AWARD NUMBER: W81XWH-15-1-0696

TITLE: Stroma-Based Prognosticators Incorporating Differences between African and European Americans

PRINCIPAL INVESTIGATOR: Michael McClelland

CONTRACTING ORGANIZATION: University of California, Irvine, CA 92697

REPORT DATE: Dec 2019

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE Dec 2019	**2. REPORT TYPE** Final	**3. DATES COVERED** 09/30/2015 - 09/29/2019

4. TITLE AND SUBTITLE

Stroma-Based Prognosticators Incorporating Differences between African and European Americans

5a. CONTRACT NUMBER

5b. GRANT NUMBER W81XWH-15-1-0696

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Michael McClelland, Dan Mercola, Mike Lilly

E-Mail: mmcclell@uci.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California, Irvine,
CA 92697

Medical University of South Carolina,
Charleston, SC 29425

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT.

Our objective was to use standard Formalin-fixed Paraffin-embedded (FFPE) tissue to identify RNAs in prostate tumor-adjacent stroma that distinguish between cancers in European Americans (EA) and African Americans (AA), and to determine which fraction of these differences were associated with DNA methylation and/or protein changes. Gleason Score, chemical relapse status, and approximate age, were used to match AA with EA patients. We found striking differences in interferon stimulated genes between AA and EA tumor-adjacent stroma, of potential value as prognostic and therapeutic targets. To establish an in vitro test-bed for these observations, it became necessary to developed a valuable resource of new normal and cancer-associated fibroblast cell cultures.The advances made in this project are the basis of a funded five year follow-up grant.

15. SUBJECT TERMS

Prostate, Stroma, Biomarkers, African American, DNA methylation, RNA, Prognosis.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified		

Table of Contents

1. Introduction……

Every prostate tumor is heterogeneous, with DNA mutations and copy number differences. This makes the identification of dependable biomarkers in tumors more difficult. In contrast, there are fewer mutations in the tumor-adjacent stroma, which nevertheless has many RNA expression changes relative to tumor-free stroma, presumably due to interactions with the tumor. The abundant tumor-adjacent stroma is a potential source of molecular prognosticators for prostate cancer progression and targets for therapeutics.

Our objective was to use standard Formalin-fixed Paraffin-embedded (FFPE) tissue to identify RNAs in prostate tumor-adjacent stroma that distinguishes between cancer in European Americans (EA) and African Americans (AA), and to determine which fraction of these differences were associated with DNA methylation and/or protein changes. Gleason Score, chemical relapse status, and approximate age, were used to match AA with EA patients. We found striking differences in interferon stimulated genes between AA and EA tumor-adjacent stroma. To establish an in vitro test-bed for these observations, it became necessary to developed a valuable resource of new normal and cancer-associated fibroblast cell cultures. The advances made in this project are the basis of a funded five year follow-up grant.

2. Keywords……

Prostate, Stroma, Prognosis, Biomarkers, African American, DNA methylation, RNA.

3. Accomplishments……..

- **What were the major goals of the project?**

The previous Annual Report provided details of milestone progress. In the no-cost extension progress was in data analysis, particularly with regards to the observations of interferon-stimulated gene differences between AA and EA, and culturing of CAFs for in vitro studies based on the reported achievements.

Specific Aim 1. Validation of the stroma-based prognostic classifier(s) for both African and European Americans.
Major Task 1: Obtain and process FFPE tissue for RNA and DNA isolation and identify prognosticators from training set.
Milestone Achieved: RNA-based prognosticator for recurrence refined based on training set. Retargeted to RNAseq Reported earlier.
Major Task 2: Perform blinded randomized test.
Milestone Achieved: Identification of prognostic power of the refined prognosticator in a randomized blinded trial. Reported earlier.

Specific Aim 2: Test for DNA methylation differences near genes that have prognostic RNA expression differences.
Major Task 1: DNA quality assessment, PCR optimization, sequencing.
This aim was altered to a global genome methylation peak identification strategy that works on degraded FFPE samples
Milestone Achieved: Determination of DNA methylation differences in genes of known prognostic or race-enriched expression profile. Reported earlier.
Specific Aim 3: Test for congruence of prognostic RNA expression differences with protein expression differences.
Major Task 1: Obtain and process FFPE tissue for protein expression analysis.
Milestone progress: This task continues to be explored through the use of Tissue Microarrays, including additional ones of which have been acquired in a collaboration with collaborators at Baylor.

