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14. ABSTRACT There is an urgent need to better understand the biology contributing to the worse prognosis observed in African American (AA) prostate cancer (PCa) patients in order to 1) predict which men are more likely to suffer from an aggressive form of the disease and 2) develop more patient-specific treatments. Because AA men are less likely to be included in key PCa studies and clinical trials, we miss many other potential genetic indicators that steer molecular mechanisms, disease outcomes, and drug response. For example, human epidermal growth factor receptor-2 (HER2) overexpression has been correlated with advanced stage, treatment resistance, and worse outcome for PCa patients. Phase II clinical trials targeting HER2 were initiated, but major limitations were the lack of enrollment of study participants with HER2+ tumors as well as poor efficacy with anti-HER2 single agents. PCa patients were not stratified by race, and racial differences in HER2 expression were not evaluated. In this study, I aim to establish that HER2 expression promotes cellular growth, clonogenicity, and migration in a racially diverse panel of PCa cell lines and tumor tissue specimens. Thus far, results suggest that the targeting of HER2 with trastuzumab inhibits viability of RC-77T/E, an AA PCa cell line, but not PC3, a European American (EA) cell line. I will also confirm whether HER2 overexpression is positively correlated with West African ancestry (WAA) in tumor tissue specimens. Preliminary RNAseq analysis of prostate tissue suggested a moderate positive correlation between HER2 gene expression and WAA (r=0.1593). Additional genotyping analysis of isolated genomic DNA that is patient-matched to a 456 Case Race Disparity TMA is underway to support these prior findings. I will also determine if HER2 overexpression correlates with disease stage, clinical features, treatments, and outcomes in PCa patients. Preliminary HER2 membrane staining of AA FFPE specimens with IHC showed 30% (3/10) scored as 2+ or equivocal. In-progress IHC analysis of 400 FFPE specimens acquired from the COHCCC Frozen Tissue Bank as well as 456 Case Race Disparity TMA acquired from the DOD PCBN will provide additional insight into the correlation with HER2 overexpression and clinical variables associated with aggressive PCa and worse outcomes. Timely completion of this and a subsequent clinical trial would have a major near-term impact in lessening the burden of PCa mortality in AA men and would provide an additional treatment option for others with HER2+ metastatic PCa.

15. SUBJECT TERMS
prostate cancer, HER2, African American, health disparities, mCRPC, West African ancestry

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INTRODUCTION: African American (AA) men suffer from increased prostate cancer (PCa) incidence and mortality.[1] Gene expression profiles confirm prominent racial differences in tumor biology, and a variety of pro-tumorigenic genes are differentially expressed in AA men.[2-5] Human epidermal growth factor receptor-2 (HER2) is a tyrosine kinase involved in the promotion of cellular division and suppression of apoptosis and is correlated with worse prognosis and treatment resistance in PCa patients, but this gene has not been evaluated in AA men.[6,7] Clinical trials targeting HER2 were previously initiated in PCa patients. There is no indication that racial differences in HER2 expression were evaluated.[8-11] There is an urgent need to better understand the biology contributing to the worse prognosis observed in AA PCa patients in order to 1) predict which men are more likely to suffer from lethal disease and 2) develop more patient-specific treatments. In this study, we seek to establish that HER2 expression promotes cellular growth, clonogenicity, and migration in a racially diverse panel of PCa cell lines; confirm whether HER2 overexpression is positively correlated with West African ancestry (WAA) in tumor tissue specimens; and determine if HER2 overexpression correlates with disease stage, clinical features, treatments, and outcomes in PCa patients.

KEYWORDS: prostate cancer, HER2, African American, health disparities, mCRPC, West African ancestry

BODY

ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Establish that HER2 expression promotes cellular growth, clonogenicity, and migration in a racially diverse panel of PCa cell lines.

Major Task 1: Quantify basal HER2 gene and protein levels.
Target Dates: 1-6 months
Percentage of Completion: 50%

Major Task 2: Evaluate whether HER2 overexpression alters cell growth, clonogenicity, and migration.
Target Dates: 6-12 months
Percentage of Completion: 0%

Major Task 3: Evaluate whether HER2 blockade alters cell growth, clonogenicity, and migration.
Target Dates: 9-15 months
Percentage of Completion: 30%

Specific Aim 2: Confirm whether HER2 overexpression is positively correlated with WAA in tumor tissue specimens.

Major Task 1: Access racially diverse tumor tissue specimens.
Target Dates: 1-15 months
Percentage of Completion: 100%

Major Task 2: Quantify HER2 in tumor tissue specimens.
Target Dates: 12-24 months
Percentage of Completion: 100%

Major Task 3: Calculate genetic ancestry of patients.
Target Dates: 12-18 months
Percentage of Completion: 10%

Specific Aim 3: Determine HER2 correlation with PCa disease stage, clinical features, treatments, and outcomes in patients.

Major Task 1: Stratify tumor tissue specimens.
Target Dates: 12-24 months
Percentage of Completion: 50

Major Task 2: Correlate HER2 expression with disease stage, clinical features, treatments, and outcomes.
Target Dates: 12-24 months
Percentage of Completion: 0%

What was accomplished under these goals?

Specific Aim 1: Establish that HER2 expression promotes cellular growth, clonogenicity, and migration in a racially diverse panel of PCa cell lines.

Major Task 1: Quantify basal HER2 gene and protein levels.

Subtask 1: Examine HER2 mRNA levels in cancer cells using qPCR.
Cell lines used: MDA-PCa-2b, PC3, DU145, 22Rv1, RC-77T/E

I am repeating qPCR with in-lab equipment (Applied Biosystems) to establish HER2 mRNA transcript levels in all cell lines. All cell lines are grown in a humidified incubator with 5% CO_2 at 37°. PC3 (Cat:CRL-1435), DU145 (Cat:HTB-81), 22Rv1 (Cat:CRL-2505) and MDA-PCa-2b (Cat:CRL-2422) cell lines were purchased from the American Type Culture Collection (ATCC). PC3, DU145, and 22Rv1 was cultured in RPMI 1640 medium (Corning, Cat:10-040-CV) supplemented with 10% fetal bovine serum (Corning, Cat:35010CV), penicillin-streptomycin (Corning, Cat:30001CI), and gentamicin (Gibco, Cat:15710064) as recommended by the supplier. MDA-PCa-2b (Cat:CRL-2422) cell line was cultured in F-12K medium (ATCC®, Cat:30–2004) supplemented with 20% fetal bovine serum (Corning, Cat:35010CV), cholera toxin (Sigma-Aldrich, Cat:C8052), epidermal growth factor (Sigma-Aldrich, Cat:E4127), *o*-phosphoethanolamine (Sigma-Aldrich, Cat:P0503), hydrocortisone (Sigma-Aldrich, Cat:H0888), selenious acid (ACROS Organics, Cat:AC19887), bovine insulin (Sigma-Aldrich, Cat:I6634), and penicillin-streptomycin as recommended by the supplier. The RC-77T/E cell line was provided by C.A. Casiano's laboratory with permission from J.S. Rhim. RC-77T/E was grown in collagen-coated treated culture dishes in keratinocyte serum-free medium in (K-SFM) supplemented with bovine pituitary extract and recombinant epidermal growth factor (Gibco, Cat:17-005-042) used for growing and maintaining the cells. 0.2% Normocin (Invivogen, Cat:ANT-NR-1) was added to the medium for all cell lines to prevent contamination by mycoplasma, bacteria, or fungi. In accordance with NIH guidelines concerning the authentication of key biological resources, I have verified the identity and purity of the cell lines by performing mycoplasma testing (**Table 1**) using MycoProbe Mycoplasma Detection Kit (Cat: CUL001B) in addition to short tandem repeat (STR) profiling provided by ATCC (**Tables 2-6**). For STR profiling, seventeen STR loci plus the gender determining locus, Amelogenin, were amplified using the commercially available PowerPlex® 18D Kit from Promega. The cell line sample was processed using the ABI Prism® 3500xl Genetic Analyzer. Data were analyzed using GeneMapper® ID-X v1.2 software (Applied Biosystems). Appropriate positive and negative controls were run and confirmed for each sample submitted.

PC3	-0.0054
DU145	0.0028
22Rv1	-0.00075
MDA	-0.0088
RC77T	-0.0073

Table 1. Mycoplasma testing O.D. Values (Calculated) to verify absence of mycoplasma. <0.05 is considered negative with no mycoplasma detected. 0.05-0.10 is considered inconclusive with sample being suspect for mycoplasma. >0.10 is considered positive with mycoplasma detected.

Cell Line
Authentication Service
STR Profile Report

FTA Barcode: STRB8073

ATCC Sales Order: SO0878071

Test Results for Submitted Sample					**ATCC Reference Database Profile**			
Locus	**Query Profile: PC3**				**Database Profile: PC-3; Prostate Adenocarcinoma; Human (Homo sapiens)**			
D3S1358	16							
TH01	6	7			6	7		
D21S11	29	31.2						
D18S51	14	15						
Penta_E	10	13	17					
D5S818	13				13			
D13S317	11				11			
D7S820	8	11			8	11		
D16S539	11				11			
CSF1PO	11				11			
Penta_D	9	11						
Amelogenin	X				X			
vWA	17				17			
D8S1179	13							
TPOX	8	9	11		8	9		
FGA	22	24						
D19S433	14							
D2S1338	18	20						
Number of shared alleles between query sample and database profile:								12
Total number of alleles in the database profile:								12
Percent match between the submitted sample and the database profile:								100
The allele match algorithm compares the 8 core loci plus amelogenin only, even though alleles from all loci will be reported when available.								
NOTE: *Loci highlighted in grey (8 core STR loci plus Amelogenin) can be made public to verify cell identity. In order to protect the identity of the donor,* ***please do not publish*** *the allele calls from all the STR loci tested. Electropherograms showing raw data are attached.*								

Explanation of Test Results
Cell lines with 80% match are considered to be related; i.e., derived from a common ancestry. Cell lines with between a 55% to 80% match require further profiling for authentication of relatedness.

☐ The submitted sample profile is human, but not a match for any profile in the ATCC STR database.

☐ The submitted profile is an exact match for the following ATCC human cell line(s) in the ATCC STR database (8 core loci plus Amelogenin):

☒ The submitted profile is similar to the following ATCC human cell line(s): CRL-1435

☐ An STR profile could not be generated.

Table 2. STR profiling performed by ATCC to verify that the PC3 sample profile provided matches (100%) the human cell line CRL-1435.

Cell Line Authentication Service
STR Profile Report

FTA Barcode: STRB8074

ATCC Sales Order: SO0878071

Test Results for Submitted Sample					ATCC Reference Database Profile			
Locus	**Query Profile: DU145**				**Database Profile: DU 145; Prostate Carcinoma; Human (Homo sapiens)**			
D3S1358	16							
TH01	7				7			
D21S11	28	30	31	33				
D18S51	12	17						
Penta_E	12	13	14					
D5S818	10	13			10	13		
D13S317	12	13	14		12	13	14	
D7S820	7	10	11		7	10	11	
D16S539	11	13			11	13		
CSF1PO	10	11			10	11		
Penta_D	9	11	13					
Amelogenin	X	Y			X	Y		
vWA	17	18			17	18	19	
D8S1179	13	14						
TPOX	11				11			
FGA	22	24						
D19S433	13	15						
D2S1338	16	19						
Number of shared alleles between query sample and database profile:								18
Total number of alleles in the database profile:								19
Percent match between the submitted sample and the database profile:								95

The allele match algorithm compares the 8 core loci plus amelogenin only, even though alleles from all loci will be reported when available.

NOTE: *Loci highlighted in grey (8 core STR loci plus Amelogenin) can be made public to verify cell identity. In order to protect the identity of the donor,* ***please do not publish*** *the allele calls from all the STR loci tested.*
Electropherograms showing raw data are attached.

Explanation of Test Results
Cell lines with 80% match are considered to be related; i.e., derived from a common ancestry. Cell lines with between a 55% to 80% match require further profiling for authentication of relatedness.

