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14. ABSTRACT

The proposed project will advance research into the biology of lethal prostate cancer (PCa) and reduce the burden of lethal PCa health disparities in Puerto Rican Hispanic/Latino men (PR H/L) through the following aims: **Aim 1:** Identify the genomic alterations and mutation signatures that characterize lethal PCa in PR Hispanic/Latino (PR H/L) men. **Aim 2:** Studying the lethal PCa phenotype in terms of overall DNA repair capacity (DRC) levels using lymphocytes as surrogate markers to develop a potential biomarker for identifying men at high-risk. **Aim 3:** To increase PCa awareness and screening in PR communities with high African ancestry and high PCa mortality rates. During this funding period, the molecular analysis for 14 prostate tumors from PR H/L men was completed: WES and RNA sequencing data are available for 11 and 14 tumors, respectively. For Aim 2, 55 samples were analyzed for DRC values including cases and controls. Significant differences were observed when comparing cases and controls ($p<0.001$). When stratifying by disease aggressiveness, significant differences were observed when comparing controls with aggressive or indolent groups ($p<0.0001$). Several oral presentations and posters regarding the data from this aim were presented at local meetings. A manuscript was published in a peer-review journal. For Aim 3, all six focus groups proposed were performed with a response rate of 105%. Key topics were identified from the focus group discussions including: access as a barrier to healthcare services (i.e. transportation limitation, limited number of specialists in the island, appointments are not available until various months, etc.).

15. SUBJECT TERMS Lethal prostate cancer, Hispanic/Latino, Puerto Rico, genomics, DNA repair, tumor, blood, community outreach, education, screening, African ancestry, mortality

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1. **INTRODUCTION:**

Puerto Rico is a United States (US) Territory located in the Caribbean with an estimated population of 3,200,000 inhabitants. Puerto Ricans are particularly vulnerable to cancer disparities because of socioeconomic inequalities. In 2020, approximately 44% of the population in Puerto Rico lived in poverty, compared with 17% of US Hispanics and 9% of Non-Hispanic Whites (NHW) living in the continental US. Chinea *et al.* (2017) reported that Hispanic/Latino (H/L) subgroups have different prostate cancer-specific mortality (PCSM) rates when compared to NHW and non-Hispanic Black (NHB) men, using data from 2000-2013 that included 486,865 men. Prostate Cancer (PCa) incidence and mortality rates in H/L men were similar to NHW; however, Puerto Rican Hispanic/Latino (PR H/L) men had significantly higher PCSM than NHW and had the highest mortality among Hispanic subgroups. In 2018, the general PCa incidence rate in Puerto Rico was 145.2 cases per 100,000 population and an age-adjusted mortality rate of 18.2 deaths per 100,000 population (Figure 1). However, at the regional level, differences in incidence and mortality were identified among those living in the south (Ponce Region) and east (Fajardo Region) parts of Puerto Rico. Most recent data shows that both areas have the highest age-adjusted mortality rate with 23.7 and 22.4 deaths per 100,000 population, respectively (Figure 1B). As shown in Figure 1, the study's target municipality, Patillas, (Aim 3) is part of the Ponce region, which is one of the two regions of Puerto Rico with the highest PCa mortality rates (19.7-23.9). The study's other two target municipalities (Maunabo and Las Piedras) are part of the Caguas region, which has the second highest PCa mortality rates (18.3-19.6), as shown in Figure 1. With regards to incidence, recent data also illustrates a similar scenario in which the Ponce and Caguas Health regions are the two regions with the highest PCa incidence rates (151.7- 162.6), as shown in Figure 1.

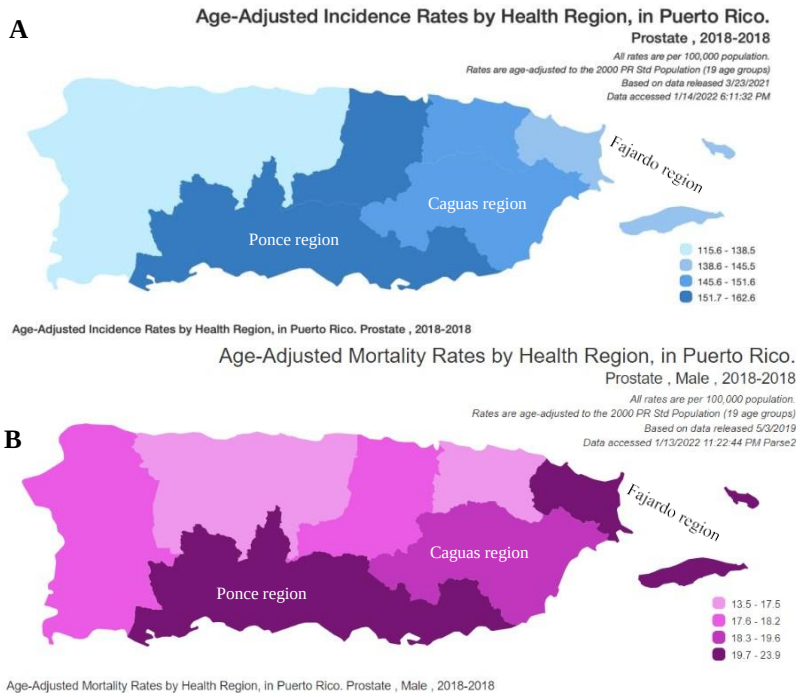


Figure 1. Age-Adjusted Incidence and Mortality Rates by Health Region in Puerto Rico- Prostate Cancer, 2018. Note: Names of regions added by research team to illustrate regions for this report. Source: Puerto Rico Cancer Registry. Tasas y Mapas (rcpr.org)

2. **KEYWORDS:**

Lethal prostate cancer, Hispanic/Latino, Puerto Rico, genomics, DNA repair, tumor, blood, community outreach, education, screening, African ancestry, mortality

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

The study team aims to significantly advance research in the biology of lethal prostate cancer (PCa) and reduce the burden of lethal PCa health disparities in Puerto Rican Hispanic/Latino (PR H/L) men. PCa is the most prevalent cancer, both in terms of incidence and mortality in Puerto Rico. The long-term goal of this study is to reduce lethal PCa disparities in PR H/L men by: 1) identifying genetic and genomic differences in the PR H/L population, the study team expects to gain insight into why PR H/L men have the highest PCa mortality among all Hispanic subgroups in the United States (US), and; 2) engaging the community as research partners, the team expects to gain knowledge as to specific social, psychological, and cultural factors that may represent barriers to PCa education and screening. These goals are presented in the SOW through the following three Specific Aims:

> **Aim 1:** Identify the genomic alterations and mutation signatures that characterize lethal prostate cancer in Puerto Rican Hispanic/Latino (PR H/L) men.
>
> **Aim 2:** Studying the lethal prostate cancer phenotype in terms of overall DNA repair capacity (DRC) levels using lymphocytes as surrogate markers to develop a potential biomarker for identifying men at high-risk.
>
> **Aim 3:** To increase PCa awareness and screening in Puerto Rican communities with high African ancestry and high PCa mortality rates.

What was accomplished under these goals?

Specific objectives for Specific Aim 1

Identify the genomic alterations and mutation signatures that characterize lethal PCa in Puerto Rican Hispanic/Latino (PR H/L) men. Hypothesis: PR H/L men with stronger African ancestry will be enriched for somatic mutations and signature profiles associated with a higher aggressiveness of lethal PCa as measured by Gleason scores and metastasis.

Specific Aim 1: Identify the genomic alterations and mutation signatures that characterize lethal prostate cancer in Puerto Rican Hispanic/Latino (PR H/L) men.	**Months**	**Percentage of completion**
Task 1: Recruitment, tissue collection, processing, and data analysis		
Subtask 1: Study start-up procedures, including approval from USAMRDC ORP HRPO and IRB prior to work involving human subjects.	1-3	100%
Subtask 2: Recruit PR H/L men with PCa (n =150), send to ORIEN for QC, WES and RNA-sequencing. Expect 10 participants per month.	3-18	31-58%
Subtask 3: DNA extraction from whole blood and GWAS array genotyping.	3-18	31-58%
Subtask 4: Request WES data to the Tissue and Data Sharing committee of the PRBB.	6, 22	Month 6 complete
Subtask 5: Genotyping of genome-wide SNP array for ancestry analysis.	6-20	40%

Major activities

The team proposed to characterize genomic alterations (genes mutated, types of mutations, mutation burden, fusion genes) and mutational signatures (the combinations of genomic changes resulting from specific mutagenesis processes) in tumors from PR H/L men, comparing tumors with favorable Gleason scores to lethal, advanced stage PCa including those with Gleason scores ≥8, and metastatic castration- resistant prostate cancer (mCRPC) both classified as high-risk by the 2020 National Comprehensive Cancer Network Guidelines for Advanced Stage Prostate Cancer (NCCN PCA, nccn.org/patients/guidelines). The ancestral composition of the cohort will also be measured to allow clustering of samples according to their African, European, and Indigenous American ancestral composition. PR H/L data will be compared to US existing datasets: non-Hispanic White (NHW) and non-Hispanic Blacks (NHB). As presented in the proposal's statement of work (SOW), this aim was divided into five subtasks which are summarized below. The table shown above presents an overview of the workflow for this aim and progress accomplished to date.

The overall progress for the first year falls within the timeline planned during the initial grant submission. Specifically, the patient consent rate is currently at 9 patients per month (the projected rate was 10 patients per month). Other subtasks are ahead of the original timeline. We have used the initial 14 RNA sequencing and 11 whole-exome sequencing (WES) datasets for establishing the analysis pipeline for somatic mutation detection, mutational signature quantification, and gene fusion identification. Preliminary outcomes of this initial analysis are presented in this report.

<u>Goals related to Subtask 1.1:</u> Study start-up procedures, including approval from USAMRDC ORP HRPO and IRB prior to work involving human subjects

Prior to the initiation of the project, as part of the Award Management process, Dr. Jaime Matta, (PI) requested PHSU IRB approval granted on February 18, 2021. This approval (PHSU Protocol #2101051235R001) was renewed on February 14, 2022 and expires on February 13, 2023. In addition, FormE2130.1a Continuing Review Submission was submitted to the Human Research Protection Office (HRPO) on February 14, 2022, together with the PHSU IRB renewal for 2022-2023.

<u>Goals related to Subtask 1.2:</u> Recruit PR H/L men with PCa (n = 150), send to ORIEN (for QC, whole exome sequencing and RNA-sequencing. Expect 10 participants per month.)

Enrollment of tumors from PCa patients is ongoing through the Puerto Rico BioBank (PRBB) protocol. In brief, the PRBB works jointly with the Urology clinical team headed by Dr. Ruiz-Deya (Co-PI) to identify eligible patients, consent, and collect biospecimens. Additional support is provided by three additional urologists based at Urocentro del Sur. The PRBB collaborates with the Oncology Research Information Exchange Network (ORIEN) for evaluation of the clinical data biospecimens and clinical data.

On July 1, 2022, a data new data abstractor (Mr. Xavier Muniz Santiago) was contracted part-time in place of Mr. Jimmanuel Melendez who was previously supporting the data abstraction needs of this project. Mr. Muniz-Santiago's qualifications include a technical degree in information technology, a Bachelor's in Nursing, and a Master's in Public Health. In addition, he has been working as a consenter for the Puerto Rico Biobank (PRBB) for over 5 months. Currently, he is receiving training in data abstraction specifically for PCa cases under the supervision of Dr. Jose Oliveras, the data concierge and lead data abstractor for the PRBB. As described in the grant proposal, the PRBB is a core facility of the PHSU-Moffitt Cancer Center (MCC) PACHE U54 Partnership.

As of August 2022, 108 PCa patients have been consented by the PRBB for the ORIEN protocol, of which 61 have been evaluated for tumor cellularity. A total of 12 samples were eliminated due to low tumor cellularity (<30%), and 3 additional samples failed QC due to low nucleic acids quality (n = 3). Evaluation of the tumor specimens for the remaining 47 consented patients is ongoing. Clinical data abstraction was completed for 45 of

the 46 consented patients that passed QC. A summary of the characteristics of these patients is presented in Table 1.

The pathological characteristics of the tumors accrued continues to show an oversampling of tumors of lower Gleason score (GS), despite our prior discussions with the clinical urology team (Figure 2). Hence, the total number of indolent cases already consented constitute one third of the total planned sample of 150. Consequently, our recruitment goal for this disease category has been achieved in year 1. In order to shift the focus to aggressive PCa cases, the study team held a discussion on July 1, 2022, to agree on the best strategy to achieve our recruitment goals. The following actions were agreed on and are being implemented:

- Rather than consenting unselected PCa patients on a rolling basis, the clinical team will target patients with aggressive disease and expand the recruitment sites to include 2 additional urology clinics in the city of Ponce and Mayaguez, PR. In addition, there are plans to contact Southern Pathology (Ponce, PR) for availability of aggressive tumor samples whose surgeries have been done at San Lucas Hospital (Dr. G. Ruiz-Deya) or in Bella Vista Hospital (Mayaguez, Dr. W. Roman). Once these tumors are identified in the first quarter of year 2, these patients will be provided a medical appointment by their respective urologists in order to obtain Informed Consent and administer the questionnaire used for the ORIEN protocol. Patients with GS ≥8 that have had undergone surgery within the past 3 years or are pending surgery will be given an appointment during which they will be invited to participate to the study by the study consenters. In the combined sites, we expect to be able to consent at least 10 patients with aggressive PCa per month.

Variables	PCa patients (n=45)
BMI [mean (SD)]	28.3 (4.0)
Smoking status [n (%)]	
Never	29 (64.4)
Former	13 (28.9)
Current	3 (6.7)
Alcohol use [n (%)]	
Never	26 (57.8)
Former	1 (2.2)
Current	18 (40.0)
PSA (ng/ml) [mean (SD)]	7.7 (4.0)
Pathological grade [n (%)]	
1	10 (22.2)
2	22 (48.9)
3	10 (22.2)
4	1 (2.2)
5	2 (4.4)
Gleason Score [n (%)][1]	
6	22 (23.7)
7 (3+4)	51 (54.8)
7 (4+3)	15 (16.1)
8	3 (3.2)
9	2 (2.2)

[1] Gleason Scores were available for all consented cases (n=93). BMI body mass index, PSA prostate-specific antigen, SD standard deviation.

Table 1. Demographic and clinical characteristics of the study participants for which data abstraction has been completed (n=45).

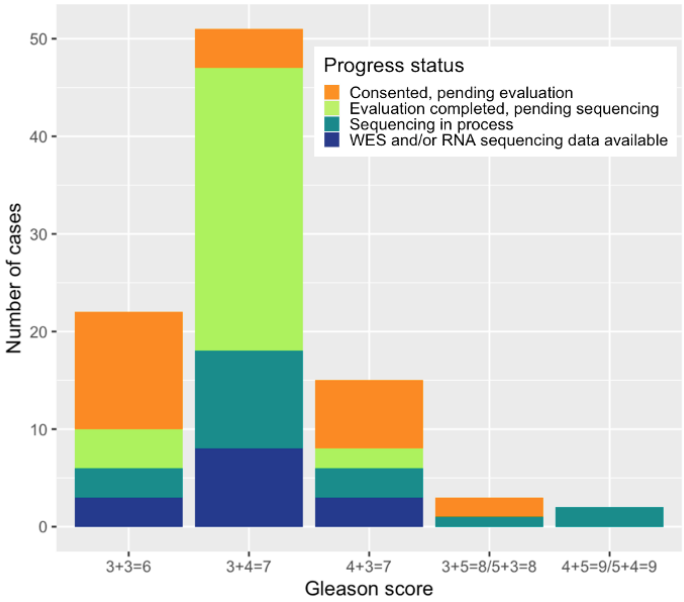


Figure 2. Distribution of the Gleason Score for tumors of consented prostate cancer patients at different stages of processing (n = 93). Cases that have been excluded for QC reasons are not included.

- Explore the possibility of using transrectal core biopsy samples rather than surgical blocks as a source of RNA/DNA. The study team has established a collaboration with the Quantitative Sciences Core (QSC) and Cancer Genomics lab at PHSU to perform a pilot study for the use of biopsy samples to perform WES. This idea is supported by recent evidence in the literature demonstrating that prostate tumor biopsy samples constitute an adequate source of material for whole genome molecular analysis (Nat Commun. 2019 Nov 20;10(1):5251. doi: 10.1038/s41467-019-13084-7). This approach will allow the team to capture aggressive and metastatic cases that are not undergoing surgery but for which biopsy samples are available. As this grant's budget does not include funds for such analysis, alternative funds have been identified though the QSC.

- On July 2022, Dr. Matta contacted by phone Mr. Mariano de Socarraz (CorePlus CEO) and explained our needs of recruitment of aggressive tumors from Dr. Ruiz-Deya's patients. A recent follow up meeting was held in August 2022 with Mr. de Socarraz, CorePlus staff, the two PIs, and the Program Manager. At this meeting, Mr. de Socarraz informed the team that CorePlus provides pathology services for transrectal core biopsies and a report of the potentially available tumors for the DoD is being prepared.

<u>Goals related to Subtask 1.3:</u> DNA extraction from whole blood and GWAS array genotyping

Once the patients are consented to participate in the study and donate their tissues, a blood sample is also collected. Germ-line DNA (gDNA) from matching blood is extracted using a standard protocol from the manufacturer (PAXgene Blood DNA system, Qiagen). The gDNA obtained is used for ancestry analysis (Subtask 1.5). Germ-line DNA is currently available for 42 patients, which corresponds to 28% of the consented patients.

<u>Goals related to Subtask 1.4:</u> Request Whole Exome Sequencing data to the Tissue and Data Sharing committee of the Puerto Rico Biobank.

Through the collaboration with ORIEN, nucleic acids are extracted from tumors and from blood. This material is used for whole exome sequencing (WES) (tumor DNA and germline DNA to control for inherited variants) and RNA sequencing (tumor RNA). The data is then shared with the study team for analysis.

- ORIEN has completed molecular analysis for a total of 14 prostate tumors from H/L men from Puerto Rico. After QC steps, WES and RNA sequencing data is available for 11 and 14 tumors, respectively.
- An additional shipment of 19 additional prostate tumors was sent to ORIEN for processing in January 2022. These samples are currently in queue or undergoing sequencing.
- Tumors for the remaining 60 consented patients are either awaiting shipment to the sequencing facility (n=13), undergoing DNA/RNA extraction, or being reviewed by the pathologist and evaluated for cellularity (n=47).
- The analysis pipelines for somatic mutations, mutational signatures and gene fusion events identification have been developed and are ready for implementation once sequencing data becomes available.
- There was an initial request for molecular data prior to month 6 of the grant and for which preliminary data is presented below. This data was also included in the 6 months technical progress report. No additional sequencing data has been requested. According to the SOW, the next request for data and analysis is scheduled for month 22. However, additional analysis including preliminary data on mutation signatures and comparisons and preliminary comparative analyses are presented below.

<u>Somatic mutations.</u> Figure 3A shows mutations identified from analyzing a subset of 177 DNA repair genes in the PCa 11 tumors for which WES data is available. This bioinformatics analysis has been performed by Dr. Jamie Teer, Co-Investigator, MCC working closely with Dr. Julie Dutil, Co-I, PHSU. Figure 3B also shows a

preliminary robust regression analysis used to identify those genes more mutated than expected by chance (above the trend line). Our current sample size of 11 PCa tumors has limited power to separate truly over-mutated genes from the background mutation rate. However, this will improve as our sample accrual continues to increase during the grant period. As of now, no common driver mutations were observed when analyzing mutations in from the WES data.