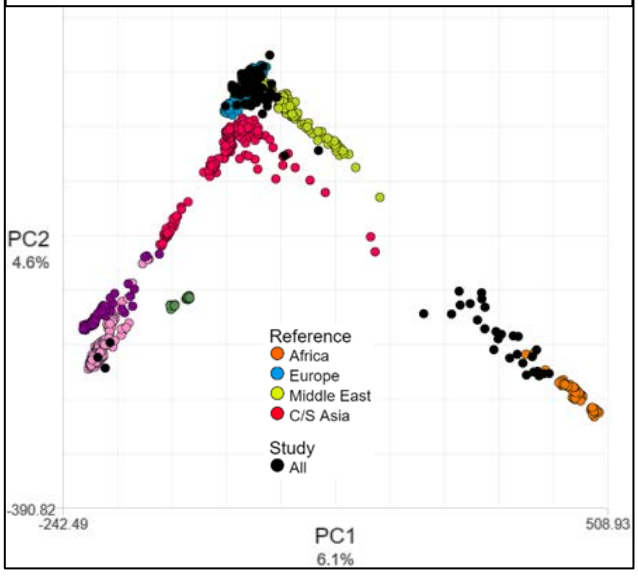
- **What was accomplished under these goals?**

Gleason Score	Total cases	AA FFPE	EA FFPE
G5	5	-	5
G6	170	28	142
G7	**343**	**83**	**260**
G8	59	29	30
G9	36	14	22
Total	613	154	459

Table 1. FFPE prostatectomy samples accumulated

Beyond what was reported in the previous Annual Reports, which exceeded our goal for sample accumulation, and allowed us to match AA and EA patients even better than planned, we have continued to add to the collection during the no-cost extension, particularly, Gleason 7 that are critical in determining treatment (highlighted in **blue** in **Table 1**). We have also accumulated additional fresh frozen tissues and cultured these for tumor-adjacent stroma, in collaboration with Dr. Xiaolin Zi at UCI.

Fig 1. SNP classification of RNA samples by race



One advantage of using sequencing instead of multiplex PCR, which was our original plan, is that we have been able to determine the proportion of African and European ancestry of each sample. This allows us to check for chain-of-custody errors, and also allows mixed race samples to be used as a continuous variable in a multivariate analysis. We used LASER (http://csg.sph.umich.edu/chaolong/LASER/index.html), which provides an estimate of geographic origin based on 632,958 SNPs. The subset of SNPs present in RNA-Seq is more than sufficient to classify at the level required. In the no-cost extension we have analyzed almost 200 additional samples, including both tumor-adjacent

stroma and tumor-enriched samples. A projection of a subset of this data is presented in **Figure 1**. Black dots represent patients in our dataset. All but four self-identifications were accurate, and mixed race individuals were easy to identify and semi-quantitate.

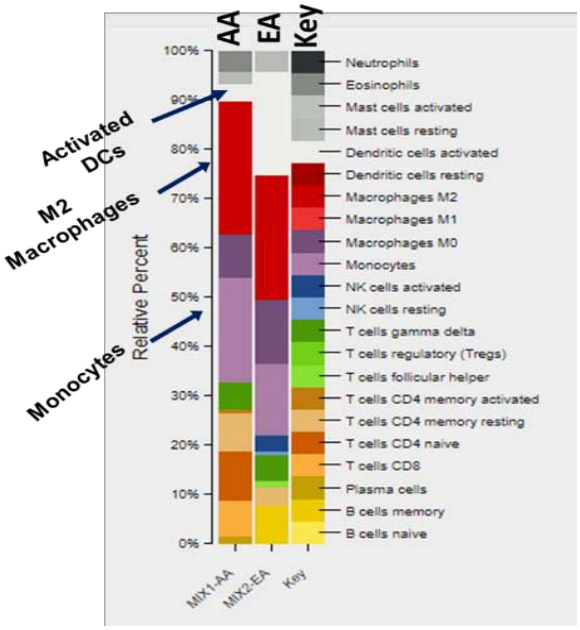
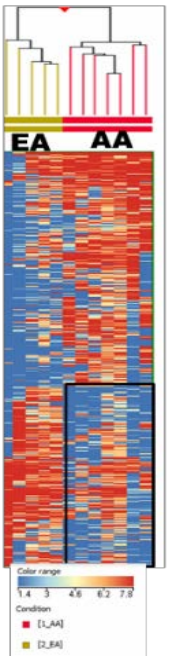


Figure 2. CIBERSORT analysis of immune cell compositions in AA and EA tumor-adjacent stroma. The frequency of infiltrated immune cell types was estimated using gene expression profiling of tumor-adjacent stroma AA and EA PCa patients. Results are reported as relative fractions normalized to 100% across all immune cell subsets.

We used a computational approach, CIBERSORT to estimate the relative fractions of 22 immune cell types in AA and EA PCa tumor-adjacent stroma using RNA transcripts (RNAseq). Interestingly, the frequency of activated DCs in tumor-adjacent stroma of EA was 7-fold higher than in tumor stroma of AA patients (21% vs 3%) (**Figure 2**). Dendritic cells (DCs) are the major antigen presenting cells of the immune system, which initiate and regulate T cell responses and mount an immune response against the tumors. Dendritic cells also produce type I interferon. A weaker anti-tumor immune response in PCa tumor stroma of AA patients may therefore be caused by a relative scarcity of Ag presenting cells (i.e., DC cells).

Of interest, we have demonstrated that particular immunological pathways are generally down-regulated in stroma of AA versus EA in FFPE tissues (**Figure 3**), and one of these classes; interferon-stimulated genes (ISGs), is reiterated in cultured cancer-associated fibroblasts (CAFs) (**Figure 4A** and **4B**). Furthermore, this pathway is reactivatable by global dysregulation, in vitro (data in preparation). As a number of dysregulators are approved anti-cancer agents, this result may lead directly to a therapeutic strategy.

