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14. ABSTRACT

African-Americans (AA) have a higher incidence of prostate cancer (PCa) and higher mortality. Stromal and epithelial inflammatory processes have a fundamental role in carcinogenesis and may predict PCa clinical progression. Discovery of novel variants as well as re-discovery of known variants deliver new opportunities for therapeutic advances such as new drug targets and personalized therapy. We hypothesize next-generation whole genome sequencing, paired with new methodologies of intratumoral phylogenetic analyses, will yield pivotal information in elucidating the key genes involved evolution of PCa from precursor inflammatory lesions in AA men. During this research period, extensive translational research training has been completed including both clinical training as well as training in bioinformatics. The most significant outcome of the present study has been the assemblage of a robust database of clinical annotation and follow-up for early stage prostate cancer patients treated at Tulane University Medical Center. This database combined with genetic data produced by this study may empower patients and doctors to make personalized treatment decisions and ultimately improve treatment outcomes. Similarly, as product of training, research database and ongoing genetic analyses, the PI has had the opportunity to collaborate on numerous publications and conference presentations.

15. SUBJECT TERMS
prostate cancer, African-American, chronic inflammation, next-generation sequencing, translational research, genetics, disparity

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1. **INTRODUCTION:** African-Americans (AA) have a higher incidence of prostate cancer (PCa) and higher mortality. Stromal and epithelial inflammatory processes have a fundamental role in carcinogenesis and may predict PCa clinical progression. Prostate inflammation is present in a higher proportion of AA men. Comprehensive identification of the very early genetic alterations associated with progression from inflammation to PCa remains elusive. Understanding and identifying the very early genetic events driving chronic inflammation and development of PCa in AA men may provide insight into disease disparity and clinically actionable opportunities to improve treatment of chronic inflammation, PCa prevention, early detection of high risk PCa, and identification of genes and pathways which may influence PCa recurrence and metastasis. Discovery of novel variants as well as re-discovery of known variants deliver new opportunities for therapeutic advances such as new drug targets and personalized therapy. We hypothesize next-generation whole genome sequencing, paired with new methodologies of intratumoral phylogenetic analyses, will yield pivotal information in elucidating the key genes involved evolution of PCa from precursor inflammatory lesions in AA men.

2. **KEYWORDS:** prostate cancer, African-American, chronic inflammation, next-generation sequencing, translational research, genetics, disparity

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**
  - Specific Aim 1&2: To independently characterize intraprostatic somatic mutations in prostate tissue derived from tumor and chronic inflammation.
    - Major Task 1: Obtain prostate tissue samples. 90% complete.
    - Major Task 2: Whole-exome sequencing of prostate tissue derived from tumor and chronic inflammation, and germline DNA. 15% complete.
    - Major Task 3: Perform genetic analyses characterizing somatic mutations in prostate tissue samples. 5% completed.
  - Specific Aim 3: To compare and phylogenetically model intraprostatic genetic changes between prostate tissue with chronic inflammation and PCa.
    - Major Task 1: Compare and contrast genetic changes between prostate tissue with chronic inflammation and PCa. 0% complete.

- Major Task 2: Confirm significant genetic alterations in an independent validation cohort. 20% completed.

- **What was accomplished under these goals?**
- Specific Aim 1&2: To independently characterize intraprostatic somatic mutations in prostate tissue derived from tumor and chronic inflammation.
    - Major Task 1: Obtain prostate tissue samples. 90% complete. IRB was successfully submitted and approved. A total of 113 patient samples were clinically annotated and screened for inclusion in this study. 52 African American patients with evidence of both chronic inflammation and PCa were identified. 20 patient samples were submitted for review by a pathologist and for tissue extraction with laser capture microdissection. Statistical analyses were performed to assess any significant clinical differences between and within both the African American and Caucasian patient cohorts.

      The study sample consisted of 61 African American (AA) and 52 Caucasian (CA) PCa patients who underwent radical prostatectomies (RP) at Tulane Hospital between 2013 and 2015. The presence or absence of chronic inflammation (CI) was determined based on reviews of RP pathology reports from multiple pathologists. Other clinical data was extracted from both biopsy and RP pathology reports. The study examined the relationship between CI, race, percent of positive cores, extraprostatic extension, PSA, PSA density, urinary PCA3 and TMPRSS2, and prostate size (g). Pearson's chisquare, Fisher's exact, and KruskalWallis tests were used to analyze categorical, noncontinuous data; ANOVA tests were used to analyze continuous data. Differences between biopsy and surgical/pathologic Gleason scores and clinical/pathological stages were also assessed. 94 patients (52 AAs and 42 CAs) had CI to some degree and 19 did not (9 AAs and 10 CAs). There was no difference in rate of CI between AA and CA patients ($P = 0.526$). Among all patients sampled, AAs had higher percentages of positive cores ($P = 0.005$), PCA3 copy levels ($P =0.004$), and PCA3 scores ($P <0.001$), lower TMPRSS2 scores ($P =0.039$), and were more likely to have "high" or "intermediate" NCCN risk strata ($P =0.010$). Among patients with CI, AAs were more likely than CAs to have extra-prostatic extension ($P=0.026$) and less

likely to have undergone a prior prostate biopsy (P =0.043). Patients without CI were more likely than patients with CI to have positive tumor margins (P =0.035) and SV invasion (P =0.013). There were no significant relationships between race and CI, and changes in either total Gleason score or stage from biopsy to RP. Ultimately, this review of Tulane patients showed that AAs and patients without CI had more advanced forms of PCa though this is possibly due to PSA detection biases (See Appendices 1-3 for additional detailed results). This review of clinical pathology and patient population at Tulane hospital has been instrumental toward the project goals and characterization of chronic inflammation for this cohort.

    - Major Task 2: Whole-exome sequencing of prostate tissue derived from tumor and chronic inflammation, and germline DNA. 15% complete. Quality control and preliminary DNA extractions are currently in progress.
    - Major Task 3: Perform genetic analyses characterizing somatic mutations in prostate tissue samples. 5% completed. In preparation for analyses, training in necessary techniques and applications is ongoing.
- Specific Aim 3: To compare and phylogenetically model intraprostatic genetic changes between prostate tissue with chronic inflammation and PCa.
    - Major Task 1: Compare and contrast genetic changes between prostate tissue with chronic inflammation and PCa. 0% completed.
    - Major Task 2: Confirm significant genetic alterations in an independent validation cohort. 20% completed. Clinical annotation and sample identification is ongoing in preparation for validation cohort; data, including presence of chronic inflammation, is being assembled for patients who have undergone radical prostatectomy at Tulane hospital.

- **What opportunities for training and professional development has the project provided?**
- There have been many opportunities for training and professional development. The PI completed a two week surgical pathology rotation in the Tulane Pathology department. This training focused on interpreting data presented in pathological reports, distinguishing between benign and neoplastic tissue, understanding histopathological grading of PCa and identifying chronic inflammation in prostate tissue. In addition to basic training in pathology, the PI has spent time in developing experience in clinical annotation of PCa. This includes

direct experience in patient care through participation and observation during outpatient clinic appointments in both the Tulane Cancer Center and Urology clinics. Specifically, through direct interaction with both patients and physicians as well as evaluation of medical records, the PI is learning the clinical subtleties of treatment and management of PCa, current trends in PCa treatment and detection, and the diversity of the patient population, from newly diagnosed patients to end-of-life decision making. This clinical training has been invaluable for continued training as a translational researcher.

The PI also attended monthly Prostate Interest Group (PIG) meetings; these meeting consist of a multi-disciplinary group of basic scientists and clinicians focused on PCa research. These meetings have been essential for establishing intra- and inter-institutional collaborations, as well as an expert panel for assistance with the current project. The PI also attends a monthly tumor board focused on genitourinary malignancies. Aside for prostate specific meetings, the PI also regularly attends seminars hosted by the Louisiana Cancer Research Center (LCRC) and Louisiana State University Health Sciences Center Genetics Departmental Seminar series. Professional development has also included attendance and poster presentation at Genitourinary (GU) Cancers Symposium in San Francisco.

