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14. ABSTRACT

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.

15. SUBJECT TERMS
None listed.

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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.

o 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 have been 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 CIRB approval for protocol # 10191, DF/HCC protocol # 20-661 and the IRB approval thereof, and HRPO approval are attached.

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.

Biomarker conference call with NCI/CTEP (Charles Kunos, Jeff Moscow, Tracy Lively, Melissa-McKay-Daily, Chaz Stephens) on January 26, 2021 confirmed plan to complete the biomarker studies detailed in the protocol including whole exome sequencing to assess genetic features correlating with response to carboplatin + docetaxel and to clinical benefit in both arms of the study. Svetlana Nazarenko in the CTEP Regulatory Affairs Branch (RAB) was notified and asked to prepare and route the biospecimen transfer agreement for transfer of these specimens to the NCI Molecular Characterization (MoCHa) laboratory for whole exome sequencing.

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

The material transfer agreement with the Broad Institute for analyses of these specimens was finalized. In order to finalize this agreement, the circulating cell-free DNA sequencing will be performed as “fee-for-service” at the Broad Institute with the raw data deposited in a secure database for analysis by the laboratory of Dr. Eliezer Van Allen.

Pilot projects are ongoing for analysis of circulating cell-free DNA specimens from banked plasma specimens from patients treated with standard of care Radium-223 and docetaxel using the 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.

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 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%.

Preliminary metrics from sequencing suggest excellent target recovery of > 90%. However, recovery of DNA duplexes is limited at this Mean Target Coverage per Figure 1.

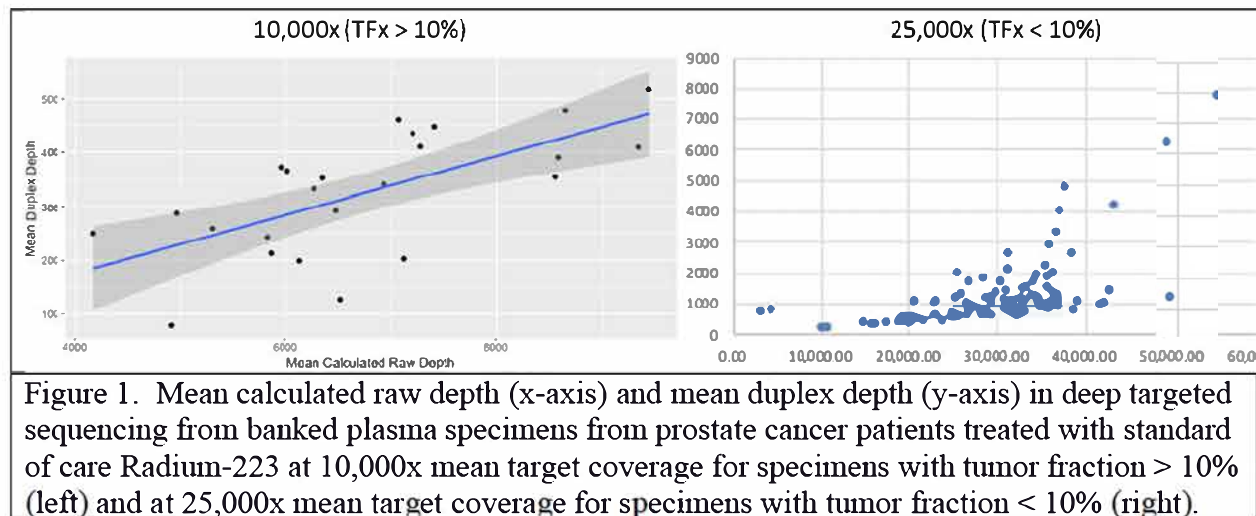

Figure 1. Mean calculated raw depth (x-axis) and mean duplex depth (y-axis) in deep targeted sequencing from banked plasma specimens from prostate cancer patients treated with standard of care Radium-223 at 10,000x mean target coverage for specimens with tumor fraction > 10% (left) and at 25,000x mean target coverage for specimens with tumor fraction < 10% (right).

All cfDNA specimens sequenced at 10,000x MTC and most cfDNA specimens sequencing at 25,000x MTC demonstrate mean duplex depth < 1000x, which is suboptimal for high confidence calling of genetic events present at low abundance in the circulation based on duplex consensus. Single strand consensus mutation calling can be used to detect these less abundant events but with less confidence with regards to error suppression. As such, all cfDNA specimens planned to be sequenced for this project will be sequenced at > 25,000x no matter the calculated tumor fraction. Once cfDNA sequencing from these biospecimens is completed, genetic features will be correlated with response to clinical outcomes as for whole exome sequencing as described in Major Task 2.

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.

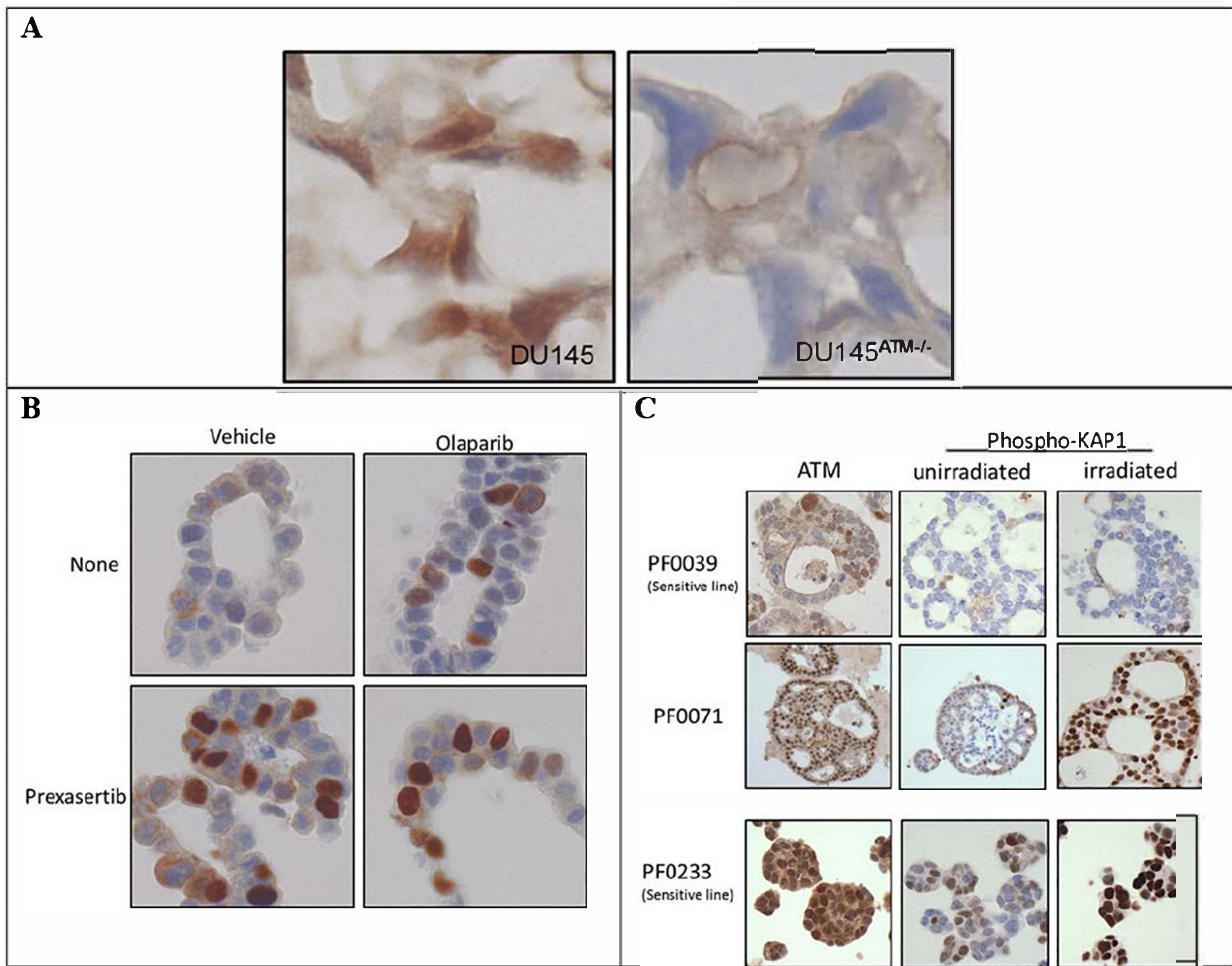

Figure 2. A. ATM staining in ATM wild-type (left) and ATM CRISPR k/o (right) DU145 cells. **B.** Phospho-KAP1 staining in DF59 high grade serous ovarian cancer PDX model with Olaparib, prexasertib or the combination. **C.** Phospho-KAP1 staining in PDX models. PF0039 is ATM-deficient and sensitive to ATR inhibition; KAP1 staining is not increased with irradiation (due to absence of ATM kinase). PF0233 is ATM wild-type with high basal levels of phospho-KAP1 and is sensitive to ATR inhibition; PF0071 is ATM wild-type but with low basal level of phospho-KAP1 and is resistant to ATR inhibition.

We have also optimized the ATM immunohistochemistry assay with the Abcam (Y170) Ab32420 antibody using immortalized fibroblasts from an ataxia telangiectasia patient complemented with exogenously expressed ATM. ATM staining in ATM wild-type (left) and ATM-/- (CRISPR knockout) DU145 cells is shown in Figure 2A. ATM staining is analyzed as follows: 1) ATM is a nuclear protein and expression of ATM is not known to be regulated by the cell cycle 2) ATM scoring: If > 50% of tumor cells stain positive for ATM, the sample is ATM positive, else, the sample is ATM negative 3) Infiltrating lymphocytes and tumor associated stroma are usually ATM positive and can be used as an in-situ reference for staining performance and scoring e.g. variation in tissue processing and its effect on staining.

Since submission of the initial grant application to the DoD, we have added staining for phospho-KAP1 as one of the correlative studies on this protocol. KAP1 (KRAB [Kruppel-Associated Box Domain]-Associated Protein 1) is a protein that in humans is encoded by the TRIM28 gene. KAP1 is phosphorylated by ATM in response to DNA damage, and IHC for phosphorylated KAP1 was optimized using rabbit monoclonal from Cell Signaling clone C42G12. We have demonstrated that phosphorylation of KAP1 increases in response to the PARP inhibitor olaparib, the CHK1 inhibitor prexasertib, and the combination in the DF59 high grade serous ovarian cancer PDX model (Figure 2B). Unpublished data from the laboratory of Dr. Alan D'Andrea suggests that high basal levels of p-KAP1 (which is an indicator of DNA replication stress) in unirradiated tissue may also predict for sensitivity to an ATR inhibitor (Figure 2C).