We have two manuscripts in preparation based on these data.



Immune response pathways associated with down regulated genes in AA compared to EA tumor stroma

Pathway	P value
Metabolism of amino acids and derivatives	1.18E-08
Integrin mediated Cell Adhesion	1.62E-06
Regulation of toll-like receptor signaling	7.41E-06
IL-1 signaling	1.46E-05
Focal Adhesion	1.89E-05
APC -mediated degradation of cell cycle protein	4.18E-05
Cell Cycle Checkpoints	1.23E-04
Cytokines and Inflammatory Response	1.76E-04
Signaling by NOTCH2	2.26E-04
Mitotic Metaphase and Anaphase	2.90E-04
Elastic fibre formation	3.44E-04
Protein folding	3.66E-04
Interleukin-1 signaling	4.61E-04
IL-5 Signaling	5.39E-04
Interleukin-11 Signaling	5.39E-04
Structural Pathway of Interleukin 1 (IL-1)	7.44E-04
IL-3 Signaling	8.09E-04
Regulation of Apoptosis	9.46E-04
Extracellular matrix organization	1.10E-03
Leptin signaling	1.30E-03
Proteasome Degradation	1.30E-03
AGE-RAGE	1.50E-03
Regulation of DNA replication	1.60E-03
L1CAM interactions	1.70E-03
M-G1 Transition	2.00E-03
Apoptosis Modulation and Signaling	2.10E-03
Selenium Micronutrient Network	2.20E-03
Regulation of mRNA Stability	2.20E-03
Class I MHC mediated antigen processing	2.30E-03
Apoptosis	2.40E-03
Androgen receptor signaling	2.60E-03
TCR Signaling	2.70E-03
Signaling by the B Cell Receptor	5.40E-03

Figure 3. Genes with reduced expression in tumor-adjacent stroma of AA: Black box in bottom right of heat map. Right: immune system pathways are highlighted in blue font.

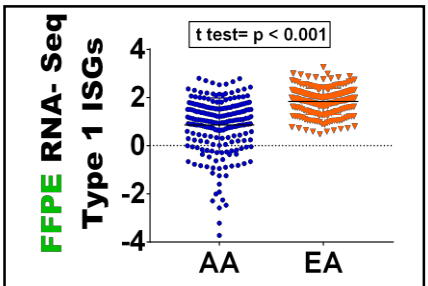


Figure 4A: 248 IFN-stimulated genes were expressed at statistically significantly lower levels in AA vs EA tumor stroma. Y axis represents normalized log2 read ratios.

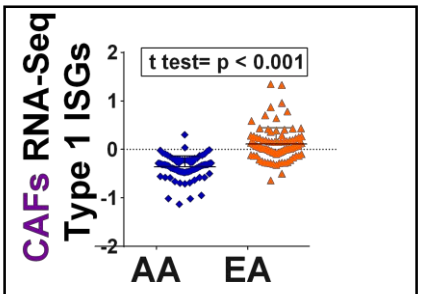


Figure 4B. Expression of 248 ISGs in AA and EA PCa CAFs.

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

Farah Rahmatpanah, the primary bench researcher, attended a total of eight conferences and presented posters and invited talks.

In addition, many others participated in this project for training:

- Nidhi Pradip Sheth. Bio199 Public health student, UCI. 2017-2019
- Parsa Rahmantpanah. Undergraduate student. UCI. 2015-2018.
- James Nguyen. Bio199 UCLA summer student. 2018
- Seunghyun Hwang, MD. Visiting scholar. 2018-2019.
- Jhannell Hannah Dioquito. Bio199 Biological Sciences student, UCI. 2017-2019.
- Gabriela De Robles. Bio199 Biological Sciences student. UCI. 2018-2019.
- Pavneet Randhawa. Bio199 Biological Sciences student, UCI. 2018-2019.
- Sundeep Pahal. Bio199 Biological Sciences student, UCI. 2018-2019.
- Vinay Kumar. UCLA Bioinformatics student. 2019.

The following people donated time and resources to pay for the creation of cultured cancer-associated fibroblasts (CAFs) and the Luminex assays that proved AA and EA cancer stroma are different, even *in vitro*, or contributed TMAs for IHC studies. We are now also including these CAFs in our protein biomarker screening.

- Xiaolin Zi, MD, PhD, Professor, Department of Urology.
- Anshu Agrawal, PhD, Assistant Adjunct Professor, Department of Medicine.
- Sudhanshu Agrawal, PhD, Specialist, Department of Medicine, Immunology.
- Liankun Song, Postdoctoral Scholar (Zi lab).
- Michael Ittmann, Baylor school of Medicine. TMAs

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

  Not repeating activities reported in the previous Annual Reports, in this no-cost extension:

- Talk at two public events for the American Cancer Society.
- Invited talk at Experimental Biology conference.
- Invited talk at Pathology and Laboratory Medicine seminar series.
- Talk at Hollings Cancer Center, Developmental Therapeutics program meeting.