An important aspect of training during this reporting period has been regulatory training and project development. The PI has received valuable training and assistance from the Tulane Office of Clinical Research (OCR). This has provided training essential for successful IRB development and submission. Additional training in regulatory compliance, HIPAA privacy, and budgeting have also been completed.

The PI has had opportunities for professional development including teaching and supervisory roles. Specifically, the PI has given guest lectures for the Population Genetics course at Louisiana State University Health Sciences Center in the Department of Genetics. In the lab, the PI has directly supervised and assisted with research projects for medical students, and graduate students. This supervision has included project design, guidance, technical assistance, meeting presentation, and ultimately publication.

- **How were the results disseminated to communities of interest?**

  Nothing to Report.

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

- Since this research is funded in part by the Tulane Prostate Cancer Research Fund, results will be presented at various outreach opportunities, fundraising, and educational events tailored for PCa patients. This includes presentations to monthly PCa support meetings, PSA screening events in conjunction with Tulane Urology and Tulane Cancer Center educational events. Additional opportunities for dissemination of this work include presentation of these results at interdepartmental seminars, namely the Tulane Department of Genetics.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
- A significant product of this research has been the creation of a very thorough and well annotated clinical history and mechanism for ongoing follow-up of African American PCa patients. This database contains and will contain a powerful translational dataset which combines clinical, pathological and genetic data. Specifically, as a disproportionately affected population which has been underrepresented in PCa genetic studies, the present study tools may give innovative genetic insights into both prostate inflammation and PCa in African American men. Since chronic inflammation has been linked with aggressive PCa, the DNA changes identified may potentially provide an early diagnostic profile for distinguishing aggressive from non-aggressive PCa in newly diagnosed men. This type of genetic knowledge combined with detailed clinical annotation and long-term follow-up may empower patients and doctors to make personalized treatment decisions and ultimately improve treatment outcomes.

- **What was the impact on other disciplines?**
  Nothing to report.

- **What was the impact on technology transfer?**
  Nothing to report.

- **What was the impact on society beyond science and technology?**
  Nothing to report.

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**

  Nothing to report.

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - The first problem encountered was delays with the IRB submission and approval. The IRB application and protocol was prepared and submitted in a timely fashion; however, due to the high volume of submissions to the institutional IRB, the full approval process required more time than was previously estimated. Subsequent submission to Tulane Medical Center IRB committee proceeded without incident. To mitigate these delays, limited preliminary data acquisition, clinical training, and assessment were performed in anticipation of IRB approval.

    The second major delay occurred at the beginning to 2016 with the departure of laboratory staff. Specifically, instructor Ratish Gambhira left the Sartor laboratory in February. He was the primary source for training and assistance in the molecular techniques necessary to complete the study design. To mitigate this problem, additional laboratory staff were hired and alternative molecular training opportunities were located. Though resolved, this delay significantly impacted progression on the proposed study design; however, despite this disruption, the PI was able to complete additional training in other disciplines as well as accrue valuable clinical training.

- **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.

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**
  - **Journal publications.** (See Appendix 4 for journal publication abstracts)
    McKay RR, Jacobus S, Fiorillo M, **Ledet EM**, Cotogna PM, Steinberger AE, Jacene HA, Sartor O, Taplin ME. **Radium-223 Use in Clinical Practice and Variables Associated With Completion of Therapy**. Clin Genitourin Cancer. 2016 Aug 20. pii: S1558-7673(16)30249-X. doi: 10.1016/j.clgc.2016.08.015.

    Vasudevamurthy AK, **Ledet E**, Garvey C, Lewis BE, Sartor O. **Estrogen-Mediated Activation of H875Y Androgen Receptor Mutation in a Prostate Cancer Patient**. Clin Genitourin Cancer. 2016 Jul 22. pii: S1558-7673(16)30218-X. doi: 10.1016/j.clgc.2016.07.015.

    Liu X, **Ledet E**, Li D, Dotiwala A, Steinberger A, Feibus A, Li J, Qi Y, Silberstein J, Lee B, Dong Y, Sartor O, Zhang H. **A Whole Blood Assay for AR-V7 and ARv567es in Patients with Prostate Cancer**. J Urol. 2016 Jul 20. pii: S0022-5347(16)30914-4. doi: 10.1016/j.juro.2016.06.095.

    Feibus AH, Sartor O, Moparty K, Chagin K, Kattan MW, **Ledet E**, Levy J, Lee B, Thomas R, Silberstein JL. **Clinical Use of PCA3 and TMPRSS2:ERG Urinary Biomarkers in African-American Men Undergoing Prostate Biopsy**. J Urol. 2016 Oct;196(4):1053-60. doi: 10.1016/j.juro.2016.04.075.

    Steinberger AE, **Ledet EM**, Luk E, Cotogno P, Stolten M, Desmond D, Feibus A, Silberstein J, Sartor O.
    Characterizations of Clinical and Therapeutic Histories for Men With Prostate Cancer-Specific Mortality. Clin Genitourin Cancer. 2016 Apr;14(2):139-48. doi: 10.1016/j.clgc.2015.11.003.

Stolten M, **Ledet E**, Dotiwala A, Luk E, Sartor O. **Alternative Digit Ratios and Their Relationship to Prostate Cancer**. Clin Genitourin Cancer. 2016 Apr;14(2):149-52. doi: 10.1016/j.clgc.2015.11.005.

Zhang G, Liu X, Li J, **Ledet E**, Alvarez X, Qi Y, Fu X, Sartor O, Dong Y, Zhang H. **Androgen receptor splice variants circumvent AR blockade by microtubule-targeting agents.** Oncotarget. 2015 Sep 15;6 (27):23358-71.

- **Books or other non-periodical, one-time publications.**
  Nothing to report.

- **Other publications, conference papers, and presentations.** (See Appendix 5 for conference presentation abstracts)
  Chowdry R, **Ledet EM**, Phelan M, Sartor O. **MLL Translocation in Two Castrate Resistant Prostate Cancer (CRPC) Patients**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.*

  Ernst E, **Ledet EM**, Feibus A, Silberstein J, Sartor O. **Race, Inflammation, and Prostate Cancer: A Comparison of African Americans and Caucasians**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.

  Steinberger A, **Ledet EM**, Feibus A, Premkumar V, Dotiwala A, Stolten M, Lewis B, Sartor O. **Sequencing of treatments in metastatic CRPC for patients who have completed all therapeutic interventions**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.*

  Ledet EM, Miller P, Gambhira R, Dotiwala A, Sartor O. **Characterization of plasma derived and urinary exosomal microRNA from metastatic CRPC patients**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.

  Stolten M, **Ledet EM**, Guccione J, Feibus A, Lewis B, Silberstein J, Sartor O. **Evaluating Abiraterone Responses in African Americans With Metastatic CRPC**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.

Garvey C, Cotogno P, Ernst E, **Ledet EM**, Sartor O. **Clinical differences between African Americans (AA) and Caucasians (CA) with and without family history (FH) of prostate cancer**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.

Guccione J, **Ledet EM**, Stolten M, Steinberger A, Chow L, Cotogno P, Lewis B, Sartor O. **Early Assessment of PSA response in CRPC Patients Treated with Enzalutamide (Enza) or Abiraterone (Abi).** Poster. 2016 Genitourinary Symposium. San Francisco, CA.

Chow L, **Ledet EM**, Steinberger A, Guccione J, Sartor O. **Body mass index at mCRPC, weight change and survival in advanced prostate cancer**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.

Gambhira R, **Ledet EM**, Dotiwala A, Mandal D, Sartor O. **Copy number variations in AR associated and DNA repair genes from plasma cell free DNA of metastatic CRPC patients**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.

