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PRINCIPAL INVESTIGATOR: Atish Choudhury, MD, PhD

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Atish Choudhury, MD, PhD
Email: Achoudhury@partners.org
Kent Mouw, MD, PhD
Email: Kent_Mouw@dfci.harvard.edu

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Dana-Farber Cancer Institute
450 Brookline Avenue
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Alterations in DNA damage repair (DDR) genes are common in metastatic castration-resistant prostate cancer (mCRPC), and are implicated in responses to carboplatin, PARP inhibitors and immunotherapeutics. Inhibitors of the ATR kinase, which is involved in the DDR response, have been demonstrated to have synergistic activity with platinum compounds in preclinical models. We therefore conducted a Phase 2 study of the ATR inhibitor M6620+carboplatin vs. docetaxel+carboplatin in mCRPC (NCI protocol # 10191, NCT03517969). The trial mandates pre-treatment tumor biopsy and research blood collections for circulating cell-free DNA (cfDNA) analyses pre-treatment, every 3 cycles on treatment and at end of study. This proposal is for biomarker studies from these biospecimens and for functional studies in model systems to define genetic correlates of response and resistance to therapy.

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Molecular and genetic determinants of response to carboplatin with or without an ATR inhibitor (M6620) in mCRPC

PI: Atish Choudhury, MD PhD

Co-PI: Kent Mouw, MD PhD

1. INTRODUCTION

Alterations in DNA damage repair (DDR) genes are common in metastatic castration-resistant prostate cancer (mCRPC), and are implicated in responses to carboplatin, PARP inhibitors and immunotherapeutics. Inhibitors of the ATR kinase, which is involved in the DDR response, have been demonstrated to have synergistic activity with platinum compounds in preclinical models. We therefore conducted a Phase 2 study of the ATR inhibitor M6620+carboplatin vs. docetaxel+carboplatin in mCRPC (NCI protocol # 10191, NCT03517969). The trial mandates pre-treatment tumor biopsy and research blood collections for circulating cell-free DNA (cfDNA) analyses pre-treatment, every 3 cycles on treatment and at end of study. This proposal is for biomarker studies from these biospecimens and for functional studies in model systems to define genetic correlates of response and resistance to therapy.

2. KEYWORDS

Prostate cancer, carboplatin, ATR, VX-970, M6620, berzosertib, castration resistant, DDR, HRR

3. ACCOMPLISHMENTS:

For NCI protocol # 10191, patients previously treated with at least one secondary hormonal therapy and taxane underwent mandatory pre-treatment biopsy and were randomized 1:1 to receive Arm A (docetaxel 60 mg/m2 day 1 + carboplatin AUC 4 day 1) or Arm B (M6620 90 mg/m2 days 2,9 + carboplatin AUC 5 day 1) every 21 days. Patients randomized to Arm A who were not candidates for docetaxel received carboplatin AUC 5 monotherapy. Stratification factors were 1) prior PARP inhibitor (yes vs. no) and 2) evaluable disease by RECIST 1.1 (yes vs. no). Patients on Arm A crossed over to Arm B (M6620+carboplatin) at the earlier of PSA or radiographic progression. The primary endpoint was overall response rate (ORR; PSA reduction by ≥ 50% or radiographic response by RECIST 1.1). Secondary endpoints included time to PSA progression, radiographic PFS (rPFS), PFS by PCWG3 criteria, and adverse events (AEs) in each arm. Planned enrollment was 136 patients (for 130 to be treated), with interim analysis for futility after 65 patients were treated.

Seventy-three patients were randomized between 6/2019 and 7/2020; 34 patients were treated on Arm A (26 carboplatin+docetaxel; 8 carboplatin alone) and 31 on Arm B. Median number of prior systemic therapies (excluding ADT, 5α-reductase inhibitors, 1st generation antiandrogens) was 4 (range 2-8). Median treatment duration was 3 cycles, and 4 patients in each arm discontinued for AEs. Grade 3 or higher treatment-related AEs (TrAE) were seen in 13(38%) patients in Arm A and 21(68%) in Arm B. Patients in Arm B had greater frequency of grade 3-4 thrombocytopenia (8[26%] vs. 3[9%]). 1 pt in Arm B had grade 5 sepsis attributed to study treatment. ORR was 15% in Arm A (5/34; 5/26[19%] in patients who received carboplatin+docetaxel) and 0% in Arm B (0/31). 14 patients in Arm A crossed over, with no subsequent responses seen. Median rPFS was 2.1(95% CI:2.0,3.2) mo in Arm A and 2.4(1.9,4.2) mo in Arm B. At planned interim analysis, trial enrollment and crossover to Arm B were halted due to futility.

At the time of this writing, biospecimens from trial participants have been analyzed as detailed below, with correlation with clinical outcomes pending.

What were the major goals of the project? / What was accomplished under these goals?

Specific Aim 1: To correlate genetic and molecular features from pre-treatment tumor biopsy and cfDNA with clinical outcomes for M6620+carboplatin and docetaxel+carboplatin

Major Task 1: IRB and HRPO approval

The biomarker analyses from tumor and blood specimens from participants in the clinical trial are included in the study protocol and were approved by the Central IRB (CIRB). In addition, a secondary use protocol that includes only those activities funded by the DoD (as referenced in the approved Statement of Work) was written and has been approved by the Dana-Farber/Harvard Cancer Center (under DF/HCC protocol # 20-661) and by HRPO. Letters documenting continuing CIRB approval for protocol # 10191, IRB approval for DF/HCC protocol # 20-661 and HRPO approval were attached in previous versions of this Technical Report.

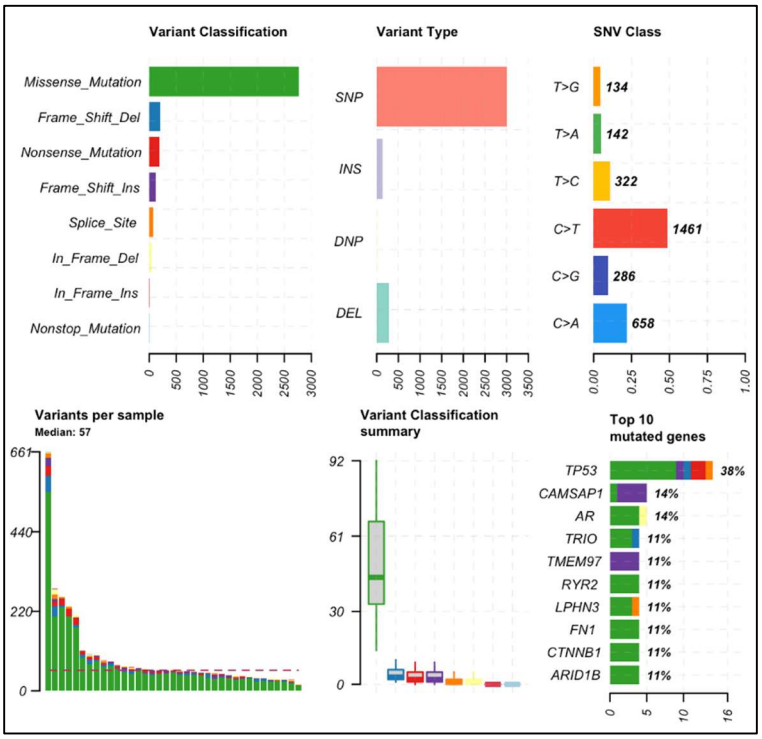


Figure 1. Summary of somatic variants (based on MuTect2) from paired tumor/normal whole exome sequencing of 37 tumor biopsy specimens after filtering by blacklist_freq.txt, blackListedGenes.txt, and for 10% variant allele frequency (VAF)

Major Task 2: Whole exome sequencing analysis of pre-treatment tumor biopsy specimens

While pre-treatment biopsy was mandatory for trial participation, this requirement was waived during the COVID-19 pandemic during a time when research biopsies were not being performed at many institutions. Of the 73 randomized patients, 68 patients had pre-treatment biopsies performed and sent to the NCI Biorepository for analysis.

DNA and RNA were successfully extracted from the pre-treatment specimens, though three of these cases initially had low yield so required coring and re-extraction. After undergoing additional quality check steps, 39 of the 65 treated patients had extracted DNA felt adequate for whole exome sequencing (WES). One of the 39 DNA specimens failed sequencing and another did not have matched germline available, so 37 tumor/normal pairs were available for mutation calling as summarized in Figure 1.

