Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Thomas Jefferson University

2. **Reporting Period (start and end date of grant award period):** 1/1/2011-12/31/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Theodore F. Taraschi, PhD

4. **Grant Contact Person's Telephone Number:** 215-955-3900

5. **Grant SAP Number:** 4100054872

6. **Project Number and Title of Research Project:** Project 2 – Cell Cycle Regulation in Prostate Cancer

7. **Start and End Date of Research Project**: 1/1/2011-6/30/2014

8. **Name of Principal Investigator for the Research Project:** Karen Knudsen, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 534,390.73

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Knudsen	Professor	3% Yr 1, 33% Yr 2, 10% Yr 3	$110,604.81
Ostrander	Research Tech	2% Yr 1, 10% Yr 2, 5% Yr 3	$6,428.48
Balasubramaniam	Student	50% Yr 2, 38% Yr 3	$27,038.40
Urban	Student	30% Yr 2	$12,908.85
Goodwin	Student	50% Yr 2	$17,463.89
De Leeuw	PostDoc	21% Yr 2	$8,806.56
Potoczek	Histology Tech	1% Yr 2	$3,285.15
Mankame	PostDoc	50% Yr 2	$26,169.86

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No_____X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____ No____X_____

If yes, please describe your plans:

12. Future of Research Project. What are the future plans for this research project?

None

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No___X_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No___X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No____X_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not

print as boxes (☐) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Project Title and Purpose

Cell Cycle Regulation in Prostate Cancer - In the United States, prostate cancer (PCa) is the most frequently diagnosed malignancy, and the second leading cause of cancer death, in men. The morbidity associated with this tumor type is attributed to ineffective means in combating advanced disease. Local PCa can be definitively treated by radical prostatectomy or radiation therapies. However, disseminated PCa is largely resistant to standard cytotoxic chemotherapeutics. The androgen receptor (AR) is the therapeutic target of disseminated disease. As such, first line therapies for disseminated disease are centered on the addiction of this tumor type to androgen. This project will utilize the combined knowledge of AR and cell-cycle based cytotoxic action, to design strategies for maximal PCa cell death.

Project Overview

This project builds on previous data, which show that AR activity can be effectively manipulated to sensitize cells to taxane-based cell death. The data demonstrate that the ability of AR to induce G1 progression is critical for selected cell-cycle based cytotoxic interventions. This concept will be challenged in this project, which will utilize the combined knowledge of AR and cell-cycle based cytotoxic action, to design strategies for maximal PCa cell death.

Goal: Delineate mechanisms to enhance PCa cell death through coordinated manipulation of taxane sequence, AR activity, and p53 status.

Aim 1: Determine the impact of taxanes during intermittent hormone therapy.

Aim 2: Determine the p53 requirement for androgen-enhanced taxane cytotoxicity.

Objective: These principles will challenge the impact of sequence and timing on taxane-based action during intermittent hormone therapy and the contribution of p53 status to this response.

Expected Research Outcomes and Benefits

This laboratory has shown that the G1-S progression function of AR sensitizes PCa cells to the cytotoxic effects of taxanes. This suggests that this function of AR could be manipulated during intermittent hormone therapy, to enhance therapeutic response and prolong or prevent tumor progression. This project addresses this innovative hypothesis directly, by dissecting the optimal cycling sequence of taxanes and AR ablative strategies using model systems reflective of naïve tumors. This research will distinguish the responses of wild-type and mutant AR. Moreover, the contribution of p53 status to this process will be determined, as taxane-induced p53 tumor suppressor activation is enhanced by active AR. Thus, the project will determine which cohorts of patients (dependent on p53 and AR status within the tumor) may most benefit from combinatorial therapy. Lastly, the ability of pharmacological agents that activate endogenous p53 to replace or enhance the ability of AR to sensitize cells to taxanes will be determined, thus potentially identifying a novel mode of adjuvant intervention with the taxanes. Project outcomes have the capacity to markedly alter/improve therapeutic strategies to treat the most deadly forms of PCa before the stage of hormone resistance is reached.

Summary of Research Completed:

Cabazitaxel shows enhanced anti-proliferative and pro-cytotoxic effects in CRPC
To assess the relative impact of cabazitaxel (CBTX) and docetaxel (DCTX) on prostate cancer cell growth and survival, analyses were performed in both hormone-therapy sensitive (LNCaP) and CRPC (C4-2) model systems. Cells were treated with an increasing dose of each agent (0.1-1nM) for 48h, followed by a washout, and cultured for 48h in the absence of drug. Cell viability was quantified through cell counting. As shown in Figure 1A, CBTX and DCTX showed relatively equivalent effects in hormone-therapy sensitive cells (left), with IC50 values of 0.220nM for CBTX and 0.319nM for DCTX. However, CBTX (IC50=0.142nM) resulted in markedly enhanced anti-tumor effects as compared with DCTX (IC50=0.269nM) in CRPC cells (right). Concordantly, bivariate flow cytometric analyses, monitoring both cell cycle position (via propidium iodide, PI) and progression through S-phase (via uptake of bromodeoxyuridine, BrdU), revealed a lack of S-phase entry after 16 and 48 hours of CBTX treatment in both cell types; this was more pronounced in CRPC cells (Figure 1B, top, quantified in 1C). Similar effects were observed with DCTX (not shown), but overall effects were not as dramatic as those observed with CBTX. Thus, CBTX demonstrates an enhanced anti-proliferative effect in CRPC cells.

In addition to the observed effects on cell cycle, the sub-G1 content was enhanced in CRPC cells treated with CBTX as compared with hormone-therapy sensitive models (Figure 1B, bottom). Sub-G1 quantification revealed that CBTX is more effective in CRPC cells at inducing sub-2N DNA content (Figure 1D), eliciting almost double the effect of DCTX after 48h in C4-2 cells. Moreover, CBTX effectively induced apoptosis in C4-2 cells, as measured by PARP cleavage (not shown). Together, these data suggest that CBTX shows enhanced anti-proliferative and pro-cytotoxic effects as compared with DCTX.

Cabazitaxel promotes defective mitosis
To address the underlying means by which CBTX exhibits enhanced anti-tumor effects, the impact on nuclear integrity was assessed. Cells were fixed after 16h of exposure, and nuclear features assessed after DAPI staining (Figure 2A). Visualization on a confocal microscope demonstrated a modest enrichment of mitotic figures after 16h (quantified in Figure 2B, left); however, the evidence of defective mitoses was apparent in the asymmetric appearance of segregating nuclei. Pyknotic nuclei were also observed (Figure 2A), as quantified in Figure 2B (middle), demonstrating a significant increase after 48h CBTX exposure, further supporting the contention that CBTX exerts cytotoxic effects.

Given the known function of taxanes in serving as microtubule poisons and perturbing cytoskeletal integrity, we stained treated cells with fluorescently labeled phalloidin to visualize the cellular architecture. Actin staining revealed a reduction in cytoplasmic volume in all cells (Figure 2B). These analyses also allowed for clear definition of cell borders, revealing enhanced presence of multinucleate cells in CRPC cells treated with CBTX, likely as a result of aberrant mitosis. Multinucleate LNCaP and C4-2 cells were quantified in at least four duplicates for CBTX versus control treated samples, which demonstrated a highly significant increase in polynuclear cells after 16h and 48h (Figure 2B right). Together, these data support the hypothesis that CBTX shows an enhanced capacity to disrupt cell architecture and foster defective mitoses.