4. Impact……..…….

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

We have made what may be a major contribution to the problem of obtaining high quality data from archived FFPE samples that were beyond the capabilities of array cards. In a slow progressing disease, like prostate cancer, where samples of interest for training and validation are necessarily old, this improvement is pivotal to future success of RNA and DNA biomarkers, which then lead to protein biomarkers. The data we have obtained, is a potential goldmine for the community to validate *their markers* on data from these same samples with long-term clinical follow up (see GEO RNAseq deposition, Section 6 below). We are preparing data for additional releases.

- **What was the impact on other disciplines?**

The ability to screen very old FFPE samples is especially of interest for prostate cancer, but this is not the only disease where such ability will be important.

The unmethyl-CpG binding protein has been little used by others, and we find it is exquisitely specific to CpG islands, whereas the methyl-CpG binding protein assays methylation in a lot of the genome due to a requirement for fewer methylated-C in a DNA fragment for successful capture. The difference in the spectrum of capture of the mCpG and unmethylated-CpG binding proteins may be exploitable in any system where differential methylation is of interest. The fact that most old FFPE samples can yield enough DNA for only a very limited set of bisulfite sequencing reactions, means that CpG binding protein capture methods probably have a long-term future.

- **What was the impact on technology transfer?**

  - Our results from highly degraded FFPE samples may be patentable.
  - We will protect the prognosticators and hope to transfer them to a commercial product. However, we need to further validate our final set of data, first.
  - The pathways identified in these experiments may be targetable for therapeutics.

- **What was the impact on society beyond science and technology?**

  Nothing to report.

5. Changes/Problems……

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

As reported three years ago, so many samples were of inadequate quality for the array cards that we explored using oligo capture and sequencing for RNA, and binding proteins for capture for DNA methylation. In the second and third years, this was remarkably successful; succeeding with far less RNA and of lower quality than the array cards required. This led to the discoveries outlined.

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

As reported three years ago, the quality in the oldest FFPE samples was a problem, but we learned how to deal with it, as explained above, with only a moderate delay.

- **Changes that had a significant impact on expenditures**

The fall in the cost of Illumina sequencing opened an opportunity for low quality samples that would otherwise have been prohibitively expensive. This has allowed a greater fraction of our huge effort on processing clinical samples to ultimately result in data. Although downstream bioinformatics is more labor-intensive than planned, the much richer dataset is a precious boon, generating a unique resource.

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

  None

- **Significant changes in use or care of human subjects**

  None

- **Significant changes in use or care of vertebrate animals.**
  Not applicable

- **Significant changes in use of biohazards and/or select agents**
  None

6. Products……

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

Rahmatpanah, Farah, Zhenyu Jia, Bozhao Men, Parsa Rahmatpanah, Sepideh Madahian, Michael McClelland, and Dan Mercola. 2016. "*Methylation correlates with suppressed expression of immunomodulatory genes in the tumor-adjacent stroma of African American Prostate Cancer compared patients of European American ancestry.*" ASBMB meeting annual meeting (EB 2016), San Diego, CA, http://www.fasebj.org/content/30/1_Supplement/1053.7?related-urls=yes&legid=fasebj;30/1_Supplement/1053.7

Lernhardt, W., Fiedler, F. Lasitschka, H. Kremling, F. Zinnhammer, F. Autschbach, D. Mercola, K. Schütze, J. Rassweiler. 2016. *Raman micro-spectroscopy: Potential for diagnosis and prediction of prostate cancer outcome.* Meeting of the EAU Section of Uro-Technology (ESUT), Athens, Greece.
http://www.eusupplements.europeanurology.com/article/S1569-9056(16)15111-5/fulltext

Lilly, Michael B. et al., *Lycopene and Docetaxel in PCa: regulation of angiogenesis by lycopene.* Am Inst CA Research, Bethesda, MD. 2016.

Rahmatpanah, Farah, Kathleen McGuire, Michael McClelland, Dan Mercola. 2016. *The use of whole genome methylation scanning to define genes preferentially suppressed in African American prostate Cancer*. The Science of Cancer Health Disparities in Racial/ Ethnic Minorities and the Medically Underserved. AACR meeting. Fort Lauderdale, FL. AACR program and proceeding. 2016.
http://cebp.aacrjournals.org/content/26/2_Supplement/B04

Lilly, Michael B. et al., *Circulating tumor (ct)-DNA profiling for potentially actionable targets in prostate cancer (PCa).* Meeting on Urological Cancers, Milan, Italy. 2016.
http://www.eusupplements.europeanurology.com/article/S1569-9056(16)30376-1/pdf

Lilly, Michael B. et al., *Profiling of circulating tumor (ct)-DNA for potentially actionable targets in prostate cancer.* Orlando, FL, ASCO. 2017.
http://ascopubs.org/doi/abs/10.1200/JCO.2016.34.15_suppl.5035

Lilly, Michael B. et al., *Circulating tumor (ct)-DNA alterations in metastatic castration-resistant prostate cancer (mCRPC): Association with outcomes and evolution with therapy.* Orlando, FL, 2017.