Gene fusions. The *TMPRSS2:ERG* gene fusion is the most frequent alteration in PCa. It leads to the overexpression of the E26 Oncogene homolog (ERG) as the result of a fusion with a prostate-specific and androgen-response gene (*TMPRSS2)*. This fusion was detected in only 2/14 (14%) of samples. While it is premature to perform meaningful statistical comparisons given the current sample available, the team has begun exploring these results in the context of existing cohorts. As shown in Figure 3C, the frequency of the *TMPRSS2:ERG* fusion in the prostate tumors from PR H/L patients shows intermediate values when compared to tumors from Black and White patients in the 2021 MSK Racial Differences in Prostate Cancer cohort (https://www.cbioportal.org/). The team will continue to investigate gene fusions in this population as more data is received and will also apply additional fusion detection approaches to confirm these findings.

Mutational signatures. Analysis of the mutational signature has revealed that 1/11 (9%) of the prostate tumors shows a moderate signal for the SBS3 signature (*Nature* 2013 **500,** 415–421, https://doi.org/10.1038/nature12477), which is characterized by small indels and genome rearrangements due to abnormal double strand break repair and indicative of defective homologous recombination-based DNA damage repair.

Goals related to Subtask 1.5: Genotyping of genome-wide SNP array for ancestry analysis.

Using DNA extracted from blood, genome-wide genotyping is performed using the Affymetrix UK Axiom Biobank array. This data allows accurate estimation of the genetic ancestry for each patient. Ancestry analysis allows the team to quantify the contribution of the genome from each ancestral population and to cluster individuals based on their genetic characteristics.

In February of 2022, we reported the successful genotyping of 6 samples. Figure 4 shows the clustering of the study patients. Based on these initial 6 samples, on average, the nuclear genome of individuals is composed of 62.1% European (range 44.8% to 75.5%), 20.7% African (AFR, range 11.0% to 41.6%) and 16.1% Indigenous American ancestry (range: 12.3% to 20.2%). As expected, the patients cluster with the 1000 Genomes Puerto Rico population. It is noteworthy that the patients analyzed to date represent the full spectrum of ancestry composition observed in Puerto Rico including individuals of high African ancestry, overlapping admixed Africans from the Caribbean.

As of July 23, 2022, germline DNA was extracted and evaluated for quality and quantity for samples from an additional 42 patients and is currently being genotyped at Affymetrix facilities using the UK Axiom Biobank array. Genome-wide genotyping array data for those additional samples is expected to be received and analyzed by October 2022.

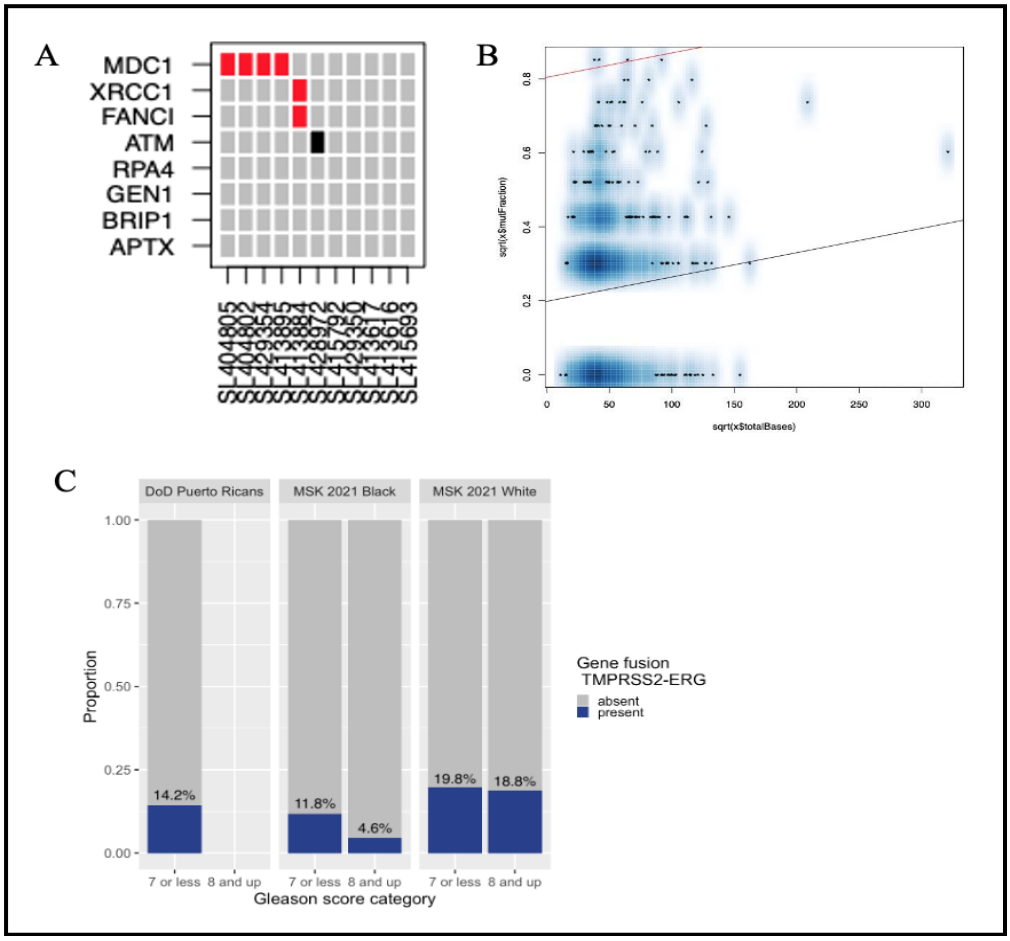


Figure 3. Mutation analysis in prostate tumors from Puerto Rican Hispanic/Latino men. **(A)** Oncoprint of the mutations detected in a subset of 177 DNA repair genes. The red boxes represent the genes/samples for which a missense mutation was observed, and the black boxes are those for which a truncating mutation was identified. **(B)** Mutation rate vs. gene size. The regression line (black) shows the average mutation rate across all genes, and the dots above the line are over-mutated. Standardized residual of 2 (red) indicates genes of interest for which there would be a statistically significant increase in mutation burden. (**C**) Frequency of the TMPRSS2-ERG gene fusion in PCa from Puerto Rican Hispanics (this study) and PCa tumors from Black (n=155) and White (1,688) patients of the MSK Race Difference in Prostate Cancer study after grouping by Gleason score category (N Engl J Med 2020; 383:1083-1085, data obtained through cBioportal, https://www.cbioportal.org/).

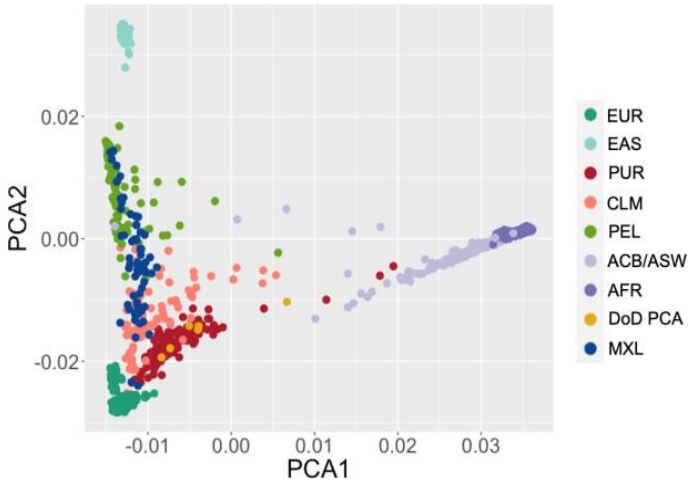


Figure 4. Visualization of the population structure from germ-line genetic variation using the first two components from principal component analysis for the study prostate cancer patients in reference to populations of the 1000 Genomes Project. Patients from the current study are represented in yellow and labeled DoD PCA. Reference 1000 Genomes populations are: ACB African Caribbean in Barbados, AFR African, ASW Americans of African Ancestry in SW US, CLM Colombian in Medellin Colombia, EAS East Asians, EUR Europeans, Mexican Ancestry from Los Angeles, California, PEL Peruvians from Lima Peru, and PUR Puerto Ricans from Puerto Rico. PC principal component

Specific objectives for Specific Aim 2

Studying the lethal prostate cancer phenotype in terms of overall DNA repair capacity (DRC) levels using lymphocytes as surrogate markers to develop a potential biomarker for identifying men at high-risk. Hypothesis 1: PR H/L men with PCa will have lower DRC levels than controls without PCa in terms of three repair pathways associated with PCa. Hypothesis 2: Varying levels of overall DRC will be detected among the subgroups of PR H/L men with PCa based on Gleason score.

Specific Aim 2: Studying the lethal prostate cancer phenotype in terms of overall DNA repair capacity (DRC) levels using lymphocytes as surrogate markers to develop a potential biomarker for identifying men at high-risk.	**Months**	**Percentage of completion**
Task 2: Blood collection and lymphocyte isolation for DRC experiments		
Subtask 1: Recruit PR H/L men with (n=124) and without (n=31) PCa.	3-12	75%
Subtask 2: Draw blood to isolate lymphocytes for storage.	6-12	75%
Subtask 3: Measure DRC levels through the nucleotide excision repair pathways using the CometChip assay.	7-33	44%
Subtask 4: Measure DRC levels through the homologous recombination pathway using the CometChip assay.	7-33	5%
Subtask 5: Data analysis.	7-36	30%
Subtask 6: Prepare abstracts and manuscripts based on Aim 2 data.	33-36	50%

<u>Goals related to Subtask 2.1:</u> Recruit PR H/L men with (n=124) and without (n=31) PCa.

During this reporting period the major goal was to continue with the recruitment of PR H/L men with (cases) and without (controls) PCa. Cases included men with pathologically confirmed primary tumors with: (1) Gleason 6, (2) Gleason 7, and (3) Gleason >8; including a study group of (4) metastatic-castrate resistant prostate cancer (mCRPC). Controls recruited were men of 50 years of age or older, with normal results on the Digital Rectal Exam (DRE), and normal PSA (prostate specific antigen) levels (<4 ng/mL).

A total of 49 participants were recruited and their blood was collected for lymphocyte isolation. During this reporting period, we were able to recruit participants for all study groups including: Gleason ≤6 (n=7), Gleason 7 (n=4), and Gleason ≥8 (n=6), mCRPC (n=2) and controls (n=14). The remaining 16 participants are still on the pipeline for data abstraction. The recruitment sample distribution is described on **Figure 5**. As can be observed in **Figure 5**, the total recruitment until this reporting period is 116 participants.

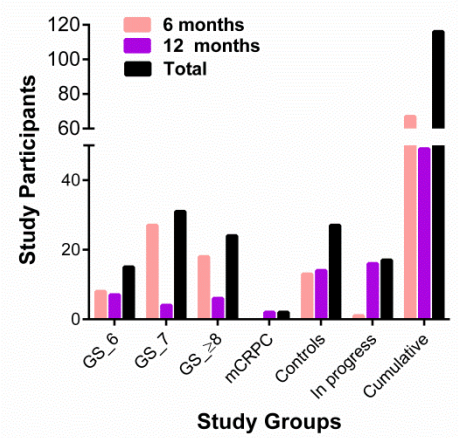


Figure 5. Recruitment of study participants at 6 and 12 months for Aim 2. Bars represent the number of participants recruited for each study group including: GS6, GS7, GS≥8, mCRPC, and controls. Participants with clinical data abstraction pending are included as In progress. Cumulative recruitment for each study groups is also included. The total recruitment for each category is represented in black bars.

<u>Goals related to Subtask 2.2:</u> Draw blood to isolate lymphocytes for storage.

This subtask included the blood collection from the study participants recruited with the collaboration of the PRBB at Dr. Gilberto Ruiz-Deyá's clinical practice and at Urocentro del Sur. The blood samples were drawn by the study consenters (Drs. Natasha Moreno and Mara Vega) and collected by Dr. Jaime Matta's laboratory staff (Dr. Carmen Ortiz and Jarline Encarnación, MS). Freshly collected blood samples were transported in ice from

San Lucas Hospital and processed at the Matta laboratory at PHSU using BD Vacutainer® CPT™ Mononuclear Cell Preparation Tubes. The lymphocytes obtained from each blood sample were preserved using the appropriate freezing media and stored at the -80° C freezer at the Matta laboratory.

<u>Goals related to Subtask 2.3:</u> Measure DRC levels through the nucleotide excision repair pathways using the CometChip assay.

The goal of this subtask is to measure the DNA repair capacity, through the nucleotide excision repair (NER), of the study participants using lymphocytes as surrogate markers. To achieve this goal, the lymphocytes previously frozen and stored were thawed and seeded in cell culture flasks for cell growth and expansion. After allowing the cells to grow for a week, the cells are counted to measure cell concentration and viability before performing the DNA repair measurements. After ensuring that the cell cultures for each participant have the appropriate viability (>70%) and concentration (>$4x10^5$ cells/mL), the DNA repair experiments were performed using the CometChip assay as described in the grant application. The activities for this subtask began during the past reporting period and have continued consistently during this period.

<u>Goals related to Subtask 2.4:</u> Measure DRC levels through the homologous recombination pathway using the CometChip assay.

The main goal for this subtask is the assessment of DNA repair capacity levels in the study participants through the homologous recombination pathway. During this reporting period, we began working with the cultures of the cell lines that will be used as internal controls for the assay. These cell lines have knockdown in key genes involved in the homologous recombination pathway. These valuable cell lines were previously acquired from Trevigen, Inc. Currently, these cell lines are being expanded and frozen aliquots are being prepared for future experiments. Since Trevigen, Inc. is no longer distributing these cell lines, it is important to prepare frozen stocks to assure that we have cell lines available to complete the DNA repair experiments proposed.

<u>Goals related to Subtask 2.5:</u> Data analysis.

During this reporting period, this subtask included the analysis of the data collected from the DNA repair capacity measurements performed through the NER pathway. The data analysis focused on the epidemiological and clinical data of the participants included in the DNA repair experiments. In addition, comparisons of DNA repair capacity values were performed between PCa cases and controls. Moreover, the DNA repair capacity values were compared among controls, and men with aggressive and indolent PCa. A general linear model analysis was performed to understand the contribution of some biological factors such as age, body mass index (BMI), and prostate-specific antigen (PSA) levels, to the differences detected in DNA repair capacity values among study groups.

<u>Goals related to Subtask 2.6:</u> Prepare abstracts and manuscripts based on Aim 2 data.

Although this subtask was planned for months 33-36, we achieved significant progress during this funding period. Under this subtask, four abstracts were prepared and presented at the 17th PHSU/PRI Annual Scientific Conference/1st RCMI Symposium in Health Disparities held on May 7, 2022. Two of these abstracts were also presented at the Florida Society for Clinical Oncology (FLASCO) meeting held in San Juan, PR in August 5-6, 2022. In addition, the first manuscript for the data collected on this aim was recently published at *Cancers* (IF: 6.639).

What was accomplished under these goals?

Major activities

- During this reporting period, we have been actively recruiting PCa cases and controls at both recruitment sites: Dr. Ruiz-Deyá's clinical practice and UroCentro del Sur. The recruitment logistics

that were coordinated along with the PRBB have been effective to continue with participant recruitment.

- Regarding DNA repair experiments, a total of 55 study participants including PCa cases (n=41) and controls (n=14) were successfully included in the experiments and DNA repair values were successfully measured. Additionally, 13 participants were included in the experiments with DNA repair data pending to be analyzed.

- To begin with the phase of measuring DNA repair through homologous recombination, the cell lines that will be used as internal controls were grown. These cell lines, previously acquired from Trevigen, Inc., have knockdown on different genes involved in this pathway including: *XRCC3* and *RAD51C*. Also, a knockdown control cell line is also being expanded.

- As a result of different analyses performed with the data from the study participants, four abstracts were prepared and presented at the 17th Ponce Research Institute Annual Scientific Conference held on May 7, 2022 at the Ponce Hilton. These meeting was attended by over 400 participants from the island:

    - Abstract 1: Overall DNA repair capacity as a potential tool to improve prostate cancer diagnosis (1st Award for Oral Presentation in Basic Sciences Category).
    - Abstract 2: Re-examining the Application of Prostate-Specific Antigen levels to Distinguish between Aggressive and Low-Risk Prostate Cancer (1st Award for Oral Presentation in Clinical Studies Category).
    - Abstract 3: Evaluation of Genetic Variants in Puerto Rican Men with Prostate Cancer.
    - Abstract 4: Clinical Features and Distribution of Aggressive Prostate Cancer in Puerto Rican Men: A Preliminary Assessment (2nd Award for Oral Presentation in Clinical Studies Category).

- From the data collected and analyzed in Aim 1, a manuscript was recently published (June 25, 2022) in the journal *Cancers* (IF: 6.639). The first author is Dr. Carmen Ortiz, Postdoctoral Researcher and Program Manager.

- During this summer (2022), two first-year MD students completed a research rotation through the MD Summer Research Program from the PRI. These students successfully presented their research project internally at PHSU/PRI. In the previous summer (2021) three first-year MD students completed research rotations through the same program. These students presented their research project internally and at the 17th Ponce Research Institute Annual Scientific Conference where two of them obtained oral presentation awards.

Significant results or key outcomes

- A total of 49 participants were recruited during this reporting period including PCa cases (n=35) and controls (n=14). During this reporting period, we were able to recruit the first 2 patients for the mCRPC group. This is significant since these mCRPC patients are difficult to identify and require the collaboration of the urologist and the oncologist to collect the necessary information for the final diagnosis.

- For the DNA repair measurements, the lymphocytes from 68 participants were successfully grown and expanded to perform the experiments. Of the 68 participants, the analysis of 55 was completed while the 13 remaining participants are still in the pipeline for analysis. The DNA repair measurements were performed using the CometChip technology standardized and adapted by Dr. Ortiz to measure DNA repair through the NER pathway.

- A total of 55 samples (controls=14, PCa cases=41) were analyzed using the CometChip assay for DNA repair capacity. The mean DNA repair capacity (DRC) value for the control group was 20.66% (±7.96%) while the mean DRC for the prostate cancer cases was 8.41% (±4.88%). Men with PCa had a 59% reduction in DRC levels compared to controls. To assess these differences in DRC levels between cases and controls, the Mann–Whitney U test was performed. Significant differences were found when comparing the average DRC levels between cases and controls ($p<0.001$) (**Figure 6A**).

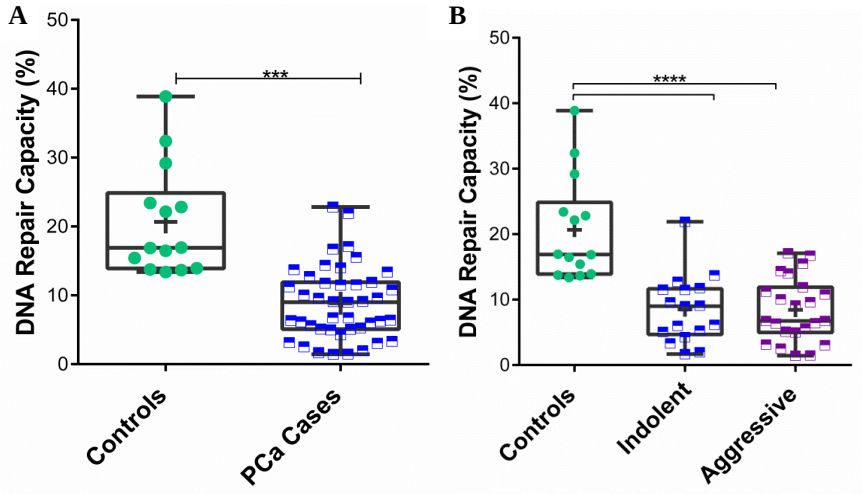


Figure 6. DNA repair capacity levels in prostate cancer cases and controls, and cases stratified by disease aggressiveness measured in terms of NER pathway. **(A)** Sample distributions using the DRC values for PCa cases (n=41) and controls (n=14). **(B)** Based on their Gleason scores, the tumors from PCa cases were stratified into indolent (n=17) and aggressive (n=23). Each box and whiskers represent the median and range values for a study group. Dots and squares represent the individual DRC values for study participants. Mean DRC value for each group is represented with a plus (+) sign. Asterisks denote statistical significance: (***) $p<0.01$ (Mann–Whitney U) and (****) $p<0.001$ (Kruskal-Wallis).