Taxane action is independent of effects on AR localization
Taxanes have been reported to affect AR localization and could thereby potentially block prostate cancer proliferation (1-3). In these previous studies, supra-clinical doses (micromolar) levels of taxanes were used; however, the *in vitro* IC50-values for these drugs are in the nanomolar range(4, 5). Thus, it is imperative to determine whether the reported effects occur at doses that are robustly cytostatic and cytotoxic, and are clinically attainable. As shown, using doses and time points sufficient to both suppress proliferation and induce cell death, endogenous AR remains nuclear in the presence of androgen-replete media (Figure 3A). To determine whether these effects hold true under conditions that mimic castration, parallel studies were conducted in the presence of charcoal-dextran-treated serum (CDT). As expected, androgen deprivation alone resulted in loss of nuclear AR enrichment in hormone-therapy sensitive cells; however, in CRPC cells, AR is retained in the nucleus even under castrate conditions (Figure 3B). Taken together, the enhanced cytotoxic and cytostatic effects of CBTX appear to be independent of AR subcellular regulation.

Cabazitaxel exerts enhanced anti-tumor effects in human tumors
Given desirable cytostatic and cytotoxic effects of CBTX in model systems, the anti-tumor effects were further assessed using next-generation, *ex vivo* tumor explants that allow for determination of effects on the complex 3D-tumor microenvironment. Here, fresh tumor material was obtained from radical prostatectomy of high volume disease, and tissue slices randomized into control or taxane-treated arms. As has been previously reported, these tumor slices retain the salient features of the tumor at the time of resection (including AR expression and proliferative capacity)(6, 7) (Figure 4A). These studies allow for intrinsically controlled analyses of taxane effects within the same tumor. Tumors were harvested after 6 days of treatment, formalin-fixed and paraffin-embedded. Standard hematoxylin and eosin (H&E) staining confirmed retention of the tumor microenvironment (Figure 4B left). Immunohistochemistry (IHC) to assess the proliferative indices (using an antibody to Ki67) was performed in parallel, for which representative examples are shown in Figure 4B (middle), and quantified in Figure 4C. Strikingly, this direct comparison in fresh tumor explants confirmed that CBTX exerts a markedly enhanced cytostatic response as compared with DCTX. Caspase-3 staining revealed an increasing trend that did not reach statistical significance, likely due to the limited sample number (n=3; Figure 4C). Finally, assessment of AR localization in response to drug treatment revealed retention of nuclear AR in the tumor cells (Figure 4D). Thus, similar to what was observed *in vitro*, the anti-tumor effects of CBTX appear to occur independently of altered AR localization. Collectively, these data identify an enhanced capacity of CBTX to elicit anti-proliferative and pro-apoptotic events in primary human tumors.

Tumors that progress to CRPC by RB loss show hypersensitivity to cabazitaxel
While the above studies suggest that CBTX harbors properties that are highly desirable in the clinical setting, a major hurdle is to identify tumor subtypes that would most benefit from treatment with the agent. It has been previously demonstrated that loss of retinoblastoma tumor suppressor protein (RB) or function occurs with high frequency in CRPC; this event alone can promote bypass of hormone therapy (8). By contrast, we and others have shown that RB loss compromises selected DNA damage checkpoints and can result in sensitization to a subset of chemotherapeutics, including docetaxel(9, 10). These findings put forward the provocative hypothesis that prostate cancers that achieve castration-resistance via loss of RB may be

exquisitely responsive to taxanes. To assess this, isogenic pairs of LNCaP cells with control shRNA or shRNA directed against RB (shCon1 and shRB1 cells, respectively) (8) were initially assessed *in vitro* for differential response to CBTX. In these studies, shRB1 cells demonstrated a modest sensitization to CBTX as compared with DCTX (Figure 5A). To challenge this *in vivo*, cells were subcutaneously injected into male athymic nude mice; when the xenograft tumors (n=5 per group) reached a size of 100-150mm^3, the mice were surgically castrated, allowing a week of recovery before starting 8mg/kg cabazitaxel treatment by intraperitoneal (IP) injections twice a week (Figure 5B schematic). Tumor volumes were monitored three times a week with caliper measurements. As shown, a remarkably enhanced tumor-suppressive effect was observed in the shRB1 tumors (Figure 5B left). Normalization of individual tumor volumes at 14 days after start of treatment compared with their respective volumes at t=0 shows a significant difference in cabazitaxel response between the shCon1 tumors (n=4) and the shRB1 tumors (n=5) (Figure 5B right). Notably, the fifth mouse growing an LNCaP shCon1 tumor had to be sacrificed before this time point because the tumor volume had already exceeded 800mm^3, despite cabazitaxel administration. These *in vivo* findings robustly support the concept that RB-deficient tumors are hypersensitive to treatment with CBTX.

To further probe the underlying basis of the observed enhanced effect in RB-deficient tumors, we performed histopathological analyses. Through H&E and Ki67 IHC analyses, shCon1 tumor cells showed the expected accumulation of mitotic figures, indicating an appropriate cell cycle arrest in metaphase as a result of CBTX treatment. By contrast, shRB1 tumors elicited fewer mitotic figures (Figure 5C, quantified in 5D top), consistent with previously reported "mitotic slippage" in cells that lack cell cycle checkpoints(11). Quantification of Ki67 positive cells in three (40x) images per tumor slide confirmed a strong reduction in proliferative cells in the shRB1 tumors versus shCon1 (Figure 5D bottom). Taken together, the *in vivo* data suggest that RB-deficient tumors are hypersensitive to CBTX and support the postulate that RB should be developed as a biomarker to identify tumors that may be most responsive to taxanes.

Cabazitaxel displays novel actions in prostate cancer cells by expression profiling

While the above studies identify novel, anti-tumor effects of CBTX and putative biomarkers to identify tumors that would be most responsive to this taxane, we performed additional studies to further uncover the molecular basis of divergent CBTX function. Hormone therapy-sensitive cells were treated for 16h with either CBTX or DCTX, in presence or absence of steroid hormones, and subjected to a genome-wide mRNA analysis. The resulting expression data were filtered to remove low-expressing genes to reduce background and false positive hits. Triplicate expression values were averaged, after which samples from the individual taxanes (CBTX or DCTX) were each normalized to the corresponding values for control treated samples, either with or without steroids. Differentially expressed genes were selected by a student's t-test ($p<0.05$) and compared between different treatments, as visualized by a Venn diagram (Figure 6A). Complete gene lists and fold alterations for each gene cluster have been calculated. Notably, distinct gene expression outcomes were readily apparent between the two taxanes. Further, these analyses revealed divergent effects of the agents in cells that are cycling (steroid replete) versus those that were arrested via steroid depletion (androgen deprived). These findings suggest, as expected based on the data above, that CBTX exerts differential effects as compared with DCTX and that the proliferative status of the tumor cell can alter downstream biological effects. Genes most altered (up- or down-regulated) by cabazitaxel in the absence or

presence of steroids are displayed in tables in Figure 6B.

