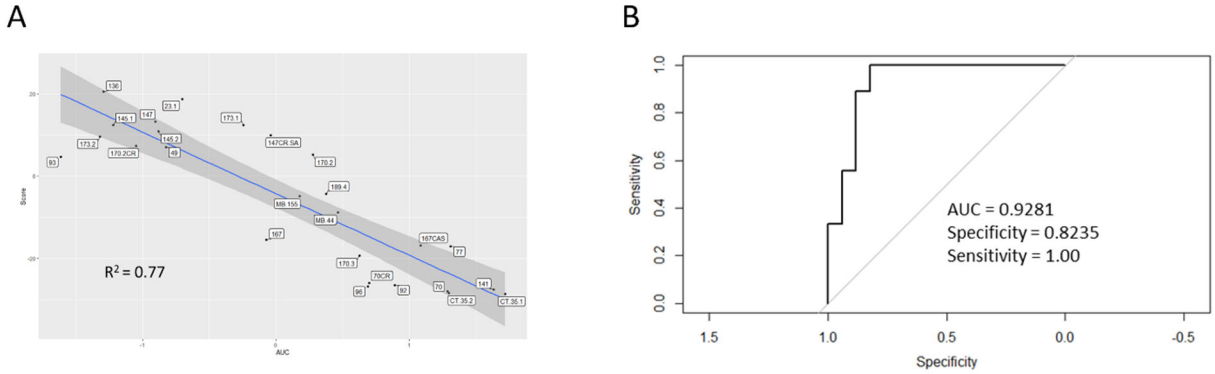

Figure 3: A) Linear regression showing a continuous relationship between a generated gene signature predicting drug sensitivity and AUC response to docetaxel. B) Bootstrapped Receiver Operations Curve (ROC) showing the projected ability of the signature score to identify predicted sensitivity to docetaxel for tumors with 82.35% specificity and 100% sensitivity.