- [ ] The submitted sample profile is human, but not a match for any profile in the ATCC STR database.
- [ ] The submitted profile is an exact match for the following ATCC human cell line(s) in the ATCC STR database (8 core loci plus Amelogenin):
- [X] The submitted profile is similar to the following ATCC human cell line(s): HTB-81
- [ ] An STR profile could not be generated.

Table 3. STR profiling performed by ATCC to verify that the DU145 sample profile provided matches (95%) the human cell line HTB-81.

Cell Line
Authentication Service
STR Profile Report

FTA Barcode: STRB8075

ATCC Sales Order: SO0878071

Test Results for Submitted Sample					ATCC Reference Database Profile			
Locus	**Query Profile: 22RV1**				**Database Profile: 22Rv1; Prostate Carcinoma; Human (Homo sapiens)**			
D3S1358	15							
TH01	6	9.3			6	9.3		
D21S11	30							
D18S51	13	14						
Penta_E	5	13						
D5S818	11	13			11	12	13	
D13S317	9	12			9	12		
D7S820	9	10	11		9	10	11	
D16S539	12				12			
CSF1PO	10	11			10	11		
Penta_D	9	12						
Amelogenin	X	Y			X	Y		
vWA	15	21			15	21		
D8S1179	12	13	14					
TPOX	8				8			
FGA	20	23						
D19S433	13	14						
D2S1338	17	18						
Number of shared alleles between query sample and database profile:								17
Total number of alleles in the database profile:								18
Percent match between the submitted sample and the database profile:								94
The allele match algorithm compares the 8 core loci plus amelogenin only, even though alleles from all loci will be reported when available.								
NOTE: *Loci highlighted in grey (8 core STR loci plus Amelogenin) can be made public to verify cell identity. In order to protect the identity of the donor,* ***please do not publish*** *the allele calls from all the STR loci tested. Electropherograms showing raw data are attached.*								

Explanation of Test Results
Cell lines with 80% match are considered to be related; i.e., derived from a common ancestry. Cell lines with between a 55% to 80% match require further profiling for authentication of relatedness.

- [ ] The submitted sample profile is human, but not a match for any profile in the ATCC STR database.
- [ ] The submitted profile is an exact match for the following ATCC human cell line(s) in the ATCC STR database (8 core loci plus Amelogenin):
- [X] The submitted profile is similar to the following ATCC human cell line(s): CRL-2505
- [ ] An STR profile could not be generated.

Table 4. STR profiling performed by ATCC to verify that the 22Rv1 sample profile provided matches (94%) the human cell line CRL-2505.

Cell Line Authentication Service
STR Profile Report

FTA Barcode: STRB8076

ATCC Sales Order: SO0878071

Test Results for Submitted Sample					ATCC Reference Database Profile			
Locus	**Query Profile: MDA-PCa-2b**				**Database Profile: MDA PCa 2b; Prostate Adenocarcinoma; Human (Homo sapiens)**			
D3S1358	14	17						
TH01	8	9			8	9		
D21S11	33.2							
D18S51	16	17						
Penta_E	8	14						
D5S818	12	13			12	13		
D13S317	11	12			11	12		
D7S820	8	10			8	10		
D16S539	10	11			10	11		
CSF1PO	10	12			10	12		
Penta_D	11	13						
Amelogenin	X	Y			X	Y		
vWA	16				16			
D8S1179	13	14	15					
TPOX	8	11			8	11		
FGA	20	21	22					
D19S433	13.2	14.2	16					
D2S1338	22	24						
Number of shared alleles between query sample and database profile:								17
Total number of alleles in the database profile:								17
Percent match between the submitted sample and the database profile:								100

The allele match algorithm compares the 8 core loci plus amelogenin only, even though alleles from all loci will be reported when available.

NOTE: *Loci highlighted in grey (8 core STR loci plus Amelogenin) can be made public to verify cell identity. In order to protect the identity of the donor,* ***please do not publish*** *the allele calls from all the STR loci tested.*
Electropherograms showing raw data are attached.

Explanation of Test Results
Cell lines with 80% match are considered to be related; i.e., derived from a common ancestry. Cell lines with between a 55% to 80% match require further profiling for authentication of relatedness.

- [ ] The submitted sample profile is human, but not a match for any profile in the ATCC STR database.
- [X] The submitted profile is an exact match for the following ATCC human cell line(s) in the ATCC STR database (8 core loci plus Amelogenin): CRL-2422
- [ ] The submitted profile is similar to the following ATCC human cell line(s):
- [ ] An STR profile could not be generated.

Table 5. STR profiling performed by ATCC to verify that the MDA-PCa-2b sample profile provided matches (100%) the human cell line CRL-2422.

Cell Line Authentication Service
STR Profile Report

FTA Barcode: STRB7784

ATCC Sales Order: SO0917716

Test Results for Submitted Sample					**ATCC Reference Database Profile**			
Locus	**Query Profile: RC77T/E**				**Database Profile:**			
D3S1358	15	18						
TH01	8	9						
D21S11	28	30						
D18S51	15	17						
Penta_E	12	14						
D5S818	12	13						
D13S317	12	14						
D7S820	9	10						
D16S539	11	13						
CSF1PO	9	11						
Penta_D	2.2	5						
Amelogenin	X	Y						
vWA	15							
D8S1179	13	15						
TPOX	9	10						
FGA	22							
D19S433	13	14.2						
D2S1338	19	22						
Number of shared alleles between query sample and database profile:								NA
Total number of alleles in the database profile:								NA
Percent match between the submitted sample and the database profile:								NA
The allele match algorithm compares the 8 core loci plus amelogenin only, even though alleles from all loci will be reported when available.								
NOTE: *Loci highlighted in grey (8 core STR loci plus Amelogenin) can be made public to verify cell identity. In order to protect the identity of the donor,* ***please do not publish*** *the allele calls from all the STR loci tested. Electropherograms showing raw data are attached.*								

Explanation of Test Results
Cell lines with 80% match are considered to be related; i.e., derived from a common ancestry. Cell lines with between a 55% to 80% match require further profiling for authentication of relatedness.

- [X] The submitted sample profile is human, but not a match for any profile in the ATCC STR database.
- [ ] The submitted profile is an exact match for the following ATCC human cell line(s) in the ATCC STR database (8 core loci plus Amelogenin):
- [ ] The submitted profile is similar to the following ATCC human cell line(s):
- [ ] An STR profile could not be generated.

Table 6. STR profiling performed by ATCC to verify that the RC-77T/E sample profile provided is human, but not a match for any profile in the ATCC STR database. RC-77T/E is not available through ATCC. As such, I expected to see no match.

Following cell line authentication and mycoplasma testing, total RNA was extracted from cells using the PureLink RNA Mini Kit (Thermo Fisher Scientific, Cat. 12183025). RNA yield and quality were determined by the absorbance ratio at 260/280 nm using the NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, Cat. ND-ONE-W). Total RNA (1 µg) was reverse transcribed into cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Cat. K1622) and random hexamer primers following the manufacturer's protocol. I am currently optimizing primers for HER2 (L: gggaaacctggaactcaccta, R: ccctgcacctcctggata) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (L: gagtcaacggatttggtcgt, R: ttgattttggagggatctcg) commercially synthesized by Integrated DNA Technologies to establish HER2 mRNA transcript levels in all cell lines. Once primer optimization is complete using the ΔΔCT method, qPCR will be performed using the PowerUp SYBR Green Master Mix (Thermo Fisher Scientific, Cat. A25742) following the manufacturer's fast cycling mode protocol. qPCR amplification will be performed using the QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, Cat. A28137). Reactions will be performed in duplicates for three independent experiments and data will be normalized to the reference gene GAPDH. Fold-change in target gene expression treated cells relative to the untreated controls will be calculated.

Subtask 2: Examine HER2 protein expression in cancer cells using Western blot.
Cell lines used: MDA-PCa-2b, PC3, DU145, 22Rv1, RC-77T/E

To quantify HER2 protein expression in all cell lines, I originally proposed to use primary antibodies against HER2 and β-actin as a control for Western blot analysis with in-lab equipment (Invitrogen) using ECL chemiluminescence. As an alternative protocol, Western blot optimization using LI-COR with Total Protein Stain are underway. Briefly, equal amounts of protein from whole cell lysates (10 µg) are separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, Bolt NuPAGE 4–12%, Thermo Fisher Scientific, Cat:NW04120BOX) and transferred onto polyvinyl difluoride membranes (Millipore, Cat:IPFL00010). Western blot normalization is performed with LI-COR Revert 700 Total Protein Stain for Western Blot Normalization (Cat:926-11011). Membranes are blocked in LI-COR Intercept TBS Blocking Buffer (Cat:926-35010). Membranes are then probed individually with rabbit anti-HER2 antibody (1:1000) (Cat:50-190-774) and LI-COR IRDye 800CW goat anti-rabbit secondary antibody (1:15,000) (Cat:926-35010) and washed several times with TBS-T between each antibody application. Membranes are imaged with the Odyssey Imaging System in the 700 and 800 nm channels. Protein bands from at least 3 independent experiments will be quantified using ImageJ Software. The ratios of anti-HER2 protein bands to the Total Protein Stain will be normalized.

Major Task 2: Evaluate whether HER2 overexpression alters cell growth, clonogenicity, and migration.

Subtask 1: Generate HER2-overexpressing PC3 and RC-77T/E cell lines using lentiviral transfection method.
Cell lines used: PC3, RC-77T/E

To complete Major Task 2, Subtask 1, I am currently hiring a postdoctoral fellow. The postdoc will generate HER2-overexpressing (HER2-OE) PC3 (European American (EA)) and RC-77T/E (AA) cells using lentiviral transfection method. Briefly, cells will be plated in 6-well plates at a density of 1 – 5 x 10^5 cells/mL to produce 60% confluency in 24 hours. Pre-packaged lentiviral particles—either human HER2 (G&P Biosciences) or empty vector control (G&P Biosciences)—

with puromycin selection marker will be added to cell medium. Gene integration will be verified through qPCR and Western blot.

Subtask 2: Evaluate HER overexpression on cell growth with CellTiter-Glo Viability assay.
Cell lines used: PC3, RC-77T/E

To complete Major Task 2, Subtask 2, I am currently hiring a postdoctoral fellow. To evaluate whether HER2-OE alters cell growth compared to empty vector control, the postdoc will use CellTiter-Glo Viability assay which determines the number of viable cells by quantifying adenosine triphosphate (ATP). Briefly, cells will be plated in a 96-well plate and the CellTiter-Glo reagent added. This will be repeated at 0, 24, 48, and 72 hours. The plate will be read on a departmental Synergy H1 microplate reader (BioTek) using Gen5 software.

Subtask 3: Evaluate HER2 overexpression on clonogenicity.
Cell lines used: PC3, RC-77T/E

To complete Major Task 2, Subtask 3, I am currently hiring a postdoctoral fellow. To determine if HER2-OE alters clonogenicity, HER2-OE and empty vector control cells will be plated, and number and size of colonies will be visualized with light microscopy (10X) using Zeiss Observer Z1 located within the COHCCC Light Microscopy Digital Imaging Core (LMDIC) and quantified at 3 days with ImageJ software. Differences between empty vector control and HER2-OE cells as well as AA vs. EA cells will be analyzed using unpaired Student's t-test with GraphPad Prism 8. P values below 0.05 will be considered statistically significant.

Subtask 4: Evaluate HER2 overexpression on migration.
Cell lines used: PC3, RC-77T/E

To complete Major Task 2, Subtask 4, I am currently hiring a postdoctoral fellow. To evaluate migration of empty vector control and HER2-OE cells, a wound healing assay will be performed. Cell migration will be tracked with light microscopy (10X) at 0, 24, and 48 hours, and the wound recovery rate obtained in 6 independent experiments will be measured using Zeiss Observer Z1 located within the COHCCC LMDIC and quantified with ImageJ software. The wound area at 24 hours will be compared to the wound area at 0 hours for empty vector control cells and HER2-OE cells to determine the % wound recovery. Differences between empty vector control and HER2-OE cells as well as AA vs. EA cells will be analyzed using unpaired Student's t-test with GraphPad Prism 8. P values below 0.05 will be considered statistically significant.