To gain deeper understanding of the cellular response to CBTX, gene ontology analyses were performed on both castrate (in yellow, corresponding to the Venn clusters in Figure 6A) and steroid replete (blue) gene lists (Figure 6B). These studies revealed enrichment of genes involved in the cell cycle, M phase and mitosis, exclusively after cabazitaxel treatment (Figure 6C), consistent with the enhanced anti-proliferative effect of this agent as compared with DCTX. Both drugs affect the stress-activated MAPK signaling pathway (via MAPKKK), which plays a role in taxane resistance(12). Under androgen-deprived conditions, CBTX and DCTX share enrichment in gene transcription related pathways. However, these appear to be negatively regulated by CBTX, versus a positive impact by DCTX, which supports the hypothesis that these drugs have distinct downstream effects. DCTX, but not CBTX, demonstrates enrichment in amino acid and protein transport regulation that could be caused by differences in microtubule stabilization. While a general mechanism has been described for taxane action, these drugs may act with differing kinetics, or be less or more efficient at stabilizing the tubulin dimers, resulting in various degrees of protein transport inhibition. The gene expression data suggest that DCTX could have a stronger effect on intracellular transport; however, the biological data suggest that this is not required for cytostatic and cytotoxic effects, and is merely a bystander effect. Classical androgen receptor targets (KLK3, TMPRSS2, FKBP5, KLK2) are not affected by CBTX or DCTX and are only altered by steroid deprivation (Figure 6D). Strikingly, the hormone conditions affect the molecular response of taxanes, as demonstrated by enrichment of distinct pathways in hormone replete versus androgen-deprived media. This differential response to CBTX (or DCTX) will likely be of relevance for designing combination therapies with androgen receptor pathway targeting drugs, such as enzalutamide and abiraterone acetate. These unbiased analyses further illustrate the divergent effects of CBTX and DCTX, and provide a gene “signature” of response to CBTX in the presence and absence of androgen.

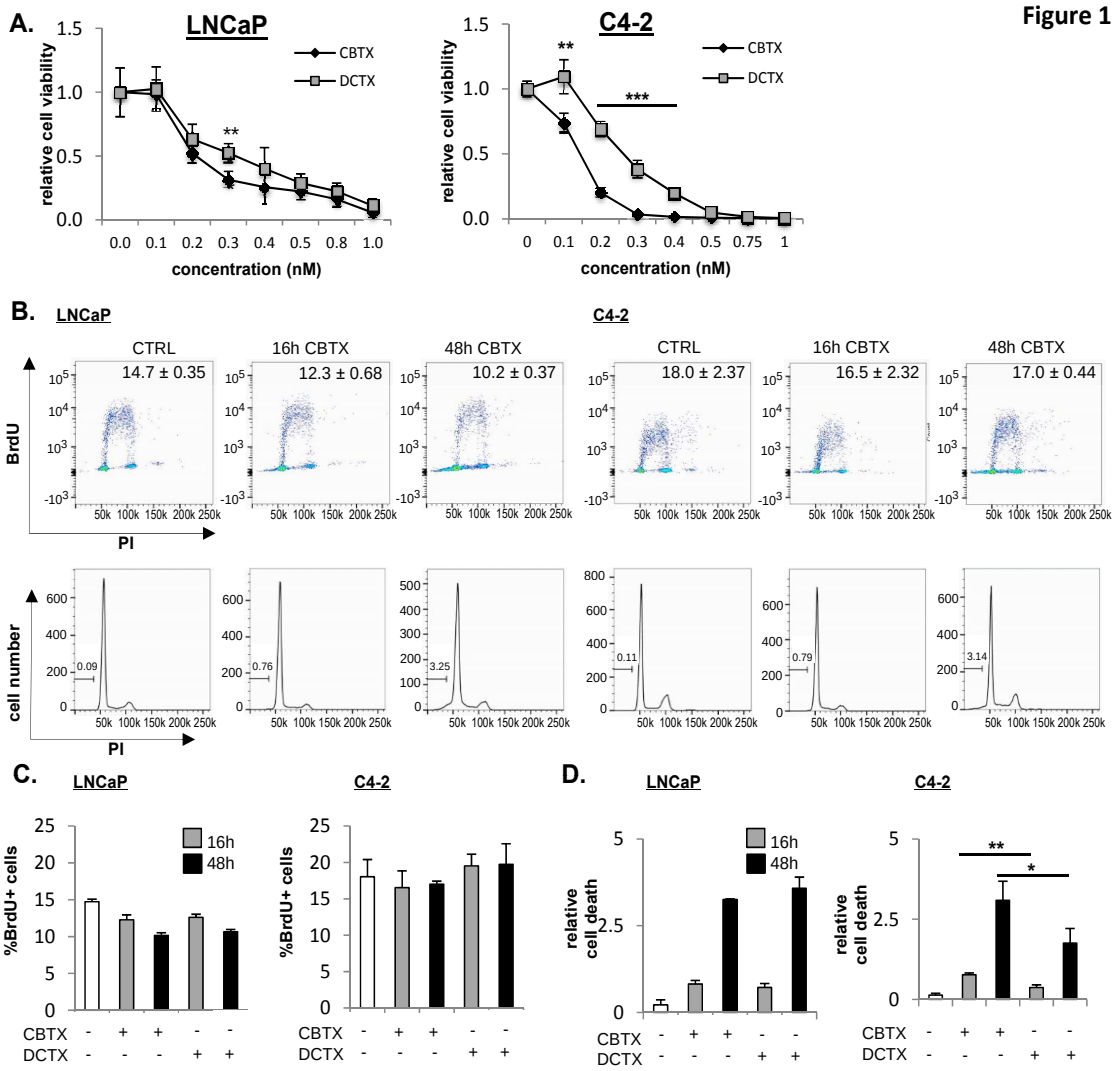


***Figure 1*: Cabazitaxel shows enhanced anti-tumor effects in CRPC.** A. Dose-dependent response to 48h cabazitaxel (CBTX) and docetaxel (DCTX) treatment and 48h fresh media without drugs was assessed in hormone therapy sensitive (LNCaP) and resistant prostate cancer cells (C4-2) by trypan blue exclusion, and cell numbers were normalized to EtOH treatment. B. Bivariate flow cytometry analyses of LNCaP and C4-2 cells treated with 1nM CBTX or control for 16 or 48h. In the top graphs, the x-axis represents relative DNA content as indicated by propidium iodide (PI) staining; the y axis shows cells undergoing active S-phase as indicated by 2h BrdU labeling. Inset values: % BrdU incorporation in viable cells (mean±s.d., from an experiment performed in biological triplicate). The bottom graphs represent the corresponding PI traces only, showing a G2M arrest, followed by cell death after CBTX exposure. C. Quantification of % BrdU+ cells in (B) and Suppl Fig 1, significant reduction is observed in taxane over control (LNCaP $p<0.05$; C4-2 $p<0.0005$ for all conditions). CBTX has a stronger effect compared to DCTX in C4-2 after 16h ($p=0.0016$). D. Taxanes induce significant cell death after 48h of CBTX or DCTX over CTRL in both LNCaP and C4-2 cells ($p<0.01$, detected as an increase in Sub-G1 content by flow cytometry (PI in B), with enhanced effects in the CRPC cells. Asterisks indicate significant differences between CBTX and DCTX at the same treatment duration, showing no significance in LNCaP, but improved efficacy for CBTX in C4-2 cells (16h 0.0042; 48h $p=0.0092$).