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, so only this paired specimen will be analyzed. 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.

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. The end of treatment specimen will be analyzed pairwise with the pre-treatment specimen - 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 generate or characterize DNA repair gene VUSs (Major Task #7) because sequencing information from clinical trial specimens is not yet available due to COVID-related delays over the past year. 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 several DNA repair deficient isogenic cell pairs (Subtask 1) and are interrogating the impact of DNA repair gene loss (Subtask #2).

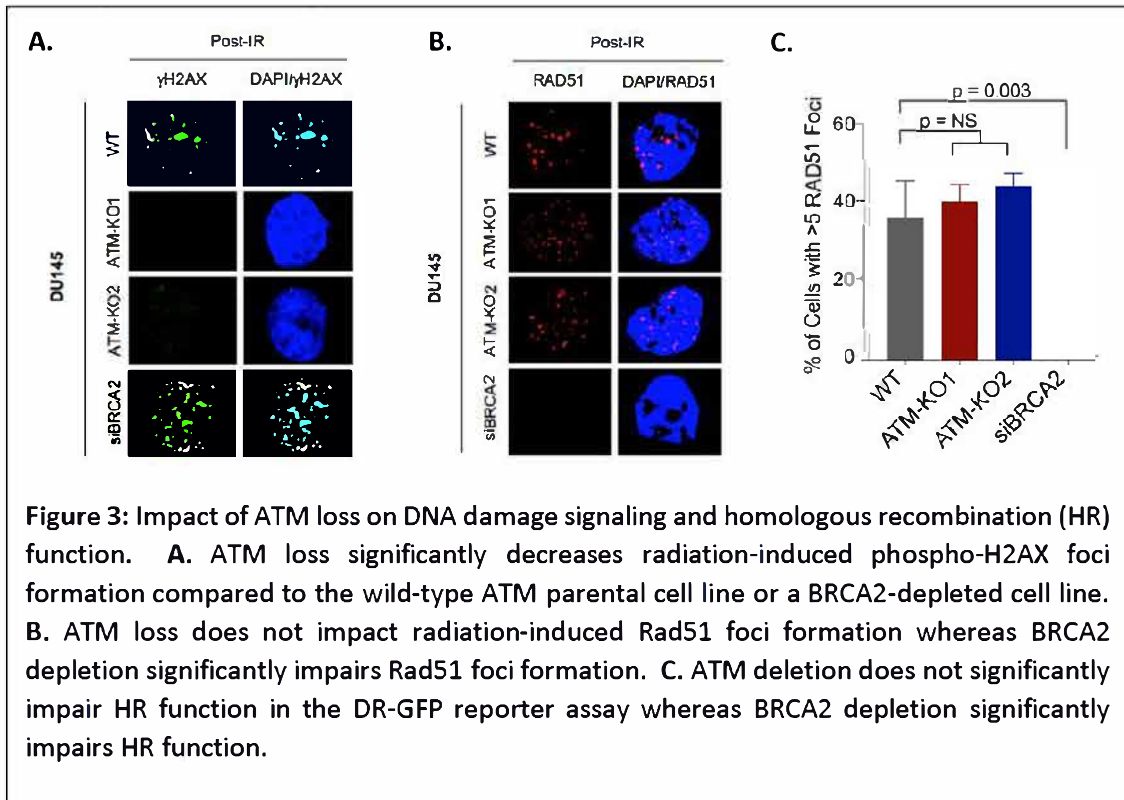

Figure 3: Impact of ATM loss on DNA damage signaling and homologous recombination (HR) function. A. ATM loss significantly decreases radiation-induced phospho-H2AX foci formation compared to the wild-type ATM parental cell line or a BRCA2-depleted cell line. **B.** ATM loss does not impact radiation-induced 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 3A). 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 3B) and no difference in HR efficiency in the DR-GFP reporter assay (Figure 3C). 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 4A, B). Interestingly, ATM loss conferred significantly increased sensitivity to ATR inhibition (Figure 4C), supporting a possible role for ATR inhibitors in the treatment of ATM-mutant prostate tumors.

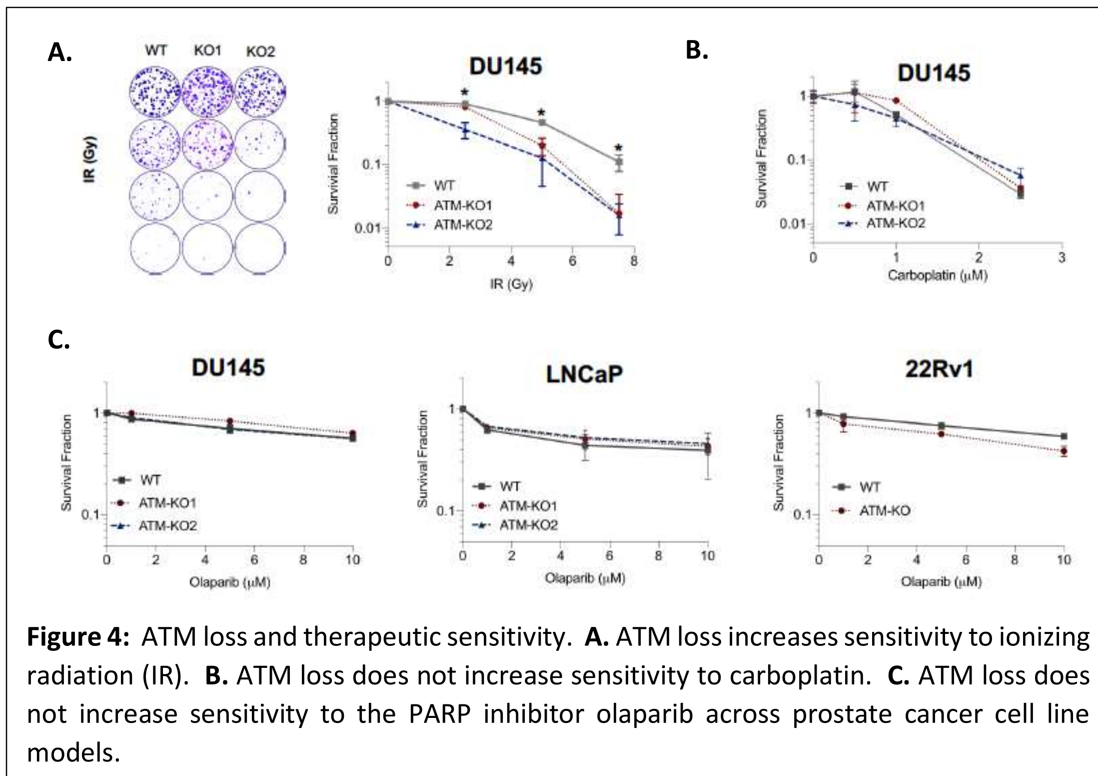

Figure 4: ATM loss and therapeutic sensitivity. **A.** ATM loss increases sensitivity to ionizing radiation (IR). **B.** ATM loss does not increase sensitivity to carboplatin. **C.** ATM loss does not increase sensitivity to the PARP inhibitor olaparib across prostate cancer cell line models.

In addition to ATM, we have also begun to study 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 preliminary data suggest that PALB2 or BARD1 loss leads to loss of HR function as measured by the DR-GFP reporter assay (Figure 5A) and also significantly increases in vitro sensitivity to PARP inhibition, with a magnitude of effect similar to BRCA2 loss (Figure 5B). Experiments are on-going to confirm this phenotype in other prostate cancer models and to extend similar

studies to other DNA repair genes of interest (such as CHEK2, RAD51, and CDK12 as listed in Major Task 8, Subtask 1).

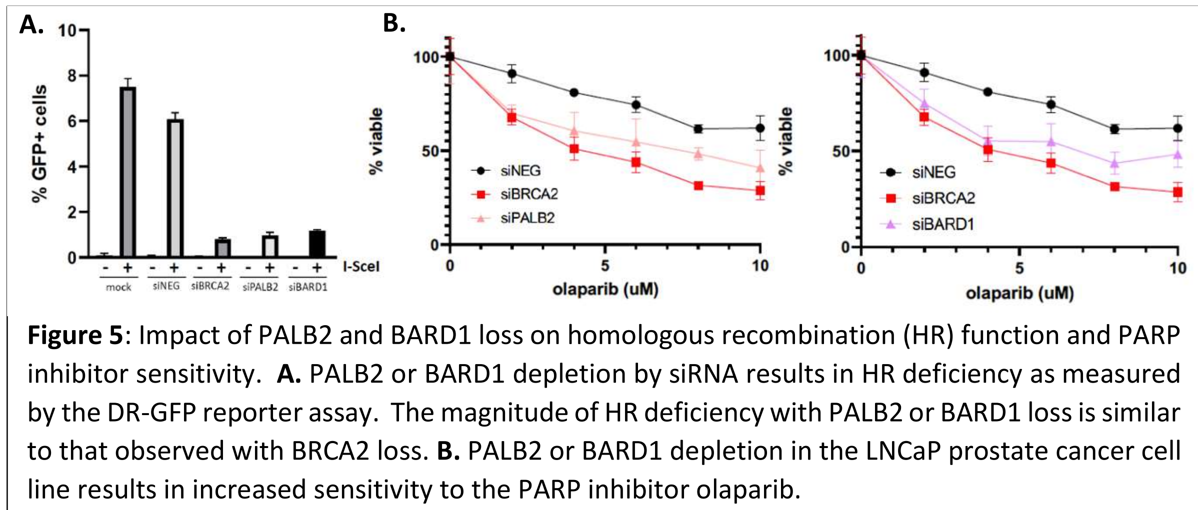

Figure 5: Impact of PALB2 and BARD1 loss on homologous recombination (HR) function and PARP inhibitor sensitivity. **A.** PALB2 or BARD1 depletion by siRNA results in HR deficiency as measured by the DR-GFP reporter assay. The magnitude of HR deficiency with PALB2 or BARD1 loss is similar to that observed with BRCA2 loss. **B.** PALB2 or BARD1 depletion in the LNCaP prostate cancer cell line results in increased sensitivity to the PARP inhibitor olaparib.

o 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.

o How were the results disseminated to communities of interest?