- To further explore the differences in DNA repair capacity within the PCa cases, stratification into aggressive and indolent disease was performed. The indolent group included cases with GS of 6 and 7 (3 + 4). The aggressive group included patients with GS of 7 (4 + 3) and higher. A total of 17 cases were classified as indolent while 23 cases were included on the aggressive group. The mean DNA repair values for the indolent cases was 8.50% (±5.14%); for the aggressive group, the mean DRC was 8.43% (±4.88%) (**Figure 6B**). Significant differences were observed when comparing the controls with the indolent group or the aggressive group ($p<0.0001$); however, no statistically significant differences were detected when the PCa groups were compared to each other.

- In order to understand whether the skewed distribution of DRC levels could be explained by other biological factors, a general linear model analysis was performed (**Table 2**). In this analysis, several continuous variables were considered, including age, BMI, and prostate-specific antigen (PSA) levels. The adjusted mean DRC level value was 20.55% (±1.60%) for the control group, a decrease of 0.11% after covariates were considered. In the PCa cases, the adjusted mean DRC level value was 8.45% (±0.89%), compared to 8.41% (±4.88%) obtained from the crude results. No significant contributions were detected from the cofactors in the linear model.

- The covariance model shows that age ($p=0.84$), BMI ($p=0.50$), and PSA levels ($p=0.27$) are not statistically significant factors in the model. Although the adjusted mean DRC values slightly vary for both groups (cases and controls), the differences in DRC are still significant after the Bonferroni correction.

- As for the tumor aggressiveness, the linear model showed variability between the crude and estimated DRC values. In aggressive cases the estimated DRC value was 9.28% (±1.23%), while for indolent cases the estimated value was 7.86% (±1.04%). Similarly to the case–control model, the age ($p=0.32$), BMI ($p=0.93$), and PSA levels ($p=0.95$) had no statistically significant impact on the model. No significant differences were detected after comparing the estimated marginal means of the tumor aggressiveness stratification ($p=0.40$).

Table 2. DNA repair capacity covariance analyses using age, BMI, and PSA levels.

Descriptive Statistics	Controls	PCa Cases	Pairwise Comparisons (p-value)	Indolent PCa Cases	Aggressive PCa Cases	Pairwise Comparisons (p-value)
Number of subjects	14	41	-	17	23	-
Dispersion Analysis						
Minimum	13.37	1.44	-	1.69	1.44	-
25% Percentile	13.90	5.04	-	4.68	4.99	-
Median	16.90	6.74	-	9.01	6.74	-
75% Percentile	24.86	11.65	-	11.65	11.91	-
Maximum	38.88	21.90	-	21.90	17.07	-
Analysis of covariance						
Mean	20.66 (7.96)	8.41 (4.88)	<0.0001	8.51 (5.14)	8.43 (4.88)	0.40
Estimated Mean [a,b]	20.55 (1.60)[a]	8.45 (0.89)[a]	<0.0001	9.28 (1.23)[b]	7.86 (1.04)[b]	0.40
Lower 95% CI	16.06	6.87	-	5.86	6.32	-
Upper 95% CI	25.26	9.95	-	11.15	10.54	-
Estimated Lower 95% CI	17.41	6.66	-	6.79	5.74	-
Estimated Upper 95% CI	23.69	10.23	-	11.77	9.97	-

a. Case–control: Covariates appearing in the model were evaluated at the following values: age=62.13, PSA=38.22, BMI=27.22.
b. Indolent–aggressive: Covariates appearing in the model were evaluated at the following values: age=63.25, BMI=29.24, PSA=51.99. A mean difference is significant at the 0.05 level (Mann–Whitney test). Adjustment for multiple comparisons: Bonferroni.

Key outcomes

- During this reporting period, we were able to successfully recruit 49 additional participants.
- A total of 55 samples were analyzed for DNA repair capacity values including cases and controls. Significant differences were observed when comparing cases and controls. When stratifying by disease aggressiveness, significant differences were observed when comparing controls with aggressive or indolent groups.
- Four abstracts were submitted and presented at the local PHSU/PRI meeting. Three of these received awards for 1st and 2nd places in their corresponding categories.
- The data analysis and experimental work performed during this funding period led to the first publication of the grant titled: "*Reduced DNA Repair Capacity in Prostate Cancer Patients: A Phenotypic Approach Using the CometChip*". This manuscript was recently published on June 2022 in the *Cancers* journal.
- We have begun with the growth and expansion of the cell lines to begin with the measurements through the homologous recombination pathway during year 2.

Specific objectives for Aim 3

To increase PCa awareness and screening in Puerto Rican communities with high African ancestry and high PCa mortality rates. Hypothesis 4: The integration of a culturally based Community-Engagement Educational Plan (CEEP) will increase PCa awareness and screening by promoting healthy behaviors through education and outreach activities among PR communities with highAfrican ancestry and PCa mortality rates.

Specific Aim 3: To increase PCa awareness and screening in Puerto Rico communities with high African ancestry and high PCa mortality rates.	Months	Percentage of completion
Task 3: Community assessment to explore knowledge, cultural beliefs, and perceived risk toward PCa (Exploratory mixed method study).		
Subtask 1: Request for institutional IRB approval prior to evaluation of men from the communities of Maunabo, Las Piedras and Patillas, PR.	1-3	100%
Subtask 2: Recruitment of participants and conduct focus groups	4-8	100%
Subtask 3: Focus group data analysis (survey).	8-10	100%
Subtask 4: Recruitment of survey participants (online).	10-15	0%*

*Survey development in process. No delays with recruitment (N=150) is expected.

<u>Goals related to Subtask 3.1:</u> Request for institutional IRB approval prior to evaluation of men from communities of Maunabo, Las Piedras, and Patillas, PR.

As stated for Aim 1, PHSU IRB initial approval was obtained on February 18, 2021. This IRB protocol included the three aims proposed for this project; therefore, it covers all aspects of the research proposed. Considerations related to the current pandemic situation and contagion rate were accounted and included within the original IRB protocol. As reported in our previous technical report, we did not anticipated nor experienced any issues in the renewal process as no amendments or changes were made to our protocol. The renewal for the IRB protocol was approved on February 14th, 2022. In addition, FormE2130.1a Continuing Review Submission was submitted to the Human Research Protection Office (HRPO) on February 14, 2022, together with the PHSU IRB renewal for 2022-2023.

<u>Goals related to Subtask 3.2:</u> Recruit participants and conduct focus groups.

Community Engagement and Educational Intervention Activities

- A community engagement and educational intervention activity was held at Maunabo and Las Piedras, on February 8th and May 10th, respectively. The purpose of this activity was to educate the community regarding PCa, including the distinction of lethal PCa, its incidence, prognosis, risk factors, and disparities thus far identified. Further, questions related to uncertainties with PCa screening procedures brought forth by the community leaders and members were discussed. Mainly, they were informed of the American Cancer Society recommendation that men should make an informed decision with their health care providers about getting screened for PCa and the relevant ages to be screened, in accordance to their risk-level (age 50 for men who are at average risk; age 45 for men at high risk – those with one first-degree relative diagnosed with PCa at an early age [younger than age 65]; and age 40 for men at even higher risk – those with more than one first-degree relative who had PCa at an early age [younger than age 65]). Participants from the community were also oriented on the incidence and mortality rates in Puerto Rico, with particular attention made to the regions within our scope of interest which have the highest mortality rates. The feedback and key outcomes of both activities is summarized below. Following that summary, a brief description of the date, number of participants and roles within the municipality of each respective activity is included.

- A key outcome of this meeting was that the individuals present expressed that the research team would gain better representation of their community if the focus groups were realized in person rather than virtually. This was also expressed by community leaders in our activity in Patillas as discussed in our

previous technical report. It was agreed that, as possible given changing COVID-19 policies and protocols, we would attempt to have all focus groups in person.

- An additional key outcome was that we identified two community members who may serve as part of our CAC (discussed in our previous report and under Task 4 in this report).

- Relevant topics of discussions brought by the community leaders and member that participated in this community engagement and educational intervention activity: **Access as a barrier to healthcare services** was openly discussed by community members. For example, factors such as lack of transportation (little to no public transportation in that area), limited number of specialists (Urologists, Oncologists) in the island, the lack of such specialist providers in Maunabo or closer municipalities, appointments are not available until various months, etc. Participants also **identified cultural beliefs** (*Machisimo*, for example) **as a potential barrier and main concern** for men to consider and be willing to get screened for PCa. Community leaders and members present in the activity emphasized the importance of addressing both issues at an individual and systemic level, including the **need to educate community members, families, health providers, and those in political and public health roles to advocate for change.**

- Upon hearing about the differences in mortality and risks associated with lethal PCa, the community leaders and members verbalized the **need to educate health providers** about the relevance and importance of disclosing this information to male patients. Some individuals disclosed not being aware that there was a lethal type of PCa, whereas most reported that this distinction was not informed by their medical providers. Further, several individuals shared that most medical providers tend to illustrate PCa as something "mild" that "won't necessarily kill you" (examples of individuals' expressions translated from Spanish to English). More so, they expressed the **need to educate vulnerable communities** (i.e., people with limited access to phone, media and internet) that may not have access to this information about lethal PCa, its incidence, risk factors, and the relevance of making an informed decision to be screened for PCa.

- The community engagement and educational intervention activity at Maunabo was held on Tuesday, February 8th, 2022. This activity was led by Dr. Julio Jiménez, MD and had the collaboration of Research Assistant (RA) Nicole M. Ryan-Nolla, Ph.D. This activity was made possible by the collaboration with our community liaison, Mrs. Paula Lebrón, who helped recruit community leaders and members through word of mouth, phone calls, emails, and snowball strategies. A total of 14 individuals (100% response rate) including cancer survivors, faith-based leaders, local business administrative personnel, community members and community leaders of Maunabo participated. Feedback provided by those in attendance was summarized in the previous bullet points.

- The community engagement and educational intervention activity at Las Piedras was held on Tuesday, May 10th, 2022. This activity was led by Dr. Julio Jiménez and had the collaboration of RA Nicole M. Ryan-Nolla, Ph.D. This activity was made possible by the collaboration with our community liaison, Mrs. Juana Castro, who helped recruit community leaders and members through word of mouth, phone calls, emails, and snowball strategies. A total of 16 individuals (94% response rate) including cancer survivors, a medical provider, spiritual leaders, community organizations leaders, local business administrative personnel, community members and community leaders of Maunabo participated. Feedback provided by those in attendance was summarized in the subpoints of the first bullet point of this section.

- Numerous phone calls and emails were made by Dr. Julio Jimenez and RA Nicole M. Ryan Nolla, Ph.D. to both community liaisons throughout the coordination of both activities.

Completion of Proposed Focus Groups at Patillas, Las Piedras and Maunabo

- A total of seven focus groups were held between December 21st, 2021, and April 27th, 2022, which yielded 63 participants in total (105% response rate, out of 60 originally proposed). Two focus groups were held at each of our three target municipalities (Patillas, Maunabo and Las Piedras, respectively). An additional focus group was held at Las Piedras due to a low response rate during our first scheduled date (March 2022). As mentioned in our previous technical report, a marked increase in COVID-19 positivity rate was observed in Puerto Rico which was suggested by our community liaison as a potential factor for the low response rate. This was supported by an increase in response rate to our second scheduled date, a month later, following the decrease in positivity rate.

- Focus groups were led by RA Luis Arroyo, MPH, MA, Dr. Melissa Marzan, DrPH, MPH, and two trained master- and doctoral-level volunteer students. A total of 63 participants were recruited (105% responsive rate), including ten PCa patients/survivors.

- Procedure: Participants were assigned to a group solely based in the order in which they arrived. Focus groups were conducted concurrently at Patillas and consecutively at Maunabo and Las Piedras. The logistics for each municipality was discussed and arranged with our community liaisons in accordance with the available locations and resources. All focus groups were held in person, at open-air spaced adhering to strict COVID-19 protocols (masks on at all times, physical distance, etc.). Concurrent groups were sufficiently separated in physical space so that the discussion held at one group did not interrupt or influence the discussion held at the other group.

- Demographic characteristics of participants: All 63 participants were residents of Patillas, Maunabo or Las Piedras, with ages ranging from 41 to 90 years (52% of participants reported to be 65 or older, n=33). With regards to civil or relationship status, participants reported to be "Married" (54%, n=34), "Single" (16%, n=10), "Living together but not legally married" (13%, n=8), "Divorced" (11%, n=7), and "Widowed" (6%, n=4). Regarding educational level, 37% (n=23) of participants reported to have completed high school or less, including eight participants (34.78%, n=8/23) who reported to have only reached some elementary grade. The other 63% (n=40) of focus group participants reported to have a degree after high school (associate degree, n=3; bachelor's degree, n=18, master's degree, n=3, doctorate's degree, n=5). Finally, 41% of our focus group participants reported to have a monthly household income less than $1,500. Demographic characteristics per municipality were included as an appendix to this report (**See Appendix A–Focus Group Participants Demographic Characteristics**).

- In general, most of the participants (76%, n=48) reported to have had at least one screening test (they chose all that may apply from a list that included PSA test, TRUS, MRI, CT, biopsy, etc.) to detect PCa in their lifetime. It is reasonable that the participants, who assisted the focus groups despite the current global pandemic and the associated fear of contagion, have a high intrinsic interest of learning more about PCa. It is also reasonable that this intrinsic interest geared most of them to have had a previous PCa screening test. For further details, please see tables 5, 6, 11, 12 & 17 & 18 for additional information regarding participants' characteristics (**See Appendix A–Focus Group Participants Demographic Characteristics**).

- Most participants reported to have never been diagnosed with PCa (78%, n=49). Ten participants (16%) that disclosed to have been previously diagnosed with PCa and only some were in remission state. Four participants (6%) opted not to respond to this question.

- Relevant topics identified from participants' responses throughout the focus groups discussion.

- An ongoing topic throughout all focus groups was cultural beliefs associated with PCa and PCa screening tests.

- The results suggest that cultural beliefs (e.g., "*prostate cancer [with regards to lethality or mortality] is not something to be concerned about*") at the individual-level, negatively influences PCa screening in Puerto Rican communities. More so, participants identified systemic barriers such as lack of health insurance. They also discussed that health providers tend to minimize the possible risks associated with PCa (e.g., "*they tell you that the mortality is low, without explaining that it [mortality] is different if you have lethal prostate cancer*"). The role of health providers and healthcare insurance companies as potential barriers in an individual's willingness to seek PCa screening continued as an ongoing topic brought forth by participants in all focus groups. Most focus group participants mentioned the importance of providing education at the individual and health care provider level about the distinction of lethal PCa versus other types of PCa, as well as the relevance of screening in accordance with a male's age and risk-level.
- Participants made verbal expressions that suggested a perceived barrier in access to PCa prevention strategies, including a lack of support from their medical providers in making informed decisions about whether to get screened for PCa. Participants disclosed that, in general, medical providers impressed to not be up to date with information regarding lethal PCa and the American Cancer Association's recommendations of screening at ages earlier than age 50, in cases of high or even higher risk for developing prostate cancer. Participants suggested that medical providers would benefit from an educational intervention activity similar and an open discussion about their beliefs.

- Another frequent topic that was commonly brought up by the participants of these two focus groups was the role that health insurance companies influence on potential barriers for access to medical providers and health services including screeners and routine follow-ups. A complete report of focus group data analyses was created by our team and included as an attachment to this report (See **Appendix B–Focus Groups Summary**).

- Monthly team meetings with Specific Aim 3 (SA3) Research Team were held virtually. Participants in these meetings included doctors Jaime Matta (PI), Julio Jiménez (SA3 Co-Investigator) and Melissa Marzan (SA3 Co-Investigator) as well as Dr. Carmen Ortíz-Sánchez (Program Manager and Postdoctoral Researcher) and both Research Assistants (RA) Luis Arroyo and Nicole M. Ryan-Nolla. These were held in the following dates: February 10th, 2022, March 10th, 2022, April 21st, 2022, May 12th, 2022, June 9th, 2022, and July 14th, 2022.

- Bi-weekly team check-in meetings held between Dr. Jiménez and RA Nicole M. Ryan Nolla were held through phone or virtually to discuss relevant administrative topics and tasks in accordance with SOW were held on Mondays at 11:00 AM, except for Holidays.

- Monthly meetings were held between Dr. Melissa Marzan and RA Luis Arroyo to review, discuss and analyze data collected from focus groups. Data was analyzed using NVivo. The team met on the following dates: February 2nd, March 2nd, April 4th, May 4th, June 8th, and July 6th.

Significant results or key outcomes

- High response rates were witnessed at our last two community engagement activities (100%, n=14/14; and 94%, n=16/17). This suggests an effective collaboration between our team and the community liaisons despite that both activities were held in person during the pandemic.

- High response rates observed in our focus groups (105%, n=63/60) is noteworthy considering the impact COVID-19 spread, as well as the impact that subsequent associated protocols and policies have had in the willingness of individuals to assist to social activities. This also suggests that the community

engagement activities were effective in informing community leaders and members of the recruitment for focus groups and in yielding a higher response than is generally documented from low-income rural areas.

- During the analyses of our focus group data, Dr. Melissa Marzan and RA Luis Arroyo identified the need to choose a more tailored theoretical model to conceptualize the information provided by focus groups participants. As mentioned before, our participants also provided a lot of feedback and information regarding barriers and disparities they have encountered in their decision making and access to healthcare services. As such, our qualitative data theoretical framework was changed from the Implementation Science Model to the **Health Equity Implementation Framework (HEIF) model**. The HEIF was developed by Woodward *et al*. (2019) after integrating and modifying two conceptual frameworks (one from implementation science and one from healthcare disparities research). Woodward and his team conducted a study to explore the feasibility of the HEIF in a historical disparity challenge–hepatitis C virus and its treatment in Black patients seeking care. They found that the HEIF was feasible for implementation researchers and identified barriers and facilitators at all levels (individual to systemic) which is framed as recipients (patient and provider factors, respectively), patient-provider interaction (clinical encounter), characteristics of treatment (innovation), and healthcare system (inner and outer context). These levels were congruent with topic domains found in the analyses of our qualitative data collection. A summary of the domains and topics discussed by focus group participants was included as an Appendix to this report (**See Appendix B–Focus Groups Summary**).

<u>Goals related to Subtask 3.3:</u> Focus group data analysis (survey).

- SA3 Team meetings were held between Dr. Melissa Marzan and RA Luis Arroyo to review, discuss and analyze data collected from focus groups. Data was analyzed using NVivo. The team met on the following dates: February 2nd, March 2nd, April 4th, May 4th, June 8th, and July 6th.

- The information from these analyses will help inform and guide selection of pre-existing surveys as well as identify additional questions that should be included to collect information that is sensible to our target population and project goal.

- A meeting will be held during Month14 between Dr. Melissa Marzan, Dr. Julio Jiménez, and both RAs Luis Arroyo and Nicole Ryan-Nolla to discuss in detail the qualitative data analyses from the focus groups. This information will help gear what information should be collected through quantitative surveys, in addition to what is suggested in the literature.