Figure 2

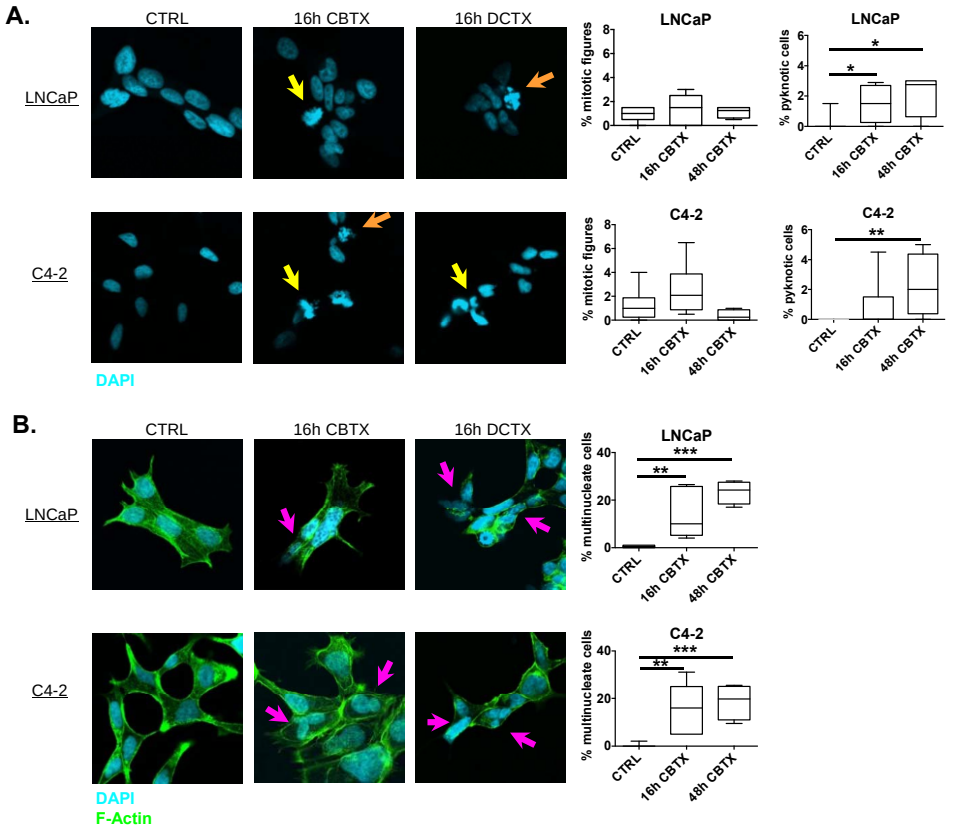


Figure 2: Cabazitaxel treatment results in increased multinucleated cells and aberrant nuclei. A. The nuclei of fixed LNCaP and C4-2 cells that were treated for 16hrs with taxanes were visualized with DAPI staining (cyan) on a confocal microscope (40x), demonstrating defective mitosis and pyknotic nuclei. B. Actin stained with Alexa Fluor 488 labeled phalloidin shows changes in cytoplasmic volume and cell morphology, as induced by taxanes after 16hrs compared to control. The zoomed images show examples of multinucleate cells in 48h treated cells, which are quantified in the whisker plots. Quantification of multinucleate cells and pyknotic cells mitotic figures in at least quadruplicate shows significant elevation of multinucleate C4-2 cells after 16h and 48h CBTX treatment, pyknotic cells after 48h. C4-2 cells show a modest, however non-significant increase in mitotic figures.

Figure 3

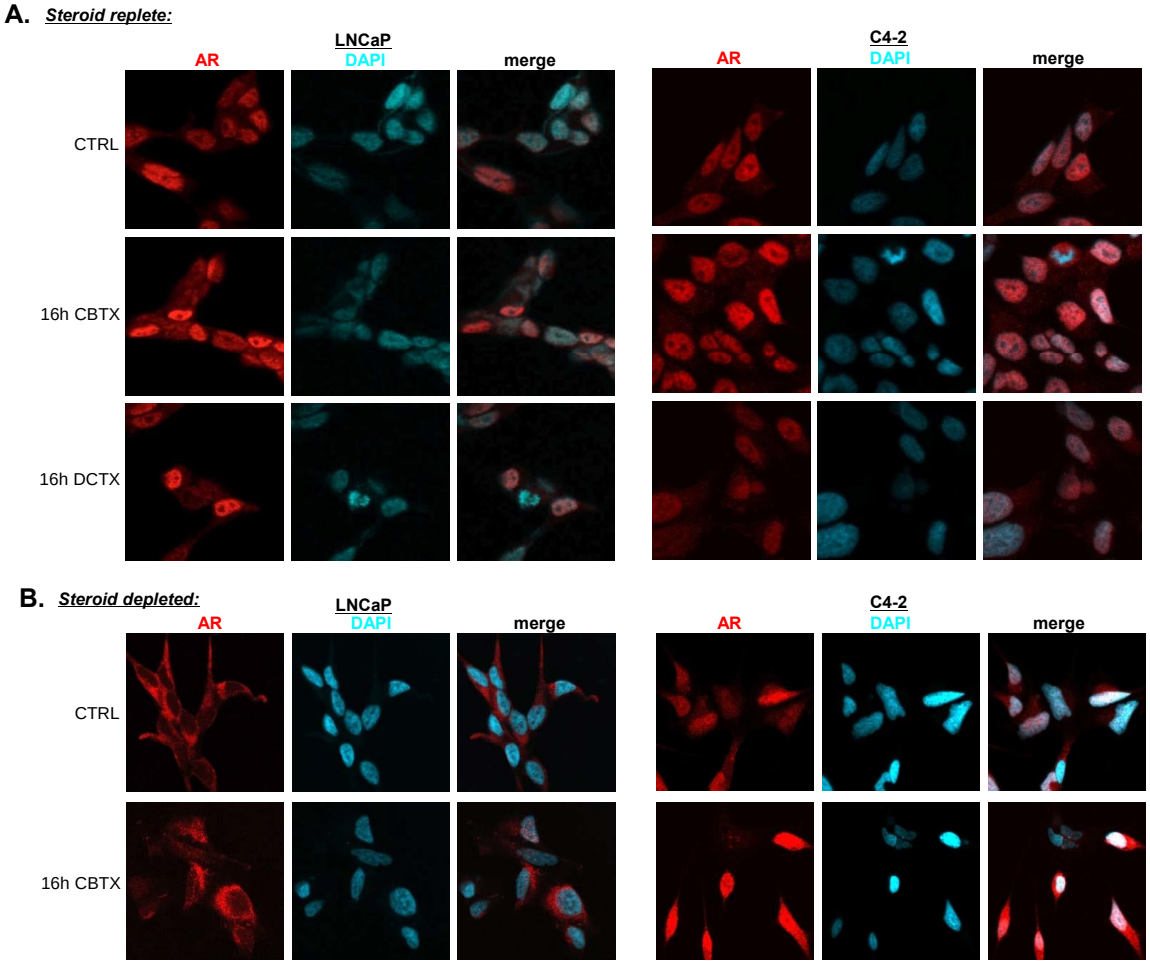


***Figure 3*: The effects of taxanes are independent of altered AR localization.** A. Immunofluorescence after 16h 1nM CBTX, DCTX or CTRL treatment demonstrates nuclear localization of AR (red) is unaffected in presence of steroids. DAPI staining (cyan) denotes cell nuclei. B. In androgen deprivation, AR resides in the cytoplasm of LNCaP cells, but remains nuclear in C4-2 CRPC cells in androgen deprivation conditions (-steroids), even after 16h cabazitaxel exposure.