http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.6_suppl.149

vanDraanen, J. M., Davidson, H. Bour-Jordan, L. Bowman-Carpio, D. Boyle, S. Dubinett, B. Gardner, J. Gardner, C. McFall, D. Mercola, T. Nakazono, S. Soares, H. Stoppler, M. Tempero, S. Vandenberg, Y.Y. Wan, S. Dry. *Assessing Researcher Needs for a Virtual Biobank.* Biopreservation and Biobanking. 2017:15:203-210. https://www.ncbi.nlm.nih.gov/pubmed/27929677

Rice, L. J., Jefferson, M., Briggs, V., Delmoor, E., Johnson, J. C., Gattoni-Celli, S., Savage, S. J., Lilly, M., Prasad, S. M., Kittles, R, Halbert, CH. 2017. *Discordance in perceived risk and epidemiological outcomes of prostate cancer among African American men*. Preventive medicine reports, 7:1-6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5423348/

Nancy L Goicochea, Maria Garnovskaya, Mary G Blanton, Grace Chan, Richard Weisbart, Michael B Lilly. 2017. *Development of cell-penetrating bispecific antibodies targeting the N-terminal domain of androgen receptor for prostate cancer therapy*. Protein Engineering, Design and Selection, 30, 12, 785–793. https://academic.oup.com/peds/article/30/12/785/4710350

Thomas Paul Slavin, Kimberly Banks, Darya Chudova, Geoffrey R. Oxnard, Justin I. Odegaard, Rebecca J Nagy, Susan L. Neuhausen, Stacy W. Gray, Massimo Cristofanilli, Angel Augusto Rodriguez, Aditya Bardia, Brian Leyland-Jones, Mike Janicek, Michael B. Lilly, Guru Sonpavde, Christine E. Lee, Richard B. Lanman, Funda Meric-Bernstam, Razelle Kurzrock, Jeffrey N. Weitzel 2017. *Identification of putative germline mutations in 10,288 patients undergoing circulating tumor DNA testing.* Journal of Clinical Oncology. 35, 15:1514-1514. http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.15_suppl.1514

Gang Liu, Jinyu Zhang, Lewis Frey, Xiao Gang, Kongming Wu, Qian Liu, Michael Lilly and Jennifer Wu. 2017. *Prostate-specific IL-6 transgene autonomously induce prostate neoplasm through amplifying inflammation in the prostate and peri-prostatic adipose tissue.* Journal of Hematology & Oncology 10:14 https://jhoonline.biomedcentral.com/articles/10.1186/s13045-016-0386-7

Guru Sonpavde, Rebecca J Nagy, A. Oliver Sartor, Gregory Russell Pond, Theodore Stewart Gourdin, Lakshminarayanan Nandagopal, Elisa M. Ledet, Neeraj Agarwal, Emma Carroll, Gurudatta Naik, Jue Wang, Mehmet Asim Bilen, Petros Grivas, Richard B. Lanman, AmirAli Talasaz, Michael B. Lilly 2017. *Circulating tumor (ct)-DNA alterations in metastatic castration-resistant prostate cancer (mCRPC): Association with outcomes and evolution with therapy.* Journal of Clinical Oncology 35, no. 6, 149-149. http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.6_suppl.149

Aditya Bardia, Thereasa A. Rich, Victoria M. Raymond, Stephen R Fairclough, A. Oliver Sartor, Michael B. Lilly, Mohammad Nezami, Sandip Pravin Patel, Benedito A. Carneiro,

Alice C. Fan, Adam Brufsky, Barbara A. Parker, Benjamin B. Bridges, Neeraj Agarwal, Benjamin Louis Maughan, Richard B. Lanman, Massimo Cristofanilli. 2018. *Landscape of BRCA1 and BRCA2 germline, somatic, and reversion alterations detectable by cell-free DNA testing among patients with metastatic breast, ovarian, pancreatic, or prostate cancer*. Journal of Clinical Oncology 36, no. 15:12097. http://ascopubs.org/doi/abs/10.1200/JCO.2018.36.15_suppl.12097

W Jiang, M Lilly and T Ou. 2018. *The Pathologic Role of Toll-Like Receptor 4 in Prostate Cancer.* Frontiers in Immunology 9:1188. PMC5998742. https://www.frontiersin.org/articles/10.3389/fimmu.2018.01188/full

Elisa Ledet, Michael B. Lilly, Guru Sonpavde, Neeraj Agarwal, Rebecca J Nagy, A. Oliver Sartor. 2018. *Comprehensive analysis of AR alterations in cell free DNA from prostate cancer patients.* Journal of Clinical Oncology 36, 314. http://ascopubs.org/doi/abs/10.1200/JCO.2018.36.6_suppl.314

Andrew W Hahn, Elisa Ledet, David D. Stenehjem, Roberto Nussenzveig, Matthew Braithwaite, Benjamin Louis Maughan, Michael B. Lilly, A. Oliver Sartor, Neeraj Agarwal AW Hahn, E Ledet, DD Stenehjem, R Nussenzveig. 2018. *Association of genomic alterations (GAs) in circulating tumor DNA (ctDNA) with progression on abiraterone acetate (AA) or enzalutamide (enza) in advanced prostate cancer.* Journal of Clinical Oncology 36, no. 15, 5048-5048. http://ascopubs.org/doi/abs/10.1200/JCO.2018.36.15_suppl.5048