Shahrzad 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.

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 has been submitted for presentation at the 2021 ASCO Annual Meeting.

o 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, the BTA is pending. Once fully executed, tumor biopsy specimens will be shipped from the NCI Biorepository to the Molecular Characterization (MoCha) laboratory for Whole Exome Sequencing (WES) and RNA-Seq. WES data will be analyzed in the laboratory of Dr. Eliezer Van Allen, and RNA-Seq data will be analyzed in the laboratory of Dr. Leigh Ellis. 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, the MTA is fully executed. Plasma specimens for circulating cell-free DNA analysis from pre-treatment specimens will be shipped from the NCI Biorepository to the Broad Institute and analyzed using the workflow described. Homologous recombination repair deficiency based on cfDNA sequencing will be correlated with clinical outcomes per the original grant application.

For Major Task 4, the MTA is fully executed. Tumor biopsy specimens will be shipped from the NCI Biorepository to the Center for DNA Damage and Repair (CDDR) laboratory for RAD51 focus formation and ATM IHC assays. We will also add phospho-KAP1 IHC per the amended protocol. We will correlate these IHC markers with clinical outcomes.

For Major Task 5, we will perform whole exome sequencing on the post-treatment biopsy specimen that was collected.

For Major Task 6, plasma specimens from end of study specimens will be shipped from the NCI Biorepository to the Broad Institute and will be sequenced per the described workflow, and 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 in Year 3 of this award.

For Major Task 8, we will create CHEK2, CDK12, FANCA, ERCC6, and RAD51C knockout cell lines, and characterize drug sensitivity mediated by loss of these genes.

4. IMPACT

o 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.

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.

o What was the impact on other disciplines?

Nothing to Report.

o What was the impact on technology transfer?

Nothing to Report.

o What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS

o 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, but both agreements have been completed at this point. This also delayed the generation of preliminary data in prostate cancer models for RAD51 focus formation and ATM immunohistochemistry shown in Figure 2. Closure of the Genomics Platform at Broad Institute delayed the generation of the preliminary data shown in Figure 1 and receiving biospecimens from this trial. The Genomics Platform is now fully operational so is ready to receive the cfDNA specimens.

- 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 are 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 as

detailed based on preliminary findings from Figure 1 above, and to fund phospho-KAP1 immunohistochemistry as detailed in the amended protocol.

6. PRODUCTS

o Publications, conference papers, and presentations

 Journal 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.

o 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

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

Nothing to Report

o Other Products

 Biospecimen collections

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

 Other

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

o 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:	0.43
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:	

Name:	Jett Crowdis
Project Role:	Computational Biologist
Researcher Identifier (e.g. ORCID ID):	0000-0003-4777-7303
Nearest person month worked:	1.00

Contribution to Project:	Jett focuses on analysis, method development, and application pertaining to identifying genomic features that correlate with mutational signature analysis of prostate cancers. His tasks also include aggregation and integration of mutational analysis for robust variant detection, as well as sharing of data across platforms.
Funding Support:	

Name:	Amruta Samant
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	5.72
Contribution to Project:	Ms. Samant 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.
Funding Support:	

o 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/Current/Pending Support

<u>Previous (Past Five Years)</u>:

ENDED
Claudia Adams Barr Program 07/01/17 – 03/31/20 N/A; completed
Dana-Farber Cancer Institute
Analysis of circulating free DNA in Hormone Sensitive Metastatic Prostate Cancer from the CHAARTED study
Goals/Aims: 1) To perform ultra low pass whole genome sequencing from banked plasma samples in the CHAARTED study to assess tumor purity (% tumor DNA content) in the blood as a prognostic biomarker; and 2) To develop a prostate cancer-specific targeted panel for next

generation sequencing at the Broad Genomics Platform and test in a subset of samples from the CHAARTED study.
Role: Principal Investigator
POC: Rachel Goldblatt; Rachel_Goldblatt@dfci.harvard.edu;

Current:

NEW
PCF-Pfizer Global Challenge Award 04/01/21 – 03/31/24 0.60 CM
Pfizer
A Phase Ia/Ib study of talazoparib in combination with tazemetostat in metastatic castration-resistant prostate cancer (mCRPC)
Goals/Aims: 1) To assess the safety and tolerability of the combination of talazoparib with tazemetostat in mCRPC patients, as well as to establish the Recommended Phase 2 Dose (RP2D); 2) To assess preliminary clinical efficacy of talazoparib with tazemetostat at the RP2D in mCRPC patients as assessed by overall response rate (ORR; PSA reduction by ≥ 50% OR radiographic response by RECIST 1.1)
Role: Principal Investigator
POC: Senior Manager, Outsourcing Lead: Jennifer Barrett; Jennifer.Barrett@pfizer.com

NEW
R01CA252468 (Ellis) 08/01/20 – 07/31/25 0.60 CM
NIH/NCI
ATR Dependency as a Novel Therapeutic Target in Lethal RB-deficient Prostate Cancer
Goals/Aims: 1) Validate the ability of DDR kinase targeting to exacerbate DDR deficiency and to generate hypersensitivity in RB-deficient prostate models; 2) Determine the correlation between RB function, HR proficiency and response to M6620+carboplatin and docetaxel+carboplatin in preclinical models and clinical samples; and 3) Evaluate synergy of ATR kinase inhibition, EZH2 inhibition, and immune checkpoint blockade therapy in pre-clinical RB-deficient prostate mouse models.
Role: Co-Investigator
POC: Program Official: Sundaresan Venkatachalam; sundarv@nih.gov

THIS AWARD
W81XWH-20-1-0057 (Choudhury/Mouw) 02/15/20 – 02/14/23 1.20 CM
DoD
Molecular and Genetic Determinants of Response to Carboplatin with or without an ATR Inhibitor (M6620) in mCRPC
Goals/Aims: 1) To correlate genetic and molecular features from pre-treatment tumor biopsy and cfDNA with clinical outcomes for M6620+carboplatin and docetaxel+carboplatin; 2) To discover genetic correlates of resistance to therapy from end-of-study cfDNA specimens and optional biopsies; and 3) To functionally characterize novel genetic alterations identified in pre- and post-treatment specimens using pre-clinical model systems.
Role: Principal Investigator
POC: Grants Specialist: Michelle Cromwell; michelle.l.cromwell.civ@mail.com;

NEW
P01CA228696 (Kantoff) 09/01/19 – 08/31/24 0.60 CM
NIH/NCI
Targeting the DNA Damage Repair Pathway in Non-Castrate Prostate Cancers
Goals/Aims: 1) To determine the association between long-term clinical outcome and pathogenic germline and somatic variants in DDR genes across different ethnic groups; 2) To develop treatment strategies for patients with germline or somatic alterations in DDR pathways; and 3) To evaluate the functional significance of different alterations in DDR genes.
Role: Co-Investigator
POC: Program Official: Kelly Filipski; kelly.filipski@nih.gov

MOUW, KENT

Previous/Current/Pending Support

Current:

NEW
U01 (PI: Abbosh) 03/01/21-02/28/26 0.30 calendar months*
Annual Direct cost
Optimization of urinary DNA deep sequencing tests to enhance clinical staging of bladder cancer patients
Goals/Aims: To perform urine cell-free DNA analysis from muscle-invasive bladder cancer patients.
Role: Subcontract Principal Investigator
Overlap: The proposed work is complementary to the K08 because it utilizes the same specimens as will be analyzed for work proposed in the K08.
POC: TBD

NEW
1R01CA252468-01 (PI: Ellis) 08/01/20 – 07/31/25 0.24 calendar months *
Annual Direct Cost: (complementary effort)
ATR Dependency as a Novel Therapeutic Target in Lethal RB Deficient Prostate Cancer
Goals/Aims: To investigate DNA repair function and sensitivity to DDR kinase inhibitors in RB-deficient prostate cancer and investigate therapeutic approaches that combine DDR kinase inhibition with novel agents for treatment of advanced prostate cancer.
Role: Co-Investigator
Overlap: The proposed work is complementary to the K08 because it will leverage similar tools to create DNA repair deficient cell lines.
POC: Program Official: Sundaresan Venkatachalam; sundarv@nih.gov

THIS AWARD
W81XWH-20-1-0057 (Choudhury/Mouw) 02/15/20 – 02/14/23 1.20 calendar months
DoD
Molecular and genetic determinants of response to carboplatin with or without an ATR inhibitor (M6620) in mCRPC

Goals/Aims:
1) to correlate genetic and molecular features from pre-treatment tumor biopsy and cfDNA with clinical outcomes for M6620+carboplatin and docetaxel+carboplatin
2) to discover genetic correlates of resistance to therapy from end-of-study cfDNA specimens and optional tumor biopsies
3) to functionally characterize novel genetic alterations identified in pre- and post-treatment specimens using pre-clinical model systems.
Role: Co-Principal Investigator
POC: Grants Specialist: Michelle Cromwell; 301-619-4024; michelle.l.cromwell.civ@mail.com

o What other organizations were involved as partners?

- NCI Biorepository, Columbus, OH (Facilities and Collaboration)
- NCI Molecular Characterization (MoCha) Laboratory, Frederick, MD (Facilities and Collaboration)
- Broad Institute of MIT and Harvard, Cambridge, MA (Facilities)

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report

9. APPENDICES

CIRB Approval of Continuing Review

Date: May 19, 2020

Study ID: 10191

Study Title: A Phase 2 Study of M6620 in Combination with Carboplatin compared with Docetaxel in Combination with Carboplatin in Metastatic Castration-Resistant Prostate Cancer

Protocol Version Date: 03/24/20

Study Chair: Atish Choudhury M.D.

At the convened meeting of the NCI Adult CIRB - Early Phase Emphasis held on May 19, 2020, the CIRB conducted its continuing review of 10191 and voted to approve for 12 months minus 1 day.

CIRB approval for this study will expire on May 18, 2021.