Significant results or key outcomes

- A detailed report of the focus groups data analyses conducted was developed by Dr. Marzan and RA Luis Arroyo. A summary of this report was included as an Appendix (See Appendix B– Focus Groups Summary). This summarized version will be used to lead efforts for a first scientific publication of our findings. This report will also guide our efforts into the quantitative phase of our project as well as help lead our efforts with regards to the Cancer Workshop that will be developed (Task 4) as part of this project.

- A strong alliance with community liaisons has been fostered during the first year of our DoD study. Consequently, this has proven to be effective in the recruitment of participants for both types of activities (community education and engagement, as well as focus groups). This alliance yielded in willingness from our community liaison to continue collaborating with the recruitment of participants for our quantitative survey phase of the project.

<u>Goals related to Task 4:</u> Develop educational plan for cancer prostate education and outreach activities.

Recruitment for our Community Advisory Committee (CAC)

- Phone calls and emails were made to prospective members of our CAC by RA Nicole M. Ryan Nolla beginning on June 4th, 2022. The established goal for the CAC is to recruit a total of seven men before Month 17 (currently on Month 13). The CAC members are to be distributed the following way, in accordance with our research proposal: two (2) PCa patient/survivor, two (2) family members or caregivers, two (2) community or spiritual based, and one (1) healthcare provider.

- Thus far, we have officially recruited the healthcare provider following his participation at the community educational intervention activity held at Las Piedras, on May 10th, 2022. The recruited provider is a general practice physician who lives and has his primary care office at Las Piedras, one of our target municipalities.

- We have already identified a total of three (3) additional interested prospective members and are in the process of official recruitment. These men participated in the community educational intervention activity held at one of our target municipalities, all of which expressed interest and willingness to participate in the CAC and were identified by our community liaisons as reliable community representatives.

- In accordance with our proposal, the CAC will collaborate in the development of the subtasks listed under Task 4 in our SOW. We expect to have our CAC recruited by September 2022 (Month 15) and our first virtual meetings during the months of September-October 2022 (Month 15-16), in accordance with our SOW plan to initiate subtasks related to Task 4 during Month 17.

<u>Conclusions</u>

These results reflect incremental achievements (continuing the ones reported on the previous report) towards aim 3: increasing PCa awareness and screening in Puerto Rican communities with high African ancestry and high PCa mortality rates. Strong alliances were successfully maintained with community liaisons from each target municipality (Patillas, Maunabo and Las Piedras). A total of 3 community liaisons worked closely with the DoD SA3 Team. Two types of main activities were conducted for this progress report period: a) two community educational intervention activities held one in Maunabo (n = 14, 100% response rate) and another in Las Piedras (n = 16, 94% response rate); and b) five additional focus groups for a total of 7 groups (n = 63 participants in total, 105% response rate). High response rates were generally witnessed for our community engagement activities and focus groups. This remains noteworthy considering the impact COVID-19 spread, as well as the impact that subsequent associated protocols and policies have had in the willingness of individuals to assist to social activities. Working with community liaisons from target municipalities was continued to be observed as an effective strategy in the recruitment and engagement of community leaders and members for both activities, as evidenced by the high responsive rate for each activity. The feedback provided by the participants (community leaders and members) was accounted when coordinating the logistics for the focus groups (day, time, place, etc.) The recruitment of participants for focus groups was successfully completed (n = 63). Data transcriptions and analysis for focus groups were also completed in accordance with the proposed SOW. A complete report of focus group data analyses was created by our team and included as an attachment to this report (See **Appendix B– Focus Groups Summary**). In lieu of the progress made thus far with regards to our SA3

SOW's tasks and subtasks, we do not anticipate any delays in the timeline established in the grant application.

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

Qualitative Research Methods and Data Analyses Training

- SA3 Team recruited two master- and doctoral-level students from the PHSU programs in public health and clinical psychology, respectively, interested in gaining research experience. An additional third volunteer (medical student, year 1, from another institution). Each volunteer student was provided an orientation by Drs. Jiménez and Marzan about the project proposal, goals, and aims. Students received additional training lead by Luis Arroyo (RA) related to the qualitative research methods (focus group) and all required preparation for community education and the conduction of focus groups.

- Training for all three volunteer students on how to transcribe audio data using NVivo was conducted on Tuesday, January 18th, 2022.

How were the results disseminated to communities of interest?

- A community engagement and educational intervention activity was conducted at each of our three target municipalities. Each activity had participants including PCa survivors, spiritual-based leaders, health provider, community members and community leaders. Participants of this activity were educated on information regarding PCa (incidence, risk factors, disparities, etc.), what is the importance of periodical screenings for PCa, what is lethal PCa, and the difference in prognosis of this type of cancer, the incidence and mortality rates of PCa in Puerto Rico with particular attention towards the rates in our region of study, as well as the relevance of our study. Questions and doubts brought by the community members were discussed and addressed. Preliminary focus group data were presented at the community activities held in Las Piedras and Maunabo, respectively (as no focus group had been conducted for the activity held at Patillas).

    - The community engagement and educational intervention activity at community was held on Thursday, December 9, 2021, at GuPRE Community Center in Patillas with a 85% response rate.
    - The community engagement and educational intervention activity at Maunabo was held on Tuesday, February 8th, 2022, with a 100% response rate.
    - The community engagement and educational intervention activity at Las Piedras was held on Tuesday, May 10th, 2022, with a 94% response rate.

- An article titled 'Scientists will research lethal prostate cancer among Puerto Ricans', about our study, was published on August 2, 2021 on the Ponce Research Institute (PRI) website (see https://www.psm.edu/investigaran-sobre-la-letalidad-del-cancer-de-prostata-entre-los-puertorriquenos-departamento-de-defensa-otorgo-1175000-para-este-estudio-sin-precedentes-en-la-isla/.

- A major local newspaper (El Nuevo Dia) published a supplement on October 14th, 2021, written by Jorge E. Pérez. This was based on an interview conducted with Drs. Matta (PI) and Ruiz-Deya (Co-PI). The focus was on the aggressive prostate cancer and ovarian cancer (Dr. Armaiz) in Puerto Rico (see: https://www.elnuevodia.com/suplementos/cancer/notas/agresivos-los-cancer-de-prostata-y-de-ovario-en-puerto-rico/).

- The study was featured in the December 2021 newsletter of the Ponce Research Institute (PRI). This newsletter is circled through all the PRI's, Ponce Health Sciences University's, Ponce Medical School Foundation's, and collaborating general hospitals and institutions mailing list (see: https://mailchi.mp/psm/pri-newsletter-volume-1-issue-11247922?e=c0b84eac55).

- Dr. Julio Jiménez is the host of a local TV program (*Tu Salud Informa*) broadcast on local PR television. Regularly, the project research team will have a space to disseminate educational information about PCa, screening, and research findings to the general public.

- The results gathered so far were presented through one oral presentation and four posters at the *17th PHSU/PRI Annual Scientific Conference/1st RCMI Symposium in Health Disparities*, May 7, 2022. This meeting was attended by over 400 participants throughout the island and some presenters from the US mainland. Details are provided below in section 6 (Products).

- Dr. Carmen Ortiz participated as a speaker in the *11th Annual Puerto Rico Oncology Symposium* held at San Juan on August 5-6, 2022. Dr. Ortiz's presentation was titled: Prostate Cancer Disparities in Puerto Rico. In her presentation, Dr. Ortiz provided information of what is known regarding disparities in the PR population along with the most recent progress on this project.

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

Aim 1

The following actions were agreed on and are being implemented for year 2:

- Rather than consenting unselected PCa patients on a rolling basis, the clinical team will target patients with aggressive disease and expand the recruitment sites to include 2 additional urology clinics in the city of Ponce and Mayaguez, PR. In addition, there are plans to contact Southern Pathology (Ponce, PR) for availability of aggressive tumor samples whose surgeries have been done at San Lucas Hospital (Dr. G. Ruiz-Deya) or in Bella Vista Hospital (Mayaguez, Dr. W. Roman). Once these tumors are identified in the first quarter of year 2, these patients will be cited by their respective urologists by appointment in order to obtain Informed Consent and administer the questionnaire used for the ORIEN protocol. Patients with GS ≥8 that have had undergone surgery within the past 3 years or are pending surgery will be given an appointment during which they will meet with the study team and be invited to participate. In the combined sites, we expect to be able to consent at least 10 patients with aggressive phenotype per month.

- Explore the possibility of using transrectal core biopsy samples rather than surgical blocks as a source of RNA/DNA. The study team has established a collaboration with the Quantitative Sciences Core (QSC) and Cancer Genomics lab at PHSU to pilot the use of biopsy samples for performing WES. This idea is supported by recent evidence in the literature demonstrating that prostate tumor biopsy samples constitute an adequate source of material for whole genome molecular analysis (Nat Commun. 2019 Nov 20;10(1):5251. doi: 10.1038/s41467-019-13084-7). This approach will allow to capture aggressive and metastatic cases that are not undergoing surgery but for which biopsy samples are available. As this grant's budget does not include funds for such analysis, alternative funds have been identified though the QSC.

- On July 1, Dr. Matta contacted by phone Mariano de Socaraz (CEO CorePlus) and explained our needs of recruitment of aggressive tumors from Dr. Ruiz-Deya's patients. CorePlus has both tumor blocks and core biopsy materials and a report of the potentially available tumors for the DoD is being prepared. A follow-up meeting with Mr. de Socaraz, CorePlus staff and the two PIs and the Program Manager has been scheduled for August, 2022.

Aim 2

- Continue with the recruitment of study participants to complete the recruitment goal of 155

participants. The coordination of the recruitment logistics with the PRBB will continue to be crucial for the achievement of this goal.

- Continue to perform the DNA repair measurements through the NER pathway.

- We will begin to perform the DNA repair measurements through the homologous recombination pathway using the CometChip technology.

Aim 3

- Continue to strengthen and solidify relationships with the target municipalities' community liaisons and leaders to ensure collaboration from the community throughout the research project.

- Continue to identify and recruit individuals for the Community Advisory Committee (CAC) who may collaborate in the recruitment and implementation of the SOW tasks for the following reporting period (analyses of quantitative data, community engagement, and dissemination).

- Identify and develop the survey that will be used for the quantitative data collection phase of SA3.

- Disseminate our research findings within the community and scientific platforms such as local tv, local newspaper, scientific articles, scientific oral/poster presentations, as well as community outreach activities.

4. IMPACT:

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

During year 1, the first 11 tumors from men with PCa were sequenced (whole exome sequencing). This represents the first molecular data for the H/L population in the US and in Puerto Rico. The first peer-reviewed manuscript was published (Aim 2) showing a 59% reduction in DNA repair capacity levels in H/L men with PCa compared to controls without PCa. A community network and focus groups were done in three communities in eastern Puerto Rico characterized by high PCa mortality and high African ancestry.

What was the impact on other disciplines?

This study, particularly information gathered through the community focus groups (Aim 3), has provided the foundation for a PCa prevention campaign in the southern and southwestern communities of Puerto Rico. This campaign is being headed by Ponce Health Sciences University Marketing and Communications Department with alliances with different organizations such as the Puerto Rico Association of Urology and the American Cancer Society.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Community leaders, health providers, community members, spiritual-based leaders, and community liaisons in each of our three target municipalities were educated on the higher incidence and mortality rates of PCa among men ages 40 and older living in their region. The impact of the information provided was manifested in strong support and alliance with our research team for the activities held at each municipality. A high response rate

was observed in all activities held thus far, including focus groups. This high responsive rate is significant considering that all activities were held amidst a global pandemic.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Regarding Specific Aim 3, a small change was made to our theoretical model for qualitative data analyses. During the analyses of our focus group data, Dr. Melissa Marzan and RA Luis Arroyo identified the need to choose a more tailored theoretical model to conceptualize the information provided by focus groups participants. As mentioned before, our participants also provided a lot of feedback and information regarding barriers and disparities they have encountered in their decision making and access to healthcare services. As such, our qualitative data theoretical framework was changed from the Implementation Science Model to the **Health Equity Implementation Framework (HEIF) model**. This change allows us to frame the data provided by participants within the levels and domains that accurately describe the reality they described in focus groups.

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

Recently, we became aware through a communication from the Puerto Rico Science and Technology Trust of an upcoming challenge for Aim 2. This challenge is related to the supply of the CometChip kits and reagents by Bio-Techne. Recently, Dr. Hans Beernink (Antibody Business Unit Leader at Bio-Techne) informed us that the company has been experiencing difficulties sourcing several components required to manufacture the higher throughput versions of the CometChip - specifically the 20 and 96 sample formats. Dr. Matta had met Dr. Beernink through Zoom in recent months ago and immediately communicated with him to explore potential alternatives to avoid any disruption on our DNA repair experiment pipeline. Although Dr. Beernink stated that the discontinuation of the CometChip will be effective on July 31, 2022, we have taken precautionary measures while we plan our transition into a different technology or company to acquire these materials. The objective is to prevent supply disruption issues that may have an impact in future progress of this aim. We have ordered 19 assay kits and reagents that will allow us to assess the DNA repair of 95 samples. Meanwhile, we have discovered the alternative of acquiring a similar product from another company, Cell Biolabs, Inc. Although their technique to measure DNA damage is the comet assay (similarly to Bio-Techne), the type of technology in which the samples are loaded is different. However, since the basis of the technique similar we do not anticipate any issues when acquiring our data. We will perform validation experiments with the three internal control cell lines using the new technology in order to confirm that the assay is performed accurately and the validity of our results is not compromised.

Changes that had a significant impact on expenditures

Nothing to report.

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

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Presentations:

1. Ortiz-Sánchez C. Prostate Cancer Disparities in Puerto Rico. *11th Annual Puerto Rico Oncology Symposium,* August 5-6, 2022.

2. Ortiz-Sanchéz C, Encarnación-Medina J, Moreno N, Park JY, Ruiz-Deya G, Matta J. Overall DNA repair capacity as a potential tool to improve prostate cancer diagnosis. *17th PHSU/PRI Annual Scientific Conference/1st RCMI Symposium in Health Disparities*, May 7, 2022 (1st Award Oral Presentation in Basic Sciences Category). Presented as a poster at *11th Annual Puerto Rico Oncology Symposium,* August 5-6, 2022.

3. Linares-Medina R, Marcial-Rodríguez J, Encarnación-Medina J, Ortiz-Sanchéz C, Moreno N, Ruiz-Deya G, Matta J. Re-examining the Application of Prostate-Specific Antigen levels to Distinguish between Aggressive and Low-Risk Prostate Cancer. *17th PHSU/PRI Annual Scientific Conference/1st RCMI Symposium in Health Disparities*, May 7, 2022 (1st Award Oral Presentation in Clinical Studies Category). Presented as a poster at *11th Annual Puerto Rico Oncology Symposium,* August 5-6, 2022.

4. Abreu C, Vergne R, Ortiz-Sanchéz C, Encarnación-Medina J, Ruiz-Deya G, Matta J. Clinical Features and Distribution of Aggressive Prostate Cancer in Puerto Rican Men: A Preliminary Assessment. *17th PHSU/PRI Annual Scientific Conference/1st RCMI Symposium in Health Disparities*, May 7, 2022 (2st Award Oral Presentation in Clinical Studies Category).

5. Marcial-Rodríguez J, Linares-Medina R, Encarnación-Medina J, Ortiz-Sanchéz C, Moreno N, Ruiz-Deya G, Matta J. Evaluation of Genetic Variants in Puerto Rican Men with Prostate Cancer. *17th PHSU/PRI Annual Scientific Conference/1st RCMI Symposium in Health Disparities*, May 7, 2022.

6. Arroyo-Andújar, LA, Hernandez, E, Ronda, G, Ryan, N, Marzan-Rodrigez, M, Jimenez, J and Matta, J. Prostate Cancer Screening: Barriers in the Prevention of Lethal Prostate Cancer in Men from the Communities of Southeastern Puerto Rico. 17th PHSU-PRI Annual Scientific Conference/1st RCMI Symposium on Health Disparities. May 7, 2022. Poster Presentation.

7. Rosa-Gil de Rubio P, Bernaschina-Rivera SA, Ortiz-Sanchez C, Encarnación-Medina J, Ruiz-Deya G, and Matta J. The Effects of Androgen Deprivation Therapy (ADT) on Lymphocyte DNA Damage and Plasma IL-10 Levels. MD Summer Research Program at PSHU/PRI, July 21, 2022. Oral Presentation.

Journal publications.

- Ortiz-Sánchez C, Encarnación-Medina J, Park JY, Moreno N, Ruiz-Deya G, Matta J. Reduced DNA Repair Capacity in Prostate Cancer Patients: A Phenotypic Approach Using the CometChip. Cancers 2022. (Impact Factor:5.9). PMID: 35804887, PMCID: PMC9264934 DOI: 10.3390/cancers14133117

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

  Nothing to report.

- **Technologies or techniques**

  Nothing to report.

- **Inventions, patent applications, and/or licenses**

  Nothing to report.

- **Other Products**
  Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Jaime Matta, PhD
Project Role: Co-PI
Researcher Identifier (e.g.ORCID ID): N/A
Nearest person month worked: 12
Contribution to Project: Dr. Matta has overseen the day-to-day operation of the DoD study. He has provided oversight and leadership, planning, coordination to all aspects of the project together with Dr. Ruiz Deya, also PI. He continually monitored all aspects of the study and intervened when necessary to ensure timely accrual and completion of the study Aims. Dr. Matta meets regularly with the co-investigators, one consultant, and key personnel for each aim in a monthly basis. He has also worked on the preparation and revision of the semi-annual and annual technical reports. He is responsible for compliance with all DoD guidelines for the use of Human Subjects (HRPO) and PHSU IRB guidelines. Matta is responsible for dissemination of results to the scientific community including presentation at seminars, national/international scientific meetings, preparation of publications for peer-reviewed journals, and regular progress reports to the DoD. He worked on the preparation of the manuscript recently published and in the summer medical student presentations. He has been involved in meetings and discussions with the research team including Dr. Ruiz-Deya and with CorePlus in developing a plan to recruit aggressive tumors during year 2.

Name: Gilberto Ruiz-Deya, MD
Project Role: Co-PI
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 12
Contribution to Project: Dr. Ruiz-Deya has been responsible for oversight of all clinical aspects of the project related to patient recruitment and interpretation of clinical data. He has been involved in the identification of study participants for

recruitment through the PRBB. Dr. Ruiz-Deya provides supervision to the study consenters (Drs. Moreno and Vega). He meets regularly with Dr. Matta to discuss the progress of the recruitment for Specific Aims 1 and 2. He has been involved in meetings and discussions with the research team and with CorePlus and one urologist from the west coast in developing a plan to recruit aggressive tumors during year 2.

Name: Carmen Ortiz, PhD
Project Role: Postdoctoral Researcher and Program Manager
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 12
Contribution to Project: Dr. Ortiz meets weekly with Dr. Matta and monthly with all of the co-investigators for Specific Aims 1 and 3. She has been collecting the blood samples at the recruitment sites once they are drawn by the study consenters. She has met regularly with the Data Abstractor from the PRBB assigned for this study. She has constructed and continuously updated the recruitment database with important information regarding the samples collected (i.e. study ID, date of recruitment, cell concentration, cell viability, recruitment site). She has performed the DNA repair capacity experiments with the participants' samples using the CometChip technology, acquired fluorescence images for each sample, and performed the quantitative analysis using the CometAssay software to finally obtain the DNA repair capacity values. She supervised and mentored the MD summer students during their summer rotations and provided feedback for their abstracts and oral presentations. She prepared the first draft of the manuscript with the data from Aim 2. Dr. Ortiz has been responsible for the preparation and submission of the required documents for the renewal of the IRB at PHSU. She prepared the posters to be presented at 11th Annual Puerto Rico Oncology Symposium, held in San Juan, PR on August 5-6, 2022.