Feibus A, Sartor O, Moparty K, Kattan M, Chagin K, **Ledet EM**, Levy J, Lee B, Thomas R, Silberstein J. **Utility of PCA3 and TMPRSS2:ERG Urinary Biomarkers in African American Men Undergoing Prostate Biopsy**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.*

Feibus A, Haney N, Boxberger J, Levy J, Libby R, Kramer J, **Ledet EM**, Moparty K, Thomas R, Lewis B, Silberstein J, Sartor O. **Pathologic upgrading on confirmatory biopsy in a racially diverse group of men on active surveillance for prostate cancer.** Poster. 2016 Genitourinary Symposium. San Francisco, CA.

- **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**
  - A detailed database and clinical annotation has been assembled for the inflammation cohort at Tulane University Medical Center. This database includes detailed annotation of pathologic data, outside medical records, genetic testing, treatment history and ongoing follow-up. This database is supplemented with biospecimen collection, DNA, and RNA derived from the patient's radical prostatectomy; once sequenced, the resulting genetic data will be linked to this clinical annotation. This database will be a continuous resource for this study as well as other clinical studies evaluating early stage PCa. Ultimately, this database may be incorporated into a larger registry which will be used to facilitate collaboration and further translational research opportunities.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Elisa Ledet, PhD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	orcid.org/0000-0003-1230-3255
Nearest person month worked:	12
Contribution to Project:	Dr. Ledet has performed completed training associated with this award and continues progress on the proposed research plan.
Funding Support:	Institutional

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

  Nothing to report.

- **What other organizations were involved as partners?**

- **Organization Name:** Louisiana State University Health Sciences Center
- **Location of Organization:** New Orleans, LA
- **Partner's contribution to the projec**t
  - **Financial support:** None
  - **In-kind support:** Partner makes software available to PI
  - **Facilities**: PI uses partner facilities for project activities
  - **Collaboration**: Training in genetic epidemiological methods was provided by Diptasri Mandal, PhD.
  - **Personnel exchanges**: None
  - **Other:** None

8. **SPECIAL REPORTING REQUIREMENTS**

- **COLLABORATIVE AWARDS:** Not applicable
- **QUAD CHARTS:** Not applicable

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report.* ***DO NOT RENUMBER PAGES IN THE APPENDICES.***

<u>Appendix 1</u>. Tulane Inflammation cohort: Demographics.

	Demographic Data					P values			
	All Patients	Patients with CI		Patients *without* CI		Among All Patients		Among Patients with CI	Among Patients *without* CI
		AA	CA	AA	CA	AA vs. CA	CI vs. NO CI	AA vs. CA	AA vs. CA
N=	113	52	42	9	10				
Age: - Median (Range)	62 (41-75)	62 (50-71)	62 (50-75)	62 (50-68)	57 (41-67)	N/A	N/A	N/A	N/A
BMI - Median (Range)	28.79 (14.27-46.75)	25.96 (14.27-41.50)	27.90 (21.62-41.41)	29.78 (23.10-36.29)	29.83 (22.90-46.75)	0.589[1]	0.116[1]	N/A	N/A
Family History of Prostate Cancer: - No - Yes - Unknown	 69 (61.1%) 28 (24.8%) 16 (14.2%)	 33 (63.5%) 12 (23.1%) 7 (13.5%)	 24 (57.1%) 11 (26.2%) 7 (16.7%)	 6 (66.7%) 2 (22.2%) 1 (11.1%)	 6 (60.0%) 3 (30.0%) 1 (10.0%)	0.559[2]	1.000[3]	0.641[2]	1.000[3]
Treatment: - RARP - AS, RARP	 105 (92.9%) 8 (7.1%)	 50 (96.2%) 2 (3.8%)	 36 (85.7%) 6 (14.3%)	 9 (100.0%)	 10 (100.0%)	N/A	N/A	N/A	N/A

RARP= Robot assisted radical prostatectomy; AS= Active surveillance ; [1] *P* value from two-way ANOVA test; [2] *P* value from Pearson chi square; [3] *P* values from Fisher's exact test

<u>Appendix 2</u>. Tulane Inflammation cohort: Analyses of clinical parameters.

	Clinical Data					P values				Explanations of Significance
	All Patients	Patients with CI		Patients *without* CI		Among All Patients		Among Patients with CI	Among Patients *without* CI	
		AAs	CAs	AAs	CAs					
N=	113	52	42	9	10	AAs vs. CAs	CI vs.NO CI	AAs vs. CAs	AAs vs. CAs	
PSA at Diagnosis: - **Median (Range)**	5.60 (1.30-26.70)	5.60 (1.90-26.70)	5.43 (2.00-22.20)	7.90 (4.07-21.33)	7.40 (1.30-21.70)	0.618[1]	0.437[1]	N/A	N/A	N/A
PSA Density: - **Median (Range)**	0.17 (0.022-2.52)	0.17 (0.060-2.52)	0.17 (0.040-0.93)	0.18 (0.13-0.82)	0.26 (0.022-0.77)	0.989[1]	0.618[1]	N/A	N/A	N/A
NCCN* Risk Classification: - **High** - **Intermediate** - **Low** - **Very Low** - **Unknown**	27 (23.9%) 54 (47.8%) 22 (19.5%) 5 (4.4%) 5 (4.4%)	12 (23.1%) 29 (55.8%) 7 (13.5%) 2 (3.8%) 2 (3.8%)	8 (19.0%) 17 (40.5%) 13 (31.0%) 2 (4.8%) 2 (4.8%)	4 (44.4%) 5 (55.6%)	3 (30.0%) 3 (30.0%) 2 (20.0%) 1 (10.0%) 1 (10.0%)	0.010[2]	0.553[3,4]	0.038[2]	0.206[3]	AAs more likely to have high/ intermediate risk classifications
Prior Biopsy - **No** - **Yes** - **Unknown**	81 (71.7%) 16 (14.2%) 16 (14.2%)	41 (78.8%) 4 (7.7%) 7 (13.5%)	26 (61.9%) 9 (21.4%) 7 (16.7%)	5 (55.6%) 3 (33.3%) 1 (11.1%)	9 (90.0%) 1 (10.0%)	0.338[2]	1.000[3]	0.043[2]	0.082[4]	Among patients with CI, CAs more likely to have undergone prior biopsy
Percent of Positive Cores: - **Median (Range)**	33 (0-100)	43 (8-100)	29 (0-100)	56 (17-100)	32 (9-83)	0.005[1]	0.211[1]	N/A	N/A	AAs- higher percentage positive cores
Positive Margins: - **No** - **Yes** - **Unknown**	75 (66.4%) 34 (30.1%) 4 (3.5%)	35 (67.3%) 16 (30.8%) 1 (1.9%)	32 (76.2%) 9 (21.4%) 1 (2.4%)	2 (22.2%) 6 (66.7%) 1 (11.1%)	6 (60.0%) 3 (30.0%) 1 (10.0%)	0.136[2]	0.035[2]	0.313[2]	0.153[3]	Patients *without* CI- more likely to have positive margins