Figure 4

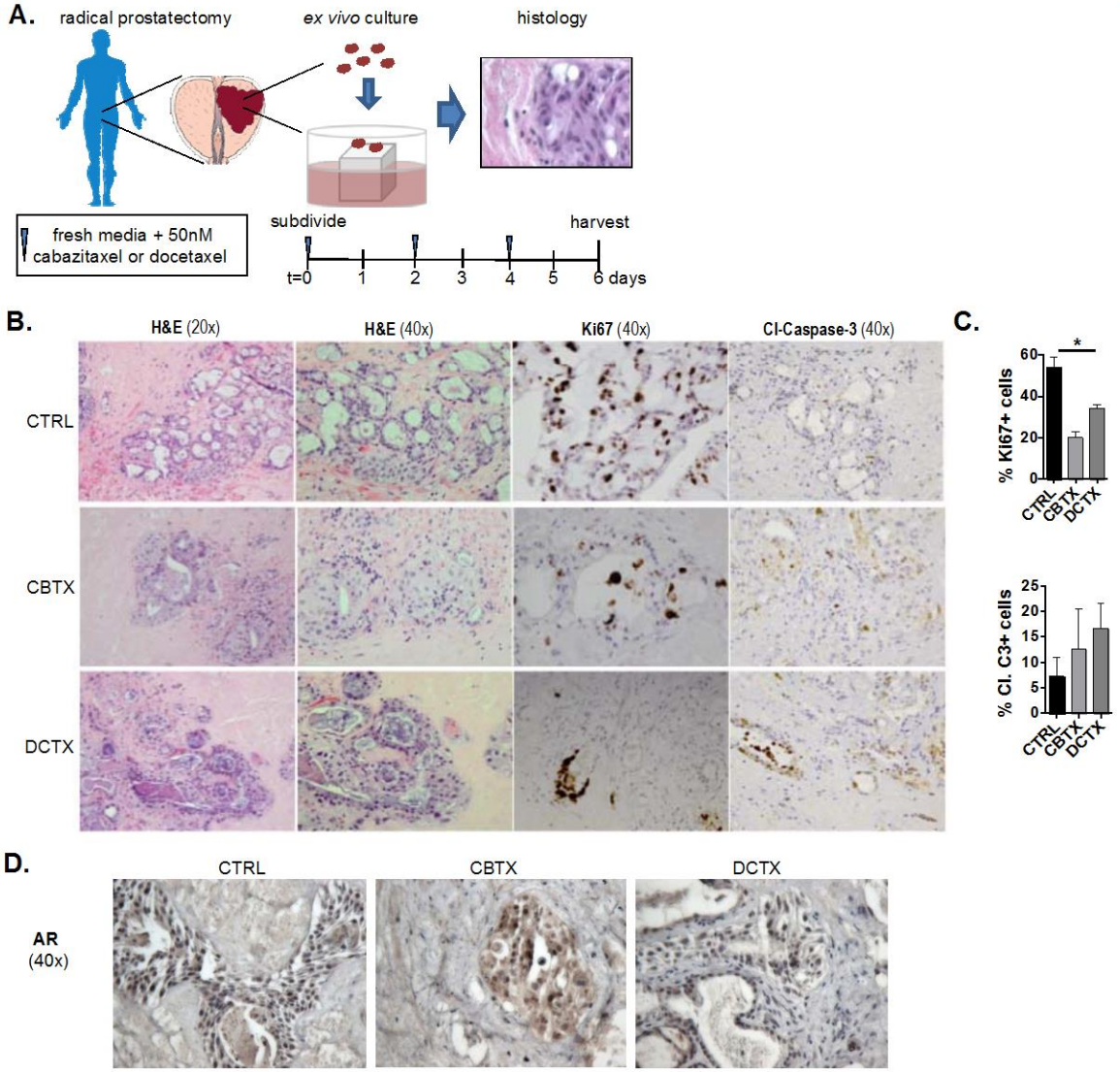


***Figure 4*: Cabazitaxel exerts enhanced anti-tumor effects in human tumors.** A. Schematic overview of the *ex vivo* explant assay. Fresh tissue obtained from radical prostatectomy is subdivided and cultured for 6 days on dental sponges submerged in cell culture media with different treatments (CTRL, 50nM CBTX or DCTX), after which tissue is formalin-fixed, paraffin-embedded, and analyzed by standardized immunohistochemistry (IHC) methods. B. H&E and IHC on explant tissues demonstrate that taxane treatment reduces tumor cell proliferation (Ki67) and induces cell death, (Caspase-3). C. Quantification of Ki67 (top) in three independent explant assays, performed on tissue from three patients, shows reduced proliferation in cabazitaxel treated samples compared to control ($p<0.05$ by Kruskal-Wallis non-parametric multiple comparison analysis). Cabazitaxel demonstrates improved drug efficacy compared to docetaxel *ex vivo*. Quantification of Caspase-3 in the three explant assays (bottom) indicates a moderate, yet non-significant increase in cell death, as induced by taxane treatment. D. AR (IHC) remains nuclear in tumor specimens after 6 days of taxane treatment.

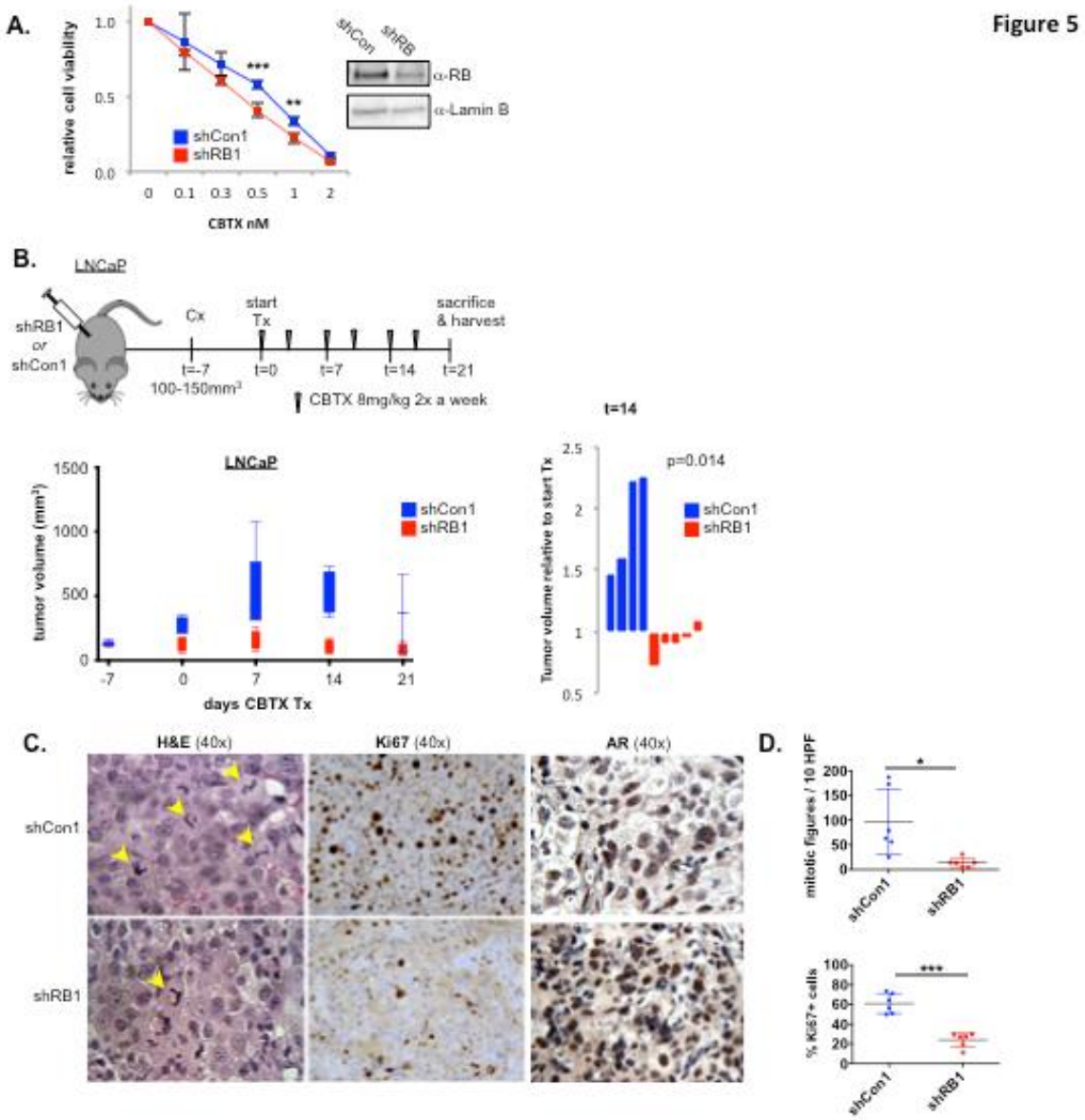
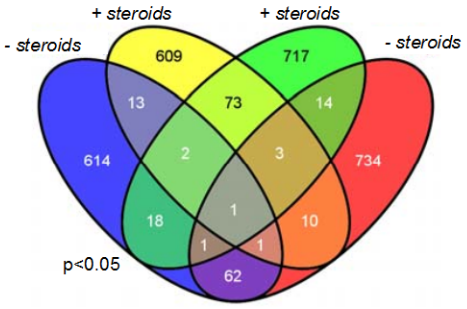