Among genes of interest related to homologous recombination deficiency, 0/37 patients had detected somatic variant in *BRCA2*, 2 had *ATM* mutations (1 insertion, 1 single nucleotide variant [SNV]), 1 had an SNV in *BRCA1* (along with deletion of *PALB2*), and 2 had SNVs in *PALB2*.

Major Task 3: Circulating cell-free DNA analysis from pre-treatment specimens

We have established a workflow for circulating cell-free DNA sequencing, which starts with ultra-low pass whole genome sequencing (ULP-WGS) library construction using a 6 base pair Unique Molecular Identifier (UMI). UMIs aid in identification of PCR duplicates to distinguish true mutations from PCR errors/sequencing errors based on consensus among reads sharing same the index. Sequencing data from ULP-WGS is used to derive tumor fraction using a previously reported computational tool called ichorCNA. The same library from

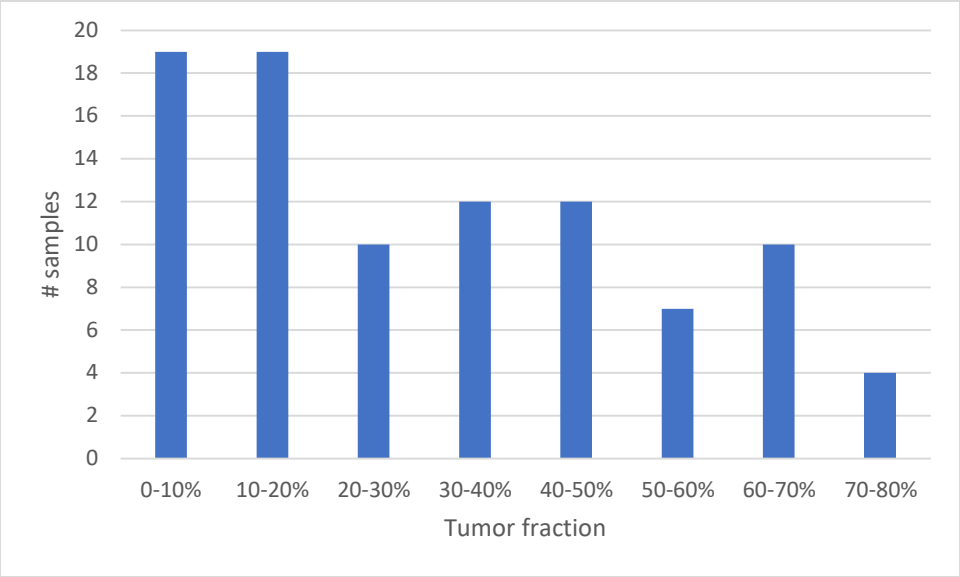


Figure 2. Distribution of tumor fraction as determined by ichorCNA for 95 specimens that underwent ULP-WGS.

ULP-WGS is selected in hybrid capture using custom targeted panel with a goal 10,000x – 25,000x mean target coverage (MTC) depending on tumor fraction, with 10,000x MTC for specimens with tumor fraction > 10% and 25,000x for < 10%.

NCI biorepository successfully isolated cfDNA from plasma for 63 of the 65 treated patients, 95 specimens in total. These underwent ULP-WGS, and tumor fraction was estimated using ichorCNA as depicted in Figure 2.

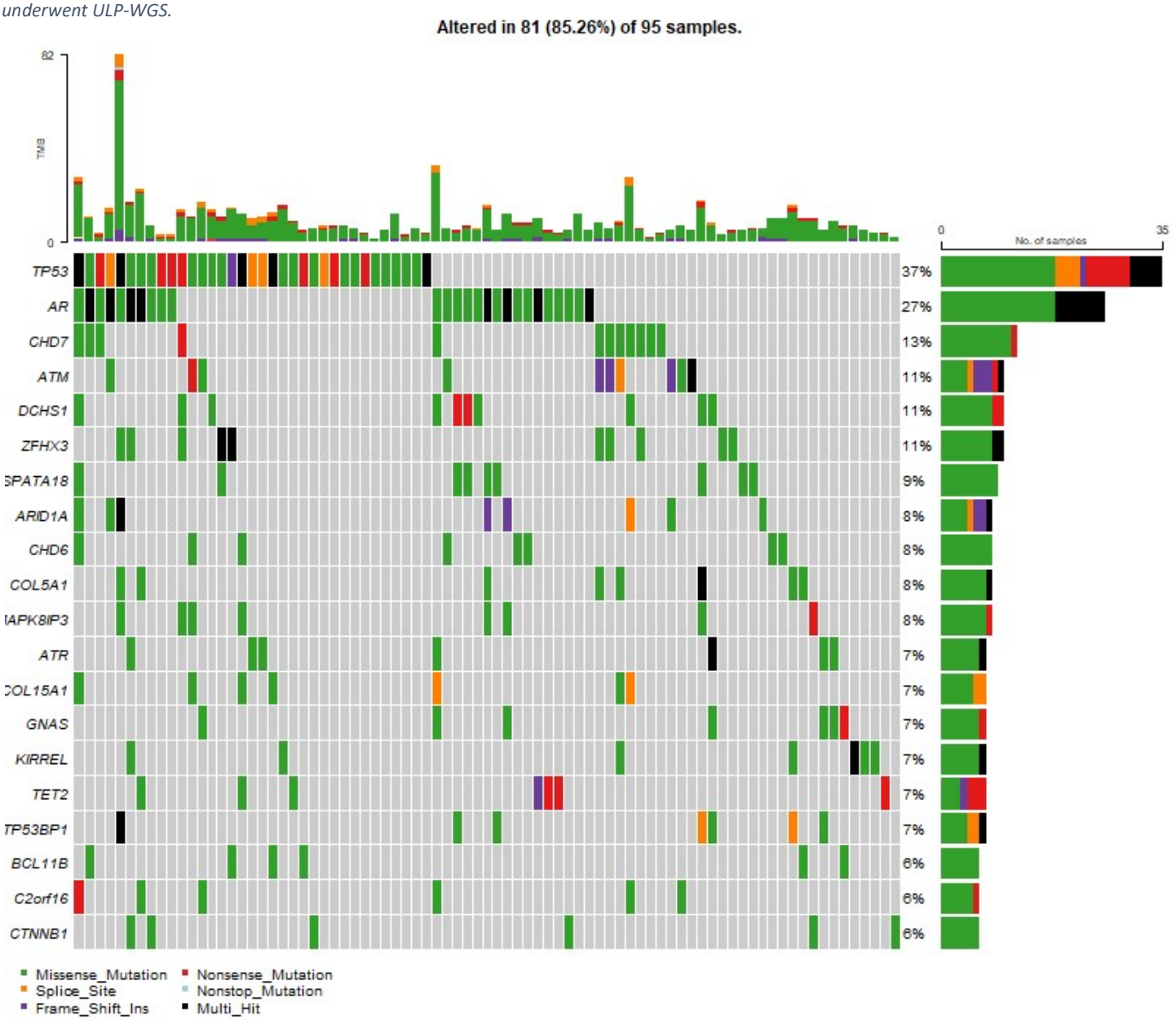


Figure 3. Co-mut plot of somatic variants detected from paired tumor-normal deep targeted panel sequencing from circulating cell-free DNA

We applied targeted sequencing panel detailed in the grant application developed in collaboration with Dr. Franklin Huang currently at University of California San Francisco. This panel includes exonic regions of 320 genes (including DNA damage repair genes, genes previously reported to be significantly mutated in prostate cancer, genes with mutations detected in African American patients), the AR enhancer, and intronic regions of ETV1, BRAF, SLC45A3, ETV4, ETV5, ERG, TMPRSS2, FOXA1, RAF1. The co-mut plot of the most commonly detected gene alterations is shown in Figure 3, and the frequency of alterations in genes involved in DNA damage repair is shown in Table 1.