Name: Jarline Encarnación, MS
Project Role: Laboratory Supervisor (Matta laboratory)
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 12, in-kind
Contribution to Project: Ms. Encarnación has provided support to Dr. Ortiz on the processes of collection of blood samples at the recruitment sites, processing of the blood samples for lymphocyte isolation and storage, and on different steps during the DNA repair experiments. She has thawed and cultured the patient's lymphocytes for the DNA repair experiments. Along with Dr. Ortiz, she supervised the MD summer students during their summer rotations. She performed the statistical analyses for the data collected on Aim 2 that were included on the manuscript. Along with Dr. Ortiz, she prepared the posters to be presented at 11th Annual Puerto Rico Oncology Symposium, held in San Juan, PR on August 5-6, 2022.

Name: Julie Dutil, PhD
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 12
Contribution to Project: Dr. Dutil has overseen and integrated all genomic analyses for Aim 1 that has been performed so far on 11 PCa tumors. Specifically, she has conducted quality control of germ-line genome-wide genotyping array data and ancestry analysis. In collaboration with Dr. Teer (MCC), she has prioritized somatic mutations of interest and compare mutation frequencies across risk groups and ethnicity/ancestry associated group. She has worked closely with Dr. Teer and participated in monthly meetings with Drs. Matta, Ortiz, and Park.

Name: Jamie Teer, PhD
Project Role: Co-Investigator, Moffitt Cancer Center
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 12
Contribution to Project: Dr. Teer has conducted quality controls of whole exome sequencing (WES) data and RNA sequencing data pertaining to the 11 PCa tumors studied so far in Aim 1. Dr. Teer has been responsible for the analysis resulting in the identification of somatic mutations of DNA repair genes in the PCa tumors from Puerto Rico. He has worked closely with Dr. Dutil and participated in monthly meetings with Drs. Matta, Ortiz, Dutil, and Park.

Name: Julio Jiménez, MD
Project Role: Aim 3 Project Co-Investigator
Nearest person month worked: 12
Contribution to Project: Dr. Jiménez has worked in leading and coordinating the general plan of Aim 3 SOW plans and tasks. He was directly involved in the revision of the Informed Consents that were submitted for the IRB amendment as well as in document templates created for this study (sociodemographic questionnaire, etc.). Dr. Jiménez has provided direction and supervision for community outreach activities. He has been directly involved on a weekly basis with consolidating the association with the community liaison for the identification and recruitment of community members, the community advisory committee (CAC) as well as prospective participants. Jimenez has also provided oversight and supervision of the two Research Assistants and student volunteers. He has provided community education in community visits and has been directly involved in the development of qualitative research instruments and protocols. Dr. Jiménez has participated in all weekly and monthly team meetings. He also worked and provided support in the preparation of this annual technical report.

Name: Melissa Marzan, DrPH
Project Role: Aim 3 Project Co- Investigator
Nearest person month worked: 12
Contribution to Project: Dr. Marzan has provided training and oversight to the RAs and has assisted Dr. Jiménez in the development of relevant reports and documentation related to Aim 3. She was directly involved in the revision

of the Informed Consents that were submitted for the IRB amendment. She has directly assisted in the qualitative data collection as co-facilitator of focus groups as well as has collaborated in the implementation of outreach educational activities. Marzan has been directly involved in the development of qualitative research strategy, instruments, and protocols, and also participated in monthly team meetings and discussions. She also worked and provided support in the preparation of this annual technical report.

Name: Nicole M. Ryan-Nolla, PhD
Project Role: Aim 3 Project Administrative and Quantitative Research Assistant
Nearest person month worked: 8
Contribution to Project: Dr. Ryan-Nolla has performed work related to community engagement and educational intervention activities as well as administrative tasks related to official documentation for project protocols, recruitment, and data collection. She has also provided support in tasks and efforts related to the coordination and recruitment of participants for all the focus groups. She has provided administrative support in the preparation of the two technical reports (Aim 3) as well as in the scheduling of team meetings and provides follow-up to pending tasks with each team member. Dr. Ryan also conducted data entry of demographic information of focus group participants.

Name: Luis A. Arroyo Andújar, MPH, MA
Project Role: Aim 3 Project Qualitative Research Assistant
Nearest person month worked: 8
Contribution to Project: Mr. Arroyo has performed work related to the development of instrument tools and data collection of focus groups. He has provided training and oversight of student volunteers for the qualitative data collection. He has also been directly involved with qualitative data collection as facilitator and/or co facilitator of focus groups. He prepared the poster for Aim 3 that was presented at the 17th Annual Scientific Conference and 1st RCMI Symposium, on May 7th, 2022, at the Hilton Ponce Golf & Casino Resort.

Name: Jong Park, PhD
Project Role: Consultant
Researcher Identifier (e.g. ORCID ID): 0000-0002-6384-6447
Nearest person month worked: 12
Contribution to Project: Dr. Park provided his services as a consultant for this project. Park and the Co-PIs work towards obtaining WES data from Hispanic patients from MCC. He attended monthly meetings related to Aim 1.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

GuPRE Community Center in Patillas, Puerto Rico, a non-profit community center ran by an alliance of community members and leaders of Patillas. Currently, the leader of this alliance is the study team's community

liaison, Dr. Ruth Reyes. Their contribution to the project has been in-kind support, facilities, collaboration, and recruitment.

Community Development Office from Las Piedras Municipality, located in Las Piedras, Puerto Rico, a local government agency that provides direct and in-kind support to Las Piedras' local community non-profits, as well as community leaders and activities. Currently our contact liaison for this alliance is Mrs. Juanita Castro. Their contribution to the project has been in-kind support, facilities, collaboration, and recruitment.

The non-profit community institution CESSA, for its acronym in Spanish (Centro Educativo Sico-Social de Ayuda Incorporada), as well as Parroquia San Isidro Labrador and Parroquia Santa María de la Cabeza. All three are in Maunabo, Puerto Rico. These local institutions work together to serve the community of Maunabo. These three institutions provided in-kind support, collaboration and aided in the recruitment of focus group participants. Parroquia San Isidro Labrador also collaborated to the project by providing facility to conduct focus groups. Our main contact in these institutions was Mrs. Paula "Sherry" Lebrón.

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS:

Reduction of Lethal Prostate Cancer Disparities in Underserved Hispanic/Latino Populations

PC200176

Award Number: W81XWH2110241

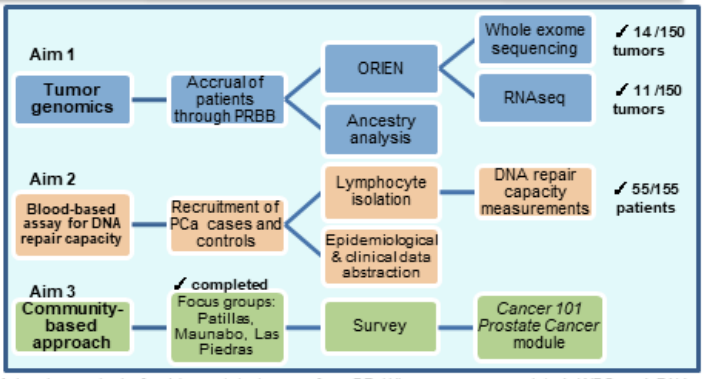
PI: Jaime L. Matta, PhD **Org:** Ponce Medical School Foundation, Inc. **Award Amount: $1,174,652**

Study Aims

- Aim 1: Identify the genomic alterations and mutation signatures that characterize lethal PCa in PR Hispanic/Latino (PR H/L) men.
- Aim 2: Studying the lethal PCa phenotype in terms of overall DNA repair capacity (DRC) levels using lymphocytes as surrogate markers to develop a potential biomarker for identifying men at high-risk.
- Aim 3: To increase PCa awareness and screening in PR communities with high African ancestry and high PCa mortality rates.

Approach

The study team aims to significantly advance research in the biology of lethal prostate cancer (PCa) and reduce the burden of lethal PCa health disparities in Puerto Rican Hispanic/Latino (PR H/L) men. PCa is the most prevalent cancer, both in terms of incidence and mortality in Puerto Rico. The long-term goal of this study is to reduce lethal PCa disparities in PR H/L men by: 1) identifying genetic and genomic differences in the PR H/L population, the study team expects to gain insight into why PR H/L men have the highest PCa mortality among all Hispanic subgroups in the United States (US), and; 2) engaging the community as research partners, the team expects to gain knowledge as to specific social, psychological, and cultural factors that may represent barriers to PCa education and screening.



Molecular analysis for 14 prostate tumors from PR H/L men was completed: WES and RNA sequencing data are available for 11 and 14 tumors, respectively. Significant differences were observed in DRC between cases and controls and by disease aggressiveness. Focus groups (6) were performed with a response rate of 105% and key topics regarding PCa were identified.

Timeline and Cost

Activities CY	21	22	23	24
Specific Aim 1				
Specific Aim 2				
Specific Aim 3				
Estimated Budget ($K)	$391K	$391K	$391K	$391K

Updated: August 10, 2022

Goals/Milestones

CY21 Goals
- ☑ IRB approval
- ☑ Begin with recruitment for Aim 1 and 2, and for focus groups (Aim 3)

CY22 Goals
- ☑ Complete with recruitment for Aim 1 and 2.
- ☑ Request WES data, perform ancestry analysis
- ☑ Measure DRC levels through NER
- ☐ Measure DRC levels through HR
- ☐ Recruitment of survey participants
- ☑ Focus groups data analysis.

CY23 Goals
- ☐ Analysis of WES, RNA-seq and ancestry data, preparation of manuscript for Aim 1
- ☐ Measure DRC levels through NER and HR
- ☐ Survey data analysis, adaptation of Cancer 101 module, and provide workshops

CY24 Goals
- ☐ Measure DRC levels through NER and HR; perform analysis and manuscript submission
- ☐ Perform interviews for evaluation of implementations strategies, interview data analysis, and manuscript preparation for Aim 3

Comments/Challenges/Issues/Concerns
- Nothing to report

Budget Expenditure to Date

Projected Expenditure: $766,269.62

Actual Expenditure: $ 408,382.38

9. APPENDICES:

Appendix A–Focus Group Participants Demographic Characteristics

Municipalities: PATILLAS, MAUNABO AND LAS PIEDRAS
Total Participants = 63

Table 1: Age range of participants from target municipalities

Age range	f	%
40-44	6	10%
45-54	8	13%
55-64	16	25%
65_	33	52%
Total	**63**	

Table 2: Relation status of participants from target municipalities

Relationship status	f	%
Living together but not legally married	8	13%
Married	34	54%
Divorced	7	11%
Single	10	16%
Widowed	4	6%
Total	**63**	

Table 3: Highest educational level of participants from target municipalities

Educational level	f	%
Doctorate's degree	5	8%
Master's degree	3	5%
Bachelor's degree	18	29%
Associate degree	11	17%
Technical degree	3	5%
High school degree	15	24%
Some elementary	8	13%
Total	**63**	

- 37% (n=23) participants reported to have completed high school or less, including eight participants who reported to have only reached some elementary grade.

Table 4: Income level of participants from target municipalities

Income level	f	%
<$500	4	6%
$501-$1,000	9	14%
$1,001-$1,500	13	21%
$1,501-$1,800	14	22%
$1,801-$2,000	5	8%
$2,001-$2,500	6	10%
>$2,501	12	19%
Total	**63**	

- 71% of the total sample reported an income level lower than $2,000
- 41% of the total sample reported an income level lower than $1,500

Table 5: Ever had a prostate cancer screening Test

Y/N Screen Test	f	%
Yes	48	76%
No	14	22%
Prefer not to say	1	2%
Total	63	

Table 6: Ever been diagnosed with prostate cancer

Y/N PCa Dx	f	%
Yes	10	16%
No	49	78%
Prefer not to say	4	6%
Total	63	

Demographic Characteristics of Focus Group Participants – PATILLAS

Municipality: PATILLAS
Total Participants = 18

Table 7: Age range of participants from Patillas

Age range	f	%
40-44	1	6%
45-54	1	6%
55-64	4	22%
65_	12	67%
Total	**18**	

Table 8: Relation status of participants from Patillas

Relationship status	f	%
Living together but not legally married	3	17%
Married	6	33%
Divorced	4	22%
Single	2	11%
Widowed	3	17%
Total	**18**	

Table 9: Highest educational level of participants from Patillas

Educational level	f	%
Doctorate's degree	0	–
Master's degree	1	6%
Bachelor's degree	3	17%
Associate degree	5	28%
Technical degree	0	–
High school degree	4	22%
Some elementary	5	28%
Total	**18**	

- 50% (n=9) participants reported to have completed high school or less, including five participants who reported to have only reached some elementary grade.

Table 10: Income level of participants from Patillas

Income level	f	%
<$500	0	–
$501-$1,000	4	22%
$1,001-$1,500	3	17%
>$1,501	11	61%
Total	**18**	

- 39% of the total sample reported an income level lower than $1,500

Table 11: Ever had a prostate cancer screening Test

Y/N Screen Test	f	%
Yes	16	89%
No	2	11%
Prefer not to say	0	–
Total	**18**	

Table 12: Ever been diagnosed with prostate cancer

Y/N PCa Dx	f	%
Yes	3	17%
No	15	83%
Prefer not to say	0	–
Total	**63**	

Demographic Characteristics of Focus Group Participants – MAUNABO

Municipality: MAUNABO
Total Participants = 25

Table 13: Age range of participants from Maunabo

Age range	f	%
40-44	1	4%
45-54	1	4%
55-64	7	28%
65_	16	64%
Total	**25**	

- For Maunabo and Las Piedras, we revised our demographic information form to include specific age. Ages ranged from 43 to 90 years old with an average age of 66.84.

Table 14: Relation status of participants from Maunabo

Relationship status	f	%
Living together but not legally married	2	8%
Married	17	68%
Divorced	2	8%
Single	3	12%
Widowed	1	4%
Total	**25**	

Table 15: Highest educational level of participants from Maunabo

Educational level	f	%
Doctorate's degree	3	12%
Master's degree	2	8%
Bachelor's degree	8	32%
Associate degree	4	16%
Technical degree	3	12%
High school degree	4	16%
Some elementary	1	4%
Total	**25**	

- 20% (n=5) participants reported to have completed high school or less, including one participant who reported to have only reached some elementary grade.

Table 16: Income level of participants from Maunabo

Income level	f	%
<$500	0	0%
$501-$1,000	4	16%
$1,001-$1,500	3	12%
$1,501-$1,800	2	8%
$1,801-$2,000	4	16%
$2,001-$2,500	4	16%
>$2,501	8	32%
Total	**25**	

- 52% (n=13) of the total sample reported an income level lower than $2,000
- 28% (n=7) of the total sample reported an income level lower than $1,500

Table 17: Ever had a prostate cancer Screening Test

Y/N Screen Test	f	%
Yes	22	88%
No	2	8%
Prefer not to say	1	4%
Total	25	

Table 18: Ever been diagnosed with prostate cancer

Y/N PCa Dx	f	%
Yes	5	20%
No	18	72%
Prefer not to say	2	8%
Total	25	

Demographic Characteristics of Focus Group Participants – LAS PIEDRAS

Municipality: LAS PIEDRAS
Total Participants = 20

Table 19: Age range of participants from Las Piedras

Age range	f	%
40-44	4	20%
45-54	6	30%
55-64	5	25%
65_	5	25%
Total	**20**	

- For Maunabo and Las Piedras, we revised our demographic information form to include specific age. Ages ranged from 40 to 78 years old with an average age of 56.70.

Table 20: Relation status of participants from Las Piedras

Relationship status	f	%
Living together but not legally married	3	15%
Married	11	55%
Divorced	1	5%
Single	5	25%
Widowed	0	–
Total	**20**	

Table 21: Highest educational level of participants from Las Piedras

Educational level	f	%
Doctorate's degree	2	10%
Master's degree	0	–
Bachelor's degree	7	35%
Associate degree	2	10%
Technical degree	0	–
High school degree	7	35%
Some elementary	2	10%
Total	**20**	

- 45% (n=9) participants reported to have completed high school or less, including two participants who reported to have only reached some elementary grade.

Table 22: Income level of participants from Las Piedras

Income level	f	%
<$500	4	20%
$501-$1,000	1	5%
$1,001-$1,500	7	35%
$1,501-$1,800	1	5%
$1,801-$2,000	1	5%
$2,001-$2,500	2	10%
>$2,501	4	20%
Total	**20**	

- 70% (n=14) of the total sample reported an income level lower than $2,000
- 60% (n=12) of the total sample reported an income level lower than $1,500

Table 23: Ever had a prostate cancer Screening Test

Y/N Screen Test	f	%
Yes	10	50%
No	10	50%
Prefer not to say	0	0%
Total	**20**	

Table 24: Ever been diagnosed with prostate cancer

Y/N PCa Dx	f	%
Yes	2	10%
No	16	80%
Prefer not to say	2	10%
Total	**20**	

Appendix B- Focus Group Summary

TITLE: Reduction of Lethal Prostate Cancer Disparities in Underserved Hispanic/Latino Populations

Abstract: Prostate cancer (PCa) is the second most common cancer in men in the United States. In Puerto Rico, prostate cancer is the most diagnosed (40%) among men and is the leading cause of mortality (18%) from cancer in men. African ancestry has been identified as a factor for increasing risk for lethal PCa. Screening tests (ST) for PCa are a crucial prevention strategy in the detection and care of these cases. However, individual and structural barriers continue to discourage men from having ST. Seven focus (7) groups were held with men (N=63) from the communities of the towns of Patillas, Maunabo and Las Piedras between December 2021 and April 2022. These focus groups were audio recorded and transcribed for analysis purpose. The analysis was conducted using the Health Equity Implementation framework (Woodward, E.V., Singh, R.S., Ndebele-Ngwenya, P., Melgar Castillo, A., Dickson, K. S. & Kirchner, J. E., 2021). A total of six (6) domains were created for the analysis: 1) Innovation factors, 2) Clinical encounters, 3) Recipient factors, 4) Context factors, 5) Societal context and 6) Strategies for disseminating information. The categories that the participants mentioned more were the following: Strategies for disseminating information (92.2%); Cultural beliefs (87.17%); Causes (54.08%); Prevention (49.96%). The categories less mentioned were the following: Private health plan (0%); Skills in managing prostate cancer issues (4.13%); and Structural facilitator (0.71%). In general, participants mention the need for more information about prostate cancer and for communities to have access to it. One of the main barriers is the machismo that limits men to undergo the rectal exam to detect prostate cancer. On the other hand, they also mention the challenges faced with medical plans and the processes to be able to get screened and request treatments. Next steps…

Keywords: lethal prostate cancer, screening test, cancer screening, Puerto Rico

AIM 3: **To increase PCa awareness and screening in Puerto Rico communities with high African ancestry and high PCa mortality rates.**

Rationale: The purpose of this activity is to explore the ways in which we can inform about PCa to communities. This activity will provide valuable information related to PCa and how to address the disparities in underserved Hispanic/Latino populations. The following tasks compose the actions needed to achieve the completion of the Activity 1.

The following tasks compose the actions needed to achieve the completion of the aim #3 for Y01.

- Task 3: Community assessment to explore knowledge, cultural beliefs, and perceived risk toward PCa (Exploratory mixed method study).
    - Subtask 1: As starting point, we will request for institutional IRB approval before starting the evaluation with men from the communities of Maunabo, Las Piedras and Patillas.
    - Subtask 2: Recruitment participants and conduct focus groups (probably online if social distancing is required).
    - Subtask 3: Focus group data analysis (inform to survey).