Extra-prostatic Extension: - **No** - **Yes** - **Unknown**	73 (64.6%) 36 (31.9%) 4 (3.5%)	30 (57.7%) 21 (40.4%) 1 (1.9%)	33 (78.6%) 8 (19.0%) 1 (2.4%)	5 (55.6%) 3 (33.3%) 1 (11.1%)	5 (50.0%) 4 (40.0%) 1 (10.0%)	0.065[2]	0.437[2]	0.026[2]	1.000[3]	Among patients with CI, AAs more likely to have extra-prostatic extension
Seminal Vesicle Invasion: - **No** - **Yes** - **Unknown**	94 (83.2%) 15 (13.3%) 4 (3.5%)	45 (86.5%) 6 (11.5%) 1 (1.9%)	38 (90.5%) 3 (7.1%) 1 (2.4%)	4 (44.4%) 4 (44.4%) 1 (11.1%)	7 (70.0%) 2 (20.0%) 1 (10.0%)	0.294[2]	0.013[3]	0.364[3]	0.247[3]	Patients *without* CI more likely to have SV invasion
Prostate Size (g): - **Median (Range)**	43.50 (22.00-160.00)	46.00 (22.00-101.00)	43.00 (28.00-99.00)	57.00 (31.00-71.00)	37.50 (23.00-160.00)	0.545[1]	0.617[1]	N/A	N/A	N/A
PSA Copies♦ - **Median (Range)**	157713 (10282-11591076)	223813 (11377-11591076)	137367 (10282-1922714)	242919 (10568-589835)	118606 (49722-4389730)	0.507[5]	0.077[5]	N/A	N/A	N/A
PCA3 Copies♦ - **Median (Range)**	8881 (192-722026)	14137 (351-722026)	6846 (192-273493)	9759 (376-6127)	4303 (535-23703)	0.004[5]	0.193[5]	N/A	N/A	AAs- higher PCA3 copy levels
TMPRSS2 Copies♦ - **Median (Range)**	12 (0-2409)	9 (0-2409)	13 (0-1843)	24 (0-327)	75 (0-762)	0.292[5]	0.223[5]	N/A	N/A	N/A
PCA3 Score♦ - **Median (Range)**	46.64 (2.80-477.00)	70.20 (14.20-477.00)	32.62 (2.80-246.64)	49.31 (13.42-173.94)	30.03 (5.00-91.21)	<0.01[5]	0.191[5]	N/A	N/A	AAs- higher PCA3 scores
TMPRSS2 Score ♦ - **Median (Range)**	8.30 (0-1532.95)	4.40 (0-297.43)	13.99 (0-1386.40)	4.14 (0-1253.09)	21.87 (0-1532.95)	0.039[5]	0.482[5]	N/A	N/A	CAs- higher TMPRSS2 scores

* NCCN= National Comprehensive Cancer Network; ♦ From urine assays; [1] *P* value from two-way ANOVA test; [2] *P* value from Pearson chi square; [3] *P* value from Fisher's exact test; [4] For NCCN Fisher's exact analysis for comparison between chronic inflammation groups, "very low" and "low" classifications grouped together and "intermediate" and "high" classifications grouped together due to low sample sizes; [5] *P* value from Kruskal-Wallis test

<u>Appendix 3</u>. Tulane Inflammation cohort: Analysis of race and chronic inflammation.

	African Americans	Caucasians	Total	P value*
Chronic Inflammation	52	42	94	0.526
No Chronic Inflammation	9	10	19	
Total	61	52	113	

* P value from Pearson chi square

Appendix 4. Journal Publication abstracts.

McKay RR, Jacobus S, Fiorillo M, **Ledet EM**, Cotogna PM, Steinberger AE, Jacene HA, Sartor O, Taplin ME. **Radium-223 Use in Clinical Practice and Variables Associated With Completion of Therapy**. Clin Genitourin Cancer. 2016 Aug 20. pii: S1558-7673(16)30249-X. doi: 10.1016/j.clgc.2016.08.015.

BACKGROUND: Radium-223 has shown clinical efficacy in metastatic castration-resistant prostate cancer. Despite improvement in quality of life and survival, practice patterns and utility of this agent outside the context of clinical trials have not been fully characterized. The primary objective in this study was to evaluate variables associated with completion of 5 to 6 radium-223 doses.
PATIENTS AND METHODS: We conducted retrospective analyses of patients who received radium-223 (n = 135). Patients were classified into 3 cohorts: 1 to 2, 3 to 4, or 5 to 6 radium-223 doses. We evaluated the association of clinical and laboratory variables with the number of cycles administered (5-6 vs. 1-4 doses).
RESULTS: Twenty-five patients (18.5%) received 1 to 2 radium-223 doses, 27 (20.0%) received 3 to 4, and 83 (61.5%) received 5 to 6. The most common reasons for treatment discontinuation included disease progression (61.5%, n = 40), patient preference (15.4%, n = 10), and toxicity (10.8%, n = 7). Factors associated with therapy completion in univariate analysis included previous sipuleucel-T treatment (P = .068), no previous abiraterone or enzalutamide treatment (P = .007), hemoglobin ≥ lower limit of normal (LLN; P = .006), white blood cell count ≥ LLN (P = .045), absolute neutrophil count (ANC) ≥ LLN (P = .049), lower alkaline phosphatase (P = .029), and lower lactate dehydrogenase levels (P = .014). Factors associated with therapy completion in multivariable analysis included previous sipuleucel-T treatment (P = .009), hemoglobin ≥ LLN (P = .037), and ANC ≥ LLN (P = .029).
CONCLUSION: Several clinical parameters are associated with radium-223 therapy completion. In general, these parameters reflect earlier disease stage. These data are hypothesis-generating and prospective testing of the optimal number of radium-223 doses is warranted.

Vasudevamurthy AK, **Ledet E**, Garvey C, Lewis BE, Sartor O. **Estrogen-Mediated Activation of H875Y Androgen Receptor Mutation in a Prostate Cancer Patient**. Clin Genitourin Cancer. 2016 Jul 22. pii: S1558-7673(16)30218-X. doi: 10.1016/j.clgc.2016.07.015. Case Report.

Clinical Practice Points:

1. Androgen receptor gene mutations have previously been observed in preclinical studies to demonstrate activation by ligands other than androgens, including estrogens. The advent of tumor genome sequencing with circulating cell-free DNA testing provides the ability to detect such mutations and better understand the behavior of individual patient's tumors.

2. The new findings from this case report describe the first-known clinical correlation to the in vitro studies previously demonstrating tumor growth in the presence of estrogens due to a mutated androgen receptor that promiscuously recognizes a number of nonandrogenic ligands. The relevance of sequencing circulating cell-free DNA testing is underscored.
3. Novel tumor genome sequence testing of circulating cell free DNA provides the potential to elucidate whether detected mutations may confer sensitivity or resistance to specific therapeutic options, thus enhancing the ability for the clinician to make more specified treatment decisions for individual patients.

Liu X, **Ledet E**, Li D, Dotiwala A, Steinberger A, Feibus A, Li J, Qi Y, Silberstein J, Lee B, Dong Y, Sartor O, Zhang H. **A Whole Blood Assay for AR-V7 and ARv567es in Patients with Prostate Cancer**. J Urol. 2016 Jul 20. pii: S0022-5347(16)30914-4. doi: 10.1016/j.juro.2016.06.095.

PURPOSE: Most prostate cancer mortality can be attributable to metastatic castration resistant prostate cancer, an advanced stage that remains incurable despite recent advances. The AR (androgen receptor) signaling axis remains active in castration resistant prostate cancer. Recent studies suggest that expression of the AR-V (AR splice variant) AR-V7 may underlie resistance to abiraterone and enzalutamide. However, controversy exists over the optimal assay. Our objective was to develop a fast and sensitive assay for AR-Vs in patients.
MATERIALS AND METHODS: Two approaches were assessed in this study. The first approach was based on depletion of leukocytes and the second one used RNA purified directly from whole blood preserved in PAXgene® tubes. Transcript expression was analyzed by quantitative reverse transcription-polymerase chain reaction.
RESULTS: Through a side-by-side comparison we found that the whole blood approach was suitable to detect AR-Vs. The specificity of the assay was corroborated in a cancer-free cohort. Using the PAXgene assay samples from a cohort of 46 patients with castration resistant prostate cancer were analyzed. Overall, AR-V7 and ARv567es were detected in 67.53% and 29.87% of samples, respectively. Statistical analysis revealed a strong association of AR-V positivity with a history of second line hormonal therapies.
CONCLUSIONS: To our knowledge this is the first study to demonstrate that PAXgene preserved whole blood can be used to obtain clinically relevant information regarding the expression of 2 AR-Vs. These data on a castration resistant prostate cancer cohort support a role for AR-Vs in resistance to therapies targeting the AR ligand-binding domain.

Feibus AH, Sartor O, Moparty K, Chagin K, Kattan MW, **Ledet E**, Levy J, Lee B, Thomas R, Silberstein JL. **Clinical Use of PCA3 and TMPRSS2:ERG Urinary Biomarkers in African-American Men Undergoing Prostate Biopsy**. J Urol. 2016 Oct;196(4):1053-60. doi: 10.1016/j.juro.2016.04.075.