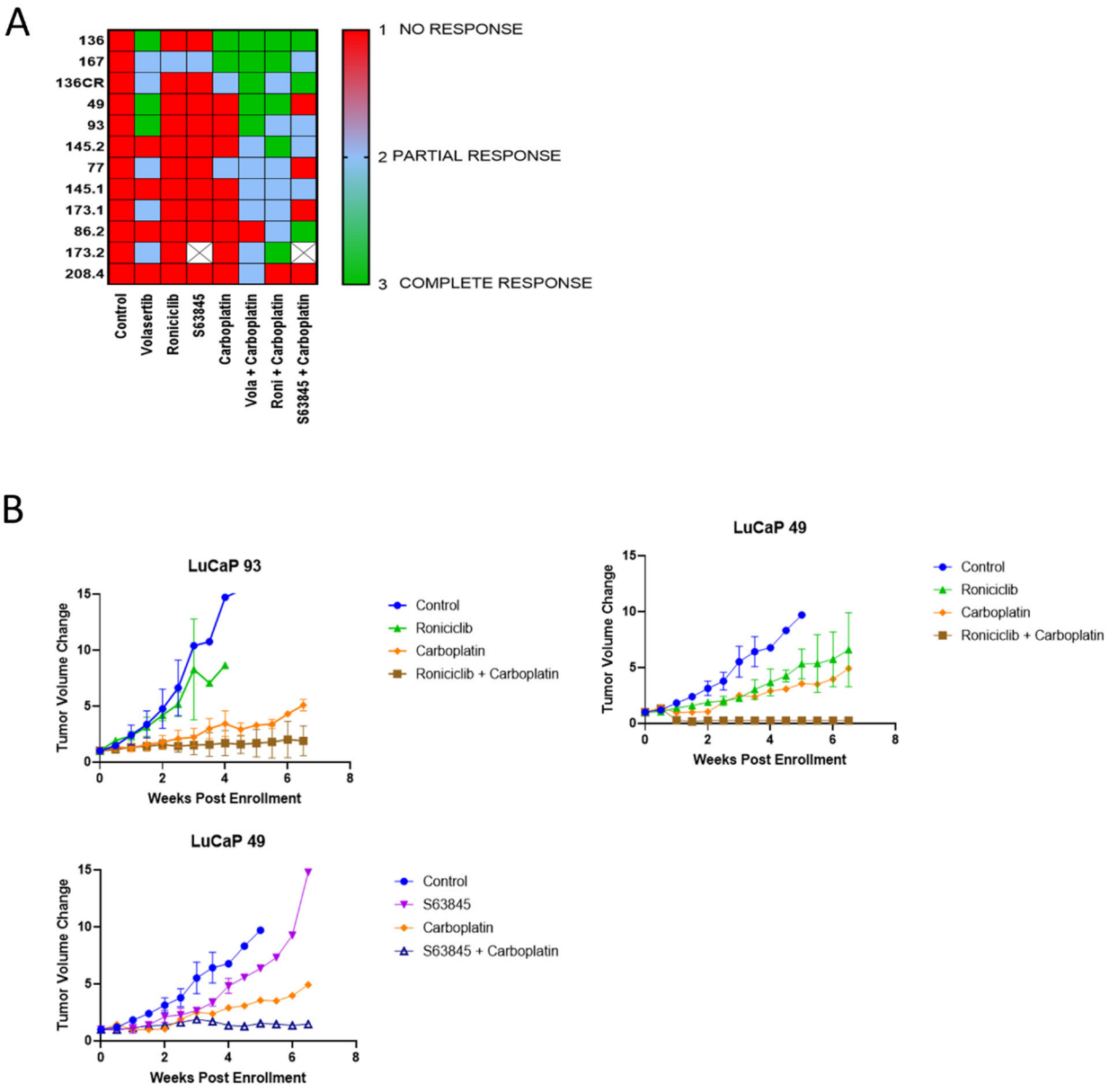

Figure 4: A) Categorical responses *in vivo* demonstrate high efficacy of combination therapy. B) Example tumor vs time response curves showing data behind categorical heatmap.

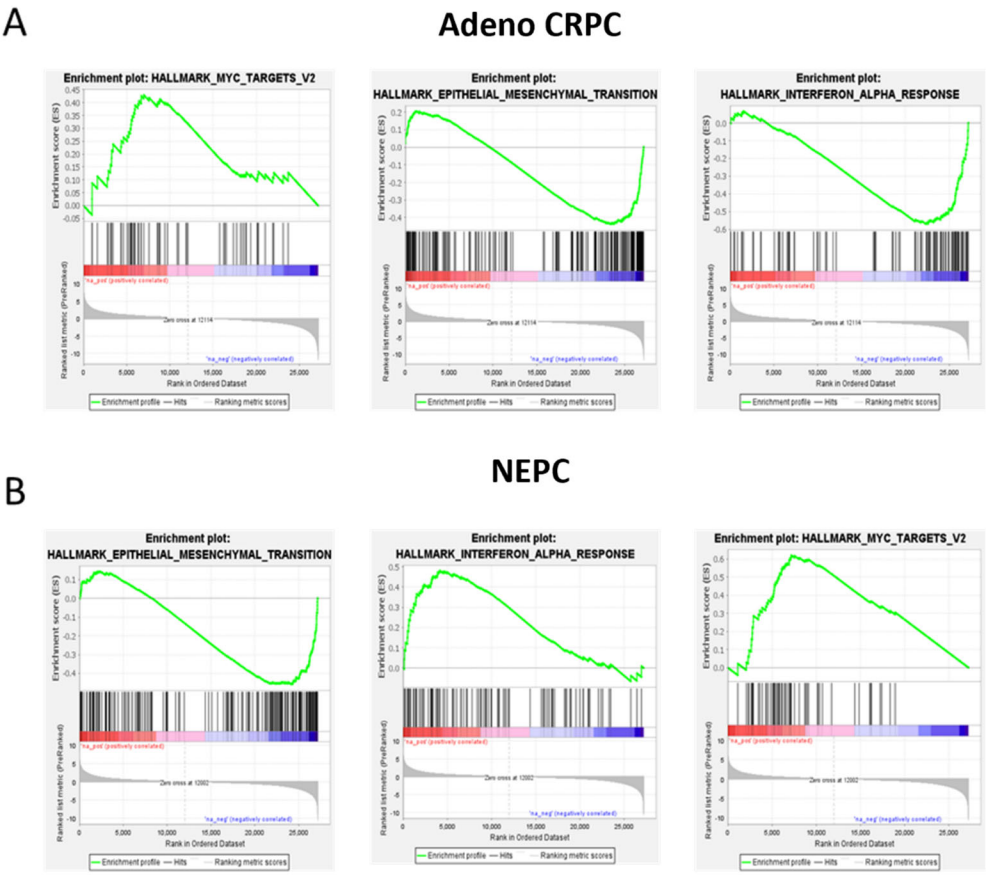

Figure 5: GSEA analysis of parental tumors. A) Examples of Hallmark gene sets enriched in adenocarcinoma castrate-resistance prostate cancer (CRPC). B) Examples of Hallmark gene sets enriched in neuroendocrine prostate cancer (NEPC). C) GSVA detects differential MEK and FGFR signaling in NEPC non-responders (NR) vs responders (R).