Table 1. Frequency of DDR gene alterations detected through targeted panel sequencing of cfDNA specimens

Hugo_Symbol	Frame_Shift _Ins	In_Frame_Del	In_Frame_Ins	Missense _Mutation	Nonsense _Mutation	Nonstop _Mutation	Splice_Site	total.	Mutated _Samples.	Percentage (out of 95 samples)
ATM	3	0	0	6	1	0	1	11	10	10.52632
PALB2	0	0	0	4	0	0	1	5	5	5.263158
CDK12	2	0	0	4	1	0	0	7	4	4.210526
CHEK2	0	0	0	5	0	0	1	6	3	3.157895
BRCA2	1	1	0	2	0	0	0	4	3	3.157895
BARD1	0	0	0	3	0	0	0	3	3	3.157895
BRCA1	0	0	0	2	1	0	0	3	3	3.157895
BRIP1	0	0	0	2	0	0	0	2	2	2.105263
RAD51C	1	0	0	0	0	0	0	1	1	1.052632

Major Task 4: RAD51 focus formation and ATM IHC assays

The RAD51 focus formation assay was described in the initial grant application and has been validated in prostate cancer model systems, and we described the ATM and phospho-KAP1 assays in prior versions of this Technical Report.

Tumor specimens were cut for immunohistochemistry at the NCI Biorepository and underwent extensive quality control. Of the 65 treated patients, 41 had tissue with adequate tumor content for IHC – these specimens were received at the Center for DNA Damage and Repair Laboratory at Dana-Farber Cancer Institute on 1/25/22. Staining for RAD51, Geminin, ATM and pKAP1 has been completed. Representative RAD51 staining results are depicted in Figure 4. This assay identified 3 tumor specimens to be HR-deficient, and 3 to be likely deficient.

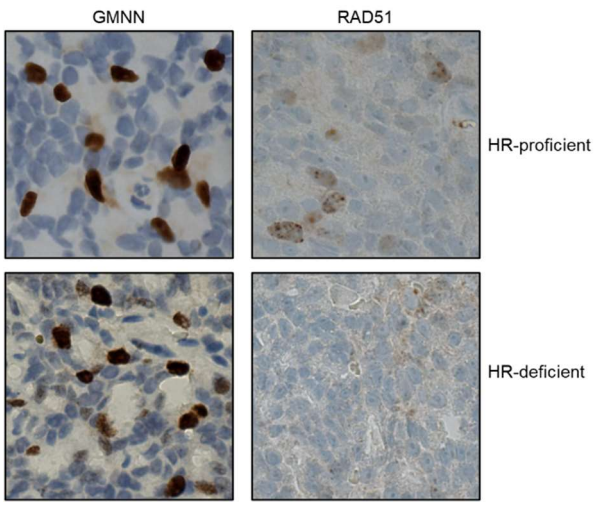


Figure 4. Representative RAD51 IHC on tumor biopsy specimens with Geminin (GMNN) control in HR-proficient (top) and HR-deficient (bottom) tumor biopsy specimens

For the ATM IHC assay, ATM staining was considered positive (Pos) if > 90% of tumor cells were positive, negative (neg) if < 5% of tumor cells were positive and heterogeneous (Hetero) if > 5% but < 90% tumor cells were positive. Representative images from this assay are shown in Figure 5A. The % pKAP1 positive cells in each sample was determined using image analysis on Aperio (Leica Biosystems). Since KAP1 is a substrate of ATM and is phosphorylated in response to DNA damage, we expect phospho-KAP1 (pKAP1) levels to be low in cells with low ATM levels compared to those with heterogeneous or high expression as is seen in Figure 5B.

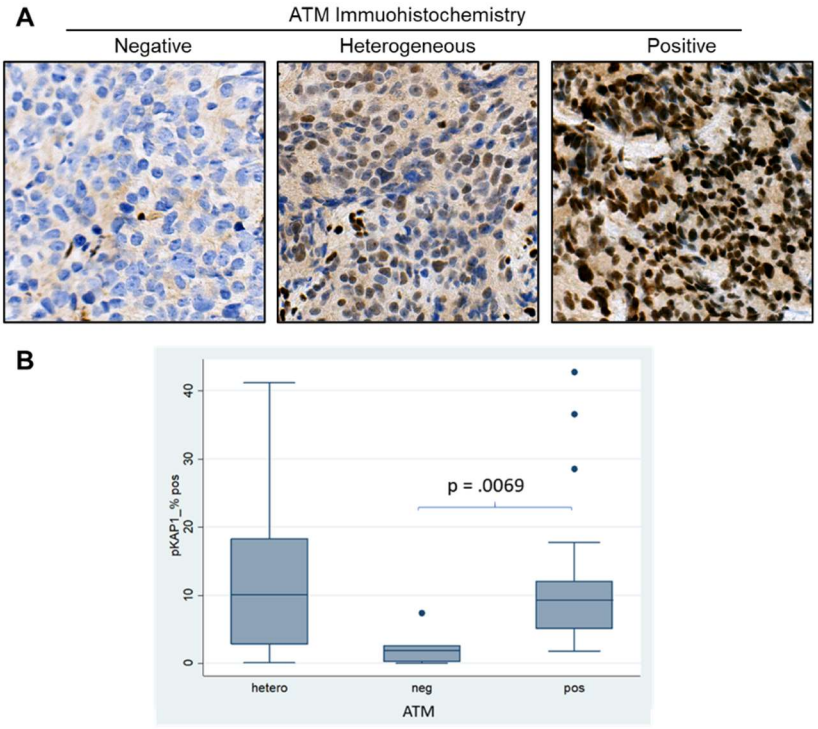


Figure 5. A. Representative images of negative, heterogeneous and positive ATM IHC. B. % positivity for p-KAP1 by ATM level by IHC.

Specific Aim 2: To discover genetic correlates of resistance to therapy from end-of-study cfDNA and optional biopsies

Major Task 5: Comparison of paired tumor biopsy specimens

Only one study participant underwent optional post-treatment biopsy in the context of ongoing COVID-19 pandemic. Unfortunately, this patient's pre-treatment specimen was not amenable to analysis so this Task is unable to be completed.

Major Task 6: Comparison of paired cfDNA specimens

Plasma was isolated for circulating cell-free DNA analysis from trial participants every third cycle of treatment (Arm B: C1D1, C4D1, C7D1, etc.; Arm A: C1D1, C4D1, etc. then C1D1[crossover], C4D1[crossover], etc.). The pre-treatment specimen will be analyzed per Major Task 3 above. A minority of patients had biospecimens collected at end-of-treatment as many patients elected for no further follow up with the trial team once they progressed on study (for example related to transition to hospice care). Of the 63 patients with pre-treatment specimens available for analysis, 26 had another specimen collected either at crossover or end-of-treatment to allow for pairwise analyses. If new genetic features are discovered in the post-treatment specimen compared to the pre-treatment, then these would be nominated as potential mediators of resistance for functional analysis.

Specific Aim 3: To functionally characterize novel genetic alterations identified in pre- and post-treatment specimens

Major Task 7: Characterization of Variants of Uncertain Significance (VUS) in DDR genes

The Mouw laboratory has not begun to characterize DNA repair gene VUSs from the trial (Major Task #7) because sequencing information from trial specimens is not yet available due to COVID-related delays. Once sequencing data from clinical trial specimens becomes available, they will analyze alterations in DNA repair genes and will prioritize recurrent and/or biologically compelling VUSs for study using the techniques outlined in Major Task #7.

Major Task 8: Characterization of drug sensitivity mediated by loss of DNA damage repair genes

Subtask 1: Generate ATM, BRCA1, BRCA2, CHEK2, PALB2, CDK12, FANCA, ERCC6, and RAD51C knockout lines

Subtask 2: Compare properties of the deficient cell lines to their parental (DNA repair proficient) cell lines

We have made significant progress towards the objectives of Major Task #8 (Characterization of drug sensitivity mediated by loss of DNA damage repair genes). We have created multiple DNA repair deficient isogenic cell pairs (Subtask 1) and are interrogating the impact of DNA repair gene loss (Subtask #2).