PURPOSE: Prostate specific antigen has decreased performance characteristics for the detection of prostate cancer in African-American men. We evaluated urinary PCA3 and TMPRSS2:ERG in a racially diverse group of men.
MATERIALS AND METHODS: After institutional review board approval, post-examination urine was prospectively collected before prostate biopsy. PCA3 and TMPRSS2:ERG RNA copies were quantified using transcription mediated amplification assays (Hologic, San Diego, California). Prediction models were created using standard of care variables (age, race, family history, prior biopsy, abnormal digital rectal examination) plus prostate specific antigen. Decision curve analysis was performed to compare the net benefit of PCA3 and TMPRSS2:ERG.
RESULTS: Of 304 patients 182 (60%) were African-American and 139 (46%) were diagnosed with prostate cancer (69% African-American). PCA3 and TMPRSS2:ERG scores were greater in men with prostate cancer, 3 or more cores, 33.3% or more cores, greater than 50% involvement of greatest biopsy core and Epstein significant prostate cancer ($p < 0.01$). PCA3 added to the standard of care plus prostate specific antigen model for the detection of any prostate cancer in the overall cohort (0.747 vs 0.677, $p < 0.0001$) in African-American men only (0.711 vs 0.638, $p=0.0002$) and nonAfrican-American men (0.781 vs 0.732, $p=0.0016$). PCA3 added to the model for the prediction of high grade prostate cancer for the overall cohort (0.804 vs 0.78, $p=0.0002$) and African-American men only (0.759 vs 0.717, $p=0.0003$) but not nonAfrican-American men. Decision curve analysis demonstrated improvement with the addition of PCA3. For African-American men TMPRSS2:ERG did not improve concordance statistics for the detection of prostate cancer.
CONCLUSIONS: For African-American men urinary PCA3 improves the ability to predict the presence of any and high grade prostate cancer. However, the TMPRSS2:ERG urinary assay does not add significantly to standard tools.

Steinberger AE, **Ledet EM**, Luk E, Cotogno P, Stolten M, Desmond D, Feibus A, Silberstein J, Sartor O. **Characterizations of Clinical and Therapeutic Histories for Men With Prostate Cancer-Specific Mortality.** Clin Genitourin Cancer. 2016 Apr;14(2):139-48. doi: 10.1016/j.clgc.2015.11.003.

BACKGROUND: Careful descriptions of men with prostate cancer (PCa)-specific mortality are scant in nontrial settings. The present retrospective review describes the clinical characteristics, timelines, and treatment histories from initial presentation to death in a cohort of men with metastatic, castrate-resistant PCa (mCRPC). Unique to the present study is the unequivocal attribution of PCa death by a single experienced clinician.
PATIENTS AND METHODS: A total of 119 patients who had been treated at Tulane Cancer Center and had died of mCRPC from 2008 to 2015 were studied through a retrospective review of the medical records.

RESULTS: The median age at diagnosis was 65 years (range, 40-85 years), and 34.4% of the patients presented with metastatic disease (stage M1). Of these patients, 56% had received definitive primary therapy, all had received androgen-deprivation therapy, and 52% had received docetaxel. The patients had received a median of 7 (1-14) systemic therapies before death. Most were secondary hormonal manipulations after the diagnosis of mCRPC (median, 4; range, 0-9). The median survival was 69 months (range, 5-270 months) after diagnosis, and the median age at death was 73 years (range, 47-95 years). The presence of metastases at diagnosis was a significant predictor of early death (hazard ratio, 4.33; P < .001), and definitive primary therapy was a significant predictor of longer survival (P < .001). The median survival for patients presenting with metastases was 39 months (range, 5-235 months) compared with 100 months (range, 6-270 months) for those with localized disease (P < .001). The median age at diagnosis between the docetaxel- and non-docetaxel-treated patients was significantly different at 62 and 71 years, respectively (P = .002).
CONCLUSION: The present retrospective analysis provides initial views clarifying the clinical characteristics of men dying of mCRPC and the therapies they received before death. Additional data are needed in multi-institutional settings to confirm these findings.

Stolten M, **Ledet E**, Dotiwala A, Luk E, Sartor O. **Alternative Digit Ratios and Their Relationship to Prostate Cancer**. Clin Genitourin Cancer. 2016 Apr;14(2):149-52. doi: 10.1016/j.clgc.2015.11.005.

BACKGROUND: The ratio of the second to the fourth digits (2D:4D) has been linked to prenatal androgen exposure and prostate cancer (PCa). The use of alternative finger ratios has been shown to be a greater indicator of sexual dimorphism when compared with the traditional 2D:4D ratio. This study aimed to assess the relationship between alternative digit ratios, racial demographics, and clinical/pathologic parameters associated with PCa.
MATERIALS AND METHODS: Digital finger length measurements were made from scanned images of hands from patients with PCa. Race, age, family history, history of metastasis, and Gleason score at diagnosis were assessed in a cross-sectional clinic-based study. Demographic and clinical parameters were analyzed with respect to various alternative finger length ratios.
RESULTS: Hand measurements were obtained in 354 white and 98 African-American patients with PCa. African-American men were more likely to have a smaller 2D:3D (P < .0001) and 2D:4D digit ratio (P < .0001) in both hands. Larger right (R)3D:5D (P = .0005), R4D:5D (P = .0014), and R2T:2D (P = .0501) digit ratios were present in African-Americans compared with whites. In exploratory analyses, African-American men with a smaller left (L)2T:2D ratio were younger at the time of PCa diagnosis (P = .0125). No relationship was found between the various digit ratios and Gleason score, the presence of metastatic disease, or family history.

CONCLUSION: Various alternative finger length ratios show strong differences between African-American and white men in this study. The potential relationship between the 2T:2D ratio and age at diagnosis in African-Americans needs additional verification.

Zhang G, Liu X, Li J, **Ledet E**, Alvarez X, Qi Y, Fu X, Sartor O, Dong Y, Zhang H. **Androgen receptor splice variants circumvent AR blockade by microtubule-targeting agents.** Oncotarget. 2015 Sep 15;6 (27):23358-71.

Docetaxel-based chemotherapy is established as a first-line treatment and standard of care for patients with metastatic castration-resistant prostate cancer. However, half of the patients do not respond to treatment and those do respond eventually become refractory. A better understanding of the resistance mechanisms to taxane chemotherapy is both urgent and clinical significant, as taxanes (docetaxel and cabazitaxel) are being used in various clinical settings. Sustained signaling through the androgen receptor (AR) has been established as a hallmark of CRPC. Recently, splicing variants of AR (AR-Vs) that lack the ligand-binding domain (LBD) have been identified. These variants are constitutively active and drive prostate cancer growth in a castration-resistant manner. In taxane-resistant cell lines, we found the expression of a major variant, AR-V7, was upregulated. Furthermore, ectopic expression of two clinically relevant AR-Vs (AR-V7 and ARV567es), but not the full-length AR (AR-FL), reduced the sensitivities to taxanes in LNCaP cells. Treatment with taxanes inhibited the transcriptional activity of AR-FL, but not those of AR-Vs. This could be explained, at least in part, due to the inability of taxanes to block the nuclear translocation of AR-Vs. Through a series of deletion constructs, the microtubule-binding activity was mapped to the LBD of AR. Finally, taxane-induced cytoplasm sequestration of AR-FL was alleviated when AR-Vs were present. These findings provide evidence that constitutively active AR-Vs maintain the AR signaling axis by evading the inhibitory effects of microtubule-targeting agents, suggesting that these AR-Vs play a role in resistance to taxane chemotherapy.

Appendix 5. Conference presentations abstracts.