The following documents were reviewed:

1. CIRB Application (PVD 03/24/20)
2. Consent Form (PVD 03/24/20)
3. Protocol Version Date 03/24/20
4. DSMC Report 02/04/20
5. GU ASCO Poster dated 2020
6. Investigator's Brochure for M6620 Version 9 dated 12/19/19
7. Investigator's Brochure for M6620 Version 9 dated 12/19/19: Summary of Change
8. Study Summary 2020

As the Study Chair, you are responsible for reporting all study-related activity and correspondence to the CIRB.

The CIRB complies with the Federal regulations 45 CFR 46, 21 CFR 50, and 21 CFR 56.

If you have any questions regarding this review, please contact the Adult CIRB - Early Phase Emphasis Coordinator at EarlyPhaseCIRB@emmes.com.

cc: Hannah Gallo, BS
Casey Pieper BA
Charlotte Pechtl BA
Deanna Hart

Notification of IRB Outcome - Approval

Date: January 08, 2021
To: Atish Choudhury, MD
From: The Office for Human Research Studies (OHRS)

On 01/07/2021 the IRB reviewed the following protocol:

IRB Protocol Number:	20-661
Type of Review:	Submission Response for Initial Review Submission Packet
Title:	Molecular and Genetic Determinants of Response to Carboplatin with or without an ATRInhibitor (M6620) in mCRPC
Principal Investigator:	Choudhury, Atish, MD
iRIS Reference Number:	386884
Review Process:	Expedite
Sponsor(s):	Department of Defense (DOD)
Submission Description:	
Participating Sites:	Dana-Farber Cancer Institute (DFCI)
Documents Revised:	Submission Components Approved

Document Type	Version	Date Approved
Submission-Initial Review Submission Packet	Version 1.0	

Current state of additional determinations (made previously or on this submission):

Risk Level:	Not Greater than Minimal Risk under 45 CFR 46 / 21 CFR 56
Expedited Review Category:	Category 5: Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis)
Waiver of Consent:	Waiver/alteration approved 46.116(c) or (d)
HIPAA:	Waiver of HIPAA Authorization for Research approved under 45 CFR164.512 (i) (2) (ii)

Comments/Recommendations:
1. The reviewer determined the criteria for waiver of HIPAA and a waiver of consent are met from the submitted materials (Secondary use application, parent protocol/consent and supporting documents)
2. This IRB approval only covers existing samples/data obtained on the parent protocol as of the approval date of this secondary

use protocol. Any additional samples/data obtained on the parent protocol in the future will require an amendment submission to the secondary use protocol for approval.

The IRB approved the protocol from 01/07/2021 to 01/06/2022 inclusive. Within 90 days prior to the expiration date you must submit a continuing review or study completion and any required attachments. If continuing review approval or study completion is not granted before the expiration date, your protocol will be put on hold.

As Principal Investigator you are responsible for the following:

1. Submission in writing of any and all changes to this protocol (e.g., protocol, recruitment materials, consent form, study completion) to the IRB for review and approval prior to initiation of the change(s), except where necessary to eliminate apparent immediate hazards to the subject(s). Changes made to eliminate apparent immediate hazards to subjects must be reported to the IRB within 24 hours.
2. Submission in writing of any and all serious adverse event(s) that occur during the course of this protocol in accordance with the IRB's policy on adverse event reporting.
3. Submission in writing of any and all unanticipated problems involving risks to subjects or others.
4. Use of only IRB approved copies of the protocol, consent form(s), questionnaire(s), letter(s), and advertisement(s) in your research. Do not use expired consent forms.
5. Informing all investigators listed on the protocol of changes, adverse events, and unanticipated problems.

If you have any questions, please contact OHRS at ohrs@dfci.harvard.edu.

cc:
Alan D D'Andrea, MD, Eliezer Mendel Van Allen, MD, Geoffrey I. Shapiro, MD, Ph.D,
Catherine Rose Curran

DF/HCC Protocol # 20-661

PROTOCOL TITLE:

Molecular and Genetic Determinants of Response to Carboplatin with or without an ATR Inhibitor (M6620) in mCRPC

Sponsor-Investigator:

Atish Choudhury, MD PhD
Dana-Farber Cancer Institute
achoudhury@partners.org

Principal Investigators (PI):

Atish Choudhury, MD PhD
Dana-Farber Cancer Institute
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VERSION NUMBER:

1.0

DATE:

October 1, 2020

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1.0 Objectives

This is a biomarker supplement to DF/HCC Protocol # 19-715 "A Phase 2 Study of M6620 in Combination with Carboplatin compared with Docetaxel in Combination with Carboplatin in Metastatic Castration-Resistant Prostate Cancer (NCI / CTEP Protocol # 10191), supporting U.S. Department of Defense CDMRP Prostate Cancer Research Program Translational Science Award PC190196 – Grant 12907103.

The specific aims of the grant related to this biomarker supplement are:
1) To correlate genetic and molecular features from pre-treatment tumor biopsy and cfDNA with clinical outcomes for M6620+carboplatin and docetaxel+carboplatin

2) To discover genetic correlates of resistance to therapy from end-of-study cfDNA specimens and optional biopsies

This biomarker supplement is a requirement of the Department of Defense Human Research Protections Office (HRPO), which requires the submission and IRB approval of a new, separate protocol that only contains the DOD funded or supported activities. The studies delineated in this biomarker supplement are all included in the primary protocol document approved by the central IRB (CIRB), and the CIRB-approved informed consent form encompasses consent to these studies.

2.0 Background

Alterations in DNA damage repair genes are common in metastatic castration-resistant prostate cancer (mCRPC), and are implicated in clinical responses to carboplatin, PARP inhibitors and immunotherapeutics. The ATR kinase is involved in the response to a wide range of DNA damage and replication stress, and ATR inhibitors have been previously demonstrated in model systems to have synergistic activity with platinum compounds, and to have activity in homologous recombination (HR)-deficient cells rendered resistant to PARP inhibitors and cells deficient for ATM expression.

We hypothesize that the ATR inhibitor M6620 in combination with carboplatin will demonstrate clinical activity in mCRPC, both in HR-deficient patients (even with PARP inhibitor resistance) and potentially HR-proficient patients due to synergistic activity of M6620 with carboplatin related to induction of replication stress. NCI protocol # 10191 is a Phase 2 study of M6620 with carboplatin compared to docetaxel with carboplatin in mCRPC (n=142 pts). This study 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 supplement is for biomarker studies from these biospecimens.

3.0 Inclusion and Exclusion Criteria

This biomarker supplement includes patients who enroll on trial 10191 and receive study treatment. Biospecimens obtained from participants who enroll but are not treated (usually due to intercurrent illness or symptomatic disease progression) will not be analyzed.

4.0 Study-Wide Number of Subjects

A total of 142 subjects will enroll on trial 10191, of whom we expect 130 patients to receive study treatment.

5.0 Study-Wide Recruitment Methods

Subjects in this biomarker study are those already enrolled on NCI/CTEP trial 10191.

6.0 Multi-Site Research

6.1 Participating sites enrolling to trial 10191 are members of the Experimental Therapeutics Clinical Trials Network (ETCTN). IRB approval for the study at all institutions is performed by the CIRB. Trial documents including the updated protocol, informed consent form (ICF) and training documents are available through the Cancer Trials Support Unit (CTSU) website.

6.2 Details of biospecimen collection and shipment to the ETCTN Biorepository for banking are delineated in the clinical trial protocol.

6.3 Biospecimens for this study will be processed and banked at the ETCTN biorepository and batched shipped as follows:

- Whole exome sequencing: DNA from one tumor biopsy core is extracted at the ETCTN biorepository and stored at -80°C. The frozen specimens are then batched shipped to the NCI Molecular Characterization (MoCha) laboratory
- RAD51 focus formation and ATM immunohistochemistry assays: Formalin fixed tumor tissue is processed and embedded upon receipt at the ETCTN Biorepository. The specimens are then batch shipped to the Center for DNA Damage and Repair Laboratory at Dana-Farber
- Circulating cell-free DNA analysis: Blood specimens in Streck DNA tubes will be processed to plasma and frozen at -80°C. The frozen plasma specimens are then batched shipped to the Broad Institute

7.0 Study Timelines

The studies will be completed in 36 months as detailed in the Statement of Work.

8.0 Study Endpoints

8.1 The primary biomarker endpoint of this study is to correlate homologous recombination repair deficiency (HRD) status (as defined by having a bone fide deleterious mutation or deletion in a gene known to be involved in homologous recombination repair detected by whole exome sequencing from treatment tumor biopsy) with response to therapy.

8.2 Exploratory endpoints include correlating the following molecular features with clinical outcomes:

- Mutational signatures of HRD from WES of pre-treatment tumor biopsy
- RAD51 focus formation and ATM immunohistochemistry from pre-treatment tumor biopsy
- HRD status/mutational signatures detected in circulating cell-free DNA

And identifying mediators of resistance of therapy through:

- Paired comparisons of sequencing results from optional tumor biopsy at the time of progression with pre-treatment specimens
- Paired comparisons of sequencing results from cfDNA at the time of progression with pre-treatment specimens

9.0 Procedures Involved

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

The proposed studies will make use of biospecimens collected through NCI protocol # 10191. This is a randomized open-label Phase 2 study of M6620 + carboplatin vs. docetaxel + carboplatin in patients with metastatic castration-resistant prostate cancer. Patients who have been treated with at least one secondary hormonal therapy and taxane-based chemotherapy will be randomized 1:1 to receive Arm A (docetaxel + carboplatin) or Arm B (M6620 + carboplatin). Patients who are ineligible for docetaxel due to 1. Allergy or intolerance to docetaxel, 2. Grade 2 neuropathy, or 3. ECOG PS=2 and are randomized to Arm Awill be treated with carboplatin as a single agent. Patients will be treated on study until the occurrence of progression by Prostate Cancer Working Group 3 (PCWG3) criteria(1), unequivocal clinical progression, unacceptable side effects, withdrawal of consent, or death. Patients will be stratified at randomization by

whether they received prior PARP inhibitor (yes vs. no) and whether they have evaluable disease by RECIST criteria at baseline (yes vs. no). A post-hoc stratification based on whether patients have a predicted deleterious mutation in a gene involved in homologous recombination repair by whole exome sequencing from a mandatory pre-treatment tumor biopsy will also be performed. Patients on the docetaxel+carboplatin arm will be allowed to cross over to the M6620+carboplatin arm at the earlier of time of PSA progression (by PCWG2 criteria(2)) or radiographic progression (by RECIST 1.1 criteria for non-osseous disease or PCWG3 criteria(1) for osseous disease) if the patient remains clinically stable and continues to meet the eligibility criteria of the protocol.