Major Task 3: Evaluate whether HER2 blockade alters cell growth, clonogenicity, and migration.

Subtask 1: Treat cells with trastuzumab to inhibit HER2.
Cell lines used: PC3, RC-77T/E

I have inhibited HER2 pharmacologically with trastuzumab (Cat: 50-187-4657) reconstituted in PBS in PC3 and RC-77T/E cells. To determine an optimal concentration of trastuzumab treatments, initial dose-dependent (0 nM to 5 μM) experiments were conducted (**Figure 1**) and evaluated with CellTiter-Glo Viability (Cat: PR-G7572) at 72 hrs. Preliminary results suggest that trastuzumab is cytotoxic to RC-77T/E cells at concentrations higher than 20 nM, but not to PC3 cells.

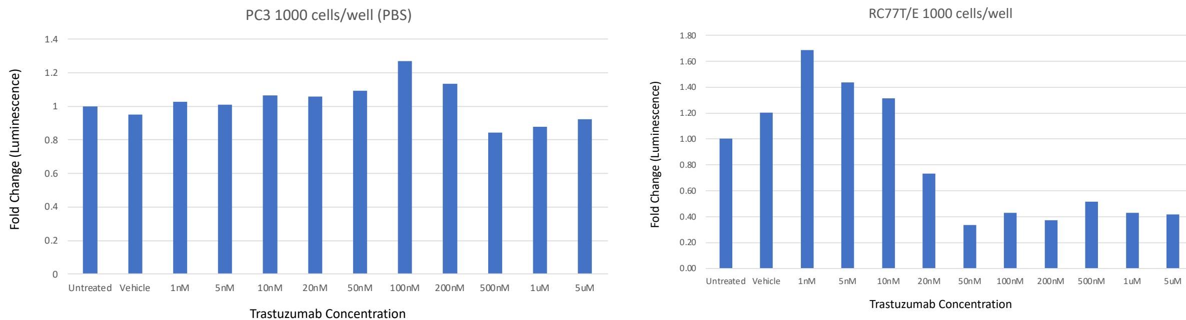


Figure 1. Fold change of viable PC3 and RC-77T/E cells in culture treated with varying concentrations of trastuzumab compared to untreated cells based on quantification of metabolically active cells using CellTiter-Glo Luminescent Viability Assay.

Subtask 2: Generate HER2 transient knockdown PC3 and RC-77T/E cell lines using siRNA.
Cell lines used: PC3, RC-77T/E

I am currently inhibiting HER2 genetically with small interfering RNA (siRNA) (Cat:6283) in PC3 and RC-77T/E cells. I am optimizing knockdown efficiency with a dose-dependent (50 nM to 100 nM oligo) and time-dependent (48 hr to 72 hr) experimental approach. I will verify efficient knockdown with Western blot analysis.

Subtask 3: Evaluate HER blockade on cell growth with CellTiter-Glo Viability assay.
Cell lines used: PC3, RC-77T/E

To evaluate whether HER2 blockade arrests cell growth, I will perform CellTiter-Glo Viability assay and compare results in untreated vs. treated cells as well as in siSD (scrambled control) cells vs. siHER2 (HER2 knockdown) cells in PC3 and RC-77T/E cells. For statistical analyses, I will use unpaired Student's t-test with GraphPad Prism 8. P values below 0.05 will be considered statistically significant.

Subtask 4: Evaluate HER2 blockade on clonogenicity.
Cell lines used: PC3, RC-77T/E

To evaluate whether HER2 blockade arrests clonogenicity, I will perform clonogenic assay and compare results in untreated vs. treated cells as well as in siSD (scrambled control) cells vs. siHER2 (HER2 knockdown) cells in PC3 and RC-77T/E cells. For statistical analyses, I will use unpaired Student's t-test with GraphPad Prism 8. P values below 0.05 will be considered statistically significant.

Subtask 5: Evaluate HER2 blockade on migration.
Cell lines used: PC3, RC-77T/E

To evaluate whether HER2 blockade arrests migration, I will perform wound healing assay and compare results in untreated vs. treated cells as well as in siSD (scrambled control) cells vs. siHER2 (HER2 knockdown) cells in PC3 and RC-77T/E cells. For statistical analyses, I will use unpaired Student's t-test with GraphPad Prism 8. P values below 0.05 will be considered statistically significant.

<u>Specific Aim 2: Confirm whether HER2 overexpression is positively correlated with WAA in tumor tissue specimens.</u>

Major Task 1: Access racially diverse tumor tissue specimens.

Subtask 1: Complete IRB approval to access tumor tissue specimens.

I have received IRB (Protocol 20619) approval from City of Hope (COH) (see below) to access tumor tissue specimens for this study from the COH Comprehensive Cancer Center (CCC) Frozen Tissue Bank as well as the Department of Defense (DOD) Prostate Cancer Biorepository Network (PCBN).

Clinical Research Protections
1500 East Duarte Road
Duarte, CA 91010-3000
Phone: (626) 218-2700
IRBeSubmit@coh.org

Institutional Review Board
Notification of Determination that Activity is Not Human Subjects Research

Date: November 04, 2020

To: Leanne Burnham, Ph.D, Principal Investigator
City of Hope - Cncr Genetics - Epigentics-BRI

From: Milda Plioplys, Director
Clinical Research Protections

COH Protocol #/Ref #: 20619 / 199945
Protocol Title: HER2 expression in men with prostate cancer
Regulatory Sponsor: City of Hope, U.S. Department of Defense (DOD)
Sponsor #:
Action Date: 11/03/2020
Action: NOT HUMAN SUBJECTS RESEARCH

The information provided for the above submission was evaluated and determined to not meet the definition of human subjects research as set forth at (45 CFR 46.102 (e)). Accordingly, IRB approval and continuing review is not required.

Please note that if any changes occur on this project, notification to the IRB is required to determine if the status of this project should be updated to be under the purview of the IRB.

Please note the following:

1. If this project is receiving funding from the Department of Defense (DOD), it will require an additional determination made by their Human Research Protections Office (HRPO) before proceeding by submitting to dha.ncr.dha-cs-mgt.mbx.hrpp@mail.mil.
2. The IRB protocol application form has been corrected. The participation of Antelope Valley and Santa Clarita have been removed as these two sites are not approved by COH to begin research at this time.

If you have questions or concerns about this submission, please contact Gwen Jorgensen or IRBeSubmit@coh.org.

Subtask 2: Access FFPE.
FFPE: 400 patients (100 biopsy negative, 100 lymph node negative, 100 lymph node positive, 100 mCRPC) [COHCCC Frozen Tissue Bank]

COH Honest Broker/Research Informatics has identified a dataset of clinical FFPE specimens from the COH Comprehensive Cancer Center (CCC) Frozen Tissue Bank previously collected from an equal amount Black/AA and White male patients at different disease stages (Group 1: normal prostate [normal prostate removed as part of cystoprostatectomy]; Group 2: prostate cancer, prostatectomy, and TNM status of T any, N0, Mx; Group 3: prostate cancer, prostatectomy, and TNM status of T any, N1, Mx; and Group 4: diagnosis of prostate cancer where tissue is NOT prostate, tissue might be bone or lymph node, lung or liver). FFPE specimens are currently being pulled by COH Pathology Core for IHC analysis.

Subtask 3: Access TMA and patient-matched DNA.
TMA: 456 Case Race Disparity TMA [DOD]

I was able to receive approval from the DOD PCBN and access the 456 Case Race Disparity tissue microarray (TMA) (please see below) as well as patient-matched DNA. As an alternative to purchasing the patient-matched DNA, the DOD PCBN negotiated with Washington University in St. Louis to be able ship patient-matched frozen cell pellets to Dr. Rick Kittles' lab for me to extract genomic DNA. An MTA agreement between Washington University in St. Louis and COH was settled (please see below), and 456 frozen cell pellets were shipped to Dr. Kittles' lab (Date of Receipt 12/08/21).

PROSTATE CANCER **BIOREPOSITORY NETWORK**

Tissue, Fluids, and Derived Biospecimens Request Application Form
Form No: 001

Application #

SECTION A: Applicant Details		
Principal Investigator (PI) Name Leanne Burnham, Ph.D.	PI Telephone 626-256-4673 Ext. 81631	PI Email lburnham@coh.org
Institution City of Hope Comprehensive Cancer Center		Department Population Sciences
Institution Address 1500 E. Duarte Rd., Duarte, CA 91010 Rick Kittles Laboratory Shapiro Building 096 Room 1038 Attn: Leanne Burnham,		Institution Type ☐ Academic/Government ☐ Commercial ☑ Non-profit
Contact Person Leanne Burnham, Ph.D.	Contact Telephone 626-256-4673 Ext. 81631	Contact Email lburnham@coh.org
Legal Contact Person (MTA purposes) **Sylvia Campos**	Legal Contact Telephone (MTA purposes) **626-256-4673 Ext. 81811**	Legal Contact Email (MTA purposes) **scampos@coh.org**

SECTION B: Billing Information		
Billing Contact Person **Claudio Carbajal**	Billing Contact Telephone **626-256-4673 Ext. 85488**	Billing Contact Email **ccarbajal@coh.org**
Billing Address **1500 E. Duarte Rd. Duarte, CA 91010**	☐ Same as Institution Address	Payment Details ☑ Purchase Order ☐ Credit Card ☐ Other
Funding Source Department of Defense Prostate Cancer Research Program Early Investigator Award	Grant ID # W81XWH2110038	Grant End Date 31 Dec 2022

SECTION C: Project Information
Project Title HER2 Expression in African American Men with Prostate Cancer
Hypothesis HER2 overexpression is positively correlated with West African ancestry and contributes to worse clinical features, treatment response, and survival outcomes.

Specific Aims

Aim 1: Establish that HER2 expression promotes cellular growth, clonogenicity, and migration in a racially diverse panel of prostate cancer cell lines.

Aim 2: Confirm whether HER2 overexpression is positively correlated with West African ancestry in tumor tissue specimens.

Aim 3: Determine if HER2 overexpression correlates with disease stage, clinical features, treatments, and outcomes in prostate cancer patients.

Protocol / Method to be used

Aim 1: Establish that HER2 expression promotes cellular growth, clonogenicity, and migration in a racially diverse panel of prostate cancer (PCa) cell lines.
Step 1.1 Quantify basal HER2 gene and protein levels in a racially diverse panel of cell lines.
Step 1.2 Evaluate whether HER2 overexpression differentially alters cell growth, clonogenicity, and migration.
Step 1.3 Evaluate whether HER2 blockade differentially arrests cellular growth, clonogenicity, and migration.