Chowdry R, **Ledet EM**, Phelan M, Sartor O. **MLL Translocation in Two Castrate Resistant Prostate Cancer (CRPC) Patients**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.*

Background: The mixed-lineage leukemia (MLL) protein is a histone methyltransferase that regulates multiple genetic elements. Chromosomal rearrangements of the MLL gene result in expression of MLL- fusion proteins that occur in a subset of acute leukemias and are associated with poor prognosis. The MLL protein complex has been shown to interact with the androgen receptor via the MLL-menin subunit and, in model systems, MLL-menin inhibition blocks CRPC growth. We describe 2 cases of metastatic CRPC with a translocation in the MLL gene. Methods: In our institution, fluorescence in situ hybridization (FISH) testing of bone marrow specimens is routinely performed using the MLL Breakapart probe from Cytocell, Ltd (Cambridge, UK). The probe consisted of an 87 kb segment labeled in red that covered a region telomeric to the MLL locus and a 168 kb segment labeled in green centromeric to MLL. The FISH assay was performed according to manufacturer's instructions. Results: MLL translocation was found coincidentally in 2 patients. The first was a 50 year old male with CRPC who had progressed on abiraterone and multiple chemotherapy regimens. A bone marrow biopsy was done to evaluate pancytopenia and pathology revealed metastatic prostate cancer marrow infiltration, without any evidence of leukemia or myelodysplasia. FISH studies revealed a rearrangement of the MLL locus using the MLL Breakapart probe. The second patient was a 77 year old male with metastatic CRPC who had also progressed through multiple hormonal and chemotherapy regimens. Bone marrow biopsy was done to evaluate thrombocytopenia and pathology revealed metastatic prostate cancer occupying nearly 100% of the marrow. Cytogenetics revealed complex karyotype and FISH was positive for MLL gene rearrangement by the same assay. There was no evidence of leukemia or myelodysplasia in either case. Conclusions: Translocation of the MLL gene is well documented in leukemia but has not been described in CRPC. Additional studies are warranted regarding the frequency and importance of this potential therapeutic target.

Ernst E, **Ledet EM**, Feibus A, Silberstein J, Sartor O. **Race, Inflammation, and Prostate Cancer: A Comparison of African Americans and Caucasians**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.

Background: African-Americans (AA) have the highest rate of prostate cancer (PCa) incidence and mortality. Studies have shown higher rates of chronic prostate inflammation in AAs compared to Caucasians (CA). In order to better understand racial disparity in PCa and chronic inflammation (CI), this study examined the effects of race and CI on clinical parameters among PCa patients. Methods: This retrospective study sample consisted

of 61 AA and 52 CA PCa patients who underwent radical prostatectomies (RP) at Tulane Hospital between 2013 and 2015. Clinical data was extracted from biopsy and RP pathology reports. The study examined the relationship between CI, race, percent of positive cores, extra-prostatic extension, PSA, PSA density, urinary PCA3 and TMPRSS2, and prostate size (g). Pearson's chi-square, Fisher's exact, and Kruskal-Wallis tests were used to analyze categorical, non- continuous data; ANOVA tests were used to analyze continuous data. Differences between biopsy and surgical/pathologic Gleason scores and clinical/pathological stages were also assessed. Results: 94 patients (52 AAs and 42 CAs) had CI to some degree and 19 did not (9 AAs and 10 CAs). There was no difference in rate of CI between AA and CA patients (P = 0.526). Among all patients sampled, AAs had higher percentages of positive cores (P = 0.005), PCA3 copy levels (P =0.004), and PCA3 scores (P <0.001), lower TMPRSS2 scores (P =0.039), and were more likely to have "high" or "intermediate" NCCN risk strata (P =0.010). Among patients with CI, AAs were more likely than CAs to have extra-prostatic extension (P =0.026) and less likely to have undergone a prior prostate biopsy (P =0.043). Patients without CI were more likely than patients with CI to have positive tumor margins (P =0.035) and SV invasion (P =0.013). There were no significant relationships between race and CI, and changes in either total Gleason score or stage from biopsy to RP. Conclusions: This study showed that AAs and patients without CI had more advanced forms of PCa (possibly due to PSA detection biases). Findings did not reveal any significant link between race and CI. Larger studies are needed to confirm these results and better understand the relationship between race, CI, and PCa.

Steinberger A, **Ledet EM**, Feibus A, Premkumar V, Dotiwala A, Stolten M, Lewis B, Sartor O. **Sequencing of treatments in metastatic CRPC for patients who have completed all therapeutic interventions**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.*

Background: The current treatment paradigm for metastatic, castrate-resistant prostate cancer (mCRPC) has rapidly changed and six therapies [abiraterone (Abi), enzalutamide (Enza), docetaxel (Doc), cabazitaxel (Cab), radium-223 (Ra-223), and sipuleucel-T (Sip-T)] have now been proven to prolong overall survival. Though sequential therapy is the norm, few studies have reported on the variety and prevalence of these agents over the course of patient's lifetime. Herein, we sought to describe the temporal frequencies of mCRPC therapies in patients who completed all of their therapies. Methods: Retrospective chart reviews were conducted on 119 patients who died from mCRPC at Tulane Cancer Center from 2008-2015 (thus completing all possible therapies). Many patients were not treated with multiple life-prolonging therapies given the timing of their death. Post-mCRPC therapies were longitudinally sequenced and a frequency table was generated for first, second, third, etc. line of therapies. Results: Median duration from initial androgen deprivation therapy to mCRPC was 29 months (range: 0-252) and 34.4% of the cohort presented with distant metastatic disease (M1)

at diagnosis. The most common front line mCRPC therapies were nilutamide, Doc, Abi, and ketoconazole (Keto) in that order. Keto, Doc, dexamethasone, and Abi were the most common second line therapies. Abi, Doc, DES, and Cab were the most prevalent third line therapies. Doc, Abi, Cab, and Ra-223 were most common fourth line therapies. The median overall survival for our cohort was 69 months (range: 5-270 months) from initial diagnosis. Conclusions: This retrospective analysis provides a temporal snapshot of the timing and frequency of treatments for men dying from mCRPC from 2008-2015. More recent patients are likely to have greater access to contemporary therapies.

Ledet EM, Miller P, Gambhira R, Dotiwala A, Sartor O. **Characterization of plasma derived and urinary exosomal microRNA from metastatic CRPC patients**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.

Background: Exosomes are nano-sized (50-100nm) vesicles derived from normal and tumor cells that function in cell-cell communication. These vesicles and their nucleic acid cargo may potentially serve as biomarkers for assessment of risk stratification and therapeutic response. The goal of this study was to characterize exosome derived microRNA (miRNA) isolated from plasma (pExos) and urine (uExos) of metastatic CRPC patients. Methods: Plasma samples were obtained from 18 mCRPC patients and 1 normal control. Following exosome isolation, RNA extraction and library prep, paired-end sequencing was performed using Illumina Hi-Seq 2000. A bioinformatics pipeline was used for data processing including alignment, duplicate removal, normalization, and variant calling. Visualization and differential analyses were performed with SNP & Variation Suite v8.x. RNA derived from uExos was amplified using whole transcriptome amplification and interrogated with Prostate Cancer (PCa) miScript miRNA PCR Array. Results: Exosomes from both plasma and urine had similar amounts of miRNA/total RNA with average 34% miRNA (range 19%-51%). pExos had larger RNA fragments (range 10-333 nt) while uExos were more highly fragmented (range 10-60 nt). The amount of miRNA and fragmentation pattern was highly variable amongst patients. In pExos, RNA from PDPK1, USP9X, MAGI2, HMGA2 and PTGFR were present and previously shown in PCa. Also in pExos, miR941-2, miR4454, miR1302-2, miR143HG and miR22HG were annotated in prostate cancer patients; these miRNA have previously been identified in cancer. In uExos miR-16-5p and miR-375 were present and are shown to be differentially regulated in prostate cancer. Expression analyses will be presented. PCR validation is ongoing. Conclusions: The identification of cancer associated miRNA in pExos and uExos may potentially serve as biomarkers in mCRPC patients. The abundance and stability of miRNA contained in exosomes may provide insight into tumor evolution and disease progression. Additional studies evaluating the clinical relevance and prognostic value of exosomal miRNA are warranted.

Stolten M, **Ledet EM**, Guccione J, Feibus A, Lewis B, Silberstein J, Sartor O. **Evaluating Abiraterone Responses in African Americans With Metastatic CRPC**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.