Figure 5: Tumors that progress to CRPC by RB loss show hypersensitivity to cabazitaxel. A. RB knockdown LNCaP cells (shRB1) show a modest sensitization in vitro to 48h cabazitaxel and 96h in fresh media (no CBTX) in culture conditions mimicking androgen deprivation therapy (ADT), but not in presence of hormones (in serum), as measured cell viability in a trypan blue exclusion assay. B. Treatment schematic for nude athymic mice, subcutaneously injected with LNCaP shRB1 or LNCaP shCon1 cells to obtain xenograft tumors in vivo (n=5 per group). Growth analysis of xenograft tumors was monitored over time of CBtX treatment (start t=0). The graph on the right denotes the sizes for each tumor at t=14 relative to the size at start of treatment (t=0), showing that only LNCaP shRB1 tumors respond to CBTX (p=0.014). C. RB proficient LNCaP xenograft tumors display decreased Ki67 staining (unpaired test: $p<0.0001$), and elevated numbers of mitotic figures after CBTX treatment. AR remains nuclear after CBTX exposure, irrespective of RB status. D. Quantification of mitotic figures (top) and Ki67 positive cells (bottom) in 10 High Power Fields (HPF, 400x) per tumor show elevated % of mitotic figures in shCon1 tumors, and a reduction in proliferating cells in shRB1 tumors.

Figure 6

A.



B. Top 25 hits

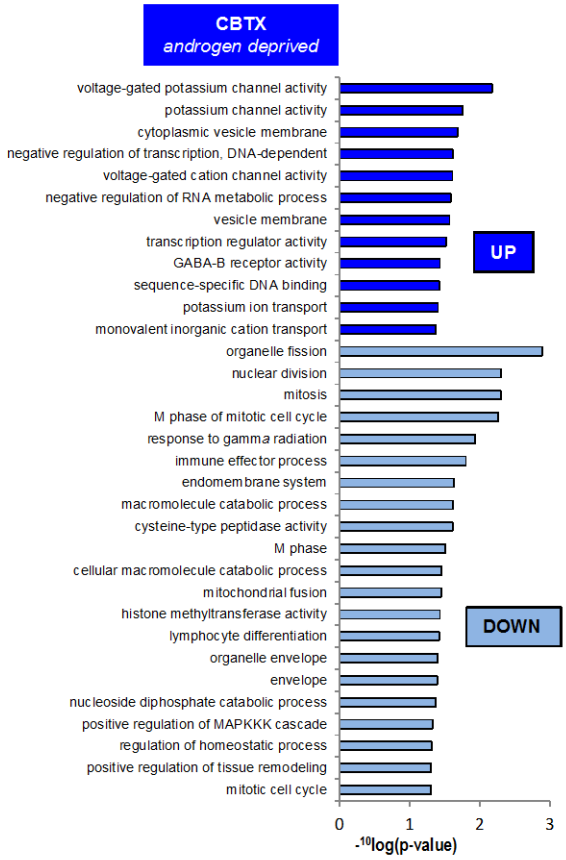
CBTX *androgen deprived*

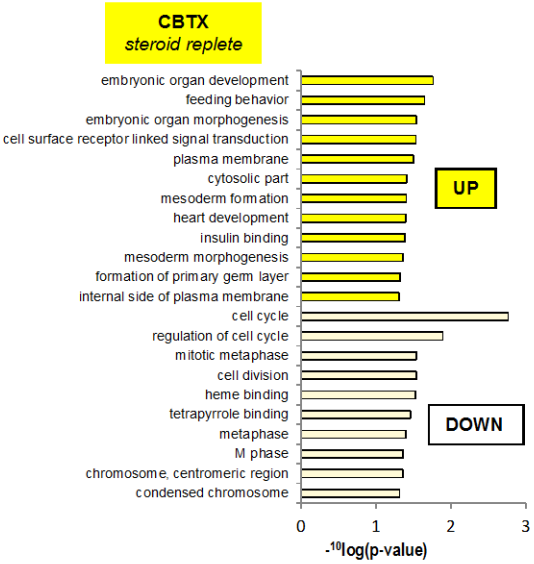
UP gene	UP fold	DOWN gene	DOWN fold
LHX3	1.7749	STARD13	-1.59
RNLS	1.6769	RMI2	-1.523
CIDEC	1.623	ZMYND10	-1.486
RELT	1.6082	GPC6	-1.473
OR10C1	1.5813	RPS6KA5	-1.469
GGT5	1.5577	ORC6	-1.468
RUNX1	1.5233	TUBGCP5	-1.463
TMEM100	1.5073	NADSYN1	-1.439
PIM1	1.4997	AFAP1L2	-1.401
IL1RAP	1.4993	CXCL1	-1.396
PRDM8	1.4561	E2F7	-1.387
C4B	1.4557	RASSF8	-1.379
KCNH6	1.4473	AKR7A3	-1.373
CGB	1.4342	COX4I2	-1.369
AREG	1.4339	CDC6	-1.367
LRRC7	1.4338	RGP1	-1.36
CSN3	1.428	CD27	-1.346
KCNJ10	1.4051	ARL4D	-1.344
KCNC3	1.4046	ANGPTL7	-1.343
OR51M1	1.4034	CCDC88C	-1.34
HEPHL1	1.3979	RAB3IL1	-1.339
PRAMEF10	1.3977	GPR89C	-1.332
GAST	1.3915	KIF23	-1.327
DEFA1B	1.3888	OIP5	-1.324
SPEG	1.3887	ALDH16A1	-1.322