Malshundria Prophet, Kun Xiao, Theodore Stewart Gourdin, Rebecca J Nagy, Lesli Ann Kiedrowski, Elisa Ledet, Guru Sonpavde, A. Oliver Sartor, Michael B. Lilly. 2018. *Identification of putative germline mutations in 10,288 patients undergoing circulating tumor DNA testing.* Journal of Clinical Oncology 36, 6, 306-306. http://ascopubs.org/doi/abs/10.1200/JCO.2018.36.6_suppl.306

Slavin TP, Banks KC, Chudova D, Oxnard GR, Odegaard JI, Nagy RJ, Tsang KWK, Neuhausen SL, Gray SW, Cristofanilli M, Rodriguez AA, Bardia A, Leyland-Jones B, Janicek MF, Lilly M, Sonpavde G, Lee CE, Lanman RB, Meric-Bernstam F, Kurzrock R, Weitzel JN. 2018*. Identification of Incidental Germline Mutations in Patients With Advanced Solid Tumors Who Underwent Cell-Free Circulating Tumor DNA Sequencing.* J Clin Oncol. https://www.ncbi.nlm.nih.gov/pubmed/?term=30339520

Farah Rahmatpanah, Sudhanshu Agrawal, Vanessa M Scarfone, Sameer Kapadia, Dan Mercola, Anshu Agrawal. 2018. *Transcriptional Profiling of Age-Associated Gene Expression Changes in Human Circulatory CD1c+ Myeloid Dendritic Cell Subset,* The Journals of Gerontology: Series A, gly106, https://academic.oup.com/biomedgerontology/advance-article/doi/10.1093/gerona/gly106/4989886

Farah Rahmatpanah, Xiaolin Zi, Anne Sawyers, Anshu Agrawal, Michael Lilly, Michael McClelland, and Dan Mercola. 2018. *The aggressive nature of prostate cancer of African Americans is correlated with massive down-regulation of many immunoregulatory genes of microenvironment*, FASEB Journal. https://www.fasebj.org/doi/10.1096/fasebj.2018.32.1_supplement.804.60

Jia Z., Lee C., Zi X., McClelland M., Mercola D. 2018. Tumor Microenvironment: *Prospects for Diagnosis and Prognosis of Prostate Cancer Based on Changes in Tumor-Adjacent Stroma*. In: Robinson B., Mosquera J., Ro J., Divatia M. (eds) Precision Molecular Pathology of Prostate Cancer. Molecular Pathology Library. Springer, Cham https://link.springer.com/chapter/10.1007/978-3-319-64096-9_16

Malshundria Prophet, Kun Xiao, Theodore Stewart Gourdin, Rebecca J Nagy, Lesli Ann Kiedrowski, Elisa Ledet, Guru Sonpavde, A. Oliver Sartor, and Michael B. Lilly. *Detection of actionable BRAF missense mutations by ctDNA-based genomic analysis in prostate cancer.* Journal of Clinical Oncology 36:6_suppl, 306-306 https://ascopubs.org/doi/abs/10.1200/JCO.2018.36.6_suppl.306

Dan Mercola, Farah Rahmatpanah, Anshu Agrawal, Zhenyu (Arthur) Jia, Xiaolin Zi, Michael B. Lilly, and Michael McClelland. 2019. *Immune-stimulatory gene expression in stroma cells of African-American prostate cancer tissues.* Journal of Clinical Oncology 37:15_suppl, e16544-e16544 https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.15_suppl.e16544

Zi X, Lilly M , Wu C, Ke Y, Ma C, Chen W, Solofff AC, Yokoyama NN, Yuan Y, Mclaren CE. 2019. A Phase I study of docetaxel plus synthetic lycopene in metastatic, castration-resistant and chemotherapy-naïve prostate cancer patients. European Urology 18: e3146 https://www.sciencedirect.com/science/article/abs/pii/S1569905619333871?via%3Dihub

Sonpavde G, Agarwal N, Pond GR, Nagy RJ, Nussenzveig RH, Hahn AW, Sartor O, Gourdin TS, Nandagopal L, Ledet EM, Naik G, Armstrong AJ, Wang J, Bilen MA, Gupta S, Grivas P, Pal SK, Lanman RB, Talasaz A, and Lilly MB. 2019. Circulating tumor DNA alterations in patients with metastatic castration-resistant prostate cancer. Cancer, 125: 1459-1469. https://acsjournals.onlinelibrary.wiley.com/doi/abs/10.1002/cncr.31959

Pannaga G. Malalur, Theodore Stewart Gourdin, Ali Roberts, and Michael B. Lilly 2019. *Circulating tumor (ct) DNA-based genomic profile of prostate cancer (PCa) patients with elevated carcinoembryonic antigen (CEA).* Journal of Clinical Oncology 37:7_suppl, 218-218 https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.7_suppl.218

Edwin Lin, Andrew W Hahn, Guru Sonpavde, Michael B. Lilly, Roberto Nussenzveig, Elisa Ledet, Sumanta K. Pal, Petros Grivas, Thereasa A. Rich, Victoria M. Raymond, A.