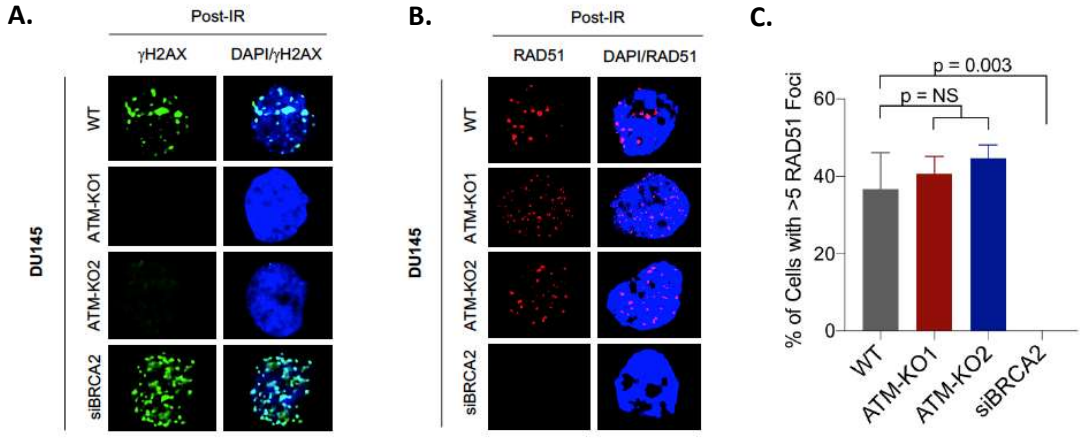


Figure 6: Impact of ATM loss on DNA damage signaling and homologous recombination (HR) function. **A.** ATM loss significantly decreases radiation-induced phospho-H2AX foci formation compared to the wild-type ATM parental cell line or a BRCA2-depleted cell line. **B.** ATM loss does not impact radiation-induced Rad51 foci formation whereas BRCA2 depletion significantly impairs Rad51 foci formation. **C.** ATM deletion does not significantly impair HR function in the DR-GFP reporter assay whereas BRCA2 depletion significantly impairs HR function.

The DNA repair gene for which we have created the most models and collected the most data is ATM. ATM is the second most commonly mutated DNA repair gene in prostate cancer (after BRCA2) and has been associated with aggressive biological and clinical features. We have deleted ATM from 3 different prostate cancer cell lines and have measured the impact of ATM loss on DNA repair capacity and sensitivity to established and emerging prostate cancer therapies. ATM loss significantly abrogates DNA damage signaling as measured by decreased formation of radiation-induced phospho-H2AX foci (Figure 6A). However, ATM loss did not directly impair homologous recombination repair activity, as evidenced by no difference in formation of radiation-induced Rad51 foci in ATM WT vs deleted cell lines (Figure 6B) and no difference in HR efficiency in the DR-GFP reporter assay (Figure 4C). Finally, we observed that ATM loss increased sensitivity to ionizing radiation but had little impact on sensitivity to PARP inhibition across prostate cancer cell line models (Figure 5A, B). Interestingly, ATM loss conferred significantly increased sensitivity to ATR inhibition (Figure 5C), supporting a possible role for ATR inhibitors in the treatment of ATM-mutant prostate tumors.

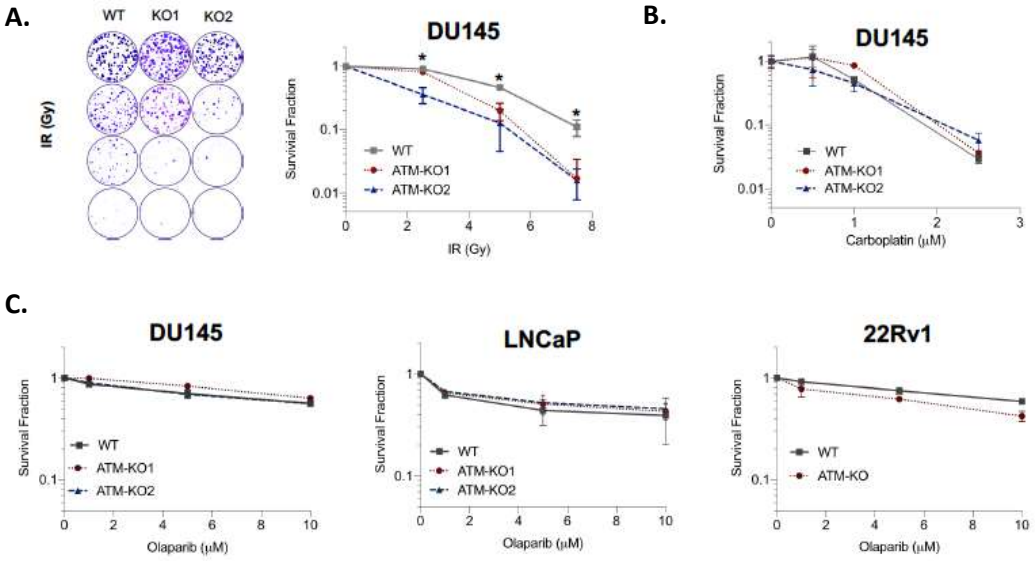


Figure 7: ATM loss and therapeutic sensitivity. **A.** ATM loss increases sensitivity to ionizing radiation (IR). **B.** ATM loss does not increase sensitivity to carboplatin. **C.** ATM loss does not increase sensitivity to the PARP inhibitor olaparib across prostate cancer cell line models.

In addition to ATM, we have also investigated the role of other DNA repair genes such as PALB2 and BARD1. PALB2 is a binding partner of BRCA2 and PALB2 loss has been associated with homologous recombination deficiency and PARP inhibitor sensitivity in breast and ovarian cancer. BARD1 is a BRCA1 binding partner and BARD1 loss or mutation has also been implicated as a homologous recombination gene of potential clinical relevance in breast and ovarian cancer. To model PALB2 and BARD1 loss, we have to date focused on using siRNAs to transiently deplete either PALB2 or BARD1 from prostate cancer cell lines. These data show that PALB2 or BARD1 loss leads to loss of HR function as measured by loss of radiation-induced Rad51 foci (Figure 8A), the DR-GFP reporter assay (Figure 8B) and also significantly increases in vitro sensitivity to PARP inhibition, with a magnitude of effect similar to BRCA2 loss (Figure 9). Findings from the PALB2 and BARD1 work were recently published: Dillon K et al. PALB2 or BARD1 Loss Confers Homologous

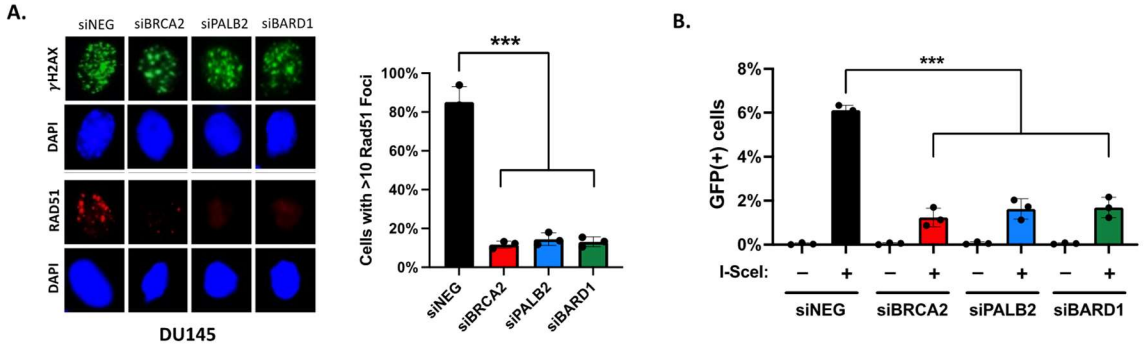
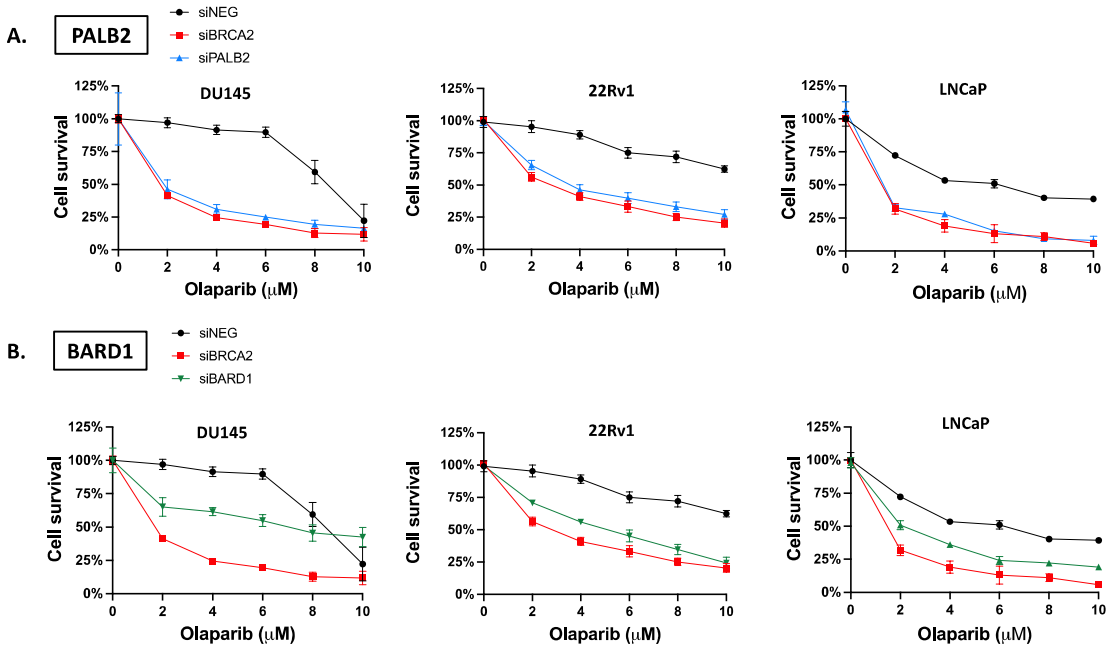


Figure 8: Loss of PALB2 or BARD1 confers homologous recombination (HR) deficiency. A. Loss of PALB2 or BARD1 results in loss of radiation-induced Rad51 foci. B. Loss of PALB2 or BARD1 results in decreased HR using the DR-GFP reporter assay.