METHOD

Design

An exploratory qualitative design using a Grounded Theory approach and the Health Equity Implementation Framework (HEIF) was conducted to guide this project (Corbin & Strauss, 2015; Strauss & Corbin, 1994).

Objectives

Focus Groups: to inform community survey and a community educational plan content and delivery methods and identify potential facilitators and barriers to intervention implementation of PCa screening among high-risk groups for lethal PCa in Puerto Rico.

Qualitative Data Collection Techniques

Participants were invited to a 90-minutes focus groups at community venues in coordination with the community leaders, faith-based organizations, and the Ponce Health Medical Science University staff. A total of n=63 key informants participated in the 7 focus groups conducted by the team. We used a semi structure qualitative interview guide for the collection data of these focus groups and the main topics discussed on these interviews were:
Innovation factors for PCa screening; clinical encounter for PCa screening, PCa screening recipient factors, context factors and strategies for disseminating information.

All focus groups were audio recorded with participant's consent. Each participant received a stipend of $30.00.

Analysis

All focus groups were audio digitally recorded. NVivo® transcription was used to transcribe all audio files. All transcriptions were submitted to review for accuracy (Poland, 2002). All qualitative data were gathered, organized, and managed using NVivo®. A content analysis using the principles of Grounded Theory was performed (Strauss & Corbin, 1994). Transcriptions were subjected to an open coding procedure (Corbin & Strauss, 2015), in which domains and categories of concepts and themes guided by the HEIF approach. Also, emerging topics were included in the analysis. Themes were compared across participants to identify commonalities and differences. The qualitative analysts were independently coding the transcripts. Discrepancies were evaluated and discussed before the coding were resumed (Corbin & Strauss, 2015).

A content analysis was conducted using the HEIF domains (see table 1). Themes regarding to identify potential facilitators and barriers to PCa screening was identified in all participants.

Content Analysis

A content analysis was conducted to identify the most and less mentioned topics of the participants. A total of six (6) domains were created for the analysis: 1) Innovation factors, 2) Clinical encounters, 3) Recipient factors, 4) Context factors, 5) Societal context and 6) Strategies for disseminating information. The categories that the participants mentioned more were the following: Strategies for disseminating information (92.2%); Cultural beliefs (87.17%); Causes (54.08%); Prevention (49.96%). The categories less mentioned were the following: Private health plan (0%); Skills in managing prostate cancer issues (4.13%); and Structural facilitator (0.71%). For more details, please see Table 1. Content Analysis for Focus Groups using Health Equity Implementation Framework.

Table 1

Content Analysis for Focus Groups using Health Equity Implementation Framework

Domain and category	N	# Occasions where category were mentioned
1. Innovation Factors		
1.a. Screening Tests		
1.a.1 Knowledge	7	43
1.b.1 Opinions	7	36
1.c.1 Reasons to don't	3	6
1.d.1 Reasons to do it	5	23
2.a. PCa Treatments		
2.a.1 Knowledge	7	37
2. Clinical Encounter		
2.a. Experience visiting a health provider	5	25
2.b. PCa information	6	24
2.c. Types of PCa screening	3	7
3. Recipients Factors		
3.a. Patient Factors		
3.a.1.Causes	7	65
3.a.2. Cultural Beliefs	7	78
3.a.3. Prevention	7	52

3.a.4. PCa general knowledge	6	14
3.b. Providers Factors		
3.b.1. Knowledge about prostate cancer	4	19
3.b.2. Skills in managing prostate cancer issues	1	1
4. Context Factors		
4.a. Inner context (Local)		
4.a.1. Structural barriers	7	47
4.a.2. Structural facilitators	2	5
4.b. Inner context (Organizational)		
4.b.1. Structural barriers (Provider)	6	30
4.b.2. Structural facilitator (Provider)	1	1
4.c. Outer context (Health system)		
4.c.1. Health System		
4.c.1.a. Public health plan	2	4
4.c.2.b. Private health plan	1	0
4.c.2. Information sources	7	33
5. Strategies for disseminating information	7	66

Note: A total of 7 focus groups were conducted (N=63).

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Appendix C- Abstracts, posters, and publications

Abstracts prepared with Specific Aim 2 data

Abstract 1:

Overall DNA repair capacity as a potential tool to improve prostate cancer diagnosis

Carmen Ortiz-Sánchez[1], Jarline Encarnación-Medina[1], Natasha Moreno[1], Jong Park[2], Gilberto Ruiz-Deyá[1], Jaime Matta[1]

[1]Department of Basic Sciences, Ponce Research Institute, Ponce Health Sciences University-School of Medicine, Ponce, PR 00716-2347, [2]Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center, Tampa, FL 33612, USA

Abstract:
Prostate cancer (PCa) is the leading type of cancer in terms of incidence (37.1%) and mortality (16.8%) in Puerto Rican (PR) men. Epidemiological studies using functional assays in lymphocytes have demonstrated that DNA repair capacity (DRC) varies among individuals, and that having low overall DRC is a significant risk factor for cancer development. The aim of this study was to evaluate variations in overall DRC levels PR men with PCa. Lymphocytes were isolated from blood samples collected from PCa cases (n=15) and controls (n=6) recruited at St. Luke's Hospital (Dr. Ruiz-Deyá's clinical practice and UroCentro del Sur) (IRB no. 2101051235R001). Clinical and epidemiological data was abstracted for each participant. DRC levels through the NER pathway were measured using the CometChip assay using UVC as a NER inductor. The mean overall DRC for controls and PCa cases were 24.41% (±4.27) and 7.48 (±1.19), respectively (p=0.0094). A Receiver Operating Characteristics (ROC) curve analysis was performed to assess whether DRC levels can be used to distinguish between cases and controls. Our results show that DRC levels measured with the CometChip assay were able to distinguish between PCa cases and controls in a binary fashion (AUC=0.966, p=0.0011). Additional analyses are currently ongoing regarding comparisons based on the Gleason score. The outcomes of this study represent an innovative step in the development of a blood-based screening test for PCa based on DRC levels. Sponsored by U54 PHSU-MCC Partnership Grant #U54CA163071, the DoD/US Army Award #W81XWH-21-1-0241, and the PRI.

A**bstract 2:**

Re-examining the Application of Prostate-Specific Antigen (PSA) levels to Distinguish between Aggressive and Low-Risk Prostate Cancer

Raymond Linares-Medina[1], Joshua Marcial-Rodríguez[1], Jarline Encarnación-Medina[2], Carmen Ortiz-Sánchez[2], Natasha Moreno[2], Gilberto Ruiz-Deyá[2], Jaime Matta[2]

[1]School of Medicine, [2]Department of Basic Sciences, Ponce Research Institute, Ponce Health Sciences University-School of Medicine, Ponce, PR 00716-2347

Abstract:
Since its discovery in the 1970s, prostate specific antigen (PSA) is commonly known as a biomarker for prostate cancer (PCa) diagnosis despite its limited specificity. There is controversy in its use; some believe the diagnosis and treatment of insignificant PCa cases has risen while PCa mortality has not been reduced. The aim of this study was to evaluate whether PSA levels can be used to distinguish between low-risk and aggressive PCa in Puerto Rican men. Medical records of PCa patients (n=55) from Dr. Gilberto Ruiz-Deyá's clinical practice at St. Luke's Hospital (Ponce, PR) were reviewed (IRB no. 2101051235R001). Men without PCa (controls) were also included in this study (n =17). PCa cases were stratified using the Gleason score into: low-risk (n=24) and aggressive (n=31). PCa patients had significantly higher PSA levels when compared to controls (p<0.0001). Although no significant differences were found between the low-risk (7.7 ng/ml) and aggressive (21.1 ng/ml) groups, the aggressive group had higher PSA levels. A Receiver Operating Characteristics (ROC) curve analysis was performed to assess whether PSA levels can be used to distinguish between study groups. PSA levels were able to distinguish between aggressive PCa and controls, and between low-risk PCa and controls. However, this was not observed when considering low-risk and aggressive PCa. This study represents the first effort to evaluate the ability of PSA in distinguishing between low-risk and aggressive PCa in patients from Puerto Rico. Sponsored by U54 PHSU-MCC Partnership Grant #U54CA163071, the DoD/US Army Award #W81XWH-21-1-0241, and the PRI.

Abstract 3:

Evaluation of Genetic Variants in Puerto Rican Men with Prostate Cancer

Joshua Marcial-Rodríguez[1], Raymond Linares-Medina, Jarline Encarnación-Medina[2], Carmen Ortiz-Sánchez[2], Natasha Moreno[2], Gilberto Ruiz-Deyá[2], Jaime Matta[2]

[1]School of Medicine, [2]Department of Basic Sciences, Ponce Research Institute, Ponce Health Sciences University-School of Medicine, Ponce, PR 00716-2347

Abstract:
Data from the Puerto Rico Cancer Registry shows that prostate cancer (PCa) is the leading cancer type in terms of incidence and mortality. Although PCa is normally characterized by a slow progression, 20-30% of cases can lead to metastasis and poor survival outcomes. Epidemiological studies have shown that first degree relatives of PCa patients have two- to three-fold increased risk of developing the disease compared to general population. The aim of this study was to perform a preliminary assessment of the frequency of genetic variants among PCa patients stratified by disease aggressiveness. A blood sample for each participant was collected at Dr. Gilberto Ruiz-Deya's clinical practice in St. Lucas Hospital (Ponce, PR), to perform the Invitae Multi-Cancer and Prostate Cancer Panel (IRB no. 2101051235R001). Genetic Variants were classified as: Pathogenic, Likely Pathogenic, Negative, and Variants of Uncertain Significance (VUS). PCa tumors were stratified based on the Gleason score as: aggressive (n=43) and low-risk (n=35). Demographic and clinical data were collected from medical records. Frequency analysis was used to assess genetic variant distribution. Significance was evaluated using χ^2 and Fisher's exact test. Almost 58% of the participants tested positive for variants. Forty VUS were found in PCa patients. Genes with highest variant frequency include: *RECQL4* and *POLD1*. Since Hispanics are underrepresented in genetic studies, it may be that some of these variants could be relevant to PCa but have been missed due to limited representation of this population. Sponsored by U54 PHSU-MCC Grant #U54CA16307, DoD/US Army Award #W81XWH-21-1-0241, and PRI.

Abstract 4:

Re-examining the Application of Prostate-Specific Antigen (PSA) levels to Distinguish between Aggressive and Low-Risk Prostate Cancer

Raymond Linares-Medina[1], Joshua Marcial-Rodríguez[1], Jarline Encarnación-Medina[2], Carmen Ortiz-Sánchez[2], Natasha Moreno[2], Gilberto Ruiz-Deyá[2], Jaime Matta[2]

[1]School of Medicine, [2]Department of Basic Sciences, Ponce Research Institute, Ponce Health Sciences University-School of Medicine, Ponce, PR 00716-2347

Abstract:
Since its discovery in the 1970s, prostate specific antigen (PSA) is commonly known as a biomarker for prostate cancer (PCa) diagnosis despite its limited specificity. There is controversy in its use; some believe the diagnosis and treatment of insignificant PCa cases has risen while PCa mortality has not been reduced. The aim of this study was to evaluate whether PSA levels can be used to distinguish between low-risk and aggressive PCa in Puerto Rican men. Medical records of PCa patients (n=55) from Dr. Gilberto Ruiz-Deyá's clinical practice at St. Luke's Hospital (Ponce, PR) were reviewed (IRB no. 2101051235R001). Men without PCa (controls) were also included in this study (n =17). PCa cases were stratified using the Gleason score into: low-risk (n=24) and aggressive (n=31). PCa patients had significantly higher PSA levels when compared to controls ($p<0.0001$). Although no significant differences were found between the low-risk (7.7 ng/ml) and aggressive (21.1 ng/ml) groups, the aggressive group had higher PSA levels. A Receiver Operating Characteristics (ROC) curve analysis was performed to assess whether PSA levels can be used to distinguish between study groups. PSA levels were able to distinguish between aggressive PCa and controls, and between low-risk PCa and controls. However, this was not observed when considering low-risk and aggressive PCa. This study represents the first effort to evaluate the ability of PSA in distinguishing between low-risk and aggressive PCa in patients from Puerto Rico. Sponsored by U54 PHSU-MCC Partnership Grant #U54CA163071, the DoD/US Army Award #W81XWH-21-1-0241, and the PRI.

Abstract 5:

Clinical Features and Distribution of Aggressive Prostate Cancer in Puerto Rican Men: A Preliminary Assessment

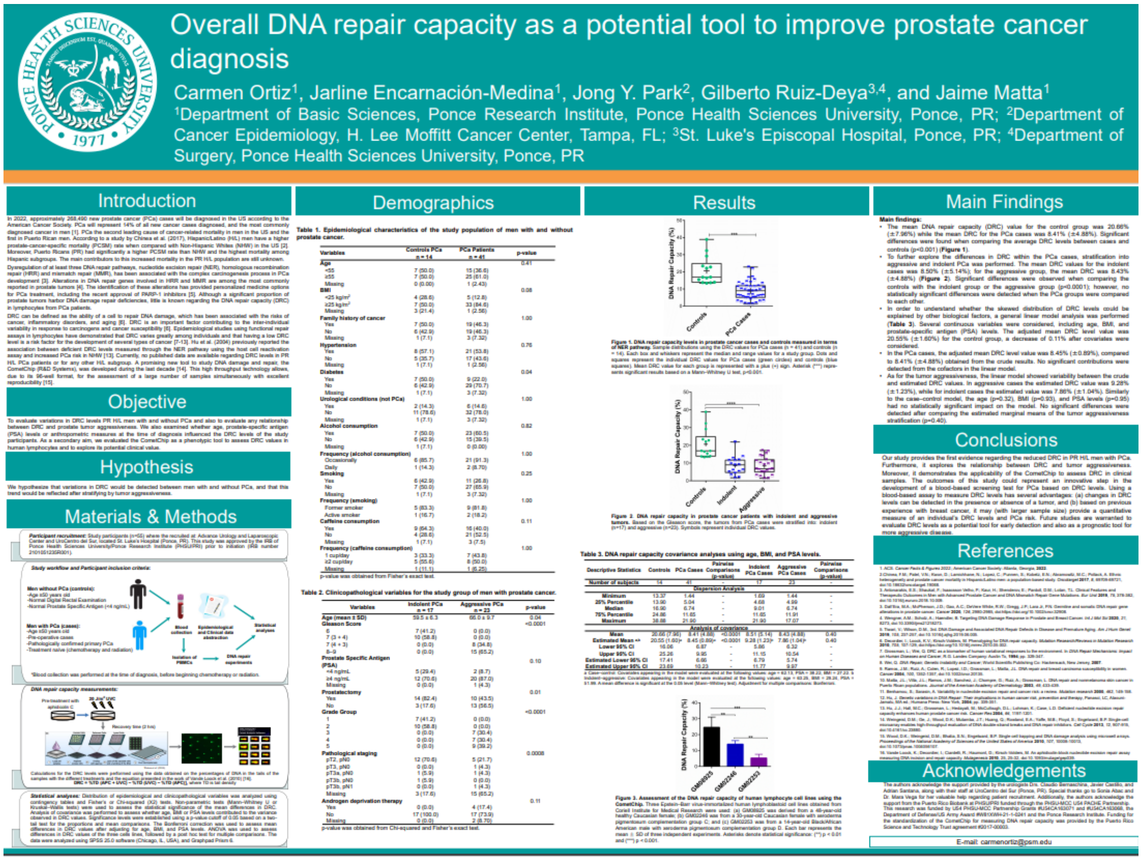
Caren Abreu[1], Ralphdy Vergne[2], Carmen Ortiz[2], Jarline Encarnación2, Gilberto Ruiz-Deyá[2], and Jaime Matta[2]

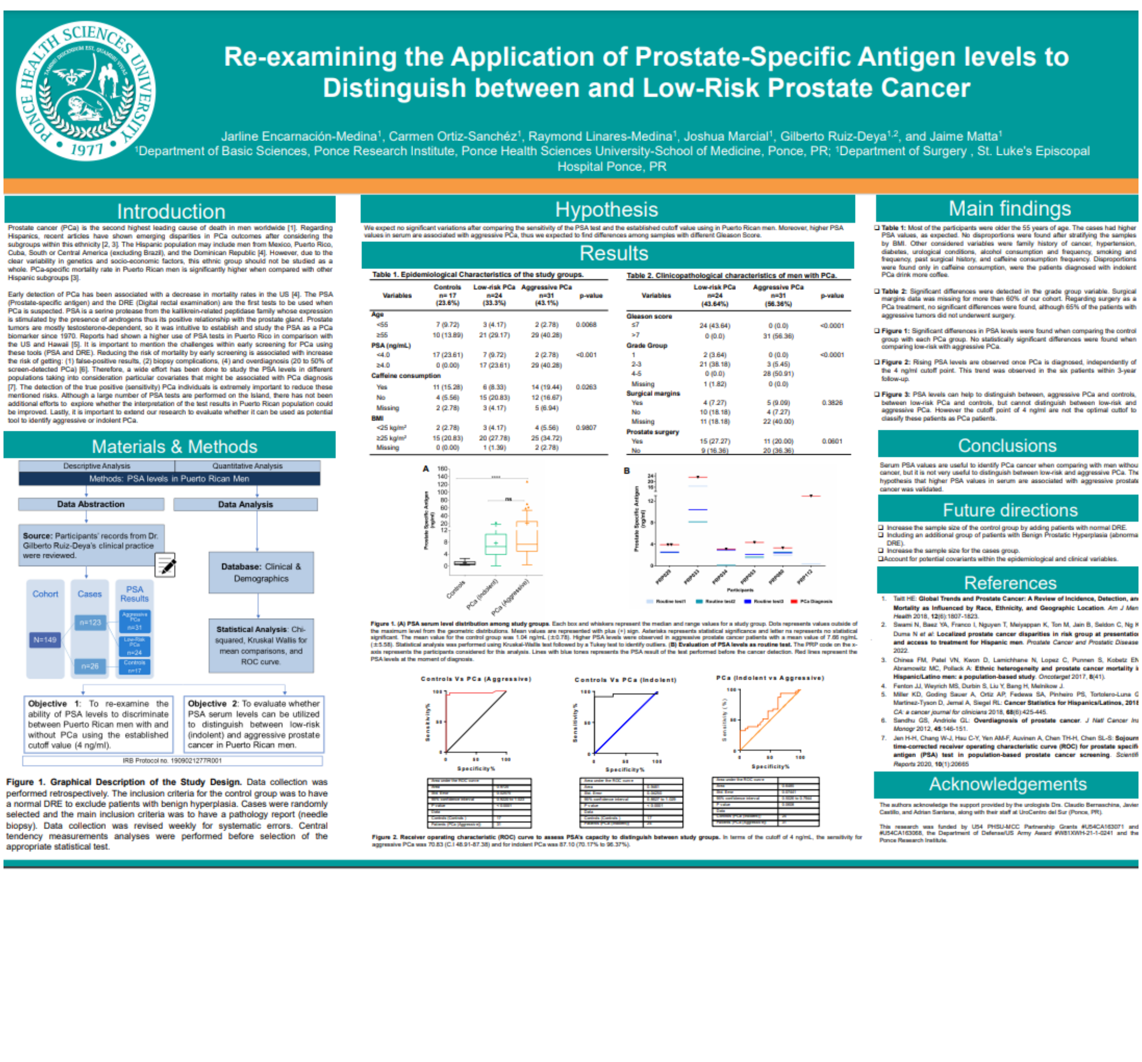
[1]School of Medicine, [2]Department of Basic Sciences, Ponce Research Institute, Ponce Health Sciences University-School of Medicine, Ponce, PR 00716-2347

Abstract:

Prostate cancer (PCa) accounts for almost 21% of the new cancer cases reported in Hispanics in the US. PCa can be classified as indolent or aggressive; the latter has been associated with metastasis and poor prognosis. Currently, little is known about the presentation of aggressive PCa in PR men. The aim of this study was to assess the distribution and clinical features of aggressive PCa cases within a subset of patients from Dr. Gilberto Ruiz-Deyá's clinical practice at St. Luke's Hospital (Ponce, PR). Clinical and epidemiological data were abstracted from PCa patients' (n=161) medical records. PCa cases were stratified as aggressive or low-risk using the Gleason score. Demographic characteristics (i.e. age, BMI) were evaluated. Clinical variables including PSA levels, grade group, pathological staging, and prostate surgery were also evaluated. Statistical significance was assessed using Chi-square and Fisher's exact tests. Most of the patients were diagnosed at ≥55 years of age, at an earlier pathological stage, and had a BMI of ≥25kg/m^2 regardless of tumor aggressiveness. Patients with indolent tumors were identified with higher frequency in our cohort (n=109, 67.70%) in contrast to patients with aggressive tumors (n=52, 32.30%). In both groups, most patients had a PSA level ≥4 ng/µl when diagnosed. Most patients with indolent tumors had radical prostatectomy, in contrast with patients with aggressive tumors. This study represents the first effort to assess the distribution of aggressive PCa cases in a clinical practice in PR. Sponsored by U54 PHSU-MCC Grants U54CA163071 & U54CA163068, and the DoD/US Army Award #W81XWH-21-1-0241.