Oliver Sartor, Mark Yandell, and Neeraj Agarwal. 2019. *Profiling of genomic alterations in MAPK/ERK signaling in a large cohort of metastatic prostate cancer (mPC) patients.* Journal of Clinical Oncology 37:15_suppl, 5032-5032
https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.15_suppl.5032

Edwin Lin, Andrew W Hahn, Guru Sonpavde, Michael B. Lilly, Roberto Nussenzveig, Elisa Ledet, Sumanta K. Pal, Petros Grivas, Thereasa A. Rich, Victoria M. Raymond, A. Oliver Sartor, Mark Yandell, and Neeraj Agarwal. 2019. *Discovery of targetable mutational signatures in advanced prostate cancer (aPC) using machine learning and next-generation sequencing (NGS) of circulating tumor DNA (ctDNA).* Journal of Clinical Oncology 37:7_suppl, 226-226
https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.7_suppl.226

Leta Ko, Michael B. Lilly, Andrew W Hahn, Roberto Nussenzveig, Marcus Marie Moses, Elisa Ledet, Charlotte Manogue, Patrick Cotogno, Brian E. Lewis, Jodi Lyn Layton, Neeraj Agarwal, A. Oliver Sartor, and Pedro C. Barata. 2019. *Genomic changes of AR in ctDNA prior to enzalutamide in men with mCRPC after abiraterone acetate.* Journal of Clinical Oncology 37:7_suppl, 320-320
https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.7_suppl.320

Farah Rahmatpanah, Gabriela De Robles, Michael Lilly, Dan Mercola, Michael McClelland, 2019. *RNA expression differences between African Americans and European Americans prostate cancers in both their tumor-adjacent stroma and in their tumors.*
AACR 2020 Abstract, See appendix

Website(s) or other Internet site(s)
None

Technologies or techniques

The use of complementary CpG/mCpG binding proteins, and demonstration of the ability to resurrect otherwise intractable samples with commercial oligo capture methods will be of wide interest as technology in upcoming manuscripts.

Inventions, patent applications, and/or licenses

Daniel Mercola, Waldemar Lernhardt, Jia Zhenyu, Michael McClelland. 2016. Stroma biomarkers for the diagnosis of prostate cancer. US20160138108A1

Patents on the initial prognosticator were initiated prior to the award of the grant. A Preliminary patent on the observation of ISGs is being prepared.

Other Products

Sequencing data has been deposited (GSE118541) and will continue to be deposited in GEO once it is validated.

7. Participants & Other Collaborating Organizations……

- **What individuals have worked on the project?**
- Effort reported is for the no-cost extension year.
- Not listed are trainees listed earlier.

Name:	*Michael McClelland*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	0000-0003-1788-9347
Nearest person month worked:	*0*
Contribution to Project:	*Supervision, data analysis, writing*
Funding Support:	*1% effort in no-cost extension*

Name:	*Dan Mercola*
Project Role:	*Co-PI*
Researcher Identifier (e.g. ORCID ID):	0000-0002-0281-9840
Nearest person month worked:	*0*
Contribution to Project:	*Supervision, data analysis, writing*
Funding Support:	*1% effort in no-cost extension*

Name:	*Mike Lilly*
Project Role:	*Co-investigator*
Researcher Identifier (e.g. ORCID ID):	0000-0001-9412-8109
Nearest person month worked:	*0*
Contribution to Project:	*Management of AA sample acquisition*
Funding Support:	*Effort is donated*

Name:	*Farah Rahmatpanah*
Project Role:	*Assistant Project Scientist*

Researcher Identifier (e.g. ORCID ID):	0000-0002-6158-1692
Nearest person month worked:	0
Contribution to Project:	*The primary bench scientist on the project*
Funding Support:	*2% effort in no-cost extension*

Name:	*Steffen Porwollik*
Project Role:	*Assistant Project Scientist*
Researcher Identifier (e.g. ORCID ID):	0000-0001-9616-614
Nearest person month worked:	*1*
Contribution to Project:	*Data management*
Funding Support:	*Effort on this grant*

Name:	*Weiping Chu*
Project Role:	*Technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Sample processing.*
Funding Support:	*Effort on this grant*

Name:	*Fred Long*
Project Role:	*Specialist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*0*
Contribution to Project:	*Database management*
Funding Support:	*0.1% Effort on this grant*

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

The following new grants were awarded.

NIH R01 CA226570 (Rahmatpanah/McClelland) 04/01/19-03/31/24

Aggressive prostate cancer of African Americans is correlated with regulation of immunoregulatory genes in stroma
Role: Co-Principal Investigator. 1.4% effort.
Major Goal: Minimize disparity in outcome of prostate cancer in African Americans
This project uses the information gained in the DOD project.