Aim 2: Confirm whether HER2 overexpression is positively correlated with West African ancestry (WAA) in tumor tissue specimens.
Step 2.1 Access racially diverse tumor tissue specimens from the COHCCC Frozen Tissue Bank and the Department of Defense Prostate Cancer Biorepository Network (DOD PCBN). We will access FFPE primary PCa specimens from the COHCCC Frozen Tissue Bank previously collected from a racially/ethnically diverse cohort of male patients at different disease stages (Group 1: biopsy negative [50 African American (AA), 50 European American (EA)], Group 2: lymph node negative at radical prostatectomy [50 AA, 50 EA], Group 3: lymph node positive at radical prostatectomy (RP) [50 AA, 50 EA], and Group 4: mCRPC [50 AA, 50 EA]). We will also access the 459 Case Race Disparity tissue microarray (TMA) (153 AA, 306 EA) collected at RP through the DOD PCBN.
Step 2.2 Quantify HER2 gene and protein expression in tumor tissue specimens. We will conduct IHC membrane staining on City of Hope Comprehensive Cancer Center (COHCCC) FFPE and DOD PCBN TMA specimens with the assistance of COHCCC Pathology Core. Tumors with absent or weak membrane HER2 staining (scored as 0 or 1+) will be regarded as HER2 negative. Tumors with intense and complete membrane HER2 staining (scored as 3+) will be regarded as HER2+. Tumors with moderate membrane HER2 staining (scored as 2+) will be regarded as HER2 equivocal and reflexively tested by fluorescent in situ hybridization (FISH) with the assistance of COHCCC Pathology Core to assess for HER2 gene amplification. A dual-probe HER2/chromosome enumeration probe 17 (HER2/CEP17) ratio >2.0 or average HER2 copy number >6.0 signals per cell will be used to classify tumors as HER2+. Data generated from each self-reported race/ethnicity will allow for detection of an effect size of 0.249 at 80% power (t-test , $\alpha = 0.05$) for intra- and inter-group differential expression analysis of 200 FFPE specimens and 0.246 at 80% power (t-test , $\alpha = 0.05$) for 456 Case Race Disparity TMA specimens.
Step 2.3 Calculate genetic ancestry proportion of study patients' DNA using validated ancestry informative markers (AIMs) through single nucleotide polymorphism (SNP) genotyping. To calculate genetic ancestry, we will obtain patient-matched DNA from COHCCC and DOD PCBN (Step 2.1). we will use a panel of AIMs developed to determine proportions of West African, European, and Indigenous American ancestry and to distinguish populations of different biogeographic origins. We will genotype isolated genomic DNA using the Sequenom MassARRAY platform with iPLEX chemistry. Individual admixture estimates will be calculated using a model-based clustering method as implemented in STRUCTURE 2.3.
Step 2.4 Compare HER2 expression to WAA proportion in the AA tumor tissue specimens. For statistical analyses, we will conduct linear regression with an 80% power to detect an effect size of 0.027 ($\alpha = 0.05$) to determine the relationship between HER2 expression (Step 2.2) and WAA (Step 2.3) in the 353 (200 FFPE, 153 TMA) (Step 2.1) AA tumor tissue specimens.

Aim 3: Determine if HER2 overexpression correlates with disease stage, clinical features, treatments, and outcomes in PCa patients.
Step 3.1 Stratify tumor tissue specimens by disease stage, clinical features, treatments, and outcomes. We will stratify FFPE primary PCa specimens from the COHCCC Frozen Tissue Bank (Step 2.1) by disease stage (Group 1: biopsy negative [100], Group 2: lymph node negative at radical prostatectomy ([100], Group 3: lymph node positive at radical prostatectomy [100], and Group 4: mCRPC [100]). The Institutional Review Board (IRB)-approved biobank includes clinical annotation; data will be abstracted related to relapse and survival. For the 456 Case Race Disparity TMA obtained through the DOD PCBN, we will request available corresponding matched clinical data upon approval for similar analysis.
Step 3.2 Evaluate HER2 expression correlation with disease stage, clinical features, treatment, and outcomes of PCa patients. For statistical analyses, we will conduct logistic regression and multivariable Cox regression with $\alpha = 0.05$ to determine the relationship between HER2 overexpression (Step 1.2) and disease stage (Step 3.1) as well as clinical features (i.e. PSA, Gleason score, lymph node status), treatments (i.e. primary hormone therapy and radiation), and outcomes (i.e. biochemical recurrence and overall survival) of PCa patients.

Name of Pathologist associated with the study (If TMA or tissue slides require microscopic examination)

Zhirong Yin, M.D.

IRB Approval Type	IRB Approval Number	IRB Approval Dates
☒ Full ☐ Expedited ☐ Exempt	20619	Nov. 4, 2020 Apr. 2, 2021

SECTION D: Biospecimen Information

Biospecimen Type	# Cases	Justification for # Cases Requested (Statistical Power Analysis)
Patient-matched DNA (matched to 456 Case Race Disparity TMA)	456	Data generated from each self-reported race/ethnicity will allow for intra- and inter-group differential expression analysis for HER2 detection of an effect size of 0.246 at 80% power (t-test , $\alpha = 0.05$) for 456 Case Race Disparity TMA specimens. For statistical analyses, we will conduct linear regression with an 80% power to detect an effect size of 0.027 ($\alpha = 0.05$) to determine the relationship between HER2 expression (Step 2.2) and WAA (Step 2.3) in the 353 (200 FFPE, 153 TMA) (Step 2.1) AA tumor tissue specimens.

Case Characteristics (optional information if you have specific requirements)

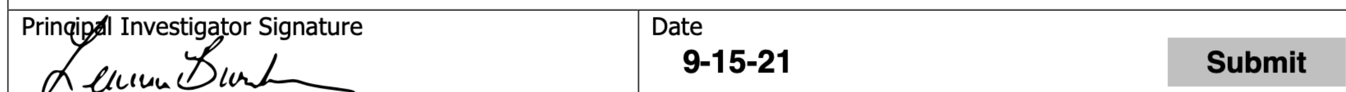
Procedure Type ☐Radical Retropubic Prostatectomy ☐Robot-Assisted Prostatectomy ☐Biopsy ☐Transurethral Resection ☐Autopsy				
Age at Surgery Min Max	Neo-adjuvant Treatment ☐ Yes ☐ No	Adjuvant Treatment ☐ Yes ☐ No	pStage Min Max	Gleason Score Min Max

Sample Preparation Details (optional information if you have specific requirements)

For Tissue			
☐ FFPE Section ☐ Core	☐ Frozen Section ☐ Touch Prep	☐ Scroll / Ribbon ☐ Other	☐ Macrodissection
☐ Malignant ☐ Other	☐ Benign	☐ Normal	☐ Tumor /Benign Pairs
For Body Fluids			
Biospecimen Minimum Volume		☐ mL	☐ uL
For Derived Biospecimens (DNA/RNA/Protein)			
Origin ☐ Frozen Tissue ☐ Plasma	☐ Fixed Tissue ☐ Serum	☐ Seminal Vesicle ☒ Buffy Coat	☐ Prostatic Fluid ☐ Seminal Vesicle Fluid
Biospecimen Minimum Amount (e.g. 100 ng total RNA) **800ng genomic DNA at 10ng/uL**			
Additional Requirements			

SECTION E: Shipping Information	
Shipping Address	☒ Same as Institution Address

We/I have read, understood and agree with the Tissue Access Policy and Conditions of Use for Tissue And Data Bank Resources. We/I agree that the samples provided by PCBN will be used for the research work detailed in the attached proposal. The material will not be used for other studies, or distributed to third parties. Tissues and their products will not be used for commercial purposes. We/I realize that there is the potential that this human biological material may contain infectious agents and, therefore, will handle it appropriately.



Principal Investigator Signature	Date **9-15-21**	**Submit**

DocuSign Envelope ID: FE3CDA79-DC5C-4C96-BE00-68E6B401B00F

Letter Agreement for the Transfer of Human Specimens
The Washington University

Whereas, The Washington University**,** One Brookings Drive, St. Louis, MO, 63130, USA (PROVIDER) possesses coded human tissue samples (MATERIAL); and

Whereas, the non-profit institution listed below (RECIPIENT) has requested from PROVIDER's investigator, Dr. Bettina Drake, the MATERIAL further described as: Frozen Cell Pellets (200um) from 456 Cases from Race Disparity TMA and HER2 IHC data on the TMA set for use in the following not-for-profit research project (PROJECT):

This pilot study is the first to investigate HER2 (ERBB2) and androgen receptor (AR/NR3C4) status in mCRPC patients inclusive of African American men. We anticipate that circulating HER2 and AR as well as ERBB2 and NR3C4 gain will be increased in African American men. Successful completion of this study will generate preliminary data to support applications for future funding to 1) fully evaluate whether HER2 contributes to the worse prognosis experienced by African American men and 2) to develop more effective treatment strategies for advanced PCa patients with increased HER2 and AR.

In response to RECIPIENT's request for the MATERIAL the PROVIDER and the RECIPIENT agree to the following before the RECIPIENT receives the MATERIAL:

1. The above MATERIAL is the property of the PROVIDER and is made available as a service to the research community. MATERIAL provided pursuant to this Agreement was collected or will be collected in accordance with the standard patient informed consent procedures of the PROVIDER in effect at the time of collection and subject to approval by the PROVIDER's IRB. MATERIAL provided to RECIPIENT by PROVIDER will not contain personally identifiable patient information and will not include "Protected Health Information" ("PHI") as defined in 45 C.F.R. Section 160.103. The parties agree that the human data and/or biospecimens transferred under this Agreement may be subject to NIH Policy NOT-OD-17-109 (the "Policy") and therefor is deemed under the Policy to be issued a Certificate of Confidentiality. Accordingly the RECIPIENT is required to adhere to the Policy and protect the privacy of the individuals from whom the data and/or biospecimens were collected in accordance with the Policy and subsection 301(d) of the Public Health Service Act.

2. THIS MATERIAL IS NOT FOR USE IN HUMAN SUBJECTS.

3. The MATERIAL will be used for not-for-profit research purposes in conduct of the above PROJECT only.

4. The MATERIAL will not be further distributed to others, outside of RECIPIENT's Scientist and RECIPIENT'S employees, medical staff, agents and representatives having a need for access to the MATERIAL in order to conduct the PROJECT, without the PROVIDER'S written consent. The RECIPIENT shall refer any request for the MATERIAL to the PROVIDER.

5. The RECIPIENT agrees to acknowledge the source of the MATERIAL in any publications and presentations reporting use of the MATERIAL.

6. The RECIPIENT agrees that it will not have or seek access to any identifiable information (such as the key to the code) under any circumstances. The RECIPIENT agrees to never use the MATERIAL or any substance derived from the MATERIAL (e.g. DNA, RNA or any information provided with the MATERIAL) to attempt to ascertain the identity of the individual from whom the MATERIAL was obtained.

7. Any MATERIAL delivered pursuant to this Agreement is understood to be experimental in nature and may have hazardous properties and may carry transmissible infectious agents. THE PROVIDER MAKES NO REPRESENTATIONS AND EXTENDS NO WARRANTIES OF ANY KIND, EITHER EXPRESSED OR IMPLIED. THERE ARE

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NO EXPRESS OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR THAT THE USE OF THE MATERIAL WILL NOT INFRINGE ANY PATENT, COPYRIGHT, TRADEMARK, OR OTHER PROPRIETARY RIGHTS. Unless prohibited by law, RECIPIENT assumes all liability for claims for damages against it by third parties which may arise from the use, storage or disposal of the MATERIAL except that, to the extent permitted by law, the PROVIDER shall be liable to the RECIPIENT when the damage is caused by the gross negligence or willful misconduct of the PROVIDER.

8. The RECIPIENT agrees to use the MATERIAL in compliance with all applicable U.S. federal, California state and local statutes and regulations.

9. The MATERIAL is provided according to the amount indicated in the provided PCBN Cost Recovery Structure document and the RECIPIENT will be further responsible for all shipping costs.

The RECIPIENT and RECIPIENT SCIENTIST must sign this letter and return one signed copy to the PROVIDER. Upon receiving this fully executed agreement, the PROVIDER will then send the MATERIAL.

RECIPIENT INFORMATION and AUTHORIZED SIGNATURE

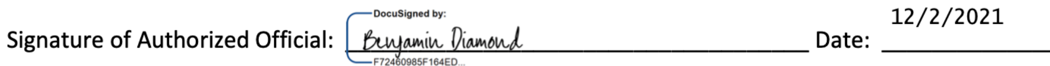
Recipient Scientist: Dr. Leanne Burnham

Recipient Organization: City of Hope National Medical Center and Beckman Research Institute of the City of Hope, each a California nonprofit, public benefit corporation.

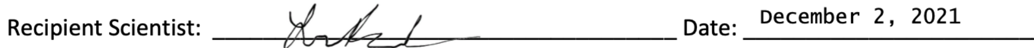
Address: 1500 E. Duarte Rd. Duarte, CA 91010

Name of Authorized Official: Benjamin Diamond

Title of Authorized Official: Senior Contract Administrator

Signature of Authorized Official:  Date: 12/2/2021

Certification of Recipient Scientist: Although not a legal party to this Agreement, I have read and understood the conditions outlined in this Agreement.