The primary objective of this trial is to investigate the response rate (CR+PR as defined by radiographic response by RECIST 1.1 or PSA decline >50% from baseline) in patients treated with a combination of M6620 and carboplatin as compared to a combination of docetaxel and carboplatin. For the primary endpoint, we anticipate a response rate of 20% in the docetaxel+carboplatin arm in this cohort of patients who have previously received at least 2 prior therapies15. If the response rate of carboplatin+M6620 is 40%, with 65 patients on each treatment arm (total N=130), there will be 80% power to distinguish a response rate of 40% versus 20% using a chi-square test (one sided a=0.05). 136 subjects will be enrolled to account for 5% of patients who never start treatment after randomization.Patients who are evaluable for both PSA and radiographic response will be considered responders if they experience a response by either criterion.

9.1.1 Whole exome sequencing analysis

Mandatory pre-treatment tumor biopsy specimens will be submitted for whole exome sequencing through the Molecular Characterization (MoCha) laboratory at the Frederick National Laboratory for Cancer Research, and will be analyzed in the laboratory of Co-Investigator Eliezer Van Allen. Exome sequencing data are processed using established analytic pipelines at the Broad Institute of MIT and Harvard (Cambridge, MA)(3). Tumor sequences and normal sequences (derived from germline sequencing from whole blood) are aligned to the hg19 human reference genome. BAM files are produced via the Picard pipeline (http://picard.sourceforge.net/), uploaded, and processed through the Firehose pipeline (http://www.broadinstitute.org/cancer/cga/Firehose). To identify somatic single-nucleotide variants, MuTect(4) is applied and reviewed with Integrated Genomics Viewer (IGV)(5). Strelka(6) is applied to detect small insertions and deletions. Artifacts from DNA oxidation during sequencing are removed using a filtered-based method(7). Oncotator(8) is used to annotate the identified variants.

For the **primary biomarker analysis**, we plan to categorize patients as homologous recombination deficient (HRD) if they are found to have a *bone fide* deleterious mutation or deletion in a gene known to be involved in homologous recombination repair from a fresh tumor biopsy collected immediately prior to trial

enrollment. Gene mutation frequencies and mean ± SD of quantitative biomarkers will be summarized by arm and in overall population at baseline and/or at end of study. We anticipate that about 75% of pre-trial specimens will be evaluable for homologous recombination deficiency (HRD) status by next generation sequencing. With a total of 98 evaluable samples, the 90% exact binominal CI width is 0.11 and 0.16 if the observed HRD event rate is 0.1 or 0.3, respectively. We hypothesize that in both arms of the study, responses to carboplatin will be enriched in patients with HRD as determined by detection of alterations in genes involved in homologous recombination repair. If 20% of patients harbor HRD, there is 83% power to detect a 30% difference in response (e.g. for a response rate 20% in HRD(-) versus 50% in HRD(+) patients) using a chi-square test (one sided a=0.05).

Beyond correlating HRD status with clinical outcomes with carboplatin in both arms of the study, we seek to better understand the relationship between particular DNA damage repair defects detected by WES and response to carboplatin +/- M6620 with in patients with metastatic CRPC. Specifically, we would hypothesize that:

1. Patients with HRD who are previously untreated with PARPi or carboplatin would be expected to respond to either docetaxel+carboplatin or M6620+carboplatin.
2. Patients with HRD who were previously treated with PARPi and are found to have a mutation in a gene involved in an alternate DNA damage repair pathway (such as nucleotide excision repair)(9) would be expected to respond to docetaxel+carboplatin or M6620+carboplatin.
3. Patients with HRD who were previously treated with PARPi and are found to have a reversion mutation in the gene originally conferring HRD (and thus no longer possessing an HRD phenotype) would not be expected to respond to docetaxel+carboplatin but could respond to the combination of M6620+carboplatin due to synergistic activity related to induction of replication stress.
4. Patients without HRD or other DNA damage repair defects would not be expected to respond to docetaxel+carboplatin but could respond to the combination of carboplatin+M6620 due to synergistic activity related to induction of replication stress.

As an exploratory analysis, we will also derive mutational signatures from whole exome sequencing data given evidence that these signatures can reveal underlying mutational processes and potential therapeutic vulnerabilities(10). Previous studies(11) have described mutational signatures associated with aging (C>T mutations at CpG dinucleotides), APOBEC activity (C>T or C>G at a TC[A/T] context), and DNA homologous recombination deficiency (BRCA-like(12)). The COSMIC database currently describes 30 signatures of mutational processes in cancer, where signature 3 has been associated with homologous recombination deficiency. Previously SNP arrays were used to identify large-scale genomic aberrations associated with homologous recombination deficiency, often induced by the loss of BRCA1 or BRCA2 function. Three such measures were identified: telomeric allelic imbalance (HRD-TAI score),(13) loss of heterozygosity profiles (HRD-LOH score),(14) and large-scale state transitions

(HRD-LST score).(15) Recently, tools have been described to derive these parameters from whole exome sequencing data(16) so we will assess whether the presence of signature 3 or high levels of HRD by the TAI, LOH and LST scores in metastatic prostate cancer specimens correlates with clinical outcomes.

9.1.2 Circulating cell-free DNA analysis

DNA derived from tumor can be detected in circulating free DNA (cfDNA) isolated from plasma from patients with metastatic cancer. We plan to perform whole genome sequencing of cfDNA at 0.1x coverage, termed ultra-low pass whole genome sequencing (ULP-WGS). The sequencing information derived from ULP-WGS can be used to identify copy number alterations (CNA) in the tumor using ichorCNA(17), a probabilistic model that is an adaptation of TITAN.(18) While other algorithms for assessing copy number changes from cfDNA have been described(19, 20), ichorCNA has the advantage of accounting for mixtures of cell populations to assess for subclonal events, while also estimating the fraction of cfDNA derived from tumor cells versus normal tissues.

A sample copy number profile derived from ULP-WGS from cfDNA from a patient with metastatic castration resistant prostate cancer (mCRPC) is shown in Figure 1B, with a copy number profile derived from whole exome sequencing of a matched metastatic biopsy shown for comparison. In this patient, we detect a focal amplification of POLA2 as well as deletions in ATM and ARFGAP3 in cfDNA, and all three of these events were also observed in the matched metastasis biopsy. Detection of an ATM deletion from a blood biopsy has potential relevance to this study, as ATM deletion is predicted to lead to vulnerability to ATR inhibition. Since metastasis biopsy is not a routinely performed clinical test in prostate cancer patients, the ability to identify copy number alterations from blood as a predictive biomarker for therapy is potentially transformative to clinical management.

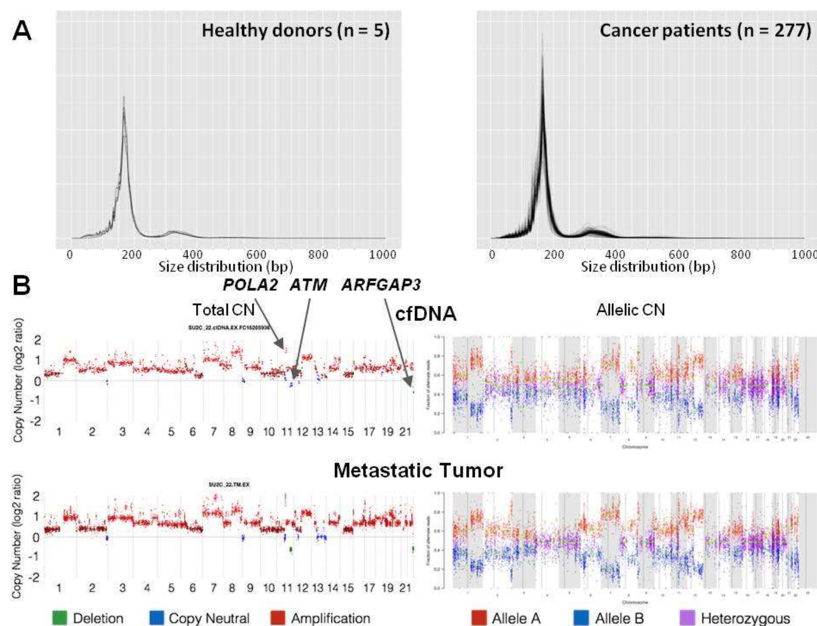

Figure 1. A. Size distribution of DNA fragments isolated from plasma from healthy donors (left) and cancer patients (right). **B.** Sample copy number profiles derived from ULP-WGS from cfDNA as compared to WES from a metastasis biopsy from the same patient.

Our model can also use ULP-WGS to estimate the fraction of circulating free DNA derived from tumor rather than normal tissues ("tumor fraction") based on the assumption that cancer cells derive from a common precursor and thus have at least one truncal copy number alteration (other alterations being subclonal). We estimate the lower limit for detection of tumor derived DNA by this method to be approximately 3%. The tumor purity estimated by ULP-WGS using ichorCNA demonstrates close concordance with that estimated from whole exome sequencing using a different method for deriving tumor fraction from somatic DNA alterations called ABSOLUTE(21), thus validating this method for quantification.

We have designed a custom bait set specifically for prostate cancer which will allow us to identify SSNVs in all genes known to be recurrently mutated in metastatic prostate cancer(22), genes involved in DNA damage repair(23), as well as sequencing of intronic and intergenic regions of genes known to be translocated or have complex structural alterations in prostate cancer (AR(24), ERG, TMPRSS2, ETV1, ETV4, SLC45A3, RAF1). Using an earlier version of this bait set and a software tool called BreaKmer(25) to analyze the first 6 cfDNA samples tested, we detected a total of 15 rearrangements and 13 indels. Importantly, these translocations and structural variants would not have been detected through current commercial assays or through WES alone. We envision that this approach allowing detection of SSNVs, CNAs, translocations and structural alterations in the same assay could become the standard cfDNA assay for clinical use in patients with metastatic prostate cancer. We believe this approach will also allow us to better sample global tumor burden than a single biopsy due to tumor heterogeneity. In addition, longitudinal monitoring of cfDNA from patients while on study will allow us to monitor clonal dynamics over time.