Background: A disparity between African American (AA) and other racial groups is documented in prostate cancer incidence and mortality. For metastatic CRPC, abiraterone (Abi) showed improvement in overall survival and gained FDA approval. However, Phase III trials enrolled mostly Caucasian (CA) patients. Documentation of Abi response rates in AA men is scant. Further characterization of Abi responses in AA men was the objective of this study. Methods: Age at diagnosis, prior enzalutamide (Enza) and/or docetaxel (Doc), and duration of Abi treatment were assessed. Baseline values at Abi initiation for alkaline phosphatase (ALP), hemoglobin (Hgb), and lactate dehydrogenase (LDH) were recorded. PSA values at baseline and throughout treatment were also logged. The velocity of PSA decline was determined by the PSA half-life (PSAHL) based on time to nadir. PCWG2 criteria were used to define PSA response and progression. Results: This was a single institution, retrospective cohort of 103 patients with mCRPC treated with Abi (n = 24 AA; n = 79 CA). Median age at diagnosis was 61.8 years and 62.4 years for AA and CA respectively. Prior Enza/Doc was 4.2%/33.3% for AA and 6.3%/29.1% for CA. Median duration of Abi therapy in AA was 207 days and 253 days for CA; neither median age or duration were statistically distinct. Median AA baseline ALP, Hgb, LDH, and PSA was 136 (range (r) = 59-653), 11.8 (r = 8.9-15.4), 256 (r = 157-401), and 59.9 (r = 4.8-1658) respectively. Median CA baseline ALP, Hgb, LDH, and PSA were 88 (r = 51-1600), 12.4 (r = 8.4-15.0), 204 (r = 100-528), and 40.6 (r = 2.5-2890) respectively. The difference in baseline lab values between AA and CA were insignificant. No statistical difference was seen in median PSAHL (AA = 55 days; CA = 64 days), or PSA decline of > 30% (AA = 50%; CA = 52%), > 50% (AA = 46%; CA = 39%), or > 90% (AA = 21%; CA = 14%). Finally, neither the median time to nadir (AA = 119 days; CA = 137 days) or progression (AA = 157 days; CA = 131 days) were significantly different. Conclusions: Comparison between AA men and CA men in mCRPC patients being treated with Abi showed no statistical difference in response rates, duration of response, or time to progression. Prospective, multi-institutional studies are needed to further assess these findings.

Garvey C, Cotogno P, Ernst E, **Ledet EM**, Sartor O. **Clinical differences between African Americans (AA) and Caucasians (CA) with and without family history (FH) of prostate cancer**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.

Background: Prostate cancer is one of the most common adult malignancies. Two well characterized risk factors for prostate cancer (PCa) are family history (FH) and race. The goal of this study was to distinguish the influence of family history and race with regards to clinically relevant covariates. Methods: In this

single-institution study, 497 PCa patients from Tulane Hospital were clinically annotated and FH was evaluated. FH was defined as having ≥ 1 first degree relative affected with PCa and/or ≥ 2 affected second or third degree relatives. There were 147 AA and 350 CA patients; 66 AA and 120 CA reported PCa FH. The following clinical factors were documented: age and PSA at diagnosis (dx), Gleason score (biopsy or radical prostatectomy), and presence of metastasis (at any time). Chi-square, ANOVA, and odds ratio tests were performed to identify potential clinical correlates with regard to FH and race. Results: Results indicate that race and FH are not independent ($p = 0.0266$), where AAs were 1.5 times more likely to have a FH of PCa than CAs (95% CI 1.0543-2.3133). On average, men with a FH of PCa were younger at dx ($p = 0.0063$). FH significantly impacted age at dx for AAs ($p = 0.036$) but not CAs. No difference in age of dx was detected between race. FH of PCa did not influence PSA at dx in either CA (6.5, 1.2-681) or AA (19.2, 1.7-500) men, however race did ($p = 0.004$). CAs with FH of PCa were more likely to be diagnosed with low or intermediate risk PCa (Gleason score ≤ 7, $p = 0.03$). Gleason scores were not significantly different between races. Overall, FH did not influence metastasis. Although, AAs ($n = 19$) were 1.61 times more likely to develop metastatic disease compared to CA men. Conclusions: For AAs, FH lowered age at dx but did not have influence on development of metastatic disease, Gleason score or PSA. For CAs, Gleason score was lower in men with a FH. Overall AAs, as compared to CAs had more metastatic disease and higher PSAs at dx. Continuing to track clinical differences between AAs and CAs with a FH of PCa may provide additional insights into underlying racial and clinical disparities.

Guccione J, **Ledet EM**, Stolten M, Steinberger A, Chow L, Cotogno P, Lewis B, Sartor O. **Early Assessment of PSA response in CRPC Patients Treated with Enzalutamide (Enza) or Abiraterone (Abi).** Poster. 2016 Genitourinary Symposium. San Francisco, CA.

Background: Abi and Enza are used in treating metastatic CRPC. Primary resistance is well described but little data exists for early treatment responders. Further characterization of patients with early PSA declines was the goal of this study. Methods: Single institution, retrospective reviews were performed on 85 CRPC patients (pts) treated with Abi or Enza. PSAs were recorded for the duration of treatment. The primary end point was to describe receiver operating characteristics (ROC) of early PSA changes, including sensitivities and specificities, as a predictor of later treatment response (defined as ≥ 50% decrease in PSA from baseline). 12 additional clinical covariates were also evaluated as treatment response predictors. Results: 38/71 pts in the Abi treatment group and 12/43 pts in the Enza treated group had a PSA treatment response at some point. Among the "eventual" Abi responders, some achieved a response early; 7/38 at 4 weeks and 20/38 at 8 weeks. Of Enza "eventual" responders, 7/12 achieved response at 4 weeks. For early responders (either 4 or 8 weeks), Abi pts median treatment time was 336 days. Enza early responders median treatment was time 303 days. For ROC

analysis of PSA changes at 4 & 8 weeks, in terms of predicting 'eventual' responders, the Abi group had an area under the curve (AUC) of 0.86 ± 0.07 and 0.93 ± 0.08, respectively. Enza ROC analysis of PSA change at 4 weeks had an AUC of 0.97 ± 0.03. Sensitivities and specificities were also described for various PSA change thresholds in each group. Ideal cut-off points for Abi at 4 weeks was a 37% decrease in PSA (sensitivity: 0.74, specificity: 0.97) and at 8 weeks was a 33% decrease (0.82, 0.97). For Enza ideal cut-off at 4 weeks was a 31% PSA decrease (0.82, 0.93). Covariate analysis of the Abi pts indicated that metastasis at diagnosis (P = 0.048) and prior taxanes predicted resistance to treatment (P = 0.019), as measured by < 50% decline in PSA. All covariates in the Enza pts failed to reach significance. Conclusions: Results suggest that early PSA changes for both Enza and Abi may be a reliable way for clinicians to predict long-term PSA response. This may allow for earlier modifications to be made in patient management for those not achieving an early PSA response.

Chow L, **Ledet EM**, Steinberger A, Guccione J, Sartor O. **Body mass index at mCRPC, weight change and survival in advanced prostate cancer**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.