Recipient Scientist:  Date: December 2, 2021

PROVIDER AUTHORIZED SIGNATURE

Name of Authorized Official: Nichole R. Mercier, PhD

Title of Authorized Official: Assistant Vice Chancellor & Managing Director

Signature of Authorized Official: Date: December 2, 2021

Major Task 2: Quantify HER2 in tumor tissue specimens.

Subtask 1: Perform IHC to detect HER2.
FFPE: 400 patients [COHCCC Frozen Tissue Bank]
TMA: 456 Case Race Disparity TMA [DOD]

The COHCCC Pathology Core will perform HER2 IHC membrane staining on COHCCC FFPE specimens that have been identified. Dr. Zhirong Yin is the research pathologist who will stain and score the COH specimens. Washington University in St. Louis has completed HER2 IHC membrane staining and scoring of the DOD PCBN TMA specimens to be analyzed by Dr. Stanley Hooker at COH. Both COH and Washington University in St. Louis will use and have used the Ventana staining protocol optimized and drafted by Dr. Yin (please see below). Data generated from each self-reported race/ethnicity will allow for detection of an effect size of 0.249 at 80% power (*t*-test , $\alpha = 0.05$) for intra- and inter-group differential expression analysis of 200 FFPE specimens and 0.246 at 80% power (*t*-test , $\alpha = 0.05$) for 456 Case Race Disparity TMA specimens.

Pathology Research Services Core
Beckman Research Institute of City of Hope

HER2 Antibody VALIDATION REPORT:

Antibody: Her 2	Species: Rabbit Monoclonal	Clone: 4B5
Titer/Concentration: pre-diluted dispenser	Catalog #: 790-2991	Localization: membrane

Status & validation from Ventana:

The Ventana Medical Systems, Inc.'s (Ventana) PATHWAY anti-HER-2/*neu* (4B5) is a FDA/CE-IVD approved HER2 IHC assays, and it has been used as reference standard method. PATHWAY anti-HER-2/*neu* is a rabbit monoclonal antibody (clone 4B5) against the internal domain of the c-erbB-2 oncoprotein (HER2).

Clone 4B5 has been shown to react with a 185 kD protein from SK-BR-3 cell lysates via Western blotting. SK-BR-3 is a breast carcinoma cell line, which has a 128-fold over expression of HER2 mRNA. The size of the band identified correlates well with that reported by Akiyama et al for HER2 protein (185 kD). Staining results in normal tissues, neoplastic tissues, and 322 cases of breast carcinoma with PATHWAY HER2 (4B5) were evaluated by Ventana.

Material and Methods:

All the specimen was fixed for 24-48 h in 10% neutral buffered formalin. The paraffin embedded tissue sections in 4μM thickness were prepared in Pathology Core at City of Hope.

PATHWAY anti-HER-2/*neu* (4B5) was used to detect HER2 antigen in sections of formalin-fixed, paraffin-embedded normal and prostate cancer tissue on the VENTANA Discovery Ultra automated immunohistochemistry slide staining device. PATHWAY HER2 (4B5) antibody binds to HER2 in paraffin-embedded tissue sections. The specific antibody can be localized by a HQ conjugated secondary antibody formulation that recognizes rabbit immunoglobulins followed by the addition of a secondary antibody-HRP conjugate. The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product (DISCOVERY ChromoMap DAB Kit). Each step is incubated for a precise time and temperature. At the end of each incubation step, the VENTANA automated slide stainer washes the sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. The detail of protocol is as the following:

Ventana Discovery Ultra HER2 IHC staining protocol

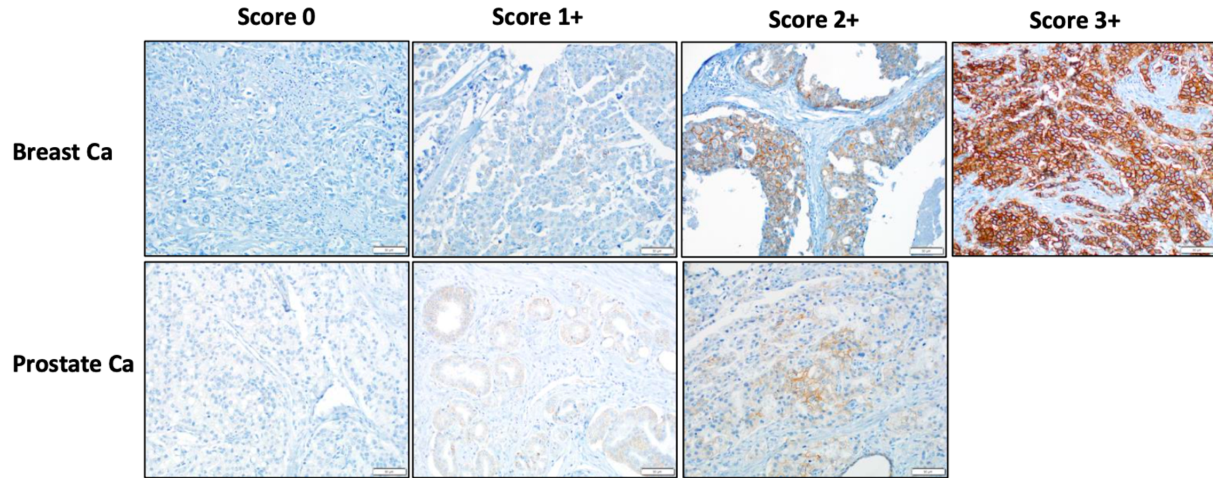
1. Load slides and reagents onto Ventana Discovery Ultra stainer.
2. Deparaffinization in EZ prep 69°C 24 minutes.
3. Cell Conditioning using Conditioner #1, Standard CC1, 95°C 64 minutes.
4. Block with Inhibitor CM, 37°C 8 minutes.
5. Incubation with HER2 primary antibody for 16 minutes.
6. Apply one drop of Discovery anti-Rb HQ and incubate for 12 minutes.
7. Apply one drop of Discovery anti-HQ HRP and incubate for 12 minutes.
8. Apply DAB kit including DAB CM, H_2O_2 CM and Copper CM, incubate 8 minutes.
9. Counterstain with Hematoxylin, incubate for 16 minutes.
10. Post counterstain with Bluing Reagent, incubate for 4 minutes.
11. Slide Cleaning.
12. Coverslipper.

Quality Control

A TMA consisting of 31 tissues of breast cancer with known positive HER2 expression, serving as positive control tissue and 7 tissues of normal breast, serving as negative control tissue used for a preliminary validation of the processing method used for staining slides with PATHWAY HER2 (4B5), and for performance controls across staining runs. A negative reagent control was also run for every specimen to aid in the interpretation of results, in which CONFIRM Negative Control Rabbit Ig is used in place of the primary antibody to evaluate nonspecific staining.
In addition, another TMA was used for testing the PATHWAY HER2 (4B5) specificity. The results showed no specific membrane staining for tonsil (1/4, focal staining of surface epithelial cells), and weak membrane staining on lung cancer (1/2) and pancreatic cancer (1/2).

Scoring of PATHWAY HER2 (4B5) on representative images

Score	ER2 Staining Assessment	Staining Pattern
0	Negative	No membrane staining is observed
1+	Negative	Faint, partial staining of the membrane in any proportion of the cancer cells
2+	Weakly Positive	Weak complete staining of the membrane, greater than 10% of cancer cells
3+	Positive	Intense complete staining of the membrane, greater than 10% of cancer cells



Zhirong Yin, MD, Ph.D

Research Pathologist
Pathology Cores & Biobanking| Shared Resources
City of Hope
Office: Familian Sciences Bldg. (#84), Lower level-L001F
Phone: (626) 218- 6840 (ext. 86840)
Email: zhyin@COH.org
1500 East Duarte Rd. Duarte, CA 91010

Subtask 2: Perform FISH to confirm overexpression in HER2 equivocal samples.
FFPE: 400 patients [COHCCC Frozen Tissue Bank]
TMA: 456 Case Race Disparity TMA [DOD]

Tumors with moderate membrane HER2 staining (scored as 2+) will be regarded as HER2 equivocal and reflexively tested by fluorescent in situ hybridization (FISH) with the assistance of COHCCC Pathology Core to assess for HER2 gene amplification. A dual-probe HER2/chromosome enumeration probe 17 (HER2/CEP17) ratio >2.0 or average HER2 copy number >6.0 signals per cell will be used to classify tumors as HER2+. Data generated from each self-reported race/ethnicity will allow for detection of an effect size of 0.249 at 80% power (*t*-test , $\alpha = 0.05$) for intra- and inter-group differential expression analysis of 200 FFPE specimens and 0.246 at 80% power (*t*-test , $\alpha = 0.05$) for 459 Case Race Disparity TMA specimens.

Major Task 3: Calculate genetic ancestry of patients.

Subtask 1: Extract genomic DNA.
FFPE: 400 patients [COHCCC Frozen Tissue Bank]
TMA: 456 Case Race Disparity TMA frozen cell pellets [DOD]

I am currently extracting and quantifying genomic DNA from frozen cell pellets which are patient-matched with the 456 Case Race Disparity TMA from DOD PCBN. I will be extracting genomic DNA from FFPE specimens accessed from the COHCCC Frozen Tissue Bank. A newly hired intern in my lab is assisting with these experiments. For genomic DNA extraction, we are using the Invitrogen PureLink Genomic DNA Kit (Cat: K1820-01).

Subtask 2: SNP genotyping using a panel of validated AIMs.
FFPE: 400 patients [COHCCC Frozen Tissue Bank]
DNA: 459 Case Race Disparity TMA [DOD]

To calculate genetic ancestry, Dr. Kittles' lab will use a panel of ancestry informative markers (AIMs) developed to determine proportions of West African, European, and Indigenous American ancestry and to distinguish populations of different biogeographic origins[12]. We will genotype isolated genomic DNA using the Sequenom MassARRAY platform with iPLEX chemistry. Individual admixture estimates will be calculated using a model-based clustering method as implemented in STRUCTURE 2.3.

Subtask 3: Compare HER2 expression to WAA.

For statistical analyses, Dr. Stanley Hooker will conduct linear regression with an 80% power to detect an effect size of 0.027 ($\alpha = 0.05$) to determine the relationship between HER2 expression and WAA in the 353 (200 FFPE, 153 TMA) AA tumor tissue specimens.

<u>Specific Aim 3: Determine HER2 correlation with PCa disease stage, clinical features, treatments, and outcomes in patients.</u>

Major Task 1: Stratify tumor tissue specimens.
FFPE: 400 patients [COHCCC Frozen Tissue Bank]
TMA: 459 Case Race Disparity TMA [DOD]

FFPE PCa specimens from the COHCCC Frozen Tissue Bank have been stratified by COH Honest Broker/Research Informatics by disease stage (Group 1: normal prostate [normal prostate removed as part of cystoprostatectomy]; Group 2: prostate cancer, prostatectomy, and TNM status of T any, N0, Mx; Group 3: prostate cancer, prostatectomy, and TNM status of T any, N1, Mx; and Group 4: diagnosis of prostate cancer where tissue is NOT prostate, tissue might be bone or lymph node, lung or liver). The IRB-approved biobank includes clinical annotation; data will be abstracted related to relapse and survival. For the 456 Case Race Disparity TMA obtained through the DOD PCBN, corresponding matched clinical data has been made available to me.

Major Task 2: Correlate HER2 expression with disease stage, clinical features, treatments, and outcomes.
FFPE: 400 patients [COHCCC Frozen Tissue Bank]
TMA: 459 Case Race Disparity TMA [DOD]

For statistical analyses, Dr. Stanley Hooker will conduct logistic regression and multivariable Cox regression with $\alpha = 0.05$ to determine the relationship between HER2 overexpression and disease stage as well as clinical features (i.e., PSA, Gleason score, lymph node status), treatments (i.e., primary hormone therapy and radiation), and outcomes (i.e., biochemical recurrence and overall survival) of PCa patients.