The cfDNA assay will be performed at the Broad Institute of MIT and Harvard. First, isolated cfDNA (which is naturally fragmented) will undergo ultra-low pass whole genome sequencing (ULP-WGS) library construction using a 6 base pair Unique Molecular Identifier (UMI) on the P7 sequencing adaptor. UMIs are random sequences of bases used to tag each molecule (fragment) prior to library amplification, thereby aiding in the identification of PCR duplicates and thus distinguish true mutations from PCR errors or sequencing errors based on consensus among reads sharing same the index. ULP-WGS results will be used to derive tumor fraction at each time point using ichorCNA.

From there, the same library from ULP-WGS is selected in hybrid capture using our custom targeted panel with a goal of 1000x mean target coverage (MTC) for cfDNA and 500x MTC for matched germline control. Standard variant calling will be carried out using MuTect, Indelocator and ConTest within the Firehose environment at the Broad. Analytical sensitivity will be assessed using cfDNA from prostate cancer patients previously sequenced through other methods with known alterations in genes present in this panel. Precision, or reproducibility, of variant identification will be assessed through concordance of variant calls across the duplicate runs of the same cfDNA library. Analytical specificity will be determined by assessing the false positive rate from duplicate runs of a control library.

For cfDNA analyses, HRD will be defined the same way as for whole exome sequencing, as the presence of a *bone fide* deleterious mutation or deletion in a gene known to be involved in homologous recombination repair. Based on our previous studies(17), we assume that ~75% of pre-treatment specimens will have adequate tumor-derived cfDNA for analysis. Thus, the statistics for correlating HRD status from cfDNA with response to therapy will be the same as for tumor biopsies as detailed above. We are unable to derive mutational signatures using data from targeted cfDNA sequencing; however, certain genomic signatures can be detected by ULP-WGS – for example a genome-wide tandem duplicator phenotype associated with CDK12 inactivation has been detected in cfDNA from prostate cancer patients using this method.(26) This will allow us to explore whether certain patterns of copy number changes detected in cfDNA may correlate with clinical responses.

9.1.3 RAD51 focus formation assay

Homologous recombination (HR) repair deficiency confers sensitivity to platinum-based chemotherapeutic agents and inhibitors of poly(ADP-ribose) polymerase (PARP). To date, the identification of tumors with impaired HR has relied on genomic features, including HR gene mutational status, an HRD mutational signature, LOH-based HRD assays or gene expression analyses defining 'BRCAness'. These tests analyze history of the tumor rather than providing a functional assessment of HR status at the time of diagnosis. Therefore, development of a functional assay for "real-time" HR status in tumors is essential to make accurate treatment decisions.

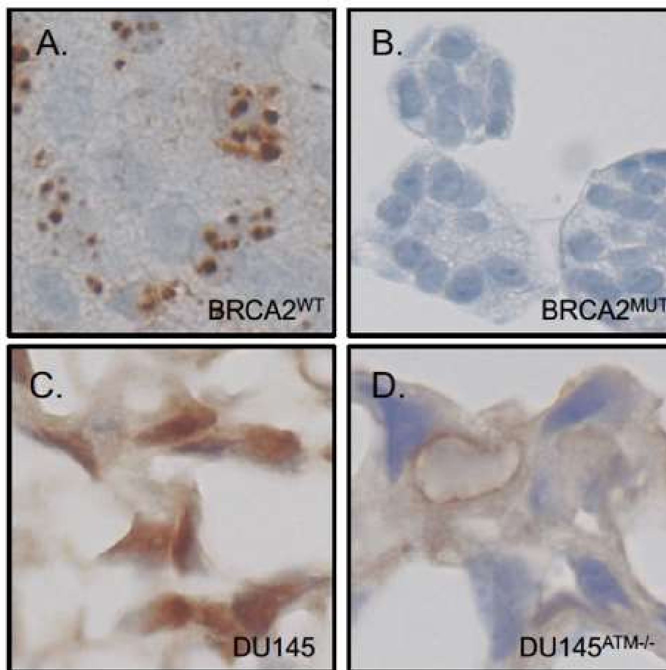

Figure 2: Rad51 and ATM Immunohistochemistry (IHC). FFPE tumor sections are stained with Rad51 or ATM antibodies. A. Prostate tumor PDX with wild-type BRCA2 displays Rad51 foci indicative of HR proficiency. B. Prostate tumor PDX with a truncating BRCA2 mutation lacks Rad51 foci, consistent with HR deficiency. C. FFPE prostate cancer cell line (DU145) with strong ATM staining. D. DU145 cells in which the *ATM* gene has been deleted using CRISPR/Cas9 gene editing lack ATM staining.

We have described a RAD51-based immunohistochemical (IHC) assay that can be used to determine HR functional status.(27) The presence or absence of RAD51 foci in cell lines and PDX models correlated with olaparib sensitivity and not with BRCA mutation status.

Therefore, tumors that are HR-deficient and PARP inhibitor-sensitive are characterized by either high RAD51 nuclear staining without foci, or absence of RAD51 staining (Figure 2). This assay may serve as a superior or complementary biomarker to homologous recombination deficient status as determined by genomic characterization (such as a predicted deleterious alteration in a gene involved in homologous recombination repair) in predicting response to carboplatin in combination with docetaxel or M6620.

For the RAD51 focus formation assay, serial sections of formalin fixed paraffin embedded (FFPE) tumor sample will be stained for RAD51 and Geminin (a marker of cells in S phase) independently. This protocol was recently approved by the Biomarker Review Committee at CTEP/NCI. Presence of RAD51 foci in tumor cells is a surrogate marker for HR proficiency. For a sample to be HR proficient, more than three RAD51 foci must be present in a minimum of one cell in three 40X fields. If RAD51 foci are absent, the sample is HR deficient if greater than 3% of the cells are Geminin positive. If there are no RAD51 foci and less than 3% of the cells are Geminin positive, the proliferation rate of the tumor is low and HR status cannot be determined. FFPE sections of a cell line block containing irradiated and unirradiated HR proficient and HR-deficient cell lines will be used as control.

The frequency of HR proficiency by RAD51 focus formation will be summarized by arm and in the overall population at baseline and/or at end of study. We anticipate that about 75% of pre-treatment specimens will be evaluable for RAD51 focus formation (i.e. that >3% of cells are Geminin positive). With a total of 98 evaluable samples, the 90% exact binominal CI width is 0.11 and 0.16 with an observed rate of HR deficiency of 0.1 and 0.3 respectively.

We hypothesize that in both arms of the study, responses to carboplatin will be enriched in patients with loss of RAD51 focus formation in this assay. If 20% of patients lose RAD51 focus formation, there is 83% power to detect a 30% difference in response (e.g. for a response rate 20% in RAD51(-) versus 50% in RAD51(+) patients) using a chi-square test (one sided a=0.05).

9.1.4 ATM Immunohistochemistry

The Ataxia Telangiectasia Mutated (ATM) and Ataxia telangiectasia and Rad3 related (ATR) serine/threonine kinases are both involved in the response to DNA damage, though ATM is primarily involved in the response to double strand breaks (DSBs) while ATR is involved in the response to a wide range of perturbations leading to DNA damage and replication defects. These kinases appear to share a synthetic lethal relationship, as cells deficient in ATM expression have been shown to be sensitive to ATR inhibition in mantle cell lymphoma(28), CLL(29) and gastric cancer(30, 31) models. In cell lines, the majority of cell lines sensitive to the ATR inhibitor BAY-1895344 were characterized by mutations in the ATM pathway.(32)

We have demonstrated that ATM-deficient human fibroblasts cell lines are sensitive to ATR inhibition, and this sensitivity can be reversed by complementation with wild-type ATM. We have also demonstrated in cell lines that ATM deficiency predicts for sensitivity to ATR inhibition, and we developed an immunohistochemistry assay for ATM demonstrating in 208 primary gastric tumors that ATM expression is absent in 11% of samples and is expressed at very low levels in 26%.(31) As ATM alterations are highly enriched in mCRPC compared with locoregional prostate cancer(33, 34), targeting ATM alterations through inhibition of ATR is a promising therapeutic strategy in mCRPC.

For assessment of ATM status, an FFPE section of the tumor sample will be stained using an antibody specific for ATM (Figure 2). Stained slides will be scanned into Aperio image scope and established algorithms will be used to determine the percentage of cells with nuclear staining intensity ranging from 0-3. The H-score will be estimated. A H-score < 5 will be considered as ATM negative. Adjacent normal tissue will serve as an internal control for the stain. FFPE sections of a cell line block containing ATM deficient cells derived from an AT patient (which lack ATM) and the ATM complemented counterpart of the same cells will be used as additional controls.

We hypothesize that patients with ATM loss by IHC will experience profound benefit to the combination of M6620 with carboplatin. We will analyze the data in three separate ways: 1) We will summarize response rate and PFS in the M6620+carboplatin and the docetaxel+carboplatin arms in the ATM-deficient and ATM-intact patients and test whether the benefit to M6620+carboplatin and/or docetaxel+carboplatin is enriched in the ATM deficient population. 2) For patients who experience an exceptional response to therapy in both arms of the study (PSA decline >90% or PFS > 12 months), we will assess if this population is enriched for patients with ATM loss as compared to the total population. 3) For the subset of patients who progress on docetaxel+carboplatin but then respond to M6620+carboplatin after crossover, we will assess if this population is enriched for patients with ATM loss as compared to the total population.

9.2 *Aim 2.* To discover genetic correlates of resistance to therapy from end-of-study cfDNA specimens and optional biopsies

We have previously performed paired analysis of whole exome sequencing (WES) data from pre-treatment and post-relapse biopsy specimens from patients with mCRPC who were treated with abiraterone and enzalutamide per the schema shown in Figure 3A(35). Kinetics of PSA responses in the seven analyzed patients are shown in Figure 3B and identified patients with intrinsic and acquired resistance to therapy. This study identified *AR* amplification and *AR* L702H mutation as correlates of intrinsic resistance to abiraterone, with the development of *MYC* amplification correlating with acquired resistance. A *CDK6* amplification was implicated in intrinsic resistance in a patient treated with enzalutamide, while a new *CDKN2A* P81L mutation correlated with acquired resistance. We have performed similar analyses from pre-treatment and post-neoadjuvant chemotherapy samples from patients treated with a cisplatin-based regimen

for muscle-invasive bladder cancer and detected alterations in cell-cycle and immune checkpoint regulation genes in post-treatment tumors.(36)

Findings from these studies demonstrate that collection and WES of paired pre/post-treatment tumor biopsy specimens is feasible, and that this WES data can yield clinically and biologically meaningful results – i.e, there is not excess "noise" in the paired comparisons leading to identification of large numbers of differential alterations without biological relevance.