Background: Body mass index (BMI) at diagnosis is associated with increased risk of fatal prostate cancer, but the link between BMI at mCRPC and cancer progression is less clear. Cachexia, often defined as involuntary weight loss > 5% over 6 months, is common in advanced cancers. The goal of this study was to examine the link between BMI at mCRPC and weight change as it relates to cancer progression, the outcomes of survival, and treatment use in a single-institution setting. Methods: 58 mCRPC patients treated at Tulane Hospital were identified, 41 of whom had an overweight BMI at mCRPC (BMI > 25) and 17 with normal BMI at mCRPC (BMI < 25). All patients had a confirmed prostate cancer death. Survival, treatment history, and percent weight change were compared according to BMI status. Rate of percent weight change was defined as the change in weight per day, from date of mCRPC diagnosis to the last treatment stop date or death date ("mCRPC days"). Linear regression, overall survival (OS), and nonparametric analyses were performed. Results: There was no significant difference between the normal and overweight BMI groups in overall survival, from date of diagnosis to death (median = 1835 days vs. 2710 days respectively). Additionally, the difference in survival from mCRPC to death was not statistically significant (median = 630 days vs. 799 days, p = 0.115). Use of Taxotere was not significantly different (47% vs. 68% respectively); however, overweight patients (n = 28) more likely received Abiraterone than normal BMI patients (n = 2) (p-value = 0.0001). The rate of percent weight change was significantly different for normal and overweight patients (mean = –0.050%/day vs. –0.019%/day, p = 0.003). Linear regression analysis showed that mCRPC days had a significant effect on percent weight change (p = 0.0109), and this effect was not significantly different between BMI groups (p = 0.6991). Conclusions: Survival after mCRPC was not significantly different between BMI groups. We observed a significant effect of mCRPC days on percent weight change, with a similar effect for both BMI groups. This

outcome is expected, as more time would allow for greater weight changes to occur. Larger studies are needed to fully evaluate these observations.

Gambhira R, **Ledet EM**, Dotiwala A, Mandal D, Sartor O. **Copy number variations in AR associated and DNA repair genes from plasma cell free DNA of metastatic CRPC patients**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.

Background: Cell-free DNA (cfDNA) present in the plasma of advanced cancer patients can reflect tumor related genetic alterations. Recent data suggests copy number variations (CNVs) in AR-associated and DNA repair pathway genes play a potential role in prostate cancer progression. Here, we performed sequencing of cfDNA from 13 mCRPC patients to evaluate its potential in elucidating tumor related genetic variations. The long-term goal of our project is to correlate cfDNA derived genetic alterations with prostate cancer progression and/or therapeutic resistance/responses. Methods: cfDNA was isolated from 13 advanced mCRPC patient plasma samples using the Qiagen circulating nucleic acid kit. 100ng of cfDNA was utilized for library construction; and the libraries were paired-end sequenced on the Illumina HiSeq 2000. The resulting data was analyzed using the GATK best practices bioinformatics pipeline and the visualized using the SNP & Variation Suite v8.x. Results: The bioanalyzer profiles of cfDNA derived from mCRPC patients is highly fragmented with an average fragment size of 306-605bp. Although, several CNVs were found across the genome, we focused analysis on CNVs related to AR associated and DNA repair genes. Our preliminary analysis of cfDNA, despite low sequencing depth, shows full or partial amplifications in AR (13/13), and other genes including FOXA1, NCOR1, NCOR2 and/or PIK3CA (7/13) and NCOR2 (10/13). For DNA repair genes partial/full amplifications were present in BRAC1, BRAC2, ATM, CDK12, MLH1 and/or MSH2 (7/13). Deletions are less reliably detected in the highly fragmented cfDNA. The majority of these CNVs have been reported in the WGS studies from metastatic CRPC tissue derived genomic DNA (cBioPortal). We are currently validating cfDNA genomic alterations by comparing it to germ line DNA derived via qPCR. Conclusions: Our preliminary study indicates that AR and DNA repair related genetic alterations could be found in the cfDNA derived from metastatic CRPC patients. This warrants more detailed examination of these cfDNA genetic alterations for identifying clinically relevant issues in mCRPC patients.

Feibus A, Sartor O, Moparty K, Kattan M, Chagin K, **Ledet EM**, Levy J, Lee B, Thomas R, Silberstein J. **Utility of PCA3 and TMPRSS2:ERG Urinary Biomarkers in African American Men Undergoing Prostate Biopsy**. Poster. 2016 Genitourinary Symposium. San Francisco, CA.*

Background: To determine the performance characteristics of urinary PCA3 and TMPRSS2:ERG (T2:ERG) in a racially diverse group of men. Methods: Following IRB approval, from 2013-2015, post digital rectal exam (DRE) urine was prospectively collected in patients without known prostate cancer (PCa), prior to biopsy. PCA3 and T2:ERG RNA copies were quantified and normalized to PSA mRNA copies using Progensa assay (Hologic, San Diego, CA). Prediction models for PCa and high-grade PCa were created using standard of care (SOC) variables (age, race, family history of PCa, prior prostate biopsy and abnormal DRE) plus PSA. Decision Curve Analysis was performed to compare the net benefit of using SOC, plus PSA, with the addition of PCA3 and T2:ERG. Results: Of 304 patients, 182 (60%) were AA; 139 (46%) were diagnosed with PCa (69% AA). PCA3 and T2:ERG scores were greater in men with PCa, ≥ 3 cores, ≥ 33.3% cores, > 50% involvement of greatest biopsy core and Epstein significant PCa (p- values < 0.04). PCA3 added to the SOC plus PSA model for the detection of any PCa in the overall cohort (0.747 vs 0.677; $p < 0.0001$), in AA only (0.711 vs 0.638; $p = 0.0002$) and non-AA (0.781 vs 0.732; $p = 0.0016$). PCA3 added to the model for the prediction of high-grade PCa for the overall cohort (0.804 vs 0.78; $p = 0.0002$) and AA only (0.759 vs 0.717; $p = 0.0003$) but not non-AA. Decision curve analysis demonstrated significant net benefit with the addition of PCA3 compared with SOC plus PSA. For AA, T2:ERG did not improve concordance statistics for the detection any or high-grade PCa. Conclusions: For AA, urinary PCA3 improves the ability to predict the presence of any and high-grade PCa. However for this population, T2:ERG urinary assay does not add significantly to standard detection and risk stratification tools.

Feibus A, Haney N, Boxberger J, Levy J, Libby R, Kramer J, **Ledet EM**, Moparty K, Thomas R, Lewis B, Silberstein J, Sartor O. **Pathologic upgrading on confirmatory biopsy in a racially diverse group of men on active surveillance for prostate cancer.** Poster. 2016 Genitourinary Symposium. San Francisco, CA.

Background: To evaluate the clinical variables associated with upgrading at confirmatory biopsy among a racially-diverse group of men with prostate cancer (PCa) who elect Active Surveillance (AS). Methods: Following IRB approval, of the more than 260 men from our multi-institutional prospective AS database we identified 140 that had undergone at least 1 confirmatory biopsy since their initial diagnosis. Patients whose diagnosis was made on TURP, had any Gleason 4 on their initial biopsy or whose initial and confirmatory biopsy were more than 2 years apart were excluded. The analysis cohort included 121 men who had Gleason Score ≤ 6, clinical stage ≤ T2a and PSA ≤ 20 ng/mL. Disease upgrading on confirmatory biopsy was Gleason score ≥ 7. Multiple variables were examined as univariate and MV predictors of upgrading. Results: We identified 121 men who fit inclusion criteria, 55 (45%) African Americans (AA) and 66 non-AA (55%) with a median follow-up of 22 months. The median age was 66, median number of biopsy cores taken at diagnostic biopsy was 12 and median time interval between diagnostic and confirmatory biopsy was 12 months. On

confirmatory biopsy, no evidence of disease was noted for 51 (42%) men (26 AA, 25 non-AA), 48 (40%) men (18, AA, 30 non-AA) had findings consistent with their initial biopsy and 22 men (11 AA, 11 non-AA) experienced upgrading at repeat biopsy. Of the 22 (18%) men who were upgraded, 18 (8 AA, 10 non-AA) upgraded to a Gleason score of 7, 3 (2 AA, 1 non-AA) were upgraded to a Gleason score of 8 and 1 (AA) had a Gleason score of 9. In univariate analysis AA race was associated with a greater number of positive cores ($p = 0.04$) and greater total prostate volume ($p = 0.03$) at confirmatory biopsy. Multivariate analysis was performed and none of the clinical variables examined (race, age, BMI, PSA, volume, PSAD, number of positive cores, total number of cores, percentage of positive cores, time between biopsies) were associated with upgrading on repeat biopsy. Conclusions: Our findings suggest that race is not associated with an increased risk of upgrading at confirmatory biopsy. AA with low-risk PCa are reasonable candidates for inclusion in most AS protocols and should not be excluded based on race alone.