Posters presented for Specific Aim 2 at the *11th Annual Puerto Rico Oncology Symposium*





Poster presented for Specific Aim 3 at the *17th PHSU/PRI Annual Scientific Conference/1st RCMI Symposium in Health Disparities*

Barriers in the Prevention of Lethal Prostate Cancer in Men from the Communities of Southeastern Puerto Rico: Preliminary Results.

Luis A. Arroyo-Andújar, MPH, MA, Eddiel Hernández-López, BA, Gustavo Ronda-Rodríguez, BA, Génesis Virella-Tirado, BS., Nicole Ryan-Nolla, PhD, Melissa Marzán-Rodríguez, DrPh, Julio Jiménez-Chávez, MD & Jaime Matta, PhD
Ponce Health Sciences University, *Ponce Research Institute, Ponce, Puerto Rico.*

INTRODUCTION

- Prostate cancer (PCa) is the second most common cancer in men in the United States.
- In Puerto Rico, PCa is the most diagnosed (40%) among men and is the leading cause of mortality (18%) from cancer in men.
- African ancestry has been identified as a factor for increasing risk for lethal PCa.
- Screening tests (ST) for PCa are a crucial prevention strategy in its detection and care. However, individual and structural barriers continue to discourage men from having ST.

PURPOSE

The purpose of this study is to be able to identify the individual and structural barriers for screening men for PCa in the communities most impacted in the southeastern of Puerto Rico. Thus, in order to develop prevention strategies that are in tune with their particular needs and contextual realities.

METHODS

- Seven focus groups (N=63 men) were held in the towns of Maunabo, Patillas and Las Piedras between December 2021 and April 2022.

Sociodemographic Data

52% (n=33) 65+ years
41% (n=26) < $18,000 Anual/family
37% (=23) High school or less
54% (n=34) Married

Recruitment

Community leaders, prostate cancer educational talks, CBOs, radio, social media, and flyers.

Procedure

- PSHU - IRB approval.
- Community leaders provided a list of potential participants.
- A team member contacted potential participants and coordinate the focus groups appointment.
- Seven focus groups (2 in Patillas, 2 in Maunabo, 3 in Las Piedras) were held with a total of 10 or more participants per group.
- The inform consent was explained and signed by all participants.
- The discussions were conducted by facilitators trained and lasted between 60 and 90 minutes

Focus groups guiding questions

To carry out the focus groups, a semi-structured question guide included: a) introduction to prostate cancer, b) beliefs and knowledge, and c) detection of prostate cancer.

Analysis

- The focus groups were recorded and transcribed for analysis purposes.
- The barriers at patients, recipients, clinical encounter, innovation, inner and outer context and societal context levels were identified using the "Health Equity Implementation".
- The Nvivo software program was used for the qualitative analysis.

RESULTS

Preliminary results show that there are barriers for men to make the informed decision to be screened for PCa. We identified the following themes: sexism, shortage of health providers, limited health plan coverage, and lack of knowledge about the causes and symptoms of prostate cancer.

LOCAL

Sexism

"...it shows in the waiting room because if I'm at the urologist and I see you come in, machismo reaches that point. Don't let them see me go in there because if you're there it's because you're going to go in there and the second step is to take off your pants in front of that guy you don't know, that doctor. I'm going to show him my buttocks or the guy sees me there with my intimate things [genitals]. That psychologically affects those who are macho. Well, I'm not going there because they're going to see me. [...] Because sexism reaches that level, that makes you retract from going to do such an important test". (FG4, Maunabo).

Lack of knowledge about the causes and symptoms of prostate cancer

[...] And I, "Gee, doctor, I feel normal, I feel fine". But as I reflected, I said "Wow, wait. He has told me twice, I come to say that I feel nothing, I feel normal". I was having -between us speaking, premature ejaculation. But since no one had told me about it, I was totally unaware of it. I think that was normal for me." (FG1, Patillas).

"If you go to the Internet or a cybernetic source there is not much information or you go to the doctor, even there, there is not much unfortunately either. It discourages you to do something" (FG4, Maunabo).

ORGANIZATIONAL

Shortage of health providers

"[...] I am seeing a urologist in Cayey who, in the area are not so many specialists and those that were, had those offices that once you arrived there were 100 people to serve in one day, that's impossible. So, the closeness of the specialists is also, it's quite poor, at least in this southeastern area. I don't know how many urologists work, but I don't think there are more than five". (GF1, Patillas).

"As long as there is no type of communication, nobody knows what type of treatment they have to take. Your primary care physician should guide you well, you know, tell you "Listen, if it gives you this or gives you that, you can go for this type of treatment" so that you can then continue your normal life as you had it." (GF1, Patillas).

Limited health plan coverage

"That now the medical plans are limiting the health of the people. Because before I could take those exams twice a year. Now, some time ago, they told me, "No, you are allowed to only once a year." So, how are they taking away people's health, that is something vital.. (GF1, Patillas)

CONCLUSIONS

- The cultural component related to sexism was a barrier consistently identified in all groups.
- Participants mentioned multiple barriers related to accessing PCa screening services. Structural barriers such as: lack of transportation, access to health coverage, limitations in health coverage, lack of information, and limited health providers.

FUTURE DIRECTIONS

The collected data will be used to develop a quantitative survey and an outreach educational plan focused on PCa detection in the southern areas of Puerto Rico.

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ACKNOWLEDGMENTS

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Manuscript published on June 25, 2022 for results from Specific Aim 2

cancers

MDPI

Article

Reduced DNA Repair Capacity in Prostate Cancer Patients: A Phenotypic Approach Using the CometChip

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Simple Summary: Prostate cancer (PCa) is the most commonly diagnosed cancer type in Hispanic men in the US. Among Hispanics, Puerto Rican (PR) men show the highest PCa-specific mortality. Various studies have shown that having low DNA repair capacity (DRC) is a significant risk factor for cancer development. The aim of this study was to evaluate variations in DRC, through the nucleotide excision repair (NER) pathway, in PR men with PCa using the CometChip. Overall, PCa cases had lower DRC than controls. When PCa cases were stratified into aggressive and indolent, controls had higher DRC than both groups. The contributions of additional factors (i.e., age and prostate-specific antigen levels) to DRC were also considered. Our data suggest that DRC levels may have the potential to discriminate between aggressive and indolent cases. Our results represent an innovative step in the development of a blood-based screening test for PCa based on DRC levels.

Abstract: Prostate cancer (PCa) accounts for 22% of the new cases diagnosed in Hispanic men in the US. Among Hispanics, Puerto Rican (PR) men show the highest PCa-specific mortality. Epidemiological studies using functional assays in lymphocytes have demonstrated that having low DRC is a significant risk factor for cancer development. The aim of this study was to evaluate variations in DRC in PR men with PCa. Lymphocytes were isolated from blood samples from PCa cases (n = 41) and controls (n = 14) recruited at a hospital setting. DRC levels through the nucleotide excision repair (NER) pathway were measured with the CometChip using UVC as a NER inductor. The mean DRC for controls and PCa cases were 20.66% (±7.96) and 8.41 (±4.88), respectively ($p < 0.001$). The relationship between DRC and tumor aggressiveness was also evaluated. Additional comparisons were performed to evaluate the contributions of age, anthropometric measurements, and prostate-specific antigen levels to the DRC. This is the first study to apply the CometChip in a clinical cancer study. Our results represent an innovative step in the development of a blood-based screening test for PCa based on DRC levels. Our data also suggest that DRC levels may have the potential to discriminate between aggressive and indolent cases.

Keywords: prostate cancer; DNA repair capacity; nucleotide excision repair; CometChip

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1. Introduction

In 2022, approximately 268,490 new prostate cancer (PCa) cases will be diagnosed in the US according to the American Cancer Society. PCa will represent 14% of all new cancer cases diagnosed, and the most commonly diagnosed cancer in men [1]. It is estimated that, in 2022, around 34,500 PCa-related deaths will occur in the US. This makes PCa the second leading cause of cancer-related mortality in men in the US and the first in Puerto Rican men. PCa is a complex disease in which multiple factors may increase the risk of its development, including age, family history of PCa, ethnicity (African ancestry), obesity, hormones and certain genetic conditions (e.g., Lynch syndrome and *BRCA1* and *BRCA2* mutations) [1]. Black men in the US and Caribbean have the highest PCa incidence rates in the world [1]. According to a study by Chinea et al. (2017), Hispanic/Latino (H/L) men have a higher prostate-cancer-specific mortality (PCSM) rate when compared with Non-Hispanic Whites (NHW) in the US [2]. Moreover, Puerto Ricans (PR) had significantly a higher PCSM rate than NHW and non-Hispanic Blacks (NHB), and the highest mortality among Hispanic subgroups. The main contributors to this increased mortality in the PR H/L population are still unknown.

Dysregulation of at least three DNA repair pathways, nucleotide excision repair (NER), homologous recombination repair (HRR) and mismatch repair (MMR), has been associated with the complex carcinogenesis process in PCa development [3-11]. Alterations in DNA repair genes involved in HRR and MMR are among the most commonly reported in prostate tumors [12-14]. The identification of these alterations has provided personalized medicine options for PCa treatment, including the recent approval of PARP-1 inhibitors [15]. Although a significant proportion of prostate tumors harbor DNA damage repair (DDR) deficiencies [11], little is known regarding the DNA repair capacity (DRC) in lymphocytes from PCa patients.

DRC can be defined as the ability of a cell to repair DNA damage, which has been associated with the risks of cancer, neurodegenerative disease, inflammatory disorders and aging [16,17]. Evidence exists that DRC is an important factor contributing to the inter-individual variability in response to carcinogens and cancer susceptibility in the general population [17,18]. Epidemiological studies using functional repair assays in lymphocytes have demonstrated that DRC varies greatly among individuals and that having a low DRC level is a risk factor for the development of several types of cancer [19-30].

The only published study that has evaluated DRC levels in lymphocytes of PCa patients was performed by Hu et al. (2004) [5]. Their results show that deficient DRC levels measured through the NER pathway using the host cell reactivation (HCR) assay in lymphocytes are associated with increased PCa risk in NHW [5]. Currently, no published data are available regarding DRC levels in PR H/L PCa patients or for any other H/L subgroup.

Decordier et al. (2010) reviewed and compared various methodologies utilized for evaluating DRC phenotypes phenotyping for DRC. Traditionally, the HCR assay with a luciferase reporter gene has been widely used to conduct large-scale population studies for different types of cancer [5,21,22,26,28,31,32]. Despite the widespread applications of the HCR assay, this technology is costly and labor-intensive, and has a limited capacity in terms of the volume of samples processed. A promising new tool with which to study DNA damage and repair, the CometChip (R&D Systems, Minneapolis, MN, USA), was developed during the last decade [33-36]. The CometChip is a high throughput technology that allows, due to its 96-well format, for the assessment of a large number of samples simultaneously with excellent reproducibility [37]. Several studies have described the potential applications and benefits of the CometChip when compared with the traditional comet assay, since it reduces experimental noise and comet-to-comet variance, and improves reproducibility [33,36,37]. Although it has been used to measure DNA damage, Ngo et al. (2021) reported that the CometChip can distinguish between DNA repair kinetics among individuals, highlighting its potential applications for future epidemiological and clinical studies [38]. Pursuant to this finding, our study represents the first report on the use of the CometChip to measure DRC levels in clinical samples, specifically from PCa patients.

The aim of this study was to evaluate variations in DRC levels PR H/L men with and without PCa and also to evaluate any relationship between DRC and prostate tumor

aggressiveness. We also examined whether age, prostate-specific antigen (PSA) levels or anthropometric measures at the time of diagnosis influenced the DRC levels of the study participants. As a secondary aim, we evaluated the CometChip as a phenotypic tool to assess DRC values in human lymphocytes and to explore its potential clinical value. This initial effort consisted of 55 samples collected as part of an ongoing case-control clinic-based study. We hypothesized that variations in DRC would be detected between men with and without PCa, and that this trend would be reflected after stratifying by tumor aggressiveness.

2. Materials and Methods

Use of Human Subjects and Institutional Review Board (IRB). This study was approved by the IRB of Ponce Health Sciences University/Ponce Research Institute (PHSU/PRI) prior to initiation (IRB number 2101051235R001). PRI has a consortium agreement with St. Luke's Hospital (Ponce, PR) where the recruitment sites are located: Advance Urology and Laparoscopic Center and UroCentro del Sur. Written informed consent from all study participants was obtained by the study nurse or physicians prior to blood sample collection. Clinical and epidemiological data were abstracted from the study participants' electronic medical records.

Study Population. Controls (men without PCa) and pre-operative PCa cases were recruited for this study. The inclusion criteria for controls were men ≥45 years of age, with normal results from the digital rectal exam (DRE), and normal PSA (prostate-specific antigen) levels (<4 ng/mL). Cases were PCa patients with pathologically confirmed primary PCa. Blood collection was performed at the time of diagnosis, before beginning chemotherapy or radiation.

Blood Collection. Blood extraction was completed by the recruitment sites' nurses. Peripheral blood lymphocytes were isolated from blood samples (6 mL) using BD Vacutainer™ Glass Mononuclear Cell Preparation Tubes (CPT). For storage, the obtained lymphocytes were suspended in 2 mL of freezing media containing 10% dimethyl sulfoxide (DMSO), 40% RPMI 1640 medium, 50% FBS and 1% antibiotic/antimycotic. Aliquots were stored in a −80 °C freezer for 1–3 weeks. The lymphocytes were then thawed in batches of five samples to perform the DRC measurements using the CometChip (R&D Systems).

Cell lines. In each DRC measurement experiment, three commercial cell lines were included as internal controls. Cell lines were purchased from Coriell Institute for Medical Research (Camden, NJ, USA). The GM08925 cell line was included as a model for normal DRC. GM02246 and GM02253 cell lines were included as models of medium and low DRC, since they have knockdowns in *XPC* and *XPD*, respectively. Lymphocytes and cell lines were grown in 88% RPMI-1640, 10% fetal bovine serum (FBS), 1% L-glutamine, 1% antibiotic/antimycotic and phytohemagglutinin. All cells were grown at 37 °C in a humidified incubator containing 5% CO_2.

DNA repair capacity (DRC) measurements. The DRC measurements were performed using the CometChip (R&D Systems, Minneapolis, MN, USA). This 96-well plate assay allows measurements of DRC levels with high reproducibility [38]. Briefly, primary lymphocytes isolated from study participants were irradiated with 20 J/m^2 ultraviolet C (UVC) light, a DNA repair inducer which preferentially activates the NER pathway. Co-treatment with 15 μM aphidicolin C (APC) for 30 min was used to allow for the accumulation of repair incisions in lymphocytes. After allowing 2 h for repair to occur, the lymphocytes were loaded on the Chip coated with low-temperature melting agarose and lysed following the manufacturer's instructions. After lysis, alkaline electrophoresis (200 mM NaOH/1 mM EDTA/0.1% Triton X-100) was performed and the chip containing the nuclei was stained with YOYO-1 (Invitrogen, Waltham, MA, USA). Ethyl methanesulfonate (EMS) was used as a positive control at a concentration of 12 mM for 4 h. Several images were acquired for each sample to capture 50 comets per sample using the EVOS M7000 (Invitrogen, Waltham, MA, USA). Images were uploaded to Comet Analysis Software (R&D Systems, Minneapolis, MN, USA) for analysis of the percentage of DNA in the tail; this is the parameter used for the assessment of single-strand DNA damage. All DRC level measurements were performed in triplicate for each study participant. Calculations for the

DRC levels were performed using the data obtained on the percentages of DNA in the tails of the samples with the different treatments and the equation presented in the work of Vande Loock et al. (2010) [39].

$$DRC = \%TD\ (APC + UVC) - \%TD\ (UVC) - \%TD\ (APC)], \text{ where TD is tail density.}$$

Statistical analysis. Analysis of variance was used to assess differences in DRC values of the three cells lines, followed by a post hoc test for multiple comparisons. Distribution of epidemiological and clinicopathological variables was analyzed using contingency tables and Fisher's or Chi-squared (X^2) tests. Non-parametric tests (Mann–Whitney U or Kruskal–Wallis tests) were used to assess the statistical significance of the mean differences from independent samples while accounting for non-normally distributed variables, such as DRC. Analysis of covariance was performed to assess whether age, BMI or PSA levels contributed to the variance observed in DRC values. Significance levels were established using a *p*-value cutoff of 0.05 based on a two-tail test for the proportions and mean comparisons. The Bonferroni correction was used to assess mean differences in DRC values after adjusting for age, BMI, and PSA levels. The data were analyzed using SPSS 25.0 software (Chicago, IL, USA), and Graphpad Prism 6 was used for graphical presentation.

3. Results

3.1. Epidemiological and Clinicopathological Variables

PCa cases were generally men over 55 years of age (61.0%) with body mass indexes (BMI) over 25 kg/m^2 (84.6%) (Table 1). Regarding comorbidities, most of the PCa cases suffered from hypertension (53.8%), but the frequency of diabetes (22%) and other urological conditions (14.6%) was low. Most of the cases reported consuming alcohol (60.5%) occasionally, and very few reported smoking (26.8%). A low frequency of caffeine consumption was reported for this group (40%). Regarding the controls, these were equally distributed across the age stratifications. Similar to the PCa cases, most of the controls had a BMI over 25 kg/m^2. Similar to the PCa cases, most of the participants in the control group suffered from hypertension (57.1%), and the frequency of urological conditions was low (14.3%). Similarly to the PCa cases, the men in the control group reported consuming alcohol (50%) occasionally. Most of the controls reported consuming more than two cups of coffee daily. Additional variables, such as family history of cancer, were also evaluated; however, no significant differences were observed between groups ($p > 0.05$).

Table 1. Epidemiological characteristics of the study population of men with and without prostate cancer.