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

This funding opportunity as a DOD Prostate Cancer Research Program Early Investigator has greatly contributed to my professional development. In May 2021, I accepted a new appointment to Assistant Research Professor in the Department of Population Sciences Division of Health Equities at City of Hope. This funding opportunity has allowed me to transition to an independent tenure-track faculty position. In January 2022, I accepted a new appointment as Assistant Professor in the Department of Population Sciences Division of Health Equities at City of Hope.

In terms of my training development, I have learned the intricacies of the IRB review process. I learned how to draft protocols, present the study, and receive endorsement from COH's Genitourinary Disease Team, and respond to IRB requests to ensure full approval. I also learned how to navigate the process of identifying samples through COH Honest Broker/Research Informatics as well as how to engage COH's Pathology Core for collaborative purposes. I discuss progress for this study weekly with my mentor, Dr. Rick Kittles. I also meet monthly to share study results and discuss goals with my mentors, Dr Rick Kittles and Dr. Tanya Dorff. I also meet with Dr. Sean Kimbro twice a year for specific feedback and direction regarding the molecular biology techniques specific to this study.

In terms of training development of others who worked on the project, I was able to hire a lab intern in August 2021 named Serene Dowiri. Ms. Dowiri has been able to learn and execute many wet lab techniques including cell culture, lysate collection, CellTiter-Glo Viability assay, genomic DNA extraction, and quantification of DNA using NanoDrop.

How were the results disseminated to communities of interest?

An overview of the goals of this study supported by this funding opportunity was disseminated to several communities of interest in virtual webinars including the following:

02/23/21 "Tackling Prostate Cancer Health Disparities at the Bench, Clinic, and Community", OncLive State of the Science Summit (Sponsored by City of Hope)

04/15/21 "What is Special About Research at City of Hope", Building Hope Toast of Hope Event, City of Hope

06/30/21 "Dine in for Hope", City of Hope

07/31/21 "Clinical Trials: An Answer to Cancer?", Cynthia Perry Ray Foundation Sistahs Can We Talk Monthly Nurses Series (Sponsored by City of Hope)

09/18/21 "Prostate Cancer in Black Men: The Latest in Screening and Treatment Options", Prostate Cancer Foundation & The West Angeles Church of God in Christ

09/23/21 "Tackling Prostate Cancer Health Disparities at the Bench, Clinic, and Community", City of Hope Community Engagement Community Advisory Board

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

During the next reporting period, I plan on completing the following underlined tasks with the methodologies described previously.

Specific Aim 1: Establish that HER2 expression promotes cellular growth, clonogenicity, and migration in a racially diverse panel of PCa cell lines.

Major Task 1: Quantify basal HER2 gene and protein levels.
Target Dates: 1-6 months
Percentage of Completion: 50%

Major Task 2: Evaluate whether HER2 overexpression alters cell growth, clonogenicity, and migration.
Target Dates: 6-12 months
Percentage of Completion: 0%

Major Task 3: Evaluate whether HER2 blockade alters cell growth, clonogenicity, and migration.
Target Dates: 9-15 months
Percentage of Completion: 30%

Specific Aim 2: Confirm whether HER2 overexpression is positively correlated with WAA in tumor tissue specimens.

Major Task 1: Access racially diverse tumor tissue specimens.
Target Dates: 1-15 months
Percentage of Completion: 100%

Major Task 2: Quantify HER2 in tumor tissue specimens.
Target Dates: 12-24 months
Percentage of Completion: 100%

Major Task 3: Calculate genetic ancestry of patients.
Target Dates: 12-18 months
Percentage of Completion: 10%

Specific Aim 3: Determine HER2 correlation with PCa disease stage, clinical features, treatments, and outcomes in patients.

Major Task 1: Stratify tumor tissue specimens.
Target Dates: 12-24 months
Percentage of Completion: 50%

Major Task 2: Correlate HER2 expression with disease stage, clinical features, treatments, and outcomes.
Target Dates: 12-24 months
Percentage of Completion: 0%

I plan on presenting results of this study at the 15th American Association for Cancer Research Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved. In addition, I plan on submitting final results for peer-reviewed publication within 120 calendar days of the award performance end date.

REPORTABLE OUTCOMES

IMPACT

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

Metastatic prostate cancer (PCa) is a lethal disease and therapeutic options are limited for patients. Since there is no cure for advanced PCa, the pursuit of therapeutic strategies to effectively treat this stage remains a critical goal. The ability to target non-androgenic growth factor signaling pathways that may promote metastasis represents a tangible and promising area of investigation. With the development of targeted therapeutic options optimized for a molecularly enriched population, there is potential to improve survival outcomes for men at high-risk for an advanced stage diagnosis. By providing an additional option to the current arsenal of available treatments, there is an opportunity to provide hope to late-stage patients and their families.

We need to better understand the biology contributing to the worse prognosis observed in African American (AA) PCa patients in order to 1) predict which men are more likely to suffer from aggressive disease and 2) develop more patient-specific treatments. Because the majority of biospecimens and patients evaluated in research studies are of European descent, we miss potential genetic indicators that steer molecular mechanisms, disease outcomes, and drug response.[13] This experimental approach is counterintuitive as AA men are more likely to suffer disproportionate PCa incidence and mortality, but are less likely to be included in key studies and clinical trials. Since recent studies have revealed better treatment response and increased overall survival in AA men receiving PCa drug treatments, there is additional incentive to decrease the current racial disparity in mortality with precision medicine.

Given that my preliminary evidence suggests a potential increase in HER2 overexpression in AA men with PCa, I predict that AA patients with metastatic disease may especially benefit from HER2-targeted treatment. This study is the first to investigate HER2 status by race in PCa patients. Successful completion of this study will allow me to apply for future funding to support a Phase II race-stratified clinical trial with a focused AA target accrual to treat HER2 positive (HER2+) PCa patients with newer, more efficient anti-HER2 drugs. A precision medicine approach may allow for the repurposing of drugs currently approved to treat HER2+ breast cancer patients and potentially improve overall survival for currently incurable stages of PCa. The ability to repurpose currently FDA-approved HER2-targeted drugs for PCa patients would also drastically reduce the transition time of expanding the availability of effective treatment from clinical trial patients to the general population. Timely completion of this and a subsequent clinical trial would have a major near-term impact in lessening the burden of PCa mortality in AA men and would provide an additional treatment option for others with HER2+ metastatic PCa.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

The potential of improving racial/minority enrollment in clinical trials has a direct influence on survival outcomes in these populations. With the appropriate focus and commitment, inclusive research studies such as this will lead to greater potential for clinical trials that diverse and inclusive thereby increasing access to cutting-edge therapies for high-risk minority populations. Continued efforts to strategically increase minority enrollment in clinical trials will ultimately help eliminate health disparities by allowing for optimized plans of treatment based on observed and documented variations in treatment response.

CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to Report

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

Nothing to Report

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

Nothing to Report

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

PRODUCTS

Publications, conference papers, and presentations

Journal publications

Nothing to Report

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

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	***Leanne Burnham***
Project Role:	*PI, Assistant Professor*
Research Identifier (e.g., ORCID ID):	*0000-0003-0856-6386*
Nearest person month worked:	*12*
Contribution to Project:	*Dr. Burnham has performed experimental work for Specific Aim 1 and 2, completed IRB application through COH to obtain approval, secured DOD PCBN specimens through MTA between COH and Washington University in St. Louis.*
Funding Support:	*Current DOD W81XWH2110038*
Name:	***Serene Dowiri***

Project Role:	*Intern*
Research Identifier (e.g., ORCID ID):	*N/A*
Nearest person month worked:	*4*
Contribution to Project:	*Ms. Dowiri has performed experimental work for Specific Aim 1 and 2.*
Funding Support:	*Current DOD W81XWH2110038*

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

There has been no change in the following active support:

01/01/21 – 12/31/23 20YOUN04
Prostate Cancer Foundation Young Investigator Award (Burnham)
Prostate Cancer Foundation
"HER2 and Androgen Receptor Signaling in Prostate Cancer" (Mentors: Rick Kittles, Ph.D. and Tanya Dorff, M.D.)
Role: PI

07/01/20 – 12/01/23 568866221
Pfizer Prostate Cancer Foundation Global Challenge Award (Dorff)
Pfizer and Prostate Cancer Foundation
"Identifying AR Characteristics that Define Populations of Patients with CSPC who Benefit from Early PARP Inhibition Therapy with Talazoparib"
Role: Co-Investigator

03/01/20 – 01/28/22 QueensCare Charitable Division (Kittles)
QueensCare Foundation
Community-Based Prostate Cancer Screenings Among Men in South LA
Co-Lead

Additional non-research active support:

11/01/21 – 10/31/22 Bank of America (Kittles)
Bank of America Charitable Foundation
Prostate Cancer Screenings in the San Gabriel Valley
Co-Lead

01/01/22 – 06/30/22 Genentech (Kittles)
Roche-Genentech/City of Hope AIR (Advancing Inclusive Research) Site Alliance
Advancing Inclusive Research in Southern California: Challenges and Opportunities
Co-Lead

What other organizations were involved as partners?

Organization Name: DOD PCBN, Washington University in St. Louis

Location of Organization: United States

Partner's contribution to the project

Financial support: Nothing to Report

In-kind support: 456 Case Race Disparity TMA; patient-matched frozen cell pellets

Facilities: Nothing to Report

Collaboration: Nothing to Report

Personnel exchanges: Nothing to Report

Other: Nothing to Report

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Nothing to Report

QUAD CHARTS: Nothing to Report

CONCLUSION

Metastatic PCa is a lethal disease and therapeutic options are limited for patients.[14-17] Since there is no cure for advanced PCa, the pursuit of therapeutic strategies to effectively treat this stage remains a critical goal. The ability to target non-androgenic growth factor signaling pathways that may promote metastasis represents a tangible and promising area of investigation. With the development of targeted therapeutic options optimized for a molecularly enriched population, there is potential to improve survival outcomes for men at high-risk for an advanced stage diagnosis. Since recent studies have revealed better treatment response and increased overall survival in AA men receiving PCa drug treatments, there is additional incentive to decrease the current racial disparity in mortality with precision medicine.[18-20] Given that our preliminary evidence suggests a potential increase in HER2 overexpression in AA men with PCa, we predict that AA mCRPC patients may especially benefit from anti-HER2 drug targeting. Successful completion of this study will generate preliminary data to support applications for future funding to 1) fully evaluate whether HER2 contributes to the worse prognosis experienced by AA men and 2) to develop treatment strategies for PCa patients, targeting HER2 overexpression with newer generation drugs that have been proven effective in other HER2+ cancer types. A precision medicine approach may allow for the repurposing of drugs currently approved to treat HER2+ breast cancer patients and potentially improve overall survival for currently incurable stages of PCa. We anticipate that successful completion of this and a subsequent clinical trial would have a major impact in reducing lethal PCa in AA men and would provide an additional treatment option for others with HER2+ mCRPC.

REFERENCES

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2 Powell, I. J. *et al.* Genes associated with prostate cancer are differentially expressed in African American and European American men. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **22**, 891-897, doi:10.1158/1055-9965.Epi-12-1238 (2013).

3 Batai, K., Murphy, A. B., Nonn, L. & Kittles, R. A. Vitamin D and Immune Response: Implications for Prostate Cancer in African Americans. *Frontiers in immunology* **7**, 53, doi:10.3389/fimmu.2016.00053 (2016).

4 Wallace, T. A. *et al.* Tumor immunobiological differences in prostate cancer between African-American and European-American men. *Cancer Res* **68**, 927-936, doi:10.1158/0008-5472.Can-07-2608 (2008).

5 Reams, R. R. *et al.* Microarray comparison of prostate tumor gene expression in African-American and Caucasian American males: a pilot project study. *Infect Agent Cancer* **4 Suppl 1**, S3, doi:10.1186/1750-9378-4-S1-S3 (2009).