CBTX *steroid replete*

UP gene	UP fold	DOWN gene	DOWN fold
NLRP13	1.81	TRAIP	-1.57
FERD3L	1.69	THSD4	-1.57
CAMKMT	1.66	OGN	-1.53
LGR6	1.65	ABCC3	-1.51
WNT3A	1.62	PTPDC1	-1.47
AP1M1	1.58	IGLJ5	-1.42
ZFAND6	1.57	FANCD2	-1.40
NAV3	1.51	ERG	-1.38
ANK2	1.51	TMCO7	-1.38
OBSL1	1.50	ALDH3A1	-1.38
IL28RA	1.50	MAPK10	-1.38
OR2AK2	1.48	LYZ	-1.36
SLC34A2	1.47	NFKBIZ	-1.35
BTLA	1.45	TIGD4	-1.35
TMEM88B	1.44	NOBOX	-1.34
WDR81	1.44	IL1A	-1.34
HTR2C	1.43	LHFP	-1.34
IFIT5	1.43	MDC1	-1.33
ECEL1	1.42	NOS1	-1.33
PNLIPRP3	1.41	ENTHD1	-1.33
TRIM15	1.40	CYP8B1	-1.32
FMOD	1.40	ZNF630	-1.31
PDZD3	1.39	SCGB2A2	-1.31
PNMA5	1.39	DNAH10	-1.31
NODAL	1.38	VAV1	-1.31

C. GO analysis (p<0.05)





D. AR targets:

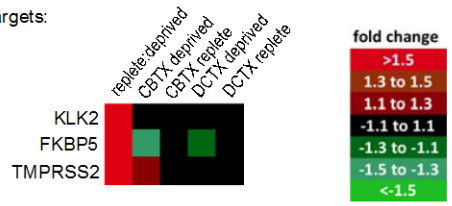


***Figure 6*: Expression profiling reveals novel action(s) of cabazitaxel in prostate cancer cells.** A. Venn diagram of a microarray analysis of p<0.05 after background filtering reveals differential response of castrate-sensitive LNCaP cells to 16hrs 1nM CBTX compared to DCTX, dependent on culture conditions: steroid rich (+) versus depleted (-) media, in triplicate. B. Top 25 significant hits of CBTX unique in androgen deprived (blue) or steroid replete (yellow) conditions are displayed with the corresponding mean fold change over control treated samples. C. David bioinformatics analysis of CBTX specific genes in castrate (blue, corresponding with Venn cluster) or steroid replete (yellow) displays GO-term enrichment of cell cycle genes, which confirms drug effects in mitosis. C. Heatmap of AR target genes: differential expression in steroid replete versus deprived (first column), and taxane versus control under different steroid conditions, as labeled.

Literature Cited

1. Darshan MS, Loftus MS, Thadani-Mulero M, Levy BP, Escuin D, Zhou XK, Gjyrezi A, Chanel-Vos C, Shen R, Tagawa ST, et al. Taxane-induced blockade to nuclear accumulation of the androgen receptor predicts clinical responses in metastatic prostate cancer. *Cancer research.* 2011;71(18):6019-29.
2. van Soest RJ, van Royen ME, de Morree ES, Moll JM, Teubel W, Wiemer EA, Mathijssen RH, de Wit R, and van Weerden WM. Cross-resistance between taxanes and new hormonal agents abiraterone and enzalutamide may affect drug sequence choices in metastatic castration-resistant prostate cancer. *European journal of cancer.* 2013;49(18):3821-30.
3. Zhu ML, Horbinski CM, Garzotto M, Qian DZ, Beer TM, and Kyprianou N. Tubulin-targeting chemotherapy impairs androgen receptor activity in prostate cancer. *Cancer research.* 2010;70(20):7992-8002.
4. Singh RK, and Lokeshwar BL. Depletion of intrinsic expression of Interleukin-8 in prostate cancer cells causes cell cycle arrest, spontaneous apoptosis and increases the efficacy of chemotherapeutic drugs. *Molecular cancer.* 2009;8(57.
5. Yang DR, Ding XF, Luo J, Shan YX, Wang R, Lin SJ, Li G, Huang CK, Zhu J, Chen Y, et al. Increased chemosensitivity via targeting testicular nuclear receptor 4 (TR4)-Oct4-interleukin 1 receptor antagonist (IL1Ra) axis in prostate cancer CD133+ stem/progenitor cells to battle prostate cancer. *The Journal of biological chemistry.* 2013;288(23):16476-83.
6. Centenera MM, Raj GV, Knudsen KE, Tilley WD, and Butler LM. Ex vivo culture of human prostate tissue and drug development. *Nature reviews Urology.* 2013;10(8):483-7.
7. Comstock CE, Augello MA, Goodwin JF, de Leeuw R, Schiewer MJ, Ostrander WF, Jr., Burkhart RA, McClendon AK, McCue PA, Trabulsi EJ, et al. Targeting cell cycle and hormone receptor pathways in cancer. *Oncogene.* 2013;32(48):5481-91.
8. Sharma A, Yeow WS, Ertel A, Coleman I, Clegg N, Thangavel C, Morrissey C, Zhang X, Comstock CE, Witkiewicz AK, et al. The retinoblastoma tumor suppressor controls androgen signaling and human prostate cancer progression. *J Clin Invest.* 2010;120(12):4478-92.
9. Sharma A, Comstock CE, Knudsen ES, Cao KH, Hess-Wilson JK, Morey LM, Barrera J, and Knudsen KE. Retinoblastoma tumor suppressor status is a critical determinant of therapeutic response in prostate cancer cells. *Cancer Res.* 2007;67(13):6192-203.
10. Bosco EE, Mayhew CN, Hennigan RF, Sage J, Jacks T, and Knudsen ES. RB signaling prevents replication-dependent DNA double-strand breaks following genotoxic insult. *Nucleic acids research.* 2004;32(1):25-34.
11. Burkhart DL, and Sage J. Cellular mechanisms of tumour suppression by the retinoblastoma gene. *Nature reviews Cancer.* 2008;8(9):671-82.
12. Hao J, Madigan MC, Khatri A, Power CA, Hung TT, Beretov J, Chang L, Xiao W, Cozzi PJ, Graham PH, et al. In vitro and in vivo prostate cancer metastasis and chemoresistance can be modulated by expression of either CD44 or CD147. *PloS one.* 2012;7(8):e40716.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown
Race:

_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
____Yes
____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two

publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Novel actions of next-generation taxanes benefit advanced stages of prostate cancer.	de Leeuw R, Berman-Booty LD, Schiewer MJ, Ciment SJ, Den RB, Dicker AP, Kelly WK, Trabulsi EJ, Lallas CD, Gomella LG, Knudsen KE.	Clinical Cancer Research	May 2014	☐Submitted ☐Accepted ☒Published
2. Consequence of the tumor-associated conversion to cyclin D1b.	Augello MA, Berman-Booty LD, Carr R 3rd, Yoshida A, Dean JL, Schiewer MJ, Feng FY, Tomlins SA, Gao E, Koch WJ, Benovic JL, Diehl JA, Knudsen KE.	EMBO Molecular Medicine	May 2014	☐Submitted ☐Accepted ☒Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____ No___X_____

If yes, please describe your plans:

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be

single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Major impact will be divided into 2 sections:

1. First, the data herein show that (i) cabazitaxel exerts stronger cytostatic and cytotoxic response compared with docetaxel, especially in CRPC; (ii) cabazitaxel induces aberrant mitosis, leading to pyknotic and multinucleated cells; (iii) taxanes do not act through the androgen receptor (AR); (iv) gene-expression profiling reveals distinct molecular actions for cabazitaxel; and (v) tumors that have progressed to castration resistance via loss of RB show enhanced sensitivity to cabazitaxel. Our combined findings show that Cabazitaxel not only induces improved cytostatic and cytotoxic effects, but also affects distinct molecular pathways when compared with docetaxel, which could underlie its efficacy after docetaxel treatment has failed in patients with CRPC. Finally, RB is identified as the first potential biomarker that could define the therapeutic response to taxanes in metastatic CRPC. This would suggest that loss of RB function induces sensitization to taxanes, which could benefit up to 50% of CRPC cases.