Recombination Deficiency and PARP Inhibitor Sensitivity in Prostate Cancer. NPJ Precision Oncology 2022; 6(1):49.

Figure 9: PALB2 or BARD1 loss leads to increased PARP inhibitor sensitivity.



Finally, we have most recently begun to investigate the role of CDK12 loss on DNA damage response and sensitivity to DNA repair-directed agents. CDK12 is a kinase that phosphorylates the C-terminal domain (CTD) of RNA polymerase II to promote transcriptional elongation. We hypothesized that CDK12 loss, which occurs in ~5% of advanced prostate tumors, would result in increased transcriptional pausing leads to replication stress due to collisions between DNA replication machinery and stalled RNA polymerase. Acute depletion of CDK12 by siRNA results in an increase in the level of cellular markers of replication stress such as increased phosphorylation of RPA, ATR, and CHK1 by immunofluorescence and/or immunoblotting as well as increased sensitivity to ATR inhibition (Figure 10). In addition we find that CDK12 depletion leads to increase in DNA:RNA hybrids (R loops) which may be responsible for creating replication stress (and resultant ATR inhibitor sensitivity) due to collisions between transcriptional machinery and the DNA replication fork. Current efforts are focused on further dissecting mechanisms of replication stress in CDK12-deficient models.

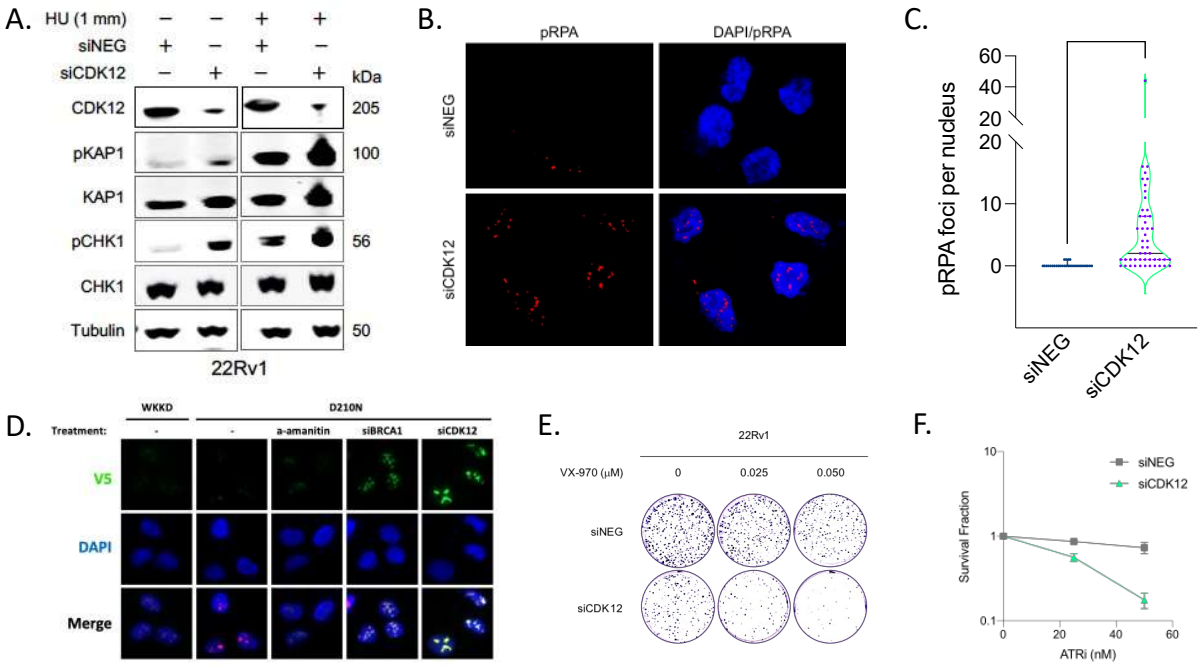


Figure 10: CDK12 loss leads to increased replication stress (RS) and ATR inhibitor sensitivity in prostate cancer cells. **A.** Protein markers of RS including pKAP1 and pCHK1 are increased following CDK12 depletion. HU treatment was used to induce RS. **B, C.** pRPA foci are higher in CDK12-depleted cells, suggesting increased RS. **D.** CDK12 depletion leads to increased R loops, a potential source of RS. V5-tagged catalytically-dead (D210N) RNaseH was used as a probe to identify R loops. WKKD is an RNaseH mutant that does not bind R loops and a-amanatin treatment prevents RNA polymerase binding (negative controls). siBRCA1 was used as a positive control for induction of R loops. **E, F.** CDK12 depletion leads to increased sensitivity to the ATR inhibitor berzosertib.

What opportunities for training and professional development has the project provided?

Shahrzad Rafiei, PhD was a post-doctoral research fellow in the Mouw lab who contributed to aims of this project. She published a first-author paper (Rafiei S, et al. ATM Loss Confers Greater Sensitivity to ATR Inhibition Than PARP Inhibition in Prostate Cancer. Cancer Res. 2020 Jun 1;80(11):2094-2100.) stemming from work related to this project. She also presented her findings as an oral abstract at the 2020 Multi-Institutional Prostate SPORE Retreat, and she won 3rd prize for best oral presentation. She began work related to Major Task #8 prior to leaving the Mouw lab to pursue a full-time position as a Senior Scientist at biotechnology company focused on developing DNA repair protein inhibitors. She was recruited and hired in large part based on the skills that she developed and utilized for this project.

From 10/1/20 through 6/30/21, Kasia Dillon, BS, a research technician in the Mouw lab, committed approximately 50% of her effort to the aims of this proposal. This has allowed her to gain additional skills in prostate cell biology, molecular cloning, and drug sensitivity assays. She was the first author of the manuscript describing results from the PALB2 and BARD1 work, and she used her experience with this project as an example of her research skillset during medical school interviews. She matriculated to the University of Massachusetts medical school in Fall 2021.

From 6/30/21 to current, Tim Hanlon, BS, a research technician in the Mouw lab, has committed approximately 50% of his effort to the aims of this proposal.

From approximately 3/1/2021 to current, Elizabeth Minten, PhD, a current Harvard Medical School student, has been a part-time contributor to this project. Dr. Minten previously studied BARD1 and BRCA1 interactions during her graduate work, and she has contributed to the aims of this project as part of her medical school thesis work.

From approximately 9/1/21 to current, David Yang, MD, a current clinical fellow in radiation oncology at Dana-Farber/Harvard Cancer Center has been analyzing data from targeted panel sequencing of cfDNA specimens from patients with metastatic prostate cancer. He has presented results from our pilot studies at the 2022 ASTRO annual meeting. From approximately 9/1/22 to current, Irbaz Riaz, MD, a medical oncologist at Mayo Clinic (Phoenix, AZ) and a Visiting Research Fellow in Oncology at Dana-Farber has been performing the analysis of cfDNA specimens from trial 10191. These studies will help Dr. Yang and Dr. Riaz launch their independent research careers.

How were the results disseminated to communities of interest?

Shahrzad Rafiei presented an oral abstract at 2020 Multi-Institutional Prostate SPORE Retreat entitled "Targeting ATM Deficiency in Prostate Cancer" for which she also won 3rd prize for best oral presentation.