6 Siampanopoulou, M., Galaktidou, G., Dimasis, N. & Gotzamani-Psarrakou, A. Profiling serum HER-2/NEU in prostate cancer. *Hippokratia* **17**, 108-112 (2013).

7 Signoretti, S. *et al.* Her-2-neu expression and progression toward androgen independence in human prostate cancer. *J Natl Cancer Inst* **92**, 1918-1925, doi:10.1093/jnci/92.23.1918 (2000).

8 Ziada, A. *et al.* The use of trastuzumab in the treatment of hormone refractory prostate cancer; phase II trial. *Prostate* **60**, 332-337, doi:10.1002/pros.20065 (2004).

9 Lara, P. N., Jr. *et al.* Trastuzumab plus docetaxel in HER-2/neu-positive prostate carcinoma: final results from the California Cancer Consortium Screening and Phase II Trial. *Cancer* **100**, 2125-2131, doi:10.1002/cncr.20228 (2004).

10 Morris, M. J. *et al.* HER-2 profiling and targeting in prostate carcinoma. *Cancer* **94**, 980-986 (2002).

11 Liu, G. *et al.* Eastern Cooperative Oncology Group Phase II Trial of lapatinib in men with biochemically relapsed, androgen dependent prostate cancer. *Urol Oncol* **31**, 211-218, doi:10.1016/j.urolonc.2011.01.002 (2013).

12 Kosoy, R. *et al.* Ancestry informative marker sets for determining continental origin and admixture proportions in common populations in America. *Hum Mutat* **30**, 69-78, doi:10.1002/humu.20822 (2009).

13 Hooker, S. E., Jr. *et al.* Genetic Ancestry Analysis Reveals Misclassification of Commonly Used Cancer Cell Lines. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **28**, 1003-1009, doi:10.1158/1055-9965.EPI-18-1132 (2019).

14 Day, K. C. *et al.* HER2 and EGFR Overexpression Support Metastatic Progression of Prostate Cancer to Bone. *Cancer Res* **77**, 74-85, doi:10.1158/0008-5472.CAN-16-1656 (2017).

15 Asmane, I. *et al.* New strategies for medical management of castration-resistant prostate cancer. *Oncology* **80**, 1-11, doi:10.1159/000323495 (2011).

16 Scher, H. I., Buchanan, G., Gerald, W., Butler, L. M. & Tilley, W. D. Targeting the androgen receptor: improving outcomes for castration-resistant prostate cancer. *Endocr Relat Cancer* **11**, 459-476 (2004).

17 Thoreson, G. R., Gayed, B. A., Chung, P. H. & Raj, G. V. Emerging therapies in castration resistant prostate cancer. *Can J Urol* **21**, 98-105 (2014).

18 Halabi, S. *et al.* Overall Survival of Black and White Men With Metastatic Castration-Resistant Prostate Cancer Treated With Docetaxel. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **37**, 403-410, doi:10.1200/JCO.18.01279 (2019).
19 (American Society of Clinical Oncology, 2019).
20 A. Oliver Sartor, A. J. A., Chiledum Ahaghotu, David G. McLeod, Matthew R. Cooperberg, David F. Penson, Philip W. Kantoff, Nicholas J. Vogelzang, Arif Hussain, Christopher Michael Pieczonka, Neal D. Shore, David I. Quinn, Eric Jay Small, Elisabeth I. Heath, Ronald F. Tutrone, Paul F. Schellhammer, Matthew Harmon, Nancy N. Chang, Stephen J. Freedland, Celestia S. Higano. Overall survival (OS) of African-American (AA) and Caucasian (CAU) men who received sipuleucel-T for metastatic castration-resistant prostate cancer (mCRPC): Final PROCEED analysis. *Journal of Clinical Oncology* **37**, 5035-5035, doi:10.1200/JCO.2019.37.15_suppl.5035 (2019).

APPENDICES: **CURRICULUM VITAE**

LEANNE BURNHAM, Ph.D.

Assistant Professor, Division of Health Equities
Department of Population Sciences
City of Hope National Medical Center
1500 E. Duarte Rd., Duarte, CA 91010

Date Prepared: January 3, 2022

I. EDUCATION

2012 University of Akron, B.S., *Cum Laude,* Biology

2018 Loma Linda University School of Medicine, Ph.D., Physiology
Graduate Degree Mentor: Carlos A. Casiano, Ph.D.

II. POSTDOCTORAL TRAINING

2018-2021 Beckman Research Institute, City of Hope, Rick Kittles, Ph.D.
2018-2020 NIH DNA Damage Response and Oncogenic Signaling (DNADRS) T32 Postdoctoral Fellow

Certifications
2018 Basic Workflow of R, Center for Informatics, City of Hope, Duarte, CA
2019- Clinical Investigative Training Program (CITP), City of Hope, Duarte, CA
CITP Clinical Trials (MEDONC531)
Adverse Events in Protocol (MEDONC400)
CITP Protocol Development (MEDONC550)
CITP Protocol Management (MEDONC551)
CITP Regulatory Processing (MEDONC562)

III. PROFESSIONAL EXPERIENCE, POSITIONS & EMPLOYMENT

2009-2011 Physician Shadow, Cleveland Clinic Glickman Urology and Kidney Institute, Cleveland, OH

2009-2011 Research Intern, Janet Houghton Colon Cancer Laboratory, Cleveland Clinic Lerner Research Institute, Cleveland, OH

2011-2012 STEM Peer Mentor, University of Akron, Akron, OH

2012-2018	Graduate Student and Research Assistant, Carlos Casiano Prostate Cancer Laboratory, Center for Health Disparities and Molecular Medicine, Loma Linda University School of Medicine, Loma Linda, CA
2012-2018	ABC/UTP Mentor, Center for Health Disparities and Molecular Medicine, Loma Linda University School of Medicine, Loma Linda, CA
2018-2021	Postdoctoral Fellow, Rick Kittles Prostate Cancer Laboratory, Beckman Research Institute, City of Hope, Duarte, CA
2019-	Community-Based Prostate Cancer Screening Coordinator, Division of Health Equities, Department of Population Sciences, City of Hope, Duarte, CA
2021-	Advancing Inclusive Research (AIR) Site Alliance Genentech-City of Hope Site Point-of-Contact, City of Hope, Duarte, CA
2021- 2022	Assistant Research Professor, Department of Population Sciences, Division of Health Equities, City of Hope, Duarte, CA
2022-	Assistant Professor, Department of Population Sciences, Division of Health Equities, City of Hope, Duarte, CA

IV. HONORS, SCHOLARSHIPS & AWARDS

1998	Chrysler Award, Stow, OH
1998	President, Jim Eliot Chapter of National Honors Society, Stow, OH
1998	Oscar Ritchie Scholar, Kent State University, Kent, OH
2008 –	Partners in Excellence Scholar, University of Akron, Akron, OH
2009 –	STEM Scholar, University of Akron, Akron, OH
2009 –	McNair Scholar, University of Akron, Akron, OH
2010 –	Verna Trushel Scholar, University of Akron, Akron, OH
2010 – 2011	Honors Scholar, University of Akron, Akron, OH
2011	Outstanding Presenter, McNair Undergraduate Research Symposium, University of Akron, Akron, OH
2012	Outstanding McNair Scholar of the Year, University of Akron, Akron, OH
2012	3rd Place, BURS Symposium Undergraduate Research, Akron, OH
2012	1st Place, UASIS Symposium Outstanding Undergraduate Student Research, Akron, OH Student-of-the-Month (Awarded by University of Akron President and Board of Trustees), Akron, OH
2012 – 2018	IMSD Fellowship, Center for Health Disparities and Molecular Medicine, Loma Linda University, Loma Linda, CA
2014 – 2016	Basic Science Dean's Award, Loma Linda University, Loma Linda
2016	AACR Scholar-in-Training, AACR Conference on the Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved, Fort Lauderdale, FL
2016	2nd Place Faculty Judge Award, Basic Science Symposium, Loma Linda University, Loma Linda, CA

2018	1st Place Student Judge Award, Basic Science Symposium, Loma Linda University, Loma Linda, CA
2018 – 2020	NCI T32 Fellow, City of Hope DNADRS Program, Duarte, CA
2019	Endocrine Society FLARE Fellow
2020	Health Equities Pilot Award, City of Hope, Duarte, CA
2020	Prostate Cancer Foundation Young Investigator Award, Santa Monica, CA
2020	Department of Defense Prostate Cancer Research Program Early Investigator Award, Washington D.C.
2021	The Cancer Health 25: Black Lives Matter

V. ACTIVE GRANTS/RESEARCH SUPPORT

01/01/21 – 12/31/22
W81XWH2110038
Department of Defense Prostate Cancer Research Cancer Program Early Investigator Award (Burnham)
Department of Defense
"Her2 Expression in African American Men with Prostate Cancer" (Mentors: Rick Kittles, Ph.D. and Tanya Dorff, M.D.)
Direct
PI

01/01/21 – 12/31/23
20YOUN04
Prostate Cancer Foundation Young Investigator Award (Burnham)
Prostate Cancer Foundation
"HER2 and Androgen Receptor Signaling in Prostate Cancer" (Mentors: Rick Kittles, Ph.D. and Tanya Dorff, M.D.)
Direct
PI

07/01/20 – 12/01/23
568866221
Pfizer Prostate Cancer Foundation Global Challenge Award (Dorff)
Pfizer and Prostate Cancer Foundation
"Identifying AR Characteristics that Define Populations of Patients with CSPC who Benefit from Early PARP Inhibition Therapy with Talazoparib"
Direct
Co-Investigator

03/01/20 – 01/28/22
QueensCare Charitable Division (Kittles)
QueensCare Foundation
Community-Based Prostate Cancer Screenings Among Men in South LA
Direct
Co-Lead

11/01/21 – 10/31/22
Bank of America (Kittles)
Bank of America Charitable Foundation
Prostate Cancer Screenings in the San Gabriel Valley

	Direct Co- Lead
01/01/22 – 06/30/22	Genentech (Kittles) Roche-Genentech/City of Hope AIR (Advancing Inclusive Research) Site Alliance Advancing Inclusive Research in Southern California: Challenges and Opportunities direct Co-Lead

COMPLETED GRANTS/RESEARCH SUPPORT

09/01/12 – 05/01/18	5R25GM060507-12 Initiative for Maximizing Student Development Fellow (De Leon) NIH/NIGMS Training Fellowship Trainee
12/01/18 – 11/30/21	5T32CA186895-04 DNA Damage Response and Oncogenic Signaling Fellow (Shen) NIH/NHLBI Training Fellowship Trainee
01/10/20 – 12/31/20	City of Hope Research Initiative Health Equity Pilot Program (Burnham/Hooker/Shuck/Termini) City of Hope Advanced Glycation End Products as Diagnostic and Prognostic Indicators of Prostate Cancer Disparities in Men of West African Ancestry Direct Co-Investigator

VI. PUBLICATIONS

Publications (peer-reviewed) 6 Total

1. Basu A, **Woods-Burnham L.,** Ortiz G., Rios-Colon L., Figueroa J., Albesa R., Andrade L.E., Mahler M., Casiano C.A. (2015) Specificity of antinuclear autoantibodies recognizing the dense fine speckled nuclear pattern: Preferential targeting of DFS70/LEDGFp75 over its interacting partner MeCP2. *Clin Immunol,* 161(2):241-50.
2. **Woods-Burnham L.**, Basu A., Cajigas-Du Ross C.K., Love A., Yates C., De Leon M., Roy S., Casiano C.A. (2017) The 22Rv1 prostate cancer cell line carries mixed genetic ancestry: Implications for prostate cancer health disparities research using pre-clinical models. *Prostate,* 77(16): 1601-1608.
3. **Woods-Burnham L.**, Stiel L., Wilson C., Montgomery S., Duran A.M., Ruckle H.R., Thompson R.A., De Leon M., Casiano C.A. (2018) Physician consultations, prostate cancer knowledge, and PSA screening of African American men in the Era of Shared Decision-Making. *Am J Mens Health,* 12(4):751-759.
4. Cajigas-Du Ross CK, Martinez SR, **Woods-Burnham L.,** Durán AM, Roy S, Basu A, Ramirez JA, Ortiz-Hernández GL, Ríos-Colon L, Chirshev E, Sanchez-Hernandez ES, Soto U, Greco C, Boucheix

C, Chen X, Unternaehrer J, Wang C, Casiano CA. (2018) RNA sequencing reveals upregulation of a transcriptomic program associated with stemness in metastatic prostate cancer cells selected for taxane resistance. *Oncotarget.* 9(54):30363-30384.