2. Clinical evidence suggests that cyclin D1b, a variant of cyclin D1, is associated with tumor progression and poor outcome; however, the underlying molecular basis was unknown. In studies guided by the above observations associated with RB pathway disfunction, novel models were created to generate a genetic switch from cyclin D1 to cyclin D1b. Extensive analyses uncovered overlapping, but non-redundant functions of cyclin D1b compared with cyclin D1 on developmental phenotypes, and illustrated the importance of the transcriptional regulatory functions of cyclin D1b *in vivo*. Data obtained identify cyclin D1b as an oncogene; cyclin D1b expression under the endogenous promoter induced cellular transformation and further cooperated with known oncogenes to promote tumor growth *in vivo*. Further molecular interrogation uncovered unexpected links between cyclin D1b and the DNA damage/PARP1 regulatory networks, which could be exploited to suppress cyclin D1b-driven tumors. Collectively, these data are the first to define the consequence of cyclin D1b expression on normal cellular function, present evidence for cyclin D1b as an oncogene, and provide pre-clinical evidence of effective methods to thwart growth of cells dependent upon this oncogenic variant.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert “None”; do not use “Not applicable.” Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Major discoveries:

1. Next generation taxanes act in a manner distinct from docetaxel

2. Identification of the first putative biomarker to stratify patients into more effective treatment regimens; it was shown that tumors defective in the RB pathway are hypersensitive to taxanes. These findings form the basis for a newly funded and initiated clinical trial assessing the impact of RB status in therapeutic response in patients with advanced prostate cancer.

3. Novel model generation of RB pathway dysfunction through genetic alteration of the RB locus revealed unexpected effects on genome integrity and the response to therapeutic DNA damaging agents.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

NAME Karen E. Knudsen	POSITION TITLE Professor, Can Bio, Urology, Rad Onc, & Med Onc, Interim Chair, Cancer Biology Director, Sidney Kimmel Cancer Center Thomas Jefferson University
eRA COMMONS USER NAME KKNUDSEN	
EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such*	

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
The George Washington University	B.S.	1990	Biology
University of California San Diego, La Jolla CA	Ph.D.	1996	Molecular Biology
Ludwig Institute for Cancer Research	Post-doc	1997-99	AR & cell cycle in PCa

A. Positions and Honors *(limited to current due to page constraints):*

University Appointments:

2007-present Member, Kimmel Cancer Center
2007-present Director, Greater Philadelphia Prostate Cancer Working Group
2010-present **Professor**, Departments of Cancer Biology (primary), Urology, and Radiation Oncology, TJU
2014-present **Professor** and **Director of Research**, Medical Oncology, TJU
2015-present **Interim Director**, SKCC
2015-present **Interim Chair**, Cancer Biology

B. Peer-Reviewed Publications: *(from>77 papers; H index 26; 1,981 citations)*

1. Burd CJ, Petre CE, Morey LM, Wang Y, Revelo MP, Haiman CA, Lu S, Fenoglio-Preiser CM, Li J, Knudsen ES, Wong J, and **Knudsen KE.** (2006) The cyclin D1b variant influences prostate cancer growth through aberrant AR regulation. *Proc. Natl. Acad. Sci.*, 103(7): 2190-5.
2. Hess-Wilson JK, Daly, HK, Zagorski WA, and **Knudsen KE.** (2006) Mitogenic action of the androgen receptor sensitizes prostate cancer cells to cytotoxic insult. *Cancer Research*, 66(24):11998-2008.
3. Sharma A, Berrera J, Knudsen ES, Morey LM, Hess-Wilson JK, and **Knudsen KE**(2007) RB status is a critical determinant of therapeutic response in prostate cancer.*Cancer Research*, 67(13):6192-203.
4. Olshavsky N, Groh E, Burd CJ, Morey L, Comstock CE, Wang Y, Revelo MP, and **Knudsen KE** (2008) Cyclin D3 impact on androgen signaling and prostate cancer, *Oncogene*.27(22):3111-21.
5. Shen H, Powers N, Saini N, Comstock CES, Sharma A, Weaver K, Revelo MP, Gerald W, Williams E, Jessen WJ, Aronow BJ, Rosson G, Weissman B, Yaniv M, Muchardt C, and **Knudsen KE** (2008) The SWI/SNF ATPase Brm is a gatekeeper of proliferative control in prostate cancer, *Cancer Research*, Dec 15;68(24):10154-62. (*selected for cover and Highlights section).
6. Comstock CES, Augello MA, PeBenito R, Karch J, Tran TH, Utama FE, Tindall EA, Wang Y, Burd CJ, Groh EM, Hoang HN, Giles GG, Severi G, Hayes VM, Henderson BE, Le Marchand L, Kolonel LN, Haiman CA, Baffa R, Gomella LG, Knudsen ES, Rui H, Henshall SM, Sutherland RL and **Knudsen KE** (2009) Cyclin D1 splice variants: polymorphism, risk, and isoform specific regulation in prostate cancer. *Clinical Cancer Research* 15(17): 5338-49. (* selected as the featured cover article)
7. Augello MA, Hickey TE, and **Knudsen KE.** (2011) FOXA1: master of steroid receptor function in cancer. *EMBO J.* Sep 20. doi: 10.1038/emboj.2011.340. PMID: 21934649

8. Schiewer MJ, Goodwin JF, Han S, Brenner JC, Augello MA, Dean JL, Liu F, Planck JL, Ravindranathan P, Chinnaiyan AM, McCue P, Gomella LG, Raj GV, Dicker AP, Brody JR, Pascal JM, Centenera MM, Butler LM, Tilley WD, Feng FY, **Knudsen KE**. (2012) Dual roles of PARP-1 promote cancer growth and progression. *Cancer Discov*. 2012 Dec;2(12):1134-1149. Epub 2012 Sep 19.
9. Goodwin JF, Schiewer MJ, Dean JL, Schrecengost RS, de Leeuw R, Han S, Ma T, Den RB, Dicker AP, Feng FY, **Knudsen KE**. (2013) A hormone-DNA repair circuit governs the response to genotoxic insult. *Cancer Discov* 2013 Sept 11 [epub ahead of print].
10. Goodwin JF, **Knudsen KE**. (2014) Beyond DNA Repair: DNA-PK Function in Cancer. *Cancer Discov* 2014 Oct;4(10):1126-1139

D. Research Support *(Current Only)*

5 R01 CA159945-03(PI Knudsen, K) 03/01/12-02/28/17
SWI/SNF dysregulation in development and disease

1 R01 CA176401-01A1 (PI Knudsen, K.) 01/14/14-12/31/19
RB function in prostate cancer progression

5 P30CA056036-14 (PI Knudsen, K.) 06/01/14-05/31/18
Translational Research in Cancer