Variables	Controls PCa *n* = 14	PCa Patients *n* = 41	*p*-value
Age			0.41
<55	7 (50.0)	15 (36.6)	
≥55	7 (50.0)	25 (61.0)	
Missing	0 (0.00)	1 (2.43)	
BMI			0.08
<25 kg/m^2	4 (28.6)	5 (12.8)	
≥25 kg/m^2	7 (50.0)	33 (84.6)	
Missing	3 (21.4)	1 (2.56)	
Family history of cancer			1.00
Yes	7 (50.0)	19 (46.3)	
No	6 (42.9)	19 (46.3)	
Missing	1 (7.1)	3 (7.32)	
Hypertension			0.76
Yes	8 (57.1)	21 (53.8)	
No	5 (35.7)	17 (43.6)	
Missing	1 (7.1)	1 (2.56)	

Diabetes			0.04
Yes	7 (50.0)	9 (22.0)	
No	6 (42.9)	29 (70.7)	
Missing	1 (7.1)	3 (7.32)	
Urological conditions (not PCa)			1.00
Yes	2 (14.3)	6 (14.6)	
No	11 (78.6)	32 (78.0)	
Missing	1 (7.1)	3 (7.32)	
Alcohol consumption			0.82
Yes	7 (50.0)	23 (60.5)	
No	6 (42.9)	15 (39.5)	
Missing	1 (7.1)	0 (0.00)	
Frequency (alcohol consumption)			1.00
Occasionally	6 (85.7)	21 (91.3)	
Daily	1 (14.3)	2 (8.70)	
Smoking			0.25
Yes	6 (42.9)	11 (26.8)	
No	7 (50.0)	27 (65.9)	
Missing	1 (7.1)	3 (7.32)	
Frequency (smoking)			1.00
Former smoker	5 (83.3)	9 (81.8)	
Active smoker	1 (16.7)	2 (18.2)	
Caffeine consumption			0.11
Yes	9 (64.3)	16 (40.0)	
No	4 (28.6)	21 (52.5)	
Missing	1 (7.1)	3 (7.5)	
Frequency (caffeine consumption)			1.00
1 cup/day	3 (33.3)	7 (43.8)	
≥2 cup/day	5 (55.6)	8 (50.0)	
Missing	1 (11.1)	1 (6.25)	

p-value was obtained from Fisher's exact test.

3.2. DNA Repair Capacity in Prostate Cancer Cases and Controls

In order to assess variations in DRC through the NER pathway among study participants, the CometChip assay was used. Through the use of UVC light, a known NER pathway inducer, the capacity to repair DNA damage through this pathway was evaluated. A total of 55 participants were included in this analysis, including PCa cases (n = 41) and controls (n = 14) (Table S1). The mean DRC value for the control group was 20.66% (±7.96%), whereas the mean DRC for the PCa cases was 8.41% (±4.88%). To assess differences in DRC levels between cases and controls, the Mann–Whitney U test was performed. Significant differences were found when comparing the average DRC levels between cases and controls ($p < 0.001$) (Figure 1).

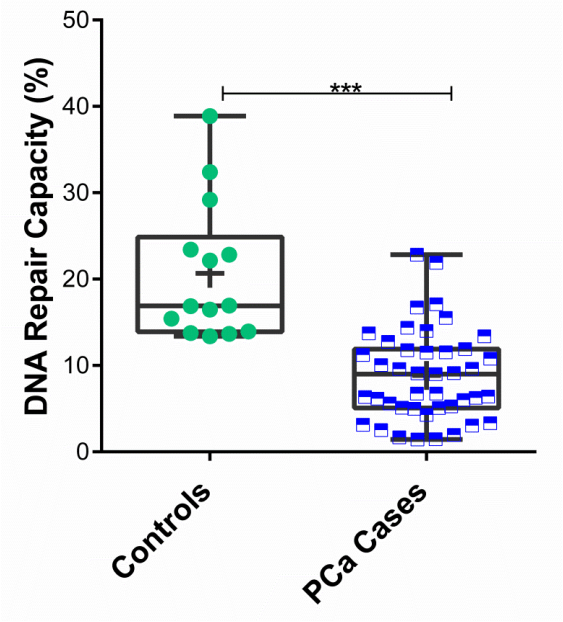

Figure 1. DNA repair capacity levels in prostate cancer cases and controls measured in terms of NER pathway. Sample distributions using the DRC values for PCa cases (n = 41) and controls (n = 14). Each box and whiskers represent the median and range values for a study group. Dots and squares represent the individual DRC values for PCa cases (green circles) and controls (blue squares). Mean DRC value for each group is represented with a plus (+) sign. Asterisk (***) represents significant results based on a Mann–Whitney U test, p<0.001.

3.3. Clinicopathological Characteristics of Prostate Cancer Patients

PCa cases were stratified into indolent and aggressive groups based on the Gleason score obtained from the pathology reports. Overall, PCa cases with indolent tumors had a mean age of 59.5 years (Table 2). Most of the participants in this group had tumors with Gleason scores of seven (3 + 4), corresponding to Grade Group 2 (58.8%) and the pathological stage of pT2 pN0. Most of these patients had PSA levels above 4 ng/mL (70.6%) at the time of diagnosis. All of the participants included in the indolent group were treatment-naïve at the time of recruitment. PCa cases with aggressive tumors were older than patients in the indolent group; their mean age was 66 years (p = 0.04). Most of the men in this group had tumors with Gleason scores of 8–9 (65.2%), corresponding to Grade Groups 4 and 5. Most of these patients had not undergone prostatectomy and had PSA levels above 4 ng/mL (87.0%). Although some of the patients in this group received androgen deprivation therapy; most of the participants had not received treatment at the time of recruitment. Most of the patients in the indolent group had undergone radical prostatectomy. In contrast, most of the patients with aggressive tumors had not (p = 0.02).

Table 2. Clinicopathological variables for the study group of men with prostate cancer.

Variables	Indolent PCa n = 17	Aggressive PCa n = 23	p-Value
Age (mean ± SD)	59.5 ± 6.3	66.0 ± 9.7	0.04
Gleason Score			<0.0001
6	7 (41.2)	0 (0.0)	
7 (3 + 4)	10 (58.8)	0 (0.0)	
7 (4 + 3)	0 (0.0)	8 (34.8)	
8–9	0 (0.0)	15 (65.2)	
Prostate Specific Antigen (PSA)			0.10
<4 ng/mL	5 (29.4)	2 (8.7)	
≥4 ng/mL	12 (70.6)	20 (87.0)	
Missing	0 (0.0)	1 (4.3)	

Prostatectomy			0.01
Yes	14 (82.4)	10 (43.5)	
No	3 (17.6)	13 (56.5)	
Grade Group			<0.0001
1	7 (41.2)	0 (0.0)	
2	10 (58.8)	0 (0.0)	
3	0 (0.0)	7 (30.4)	
4	0 (0.0)	7 (30.4)	
5	0 (0.0)	9 (39.2)	
Pathological staging			0.0008
pT2, pN0	12 (70.6)	5 (21.7)	
pT3, pN0	0 (0.0)	1 (4.3)	
pT3a, pN0	1 (5.9)	1 (4.3)	
pT3b, pN0	1 (5.9)	0 (0.0)	
pT3b, pN1	0 (0.0)	1 (4.3)	
Missing	3 (17.6)	15 (65.2)	
Androgen deprivation therapy			0.11
Yes	0 (0.0)	4 (17.4)	
No	17 (100.0)	17 (73.9)	
Missing	0 (0.0)	2 (8.70)	

p-value was obtained from Chi-squared and Fisher's exact test.

3.4. DNA Repair Capacity in Aggressive and Indolent Prostate Cancer

To further explore the differences in DRC within the PCa cases group, stratification into aggressive and indolent PCa was performed. The indolent group included PCa cases with Gleason scores of 6 and 7 (3 + 4). The aggressive group included patients with Gleason scores of 7 (4 + 3) and higher. A total of 17 PCa cases were classified as indolent, and 23 cases were included on the aggressive group (Table S2). The mean DRC for the indolent PCa cases was 8.50% (±5.14%); for the aggressive group, the mean DRC was 8.43% (±4.88%) (Figure 2). As previously mentioned, the mean DRC for the control group was 20.66% (±7.96%). Significant differences were observed when comparing the controls with the indolent group or the aggressive group ($p < 0.0001$); however, no significant differences were detected when the PCa groups were compared to each other.

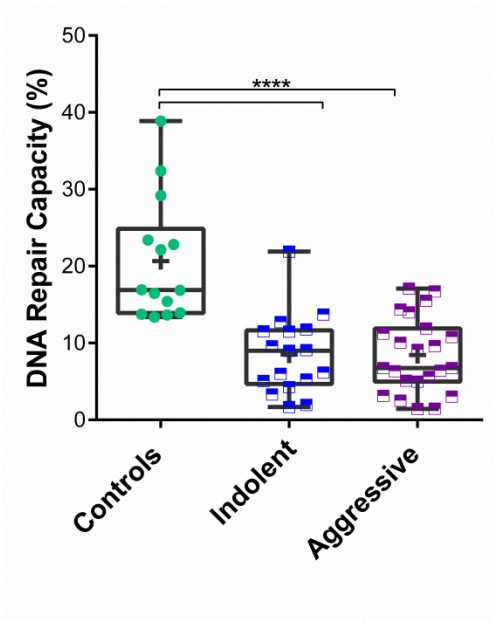

Figure 2. DNA repair capacity in prostate cancer patients with indolent and aggressive tumors. Based on their Gleason scores, the tumors from PCa cases were stratified into indolent ($n = 17$) and aggressive

(n = 23). Symbols represent individual DRC values. Mean DRC value for each group is represented with a plus (+) sign. Asterisk (****) denotes statistical significance (p = 0.001, Kruskal–Wallis test).

3.5. DRC Levels in Study Groups after Age, BMI, and PSA Level Adjustments

In order to understand whether the skewed distribution of DRC was explained by other biological factors, a general linear model analysis was performed (Table 3). In this analysis, several continuous variables were considered, including age, BMI, and PSA levels at the time of diagnosis or sample collection. The adjusted mean DRC value was 20.55% (±1.60%) for the control group, a decrease of 0.11% after covariates were considered. As for the cases, the adjusted mean DRC value was 8.45% (±0.89%), compared to 8.41% (±4.88%) obtained from the crude results. No significant contributions were detected from the cofactors in the linear model. The covariance model shows that age (p = 0.84), BMI (p = 0.50), and PSA levels (p = 0.27) are not statistically significant factors in the model. Although the adjusted mean DRC values slightly vary for both groups (cases and controls), the differences in DRC are still significant after the Bonferroni correction. As for the tumor aggressiveness, the linear model shows variability between the crude and estimated DRC values. The stratum of cases with aggressive tumors has an estimated DRC value of 9.28% (±1.23%), and the indolent stratum's value is 7.86% (±1.04%). Similarly to the case–control model, the age (p = 0.32), BMI (p = 0.93), and PSA levels (p = 0.95) had no statistically significant impact on the model. No significant differences were detected after comparing the estimated marginal means of the tumor aggressiveness stratification (p = 0.40).

Table 3. DNA repair capacity covariance analyses using age, BMI, and PSA levels.

Descriptive Statistics	Controls	PCa Cases	Pairwise Comparisons (p-Value)	Indolent PCa Cases	Aggressive PCa Cases	Pairwise Comparisons (p-Value)
Number of subjects	14	41	-	17	23	-
Dispersion Analysis						
Minimum	13.37	1.44	-	1.69	1.44	-
25% Percentile	13.90	5.04	-	4.68	4.99	-
Median	16.90	6.74	-	9.01	6.74	-
75% Percentile	24.86	11.65	-	11.65	11.91	-
Maximum	38.88	21.90	-	21.90	17.07	-
Analysis of covariance						
Mean	20.66 (7.96)	8.41 (4.88)	<0.0001	8.51 (5.14)	8.43 (4.88)	0.40
Estimated Mean [a,b]	20.55 (1.60) [a]	8.45 (0.89) [a]	<0.0001	9.28 (1.23) [b]	7.86 (1.04) [b]	0.40
Lower 95% CI	16.06	6.87	-	5.86	6.32	-
Upper 95% CI	25.26	9.95	-	11.15	10.54	-
Estimated Lower 95% CI	17.41	6.66	-	6.79	5.74	-
Estimated Upper 95% CI	23.69	10.23	-	11.77	9.97	-

[a] Case–control: Covariates appearing in the model were evaluated at the following values: age = 62.13, PSA = 38.22, BMI = 27.22. [b] Indolent–aggressive: Covariates appearing in the model were evaluated at the following values: age = 63.25, BMI = 29.24, PSA = 51.99. A mean difference is significant at the 0.05 level (Mann–Whitney test). Adjustment for multiple comparisons: Bonferroni.

3.6. Detection of Varying DRC Levels Using the CometChip

In order to determine whether our method was able to detect varying DRC levels using the CometChip, three commercially available cell lines with different DRC levels were used as internal controls. Three Epstein–Barr virus-immortalized human lymphoblastoid cell lines obtained from Coriell Institute for Medical Research (Camden, NJ, USA) were used: (a) GM08925 was derived from a 48-year-old healthy Caucasian female; (b) GM02246 was from

a 30-year-old Caucasian female with xeroderma pigmentosum complementation group C; and (c) GM02253 was from a 14-year-old Black/African American male with xeroderma pigmentosum complementation group D. We have routinely used these three cell lines for over 20 years and have established their variability in DRC levels with both the HCR assay and the CometChip. As can be observed in Figure 3, the mean DRC value for the GM08925 cell lines was 24.59% (±6.42%). As for the GM02246 and GM02253 cell lines, the mean DRC values were 14.01% (±2.20%) and 5.37 (±2.29%), respectively. Significant differences were observed when comparing the GM08925 cell lines with the GM02246 ($p < 0.01$) and GM02253 ($p < 0.001$) cell lines. Additionally, significant differences were detected when comparing GM02246 and GM02253 ($p < 0.01$). The DRC values obtained for each cell line resembled the expected results due to their genetic profiles: the highest value was detected for the GM08925 cell line, which resembles a normal DRC. Additionally, for GM02246 and GM02253, the varying levels were expected due to their genetic alterations in *XPC* and *XPD*, respectively. Therefore, our results demonstrated that the proposed method for DNA repair measurement, along with the use of the CometChip, allows for the detection of varying DRC levels in established cell lines and clinical samples.

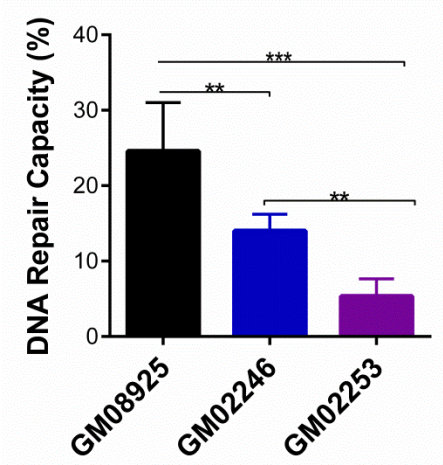

Figure 3. Assessment of the DNA repair capacity of human lymphocyte cell lines using the CometChip. Each bar represents the mean ± SD of three independent experiments. Asterisks denote statistical significance: (**) $p < 0.01$ and (***) $p < 0.001$.

4. Discussion

Prostate cancer is a complex disease, and DNA repair has been proven to play an important role both in the complex carcinogenesis process of PCa and in the biology of these tumors [3-11]. Although several studies have highlighted the importance of understanding the alterations in different DNA repair pathways in tumors, little is known regarding the functionality of DNA repair pathways in PCa patients. Moreover, the technology used to perform this phenotypic measure of DNA repair has also hindered our understanding and the application of the individual's DRC in disease development and as a potential tool for patient stratification or improving diagnosis. Therefore, our study serves a dual purpose: (1) to establish the variability of DRC in PCa patients and controls in a cohort of PR H/L men and (2) to present an additional method with which to assess DRC levels in a high throughput format that can potentially allow the field of DNA repair to continue moving forward with expanding the applications of DRC levels to a clinical setting.

Although the most commonly altered DNA repair pathways in prostate tumors are MMR and HRR, NER has also been linked to PCa risk in genetic studies [4,40-42]. NER is a very versatile pathway and is the major pathway for repairing a variety of bulky DNA lesions (adducts), such as those induced by crosslinking agents and base-damaging carcinogens [43,44]. Additionally, NER can repair helix-distorting DNA lesions generated by environmental mutagens, such as UV irradiation [45,46]. Although the preferred pathway for repairing UV-induced DNA damage is NER, recent studies have shown a non-canonical mechanism leading to the activation of the ATM pathway in noncycling cells after UV

irradiation [47,48]. Considered a "generalist" of DNA repair pathways, NER works in multiple capacities, particularly when other repair pathways exhibit reduced functionality [49].

Our main findings show that DRC, measured through the NER pathway, is reduced in men with PCa when compared to controls. Our results are similar to the findings presented by Hu et al. (2004) using the HCR assay and are consistent with the results obtained for other cancer types [21,22,28,32]. However, our study is the first to report decreased DRC in H/L PCa patients. This is extremely relevant, since H/L men have higher PCSM when compared to NHW [2]. Population studies in H/L men with PCa are very scarce in the literature and are currently underrepresented even in large genomic studies. Being that they are the second fastest growing minority in the US, our findings in this population are very relevant.

In order to further evaluate a potential relationship between DRC and tumor aggressiveness, we performed comparisons between PCa patients with aggressive and indolent tumors. Although our findings were not statistically significant, we observed a trend where PCa cases with indolent tumors had a slightly higher median DRC than participants with aggressive tumors. Interestingly, the covariance analyses showed that regardless of tumor aggressiveness, age was the major contributor in the linear model. This effect could have been due to the difference in the mean age between groups: PCa patients with indolent tumors were younger than men with aggressive tumors. Through this analysis, the trend observed in the crude results was further highlighted due to the reduced variation in the mean DRC values when age was considered. Since our method utilizes lymphocytes as surrogate markers for the individual's DRC, this finding can provide us with additional information regarding the potential role of DRC in the development of aggressive disease, which warrants additional experimentation.

Our results also show that with our experimental setup, along with the high throughput capacity of the CometChip, it is possible to detect varying levels of DRC in clinical samples. The addition of the commercial cell lines as internal controls for the assay provides a robust setup for reproducibility. Moreover, our experimental setup provides for additional robustness, since each experimental condition is analyzed in triplicate for every clinical sample, and 50 comets are evaluated for each condition. When compared to the standard HCR assay, our method is more cost-effective, less labor intensive, and could be adapted to measure multiple DNA repair pathways. Through this study, we provide the first evidence of the applicability of the CometChip as a phenotypic tool to evaluate the DRC in PCa cases and controls.

5. Conclusions

Our study provides the first evidence regarding the reduced DRC in PR H/L men with PCa. Furthermore, it explores the relationship between DRC and tumor aggressiveness. Moreover, it demonstrates the applicability of the CometChip to assess DRC in clinical samples. The outcomes of this study could represent an innovative step in the development of a blood-based screening test for PCa based on DRC levels. Using a blood-based assay to measure DRC levels has several advantages: (a) changes in DRC levels can be detected in the presence or absence of a tumor, and (b) based on previous experience with breast cancer, it may (with larger sample size) provide a quantitative measure of an individual's DRC levels and PCa risk. Future studies are warranted to evaluate DRC levels as a potential tool for early detection and also as a prognostic tool for more aggressive disease.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: DNA repair capacity values for the study cohort including Puerto Rican men with and without prostate cancer; Table S2: DNA repair capacity values for Puerto Rican men with indolent and aggressive prostate cancer.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of PHSU/PRI (IRB no. 2101051235R001, Approval date 18 February 2021).

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