Findings from ATM work were published in 2020: Rafiei S, et al. ATM Loss Confers Greater Sensitivity to ATR Inhibition Than PARP Inhibition in Prostate Cancer. Cancer Res. 2020 Jun 1;80(11):2094-2100.

The clinical trial was presented as a "Trial in Progress" at the ASCO Genitourinary Cancers Symposium and at the ASCO Annual Meeting in 2020. This trial was highlighted through UroToday: https://www.urotoday.com/conference-highlights/asco-2020/asco-2020-prostate-cancer/121932-asco-2020-a-phase-ii-study-of-m6620-in-combination-with-carboplatin-compared-with-docetaxel-in-combination-with-carboplatin-in-metastatic-castration-resistant-prostate-cancer.html

An abstract summarizing the preliminary clinical results from the clinical trial was presented as a poster at the 2021 ASCO Annual Meeting.

Findings from the PALB2 and BARD1 work were published in 2022: Dillon K et al. PALB2 or BARD1 Loss Confers Homologous Recombination Deficiency and PARP Inhibitor Sensitivity in Prostate Cancer. NPJ Precision Oncology 2022; 6(1):49.

What do you plan to do during the next reporting period to accomplish the goals?

Major Task 1 has been completed.

For Major Task 2, Whole Exome Sequencing from tumor biopsy specimens has been completed, and we will finalize categorization of tumor specimens as homologous recombination-deficient and proficient based on the sequencing results. Homologous recombination repair deficiency based on WES will be correlated with clinical outcomes (responses to carboplatin + docetaxel; clinical benefit in both arms of the study) per the original grant application.

For Major Task 3, plasma specimens for circulating cell-free DNA analysis have completed targeted panel sequencing as described. Homologous recombination repair deficiency based on cfDNA sequencing will be correlated with clinical outcomes per the original grant application.

For Major Task 4, staining for RAD51 focus formation, ATM, and phosphor-KAP1 has been completed. We will correlate these IHC markers with clinical outcomes.

Major Task 5 is unable to be completed.

For Major Task 6, paired analysis compared to pre-treatment specimens will be performed to identify genetic features that emerge over treatment to nominate potential mediators of resistance for further functional analysis.

For Major Task 7 to characterize of VUS in DDR genes, this is pending on sequencing results from Major Task 2 above so will be performed during the no-cost extension period of this award.

For Major Task 8, we have modeled loss of multiple DDR genes (ATM, BRCA2, PALB2, BARD1, CDK12). In the coming year, we will focus on additional DNA repair genes (ERCC6, RAD51C) as well as on further characterization efforts of the CDK12-deficient models.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our published finding that ATM loss confers greater sensitivity to ATR inhibition than PARP inhibition provided functional evidence supporting contemporaneous clinical observations that mCRPC patients with tumor ATM loss had very low response rates to PARP inhibition. Our findings also provided support for several on-going trials investigating ATR inhibitors in mCRPC patients with tumor ATM loss.

Our published findings that PALB2 or BARD1 loss is sufficient to confer PARP inhibitor sensitivity in preclinical prostate cancer support use of PARP inhibitors in patients with tumor PALB2/BARD1 alterations. Given the relative rarity of these alterations among mCRPC patients, the available clinical trial data did not include sufficient number of PALB2/BARD1-mutant cases to make a statistically powered determination of the potential activity of PARP inhibitors in this population.

Findings from the clinical trial provide important biological insights with regards to drugs that synergize with carboplatin chemotherapy in metastatic castration-resistant prostate cancer (mCRPC), and to guide clinical management and design future clinical trials in these patients. Specifically, our finding that the only responses seen in this study were in patients who received carboplatin and docetaxel was surprising because 1) all patients who received carboplatin plus docetaxel on this trial previously progressed on docetaxel alone and 2) the carboplatin dose given with docetaxel (carboplatin AUC 4) was lower than what was used for carboplatin with berzosertib or carboplatin alone (AUC 5).

There are no prior randomized trials of carboplatin plus docetaxel compared with carboplatin alone, so this trial provides compelling evidence that the combination of carboplatin with docetaxel is favored clinically over carboplatin with berzosertib or carboplatin alone – this finding immediately impacts clinical practice. Our biological understanding of the mechanism by which carboplatin leads to prostate cancer cell death is incomplete: the primary hypothesis of the study, that carboplatin would lead to DNA replication stress (through generation of intra- and inter-strand crosslinks between nucleotide bases) that would then sensitize prostate cancer cells to dying in response to an ATR inhibitor, even in cancers without defects in the DNA damage repair response, was not supported. One explanation for this could be that the ATR inhibitor used in this study, berzosertib, was ineffective at the dose tested. Another explanation is that prior evidence of clinical benefit of carboplatin-containing regimens was due to synergy with docetaxel through mechanisms unrelated to defects in the DNA damage repair response or induction of replication stress. Indeed, a recent study (de Porras et al. Eur Urol. 2020 Nov 2;S0302-2838(20)30778-8.) suggested that docetaxel sensitizes prostate cancer cells to carboplatin by impacting inflammatory pathways that make cells more likely to undergo cell death (apoptosis) in response to carboplatin. These findings are relevant in designing future trials of carboplatin-containing regimens in the future.

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

The clinical trial was closed to further enrollment at the time of interim analysis due to futility of the experimental regimen of carboplatin plus berzosertib. However, at least five patients who received carboplatin plus docetaxel achieved a clinical response, and a larger number of patients in both arms of the study experienced clinical benefit as demonstrated by a reduction of PSA by less than 50% or stable disease seen on imaging studies.

These clinical findings suggest the critical importance of the biomarker studies described in this grant application. For example, assessment of HRD by whole exome sequencing from tumor specimens, cfDNA sequencing, and RAD51 focus formation would help us understand whether responses were not seen with carboplatin alone or carboplatin plus berzosertib because by chance none of the patients who received these treatments actually had HRD tumors. Similarly, ATM and phospho-KAP1 IHC will help us understand if any of the patients who received carboplatin plus berzosertib had ATM loss that would be predicted to lead to sensitivity to an ATR inhibitor, or whether no responses were seen due to no patients having ATM deficiency.

We have added RNA-Seq as a planned biomarker study on tumor specimens from this trial. This will help us assess other biomarkers to predict clinical benefit from the carboplatin-based regimens that were investigated. Specifically, we will assess RB pathway loss in collaboration with the laboratory of Dr. Leigh Ellis per a funded RO1 grant. We will also assess activity of the CXCR2/BCL-2 pathway previously reported to be modulated by taxane chemotherapy to sensitize to carboplatin.

Actual or anticipated problems or delays and actions or plans to resolve them

The Mouw lab was closed completely for approximately 3 months from March through May 2020, meaning that none of the planned functional experiments could be performed during that time period. When the lab re-opened, the lab was operating at ~50% capacity for an additional ~2 months, meaning that progress was much slower than anticipated.

The Center for DNA Damage and Repair laboratory and the Genomics Platform at the Broad Institute of Harvard and MIT were closed for several months due to the COVID-19 pandemic. This delayed the finalization of the material transfer agreements for the tumor and cfDNA specimens. This also delayed the generation of preliminary data in prostate cancer models for RAD51 focus formation and ATM immunohistochemistry. The Mouw lab also experienced several important COVID-related delays in receiving necessary reagents, which significantly delayed planned experiments in several instances. This was particularly notable during the Omicron surge in early 2022, when many shipments were delayed and when several lab members were quarantined.

Closure of the Genomics Platform at Broad Institute in 2020 delayed receiving biospecimens from this trial. Preparing slides for immunohistochemistry and extraction of nucleic acids at the NCI Biorepository were also delayed due to COVID-19 related staffing shortages.

However, these issues have now been resolved: the plasma specimens for cfDNA analysis have been received and have all undergone ultra-low pass whole genome sequencing and targeted panel sequencing. Slides for immunohistochemistry have completed. DNA and RNA from pre-treatment tumor biopsy were received by the MoCha laboratory on 3/15/22 and by the NCLN laboratory at MD Anderson Cancer Center on 3/10/22, and have completed whole exome sequencing and RNA sequencing, respectively.

Changes that had a significant impact on expenditures

The investigators involved in the studies allocated the effort designated to these studies per the original budget to perform the Major Tasks detailed above. However, expenditures related to the analysis of the tumor and cfDNA biospecimens from the clinical trial were delayed due to delay in the material transfer.