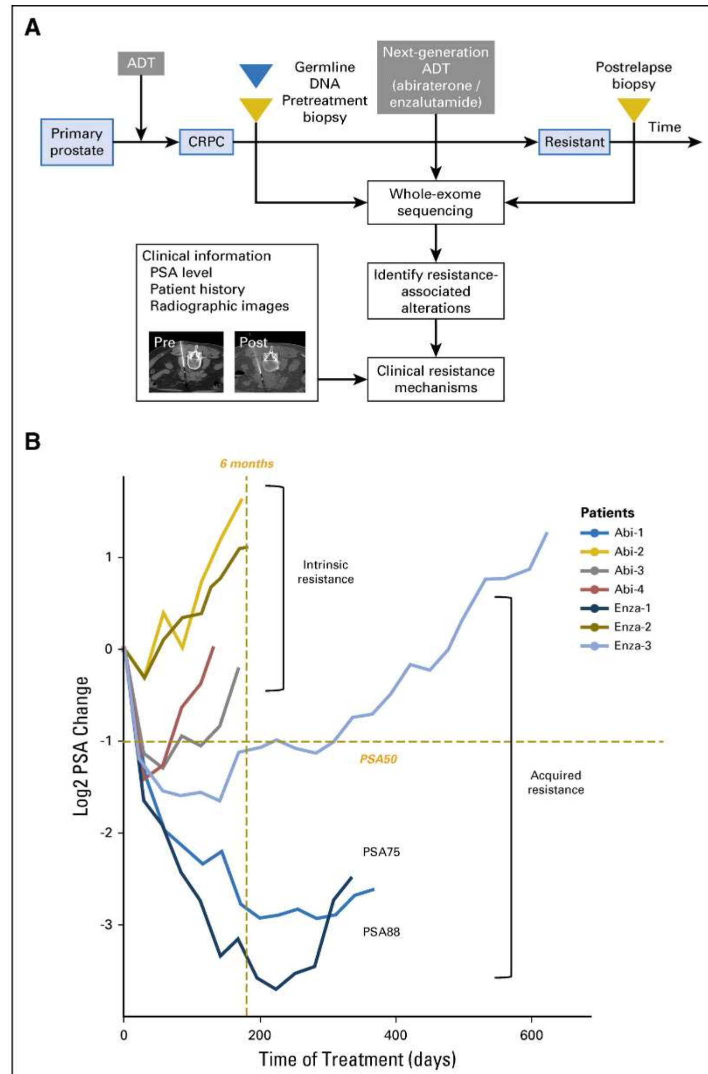

Figure 3. A. Schematic overview of paired (pre- and post-treatment) tumor biopsy collection in the context of abiraterone and enzalutamide, followed by whole-exome sequencing and computational analysis to investigate clinical resistance mechanisms. **B.** Change in PSA levels between the start of treatment (at the time of pretreatment biopsy, day 0) and the end (at the time of post-treatment biopsy) of treatment.

We plan to perform whole exome sequencing from optional end-of-study biopsy specimens at the NCI MoCha laboratory. Paired analysis of pre-treatment and post-treatment biopsies will be performed as previously described.(35, 36) We will also compare results from deep targeted next generation sequencing from plasma specimens collected at end of study with pre-treatment specimens. For the subset of patients who are randomized to the control arm of docetaxel+carboplatin, progress on treatment, and then crossover to M6620+carboplatin, we will also perform ULP-WGS and targeted sequencing at the time of crossover and compare genetic features at the time of crossover with pre-treatment and end-of-study specimens. Assuming 40~60% of patients with paired (pre- and post-treatment) biopsy available, there is 80% power to detect a 0.32~0.40 SD mean change in quantitative biomarkers between time points with n=52~78 using a paired t-test (two-sided α=0.05).

We hypothesize that a variety of different genetic features could correlate with resistance including reversion mutations restoring wild-type function of a DDR gene, activation of survival pathways, or mechanisms leading to drug metabolism or efflux.

Genetic variants that increase in abundance at the time of resistance will be prioritized for further study using PHIAL(3), which is an algorithm that takes as input somatic alterations and uses heuristics to assign clinical and biological significance to each alteration. As it is unlikely in these studies that there will be recurrent single nucleotide variants (SNVs) that drive therapeutic resistance across many patients, this algorithm will help us identify those mutations most likely to be clinically and biologically relevant by examining mutations in genes within the TARGET, COSMIC and CGC databases, while including knowledge about kinase domains, copy number directionality and two-hit pathway events. We will then functionally characterize alterations correlating with resistance.

10.0 Data and Specimen Banking, and Confidentiality

10.1 All biospecimens collected in this trial are identifiable only through their trial ID # and can only be linked to the medical records of the participants through a key stored on a secure server accessible by the clinical trial team at each participating institution.

10.2 Biospecimens banked at the ETCTN Biorepository are stored indefinitely unless a participant withdraws consent for biomarker studies (see section 12).

- After completion of planned analyses, leftover material at the MoCha laboratory, CDDR laboratory and the Broad Institute are to be returned to the ETCTN biorepository or destroyed.
- Secondary use of biospecimens is only permitted through a protocol amendment approved by NCI/CTEP and CIRB.

10.3 Genomic data will be uploaded to the database of Genotypes and Phenotypes (dbGaP) in an anonymized fashion linked to their trial ID#. Access to individual-level datasets within the controlled-access portions of the database, requires the Principal Investigator (PI) and the Signing Official (SO) at the requestor's institution to co-sign a request for data access, which will be reviewed by an NIH Data Access Committee at the appropriate NIH Institute or Center.

11.0 Withdrawal of Subjects

Subjects on this study can withdraw consent from participation in these biomarker studies at any point. Any samples collected that are located in a biobank or at a collaborating institution from a subject who has withdrawn consent will be destroyed or returned to the treating institution. Any data generated from those subjects will not be included in the final analysis or uploaded into dbGaP.

12.0 Risks to Subjects

There is a risk that someone outside of the research study could get access to subject study records or trace information in a database back to trial participants, and use the information in a way that could be harmful to them. We believe the chance that someone could access and misuse subject information is very small. However, the risk may increase in the future as people find new ways of tracing information. In some cases, this information could be used to make it harder for a research subject to get or keep a job and get or keep health insurance. There are laws against the misuse of genetic information, but they may not give full protection. Information about the laws that protect research subjects can be found at : https://www.genome.gov/10002328/

13.0 Potential Benefits to Subjects

The is no direct benefit to subjects.

14.0 Sharing of Results with Subjects

All biomarker studies described are performed for research-only use and results will not be shared with subjects.

15.0 Prior Approvals

The studies in this biomarker supplement are explicitly listed in the 10191 protocol that has already been approved by the CIRB.

16.0 Local Number of Subjects

27 of the 65 subjects treated on this protocol to date have enrolled from DF/HCC sites, so we anticipate between 50 and 60 of the 130 total subjects treated on this protocol to be enrolled from DF/HCC.

17.0 Provisions to Protect the Privacy Interests of Subjects

De-identified samples and data (clinical as well as genomic or epigenomic) collected under this protocol will be shared with collaborators performing the biomarker studies and stored in the database of Genotypes and Phenotypes (dbGaP). Specimens will be de-identified using a Trial ID # that is different from the patient's medical record number. Language regarding the acquisition and sharing of genomic data is included in the consent form for this trial. Data at both the individual level (de-identified) and aggregate level may be shared in collaboration with other researchers. Subjects may withdraw consent for biomarker studies and data sharing at any point (see section 11).

18.0 Consent Process

All biospecimens for this study are obtained from participants in NCI/CTEP trial # 10191, and the CIRB-approved protocol for this trial explicitly lists all

biomarker studies included in this supplement. The informed consent form approved by the CIRB encompasses informed consent for these biomarker studies as well (including consent to genetic testing/data sharing and information regarding risks and confidentiality), so additional informed consent is not required for this biomarker supplement.

References

1. Scher HI, Morris MJ, Stadler WM, Higano C, Basch E, Fizazi K, et al. Trial Design and Objectives for Castration-Resistant Prostate Cancer: Updated Recommendations From the Prostate Cancer Clinical Trials Working Group 3. *J Clin Oncol.* 2016;34(12):1402-18.
2. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. *J Clin Oncol.* 2008;26(7):1148-59.
3. Van Allen EM, Wagle N, Stojanov P, Perrin DL, Cibulskis K, Marlow S, et al. Whole-exome sequencing and clinical interpretation of formalin-fixed, paraffin-embedded tumor samples to guide precision cancer medicine. *Nature medicine.* 2014;20(6):682-8.
4. Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nature biotechnology.* 2013;31(3):213-9.
5. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative genomics viewer. *Nature biotechnology.* 2011;29(1):24-6.
6. Saunders CT, Wong WS, Swamy S, Becq J, Murray LJ, and Cheetham RK. Strelka: accurate somatic small-variant calling from sequenced tumor-normal sample pairs. *Bioinformatics.* 2012;28(14):1811-7.
7. Costello M, Pugh TJ, Fennell TJ, Stewart C, Lichtenstein L, Meldrim JC, et al. Discovery and characterization of artifactual mutations in deep coverage targeted capture sequencing data due to oxidative DNA damage during sample preparation. *Nucleic acids research.* 2013;41(6):e67.
8. Ramos AH, Lichtenstein L, Gupta M, Lawrence MS, Pugh TJ, Saksena G, et al. Oncotator: cancer variant annotation tool. *Hum Mutat.* 2015;36(4):E2423-9.
9. Ceccaldi R, O'Connor KW, Mouw KW, Li AY, Matulonis UA, D'Andrea AD, et al. A unique subset of epithelial ovarian cancers with platinum sensitivity and PARP inhibitor resistance. *Cancer research.* 2015;75(4):628-34.
10. Ma J, Setton J, Lee NY, Riaz N, and Powell SN. The therapeutic significance of mutational signatures from DNA repair deficiency in cancer. *Nature communications.* 2018;9(1):3292.
11. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. *Nature.* 2013;500(7463):415.
12. Alexandrov LB, Nik-Zainal S, Siu HC, Leung SY, and Stratton MR. A mutational signature in gastric cancer suggests therapeutic strategies. *Nature communications.* 2015;6:8683.
13. Birkbak NJ, Wang ZC, Kim J-Y, Eklund AC, Li Q, Tian R, et al. Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. *Cancer discovery.* 2012.
14. Abkevich V, Timms K, Hennessy B, Potter J, Carey M, Meyer L, et al. Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. *British journal of cancer.* 2012;107(10):1776.