5. **Woods-Burnham L.**, Cajigas-Du Ross C.K., Love A., Basu A., Durán A.M., Sanchez-Hernandez E.S., Martinez S.R., Ortiz-Hernandez G.L., Stiel L., Wilson C., Montgomery S., Roy S., Casiano C.A. (2018) Glucocorticoids induce stress oncoproteins associated with therapy-resistance in African American and European American prostate cancer cells. *Scientific Reports,* 8(1):15063.
6. Hooker S.E., **Woods-Burnham L.**, Bathina M., Lloyd S.M., Gorjala P., Mitra R., Nonn L., Kimbro K.S., Kittles R.A. (2019) Genetic ancestry analysis reveals misclassification of commonly used cancer cell lines. *Cancer Epidemiol Biomarkers Prev*, 28(6):1003-1009.

Publications (review articles) 2 Total

1. **Woods-Burnham L**., Stiel L., Martinez S.R., Sanchez-Hernandez E.S., Ruckle H.C., Almaguel F.G., Stern M.C., Roberts L.R., Williams D.R., Montgomery S., Casiano C.A. (2020) Psychosocial stress, glucocorticoid signaling, and prostate cancer health disparities. *Cancer Health Disparities*, doi:10.9777/chd.2020.1005.
2. Johnson J.R., **Woods-Burnham L.,** Hooker S., Batai K., Kittles R.A. (2021) Genetic contributions to prostate cancer disparities in men of West African descent. *Front Oncol.* 11.770500. doi: 10.33889/fonc.2021.770500.

Editorials and Letters 2 Total

1. **Woods-Burnham L**. (2020) Not all champions are allies in health disparities research. *Cell,* 183(3):580-582.
2. **Woods-Burnham L**., Johnson J.R., Hooker S.E., Bedell F.W., Dorff T.B., Kittles R.A. (2021) The role of diverse
populations in U.S. clinical trials. *Med,* 2(1):21-24. doi: 10.1016/j.medj.2020.12.009.

VII. INVITED SEMINARS/LECTURES/FORUMS

Intramural

01/14/12	"Pursuing STEM as a Nontraditional Student", Rosa Parks Annual Event, University of Akron
02/04/12	"Pursuing STEM as a Nontraditional Student", National TRiO Day, University of Akron
04/09/12	"Hedgehog Signaling in Colon Cancer", Talent Dividend Forum, University of Akron
02/05/18	"Glucocorticoid Signaling in Black Men with Prostate Cancer", Chalk Talk, Loma Linda University
09/15/18	"Prostate Cancer Education and Screening for Black Men", Tabahani Book Circle Worship in Pink, Imperial Heights Community Church of the Brethren (Sponsored by City of Hope)
11/10/18	"Prostate Cancer Education and Screening for Black Men", Men's Day Breakfast/Health and Wellness Forum, Metropolitan Baptist Church (Sponsored by City of Hope)
01/12/19	"Prostate Cancer Education and Screening for Black Men", CPAD Community Benefits Forum, City of Hope
04/06/19	"Prostate Cancer Education and Screening for Black Men", 10th Annual MAP Neighborhood Conference (Sponsored by City of Hope)

04/11/19 "Prostate Cancer Education and Screening for Black Men", Learning and Professional Development Week, City of Hope

06/01/19 "Prostate Cancer Education and Screening for Black Men", Omega Psi Phi and Charles R. Drew Blood Drive and Health Fair (Sponsored by City of Hope)

09/21/19 "Prostate Cancer Education and Screening for Black Men", 32nd Long Beach Jazz Festival Health and Wellness Pavilion (Sponsored by City of Hope)

10/13/19 "Prostate Cancer Education and Screening for Black Men", First Ladies Los Angeles Chapter Annual Health Day (Sponsored by City of Hope)

02/01/20 "Prostate Cancer Education and Screening for Black Men", 39th Annual Orange County Black History Parade and Cultural Fair (Sponsored by City of Hope)

07/23/20 "All of Us: A Multidisciplinary Approach to Reducing Cancer Health Disparities", KJLH Radio (Sponsored by City of Hope)

08/28/20 "My Career as a Postdoc", Irell & Manella Graduate School of Biological Sciences Symposium, City of Hope

11/18/20 "Clinical Trial Recruitment for African Americans", CISCRP Aware for All Webinar (Sponsored by City of Hope)

02/05/21 "Implications of Telemedicine for Prostate Cancer Patients", Patient Empowerment Network and Diverse Health Hub (Sponsored by City of Hope)

02/23/21 "Tackling Prostate Cancer Health Disparities at the Bench, Clinic, and Community", OncLive State of the Science Summit (Sponsored by City of Hope)

04/15/21 "What is Special About Research at City of Hope", Building Hope Toast of Hope Event, City of Hope

06/30/21 "Dine in for Hope", City of Hope

07/31/21 "Clinical Trials: An Answer to Cancer?", Cynthia Perry Ray Foundation Sistahs Can We Talk Monthly Nurses Series (Sponsored by City of Hope)

09/23/21 "Tackling Prostate Cancer Health Disparities at the Bench, Clinic, and Community", City of Hope Community Engagement Community Advisory Board

Symposia Presentations

11/11/10 "GLI vs. Smoothened (SMO) as therapeutic targets in the Hedgehog (HH) signaling pathway", ABRCMS Annual Meeting

01/06/10 "GLI vs. Smoothened (SMO) as therapeutic targets in the Hedgehog (HH) signaling pathway", McNair Undergraduate Research Symposium at UC, Berkeley

07/22/11 "GLI vs. Smoothened (SMO) as therapeutic targets in the Hedgehog (HH) signaling pathway", McNair Undergraduate Research Symposium at University of Akron

03/05/12 "GLI vs. Smoothened (SMO) as therapeutic targets in the Hedgehog (HH) signaling pathway", Choose Ohio First STEMM Showcase at COSI

04/09/12	"GLI vs. Smoothened (SMO) as therapeutic targets in the Hedgehog (HH) signaling pathway", UASIS Research Symposium
04/06/14	"Specificity of the human autoantibody response against the stress oncoprotein LEDGF/p75", AACR Annual Meeting
11/16/14	"Glucocorticoid signaling in prostate cancer cells", Loma Linda University Annual Basic Sciences Symposium
03/16/15	"Glucocorticoid signaling in prostate cancer cells", Loma Linda University Annual Postgraduate Convention
05/13/15	"PSA Screening for Black Men in Southern California", Loma Linda University Counseling and Family Sciences Conference
05/13/15	"Physician consultations, prostate cancer knowledge, and PSA screening in African American men: Are the conversations effective?", Loma Linda University Counseling and Family Sciences Conference
09/14/16	"Glucocorticoid signaling in prostate cancer cells", Loma Linda University Annual Health Disparities Research Symposium
09/26/16	"Glucocorticoid signaling in prostate cancer cells", AACR Conference on the Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved
09/26/16	"Physician consultations, prostate cancer knowledge, and PSA screening in African American men: Are the conversations effective?", AACR Conference on the Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved
09/15/17	"Glucocorticoid-mediated upregulation of stress oncoproteins associated with chemoresistance: Implications for prostate cancer health disparities", Loma Linda University Annual Health Disparities Research Symposium
04/15/18	"Glucocorticoid-mediated upregulation of stress oncoproteins associated with chemoresistance: Implications for prostate cancer health disparities", AACR Annual Meeting
11/03/18	"Genetic Ancestry Analysis Reveals Misclassification of Commonly Used Cancer Cell Lines", AACR Conference on the Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved
09/21/19	"Vitamin D Signaling of Immune-Related Genes in Diverse Prostate Cancer Cell Lines", AACR Conference on the Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved
08/10/21	"HER2 expression in African American men with prostate cancer", Eugene and Ruth Roberts Summer Student Academy Symposium
10/07/21	"Connecting under-resourced populations: A community-based prostate cancer screening intervention", 14th AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved

11/08/21	"HER2 and AR signaling in prostate cancer", 28th Prostate Cancer Foundation Annual Scientific Retreat

Extramural Invited Seminars

10/15/19	"Science as a Career", Career Day, Colony High School
10/05/20	"Dine in for Hope", I Am The Voluntourist, City of Hope
10/15/20	"Science as a Career", Career Day, Buchtel High School
01/20/21	"Health Disparities: Past, Present, and Future", Metropolitan State University, Saint Paul, MN
03/18/21	"Prostate Cancer in Black Men: A Personal and Professional Perspective", The Patient Portal
04/27/21	"Cancer Research Perspective from a Cancer Patient", CURE Magazine
09/18/21	"Prostate Cancer in Black Men: The Latest in Screening and Treatment Options", Prostate Cancer Foundation & The West Angeles Church of God in Christ

VIII. TEACHING/EDUCATION/EDUCATIONAL ACTIVITIES

Other Research Mentoring Activities

Arthur Love	High School Student	Martin Luther King High School	2013-2016
Greisha Ortiz-Hernandez	BS Student	Universidad Metropolitana	2013
Evelyn Sanchez	BS Student	California State, Northridge	2015
Isaiah Sailors	High School Student	Chaffey High School	2019
Serene Dowiri	BS Student	UCLA	2021

IX. SERVICE TO INSTITUTIONS

Loma Linda University School of Medicine

Member, ABC/UTP Summer Research Admissions Committee, 2013-2017

Member, Project C.H.A.N.G.E. Planning Committee, 2013-2018

Student Representative, Curriculum Committee, 2014-2015

Academic Vice-President, Student Council, 2016-2018

Member, Admissions Committee, 2017-2018

City of Hope

Participant, Genitourinary Disease Team, 2018-Current

Coordinator, Division of Health Equities Community-Based Prostate Cancer Screening Program, 2018-Current

09/22/18	Antelope Valley Prostate Cancer Screening, Grace Church
01/26/19	KKLA Radio Family Health Fair, First Church of the Nazarene
04/06/19	Set for Life, 10th Annual MAP Neighborhood Conference
06/08/19	Health Pasadena, Men Educating Men About Health
06/01/19	Omega Psi Phi and Charles R. Drew Blood Drive and Health Fair
09/21/19	32nd Long Beach Jazz Festival Health and Wellness Pavilion
10/13/19	Crenshaw Christian Center, First Ladies Los Angeles Chapter Annual Health Day
10/13/19	First A.M.E. Los Angeles, First Ladies Los Angeles Chapter Annual Health Day
10/19/19	Taste of Soul Los Angeles
11/16/19	Mejor Salud Pasadena
01/25/20	KKLA Radio Family Health Fair, First Church of the Nazarene
02/01/20	39th Annual Orange County Black History Parade and Cultural Fair
02/15/20	Annual Pasadena Black History Parade and Festival

Participant, Graduate Medical Education and Health Disparities Collaboration, 2020-Current

Site Point-of-Contact, Advancing Inclusive Research (AIR) Site Alliance Genentech-City of Hope, 2021-Current

Admissions Committee, Roberts Summer Academy, 2021

X. SERVICE TO PROFESSION

Professional Memberships

Societies

2012-	Member, American Association for Cancer Research, Philadelphia, PA
2018-2020	Member, Endocrine Society, Washington D.C.

Committees

2019-2020	Member, Endocrine Society Research Affairs Core Committee, Washington D.C.

Editorial and Review Experience
Reviewer:
The Prostate, American Journal of Men's Health, International Journal of Urology, Cancer Medicine, American Journal of Preventive Medicine, Frontiers in Pharmacology, Prostate Cancer Foundation

XI. PATENTS, INVENTIONS & COPYRIGHTS

None