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

The clinical trial closed to enrollment after interim analysis due to futility as detailed above. Thus, the number of biospecimens to be analyzed is from 65 patients rather than the 120 as originally projected. This will allow us to perform deeper sequencing on cfDNA specimens, and to fund phospho-KAP1 immunohistochemistry as detailed in the amended protocol.

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications

Dillon KM, Bekele RT, Sztupinszki Z, Rafiei S, Hanlon T, Szallasi Z, Choudhury A, Mouw KW. PALB2 or BARD1 Loss Confers Homologous Recombination (HR) Deficiency and PARP Inhibitor Sensitivity in Prostate Cancer. Npj Precision Oncology 2022; 6(1):49. – support from DoD listed in Acknowledgements.

Books or other non-periodical, one-time publications

Nothing to report

Other publications, conference papers, and presentations

Choudhury AD, Xie W, Parikh M, Lee D, Kessler ER, Einstein DJ, Kochupurakkal B, Mouw KW, Van Allen EM, Doyle LA, D'Andrea AD, Taplin ME, Shapiro G. A phase II study of M6620 in combination with carboplatin compared with docetaxel in combination with carboplatin in metastatic castration-resistant prostate cancer. 2020 ASCO Annual Meeting, Abstract TPS5597. 2020, Virtual Meeting. – support from DoD for biomarker studies acknowledged on poster.

Choudhury AD, Xie W, Folefac E, Lee D, Parikh M, Einstein DJ, Kessler ER, Mayer TM, McKay RR, Pace AF, Kochupurakkal B, Mouw KW, Van Allen EM, Kunos C, D'Andrea AD, Taplin ME, Shapiro G. A phase 2 study of berzosertib (M6620) in combination with carboplatin compared with docetaxel in combination with carboplatin in metastatic castration-resistant prostate cancer. 2021 ASCO Annual Meeting, Abstract 5034. 2021, Virtual Meeting. – support from DoD for biomarker studies acknowledged on poster.

Dr. Mouw presented portions of this work in a poster abstract for the 2021 and 2022 Prostate Cancer Foundation (PCF) Scientific Retreat.

Dr. Minten presented portions of this work in a poster for a Harvard Medical student research symposium in Fall 2021.

Website(s) or other Internet site(s)

https://www.urotoday.com/conference-highlights/asco-2020/asco-2020-prostate-cancer/121932-asco-2020-a-phase-ii-study-of-m6620-in-combination-with-carboplatin-compared-with-docetaxel-in-combination-with-carboplatin-in-metastatic-castration-resistant-prostate-cancer.html

Technologies or techniques / Inventions, patent applications, and/or licenses

Nothing to Report

Other Products

Biospecimen collections

Tumor biopsy specimens and blood specimens for circulating cell-free DNA analysis are stored in the NCI Biorepository.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Atish Choudhury, MD, PhD
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-9344-6631
Nearest person month worked:	1.35
Contribution to Project:	Dr. Choudhury is the overall PI of NCI protocol # 10191 and oversees all the translational studies. He is coordinating the biomarker studies on tumor and plasma specimens from this trial and correlating with clinical outcomes. He will also coordinate laboratory collaborations for functional studies of findings from these studies.
Funding Support:	

Name:	Kent Mouw, MD, PhD
Project Role:	Co-Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-7939-7343
Nearest person month worked:	0.48
Contribution to Project:	Dr. Mouw has significant experience in applying cellular and biochemical assays to study the functional implications of DNA repair pathway alterations identified in large sequencing studies. He has worked on prior studies in bladder and prostate cancer, including the functional characterization of *ERCC2* mutations identified in cisplatin-response bladder tumors. Dr. Mouw has access to a variety of cutting-edge DNA repair functional assays in the laboratory of Dr. Alan D'Andrea.
Funding Support:	5K08CA219504-03

Name:	Eliezer Van Allen, MD
Project Role:	Co- Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0002-0201-4444
Nearest person month worked:	0.12
Contribution to Project:	Dr. Van Allen, an Assistant Professor of Medicine at Dana-Farber Cancer Institute, Computational Director for the Center for Cancer Precision Medicine at Dana-Farber, and Associate Member at the Broad Institute of MIT and Harvard, is a leader in computational oncology and the molecular characterization of response and resistance to therapies of prostate cancer and immunogenomics across cancer types. He will oversee all genomic analyses related to this proposal, including integrated analysis from tumor biopsy circulating cell-free DNA specimens. He is mentoring Dr. David Yang and Dr. Irbaz Riaz in these analyses.
Funding Support:	

Name:	Tim Hanlon, BS
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	3.00
Contribution to Project:	Mr. Hanlon focuses on cell-based assays including the creation of DNA repair gene knockout cell lines as well as performing cell proliferation and drug sensitivity assays to compare properties of DNA repair-proficient and -deficient preclinical models. Mr. Hanlon's effort replaced Ms. Dillon's effort when Mr. Hanlon joined the Mouw lab and Ms. Dillon matriculated to medical school.
Funding Support:	

Name:	Jillian O'Toole
Project Role:	Research Data Specialist
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.97

Contribution to Project:	Ms. O'Toole is responsible for coordination of biospecimens that have been shipped to the Broad Institute and to the Center for DNA Damage and Repair and for sharing data related to the bioassays performed with collaborating investigators.
Funding Support:	

Name:	Amruta Samant
Project Role:	Research Tech - Senior
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.33
Contribution to Project:	Ms. Samant will perform cell-based functional experiments under the direction of Dr. Mouw. Tasks will include cloning and expression of DNA repair mutant alleles in DNA repair deficient cell lines, creating new DNA repair deficient prostate epithelial and tumor cell lines using CRISPR/Cas9 technology, and performing DNA repair and drug sensitivity assays.
Funding Support:	

Name:	Bridget Whelpley
Project Role:	Research Data Specialist
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	5.19
Contribution to Project:	Ms. Whelpley is responsible for coordinating data related to this study from the Broad Institute, the Center for DNA Damage and Repair, the MoCha laboratory and the NLCN laboratory.
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?

CHOUDHURY, ATISH

Previous:

ENDED
UM1CA186709 (Shapiro, Flaherty, Kufe) 03/01/19 – 02/29/23 0.60 CM
NIH/NCI
A Phase 2 Study of M6620 in Combination with Carboplatin compared with Docetaxel in Combination with Carboplatin in Metastatic Castration-Resistant Prostate Cancer
Specific Aims: 1) To correlate pre-treatment DNA damage repair gene mutation status from cfDNA with clinical outcomes for M6620+carboplatin and docetaxel+carboplatin; 2) To correlate a reduction in tumor fraction in cfDNA (as derived from ULP-WGS) from pre- treatment specimen to week 9 specimen with clinical outcomes; 3) To correlate pre-treatment RAD51 focus formation and ATM immunohistochemistry from mandatory pre-treatment tumor biopsies with clinical outcomes
Role: Co-Investigator
POC: Percy Ivy

ENDED
W81XWH1810489 (Huang) 09/29/18 – 09/28/22 0.12 CM
DoD
Investigating Genomic and Immunologic features of Prostate Cancer in African American Men
Specific Aims: Dr. Choudhury will work with Dr. Huang to analyze cfDNA (Aim 1) in conjunction with a comparison to genomic results from cfDNA in studies at DFCI.
Role: Co-Investigator
POC: Mirlene Andou

Current:

No changes to report

MOUW, KENT

Previous:

ENDED
CAMS 9/1/2017–8/31/2022 0.60 CM*
Burroughs Wellcome Fund
Investigating the effect of ERCC2 mutations on DNA repair capacity and chemo-radiotherapy response in muscle-invasive bladder cancer
Specific Aims: To study the mechanisms through which somatic *ERCC2* mutations impact DNA repair and treatment sensitivity in bladder cancer using a combination of cellular, biochemical, and genomic approaches.
Role: PI
Overlap: This effort is complementary to the K08 because BW funding will be used to supplement funding for sequencing of clinical bladder samples outlined in Aim #3. No salary support is being received and there are no budgetary overlaps
POC: Rolly L. Simpson Jr

ENDED
5K08CA219504-03 7/1/2017–6/30/2022 9.00 CM
NIH
Investigating the effect of ERCC2 mutations on DNA repair capacity and chemo-radiotherapy response in muscle-invasive bladder cancer
Specific Aims: 1) dissect the functional landscape of ERCC2 mutations in bladder cancer; 2) investigate the mechanistic underpinnings of ERCC2 mutations, and 3) define the association between ERCC2 mutations and chemoradiotherapy response in bladder cancer.
Role: PI
POC: Justin Birken