15. Popova T, Manié E, Rieunier G, Caux-Moncoutier V, Tirapo C, Dubois T, et al. Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. *Cancer research.* 2012:canres. 1470.2012.
16. Sztupinszki Z, Diossy M, Krzystanek M, Reiniger L, Csabai I, Favero F, et al. Migrating the SNP array-based homologous recombination deficiency measures to next generation sequencing data of breast cancer. *npj Breast Cancer.* 2018;4(1):16.
17. Adalsteinsson VA, Ha G, Freeman SS, Choudhury AD, Stover DG, Parsons HA, et al. Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. *Nature communications.* 2017;8(1):1324.
18. Ha G, Roth A, Khattra J, Ho J, Yap D, Prentice LM, et al. TITAN: inference of copy number architectures in clonal cell populations from tumor whole-genome sequence data. *Genome research.* 2014;24(11):1881-93.
19. Heitzer E, Ulz P, Geigl JB, and Speicher MR. Non-invasive detection of genome-wide somatic copy number alterations by liquid biopsies. *Molecular oncology.* 2016;10(3):494-502.
20. Ulz P, Belic J, Graf R, Auer M, Lafer I, Fischereder K, et al. Whole-genome plasma sequencing reveals focal amplifications as a driving force in metastatic prostate cancer. *Nature communications.* 2016;7:12008.
21. Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, Zack T, et al. Absolute quantification of somatic DNA alterations in human cancer. *Nature biotechnology.* 2012;30(5):413-21.
22. Armenia J, et al. The long tail of significantly mutated genes in prostate cancer. http://meetinglibrary.asco.org/content/179374-197.
23. US Food and Drug Administration CfDEaR, NDA 202-379. Clinical Pharmacology and Biopharmaceutics Review - Abiraterone Acetate. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202379Orig1s000ClinPharmR.pdf. Accessed 20 Dec 2017.
24. Henzler C, Li Y, Yang R, McBride T, Ho Y, Sprenger C, et al. Truncation and constitutive activation of the androgen receptor by diverse genomic rearrangements in prostate cancer. *Nature communications.* 2016;7:13668.
25. Abo RP, Ducar M, Garcia EP, Thorner AR, Rojas-Rudilla V, Lin L, et al. BreaKmer: detection of structural variation in targeted massively parallel sequencing data using kmers. *Nucleic acids research.* 2014;43(3):e19-e.
26. Viswanathan SR, Ha G, Hoff AM, Wala JA, Carrot-Zhang J, Whelan CW, et al. Structural Alterations Driving Castration-Resistant Prostate Cancer Revealed by Linked-Read Genome Sequencing. *Cell.* 2018;174(2):433-47 e19.
27. Kochupurakkal BS, Parmar K, Lazaro J-B, Unitt C, Zeng Q, Reavis H, et al.: AACR; 2017.
28. Menezes DL, Holt J, Tang Y, Feng J, Barsanti P, Pan Y, et al. A synthetic lethal screen reveals enhanced sensitivity to ATR inhibitor treatment in mantle cell lymphoma with ATM loss-of-function. *Molecular Cancer Research.* 2015;13(1):120-9.
29. Kwok M, Davies N, Agathanggelou A, Smith E, Oldreive C, Petermann E, et al. ATR inhibition induces synthetic lethality and overcomes chemoresistance in

TP53-or ATM-defective chronic lymphocytic leukemia cells. *Blood.* 2016;127(5):582-95.

30. Min A, Im S-A, Jang H, Kim S, Lee M, Kim DK, et al. AZD6738, a novel oral inhibitor of ATR, induces synthetic lethality with ATM-deficiency in gastric cancer cells. *Molecular cancer therapeutics.* 2017:molcanther. 0378.2016.
31. Cai M-Y, Reavis HD, Chen J-W, Ganesa C, Kochupurakkal B, Shapiro GI, et al.: AACR; 2018.
32. Wengner AM, Siemeister G, Luecking U, Lefranc J, Lienau P, Deeg G, et al.: AACR; 2017.
33. Abida W, Armenia J, Gopalan A, Brennan R, Walsh M, Barron D, et al. Prospective Genomic Profiling of Prostate Cancer Across Disease States Reveals Germline and Somatic Alterations That May Affect Clinical Decision Making. *JCO Precis Oncol.* 2017;2017.
34. Armenia J, Wankowicz SAM, Liu D, Gao J, Kundra R, Reznik E, et al. The long tail of oncogenic drivers in prostate cancer. *Nat Genet.* 2018.
35. Han GC, Hwang J, Wankowicz SAM, Zhang Z, Liu D, Cibulskis C, et al. Genomic Resistance Patterns to Second-Generation Androgen Blockade in Paired Tumor Biopsies of Metastatic Castration-Resistant Prostate Cancer. *JCO Precision Oncology.* 2017(1):1-11.
36. Liu D, Abbosh P, Keliher D, Reardon B, Miao D, Mouw K, et al. Mutational patterns in chemotherapy resistant muscle-invasive bladder cancer. *Nature communications.* 2017;8(1):2193.

E01350.1a - HRPO Approval Memorandum (Proposal Number PC190196, Award Number W81XWH-20-1-0057)

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SUBJECT: Initial Approval for the Protocol, "Molecular and Genetic Determinants of Response to Carboplatin with or without an ATR Inhibitor (M6620) in mCRPC," Submitted by Atish Choudhury, MD, PhD, Dana-Farber Cancer Institute, Boston, Massachusetts, Proposal Log Number PC190196, Award Number W81XWH-20-1-0057, HRPO Log Number E01350.1a

1. The subject protocol (version 1.0/dated 1 October 2020) was approved by the Dana Farber Cancer Institute Office for Human Research Studies (OHRS) on 7 January 2021. The U.S. Army Medical Research and Development Command (USAMRDC),Office of Research Protections (ORP), Human Research Protection Office (HRPO) reviewed the protocol and found that it complies with applicable DOD, U.S. Army, and USAMRDC human subjects protection requirements.

2. This no greater than minimal risk study is approved for the use/analysis of pre-treatment tumor biopsy from 110 patients, and germline/pre-treatment/post-treatment deep targeted sequencing of circulating cell free DNA from 110 patients.

3. The Principal Investigator has a duty and responsibility to foster open and honest communication with research subjects. The USAMRDC strongly encourages the Principal Investigator to provide subjects with a copy of the research protocol, if requested, with proprietary and personal information redacted as needed.

4. The Principal Investigator must provide the following post-approval submissions to the HRPO via email to usarmy.detrick.medcom-USAMRDC.other.hrpo-cr-documents@mail.mil. **Failure to comply could result in suspension or termination of funding.**

a. Substantive modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the HRPO for approval prior to implementation. The USAMRDC ORP HRPO defines a substantive modification as a change in Principal Investigator, change or addition of an institution (Note: HRPO review and approval of institution is required), elimination or alteration of the consent process, change in the IRB of Record, change to the study population that has regulatory implications (e.g. adding children, adding active duty population, etc.), significant change in study design (i.e. would prompt additional scientific review), or a change that could potentially increase risks to subjects.

b. A copy of the IRB continuing review approval letter must be submitted to the HRPO as soon as possible after receipt of approval. According to our records, it appears the next continuing review by

the IRB is due no later than 6 January 2022. Please note that the HRPO conducts random audits at the time of continuing review and additional information and documentation may be requested at that time.

c. The final study report submitted to the IRB, including a copy of any acknowledgement documentation and any supporting documents, must be submitted to the HRPO as soon as all documents become available.

d. The following study events must be promptly reported to the HRPO by telephone (301-619-2165), by email (usarmy.detrick.medcom-USAMRDC.other.hrpo@mail.mil),by facsimile (301-619-7803), or mail to the U.S. Army Medical Research and Development Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000.

(1) All unanticipated problems involving risk to subjects or others.

(2) Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the sponsor, or regulatory agencies.

(3) Any instances of serious or continuing noncompliance with the federal regulations or IRB requirements.

(4) The knowledge of any pending compliance inspection/visit by the Food and Drug Administration (FDA), Office for Human Research Protections, or other government agency concerning this clinical investigation or research.

(5) The issuance of inspection reports, FDA Form 483, warning letters, or actions taken by any government regulatory agencies.

e. Events or protocol reports received by the HRPO that do not meet reporting requirements identified within this memorandum will be included in the HRPO study file but will not be acknowledged.

5. **Please note**: The USAMRDC ORP HRPO conducts site visits as part of its responsibility for compliance oversight. Accurate and complete study records must be maintained and made available to representatives of the USAMRDC as a part of their responsibility to protect human subjects in research. Research records must be stored in a confidential manner so as to protect the confidentiality of subject information.

6. Do not construe this correspondence as approval for any contract or grant/cooperative agreement funding. Only the Contracting Officer/Grants Officer can authorize expenditure of funds by notice of official award documentation. It is recommended that you contact the appropriate contract/grants specialist or Contracting/Grants Officer regarding the expenditure of funds for your project.

7. The HRPO point of contact for this study is Ms. Karen Eaton, MS, Human Subjects Protection Scientist, at 301-619-9268/karen.m.eaton.ctr@mail.mil.

8. The HRPO point of contact for post-approval oversight is Mrs. Angela Urbina, Human Subjects Protection Scientist, at 301-619-2370/angela.c.urbina.ctr@mail.mil.

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Human Subjects Protection Scientist
Human Research Protection Office
Office of Research Protections
U.S. Army Medical Research and Development Command

To notify HRPO of changes to the conduct of DoD-supported research due to COVID-19:
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