Current:

AWARDED
1R01CA272657-01 07/01/2022–06/30/2027 2.40 CM
NIH
Targeting Nucleotide Excision Repair Deficiency to Improve Bladder Sparing Treatment for Muscle Invasive Bladder Cancer
Specific Aims: The major goal of this project is to dissect the cellular mechanisms that contribute to the unique properties of NER deficient tumors and to define the impact of NER deficiency on treatment response in bladder cancer
Role: PI
Overlap: None
POC: Sundaresan Venkatachalam

VAN ALLEN, ELIEZER

Previous:

ENDED
UM1CA186709 (Shapiro, Kufe, Flaherty) 09/04/2014 – 02/28/2023 0.48 CM
DFCI-BWH-Broad Institute Molecular/Biomarker Characterization Hub (Supplement)
Specific Aim 1: Provide molecular analyses for ETCTN-wide clinical trials that include integral and exploratory biomarkers or other molecular determinants of response and resistance A. Conduct CLIA-grade tumor genomic profiling of at least 120-200 patient tumor specimens from ETCTN Trials B. Perform comprehensive genomic characterization of selected paired tumor specimens obtained prior to treatment and following relapse. Specific Aim 2: Participate in ETCTN-wide consortium activities to assist in the clinical evaluation of molecular variants and driver mutations.
Role: Co-Investigator
POC: S. Percy Ivy
Overlap: N/A

ENDED
W81XWH-19-PCRP-EIRA (Hamid) 02/15/2020 – 02/14/2023 0.00 CM*
Department of Defense
PC190530 PAIR Genomic Predictors of Clinical Outcomes and Benefit of (Chemo) Hormonal Therapy in Metastatic Hormone Sensitive Prostate Cancer
The aims of this project are: Aim 1A: to determine whether TSG alterations are prognostic and/or predictive of outcome with ADT or ADT plus docetaxel in the CHAARTED trial, Aim 1B: to determine whether TSG alterations are associated with clinicopathologic features of mHSPC, and assess both prognostic and predictive associations in multivariable models in the CHAARTED trial, Aim 2: to validate the prognostic and predictive role of TSG alterations in clinicogenomic models of mHSPC in patients treated with ADT or ADT plus docetaxel in the STAMPEDE trial.
Role: Co-Investigator
POC: Michelle Cromwell, Grants Management Specialist; Address: USAMRRA
Overlap: N/A
This grant mechanism exclusively funded genomic work; no personnel have salary support from grant funds

ENDED
NIH/NCI (McDermott/Kaelin) 09/01/2021 – 08/31/2022 0.12 CM DF/
HCC Kidney Cancer SPORE
Dissecting the Spatial Patterns of Ccrcc Molecular Subtypes and Micro-Environments with Deep Learning
In this project, we will expand on our prior work and: 1) Determine the latent spatial representations of ccRCC transcriptional programs in histopathology images; and 2) Develop deep learning models that predict clinical outcomes from tumor, immune, and stromal representations in ccRCC histopathology images. Broadly, these efforts represent emerging opportunities at the intersection of genomics, multimodal histopathology, and deep learning to advance biological insights and predictive modeling in ccRCC.
Role: Co-Investigator
POC: Tara Johnston
Overlap: N/A

ENDED
W81XWH-17-1-0545 (Rosenberg/Van Allen) 09/15/2017 – 06/14/2022 0.24 CM
Department of Defense

Precision Medicine in Platinum-Treated Lethal Bladder Cancer
Specific Aims: To determine (1) the association between somatic alterations in ERCC2 and other DDR genes and clinical outcomes of patients treated with gemcitabine and cisplatin on CALGB 90601, (2) the impact of intrinsic tumor subtypes on response to chemotherapy and VEGFR blockade, and (3) the underlying biology of extreme sensitivity to gemcitabine and cisplatin (with or without bevacizumab) in outlier responses through integrated molecular and functional analyses.
Role: Partnering PI
POC: Jodi Cardoza, Grants Specialist
Overlap: N/A

ENDED
Brown Performance Group 06/04/2018 – 06/01/2022 0.12 CM
Deep Learning Models to Accelerate Translational Cancer Genomics
Aim 1: To develop a biologically informed machine learning model for outcome prediction and hypothesis generation using cancer genomics. Aim2: To apply P-net to translational and clinical cancer genomics challenges and identify novel predictive markers.
Role: Principal Investigator
POC: E-Mail: Audrey Cook
Overlap: N/A

Current:

AWARDED
LC220330 (Elmarakeby) 01/15/2023 – 01/14/2025 0.0 CM*
DoD – FY2022 Lung Cancer Research Program
Interpretable Machine Learning for Molecular Discovery in Lung Cancer
In this research we will develop a novel machine learning model that is guided by known cancer biology to identify set of features, genes, and known and novel pathways that explain the different responses and manifestations of NSCLC disease. This will help understand mechanisms of resistance in unselected populations and identify novel therapeutic targets opening the door for developing new treatments.
Role: Mentor*
POC: Danielle L. Reckley, Grants Officer and Marielena V. McGuire, Ph.D. LCRP, Program Manager
Overlap: None.

AWARDED
Dana-Farber Cancer Institute (Van Allen) 01/01/2023 – 06/30/2024 0.30 CM
Drug Discovery Translational Research Program
Dissecting Cellular Programs in Radioligand Treated mCRPC
We hypothesize that specific tumor-intrinsic and tumor-extrinsic cellular programs jointly drive therapy response following 177Lu-PSMA-617 treatment in mCRPC patients. Aim 1: Dissect the impact of 177Lu-PSMA-617 exposure on tumor reprogramming in mCRPC patients. Aim 2: Determine the tumor-immune interactions associated with therapy response in prostate cancer.
Role: Principal Investigator
POC: Henry Long, PhD, Scientific Director, Center for Functional Cancer Epigenetics, Dana-Farber Cancer Institute; Address: 450 Brookline Avenue, Boston, MA 02215-5450
Overlap: None.

AWARDED
1R50 CA265182-01A1 (Park, Jihye) 08/01/2022 – 07/31/2027 0.00 CM*

NIH/NCI
Molecular Origins and Evolution to Treatment Resistance in Genitourinary Cancers
While many cancer genomics studies have opened novel biological paradigms for tumor-intrinsic dysregulation resulting from treatment resistance and new translational impacts, the role of integrated somatic, germline, and immune systems toward treatment resistance has not been fully characterized in GU cancers. Under Unit Director Dr. Eliezer Van Allen's leadership, this interdisciplinary and highly collaborative group will continue to drive these comprehensive clinical computational research programs by utilizing novel sequencing approaches and computational methods, and by sharing these discoveries with the broader community to synergize collaboration in the cancer genomics field.
Role: Unit Leader/Mentor*
POC: Joseph Gipson; Grants Management Specialist; Address: BG 9609 Medical Center Drive RM 2W464, Rockville, MD 20850
Overlap: None.

Industry Sponsored Clinical Trials
*Funding and effort dependent on accruals for all project listed below.

AWARDED
DF/HCC - Janssen (Taplin) 07/11/2022 – 07/10/2029 0.0 CM*
Fresh Frozen Radical Prostatectomy Tumor
Collection and Single Cell Sequencing in PROTEUS Study
Goals: 1. Determine the molecular basis for resistance to neoadjuvant intensive androgen signaling inhibition and the relationship between residual disease in the prostate and metastatic disease. 2. Determine apalutamide concentration in the prostate after six months of treatment.
POC: Ademi Santiago-Walker or: Shibu Thomas, PhD, Head of Oncology Translational Research, Bladder, Janssen Research & Development, LLC; 1400 McKean Road, Spring House, PA 19477
Role: Investigator
Overlap: None.

What other organizations were involved as partners?

1. Organization Name: NCI Biorepository
   Location of Organization: Columbus, OH
   Partner's contribution to the project:
   - Facilities
   - Collaboration

2. Organization Name: NCI Molecular Characterization (MoCha) Laboratory
   Location of Organization: Frederick, MD
   Partner's contribution to the project:
   - Facilities
   - Collaboration

3. Organization Name: Broad Institute of MIT and Harvard
   Location of Organization: Cambridge, MA
   Partner's contribution to the project:
   - Facilities

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report

9. APPENDICES:

Nothing to report