NIH R03 AI139557 (McClelland) 10/01/19-09/30/21
Barcoded defined deletion mutants
Role: Principal Investigator. 1% effort.
Major Goal: Build a genetic resource

- **What other organizations were involved as partners?**
  Nothing to report

8. Special Reporting Requirements……
None

9. Appendices……
Links to works mentioned above:
http://www.fasebj.org/content/30/1_Supplement/1053.7?related-urls=yes&legid=fasebj;30/1_Supplement/1053.7
http://www.eusupplements.europeanurology.com/article/S1569-9056(16)15111-5/fulltext
http://cebp.aacrjournals.org/content/26/2_Supplement/B04
http://www.eusupplements.europeanurology.com/article/S1569-9056(16)30376-1/pdf
http://ascopubs.org/doi/abs/10.1200/JCO.2016.34.15_suppl.5035
http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.6_suppl.149
https://www.ncbi.nlm.nih.gov/pubmed/27929677
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5423348/
https://academic.oup.com/peds/article/30/12/785/4710350
http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.15_suppl.1514
https://jhoonline.biomedcentral.com/articles/10.1186/s13045-016-0386-7
http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.6_suppl.149
https://www.ncbi.nlm.nih.gov/pubmed/?term=30339520
http://ascopubs.org/doi/abs/10.1200/JCO.2018.36.15_suppl.12097
https://www.frontiersin.org/articles/10.3389/fimmu.2018.01188/full
http://ascopubs.org/doi/abs/10.1200/JCO.2018.36.6_suppl.314
http://ascopubs.org/doi/abs/10.1200/JCO.2018.36.15_suppl.5048
http://ascopubs.org/doi/abs/10.1200/JCO.2018.36.6_suppl.306
https://academic.oup.com/biomedgerontology/advance-article/doi/10.1093/gerona/gly106/4989886
https://www.fasebj.org/doi/10.1096/fasebj.2018.32.1_supplement.804.60
https://link.springer.com/chapter/10.1007/978-3-319-64096-9_16
https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.15_suppl.e16544
https://www.sciencedirect.com/science/article/abs/pii/S1569905619333871?via%3Dihub

https://academic.oup.com/biomedgerontology/advance-article/doi/10.1093/gerona/gly106/4989886
https://www.fasebj.org/doi/10.1096/fasebj.2018.32.1_supplement.804.60
https://link.springer.com/chapter/10.1007/978-3-319-64096-9_16
https://ascopubs.org/doi/abs/10.1200/JCO.2018.36.6_suppl.306
https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.15_suppl.e16544
https://www.sciencedirect.com/science/article/abs/pii/S1569905619333871?via%3Dihub
https://acsjournals.onlinelibrary.wiley.com/doi/abs/10.1002/cncr.31959
https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.7_suppl.218
https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.15_suppl.5032
https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.7_suppl.226
https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.7_suppl.320

AACR 2020 Abstract:

Farah Rahmatpanah, Gabriela De Robles, Michael Lilly, Dan Mercola, Michael McClelland

RNA expression differences between African Americans and European Americans prostate cancers in both their tumor-adjacent stroma and in their tumors

Background: Prostate cancer (PCa) of African Americans (AA) is diagnosed at an earlier median age and more advanced stage than PCa of European American (EA), and has a poorer prognosis and significantly higher mortality. 35% of AA PCa patients fall out of active surveillance, compared to 15% of EA men. Stromal cells adjacent to tumor, including reactive stroma, play a critical role in tumorigenesis of prostate cancer. We searched for differences in RNA expression in the stroma and tumor of AA compared to EA prostate cancer patients to uncover regulatory differences that might contribute to a higher rate of aggressive PCa in AA men.

Methods: RNA-Seq data was generated for tumor-adjacent stroma of prostate cancer FFPE tissues from 9 AA and 8 EA patients. RNA-Seq of tumor epithelium from 22 AA and 46 EA PCa FFPE tissues was obtained from GEO database accession GSE54460. Samples were matched clinically for Gleason score, age, and relapse status. Geographic origin was determined using the program Locating Ancestry from Sequencing Reads (LASER) which provides the fraction of ancestry based on single nucleotide polymorphisms (SNPs).The program CIBERSORT, which is an estimate of fraction of immune cells in tissues, was used to estimate the relative fractions of 22 immune cell types in each RNA-Seq sample. Pathway analysis was performed using Strand-NGS and Ingenuity Pathway Analysis (IPA) tools.

Results: PCa-adjacent stroma from 9 AA were compared to 8 EA. Similarly, tumor tissues from 22 AA were compared to 46 EA. Comparisons of AA and EA tumor-adjacent stroma identified 721 downregulated and 790 upregulated genes in AA, using a corrected p value of < 0.05. We found significant association of downregulated genes in AA tumor stroma with immune response pathways. Among upregulated genes in AA relative to EA, several metabolic pathways, signaling by TGF beta receptor complex, cytokine, and inflammatory responses, were significantly enriched.

CIBERSORT analysis, revealed M2 macrophages (i.e. immunosuppressive and proangiogenic macrophages were enriched in both AA and EA tumor stroma, and

represented 25% and 27% of screened immune cells, respectively. The frequency of activated DCs in tumor-adjacent stroma of EA was 7-fold higher than in tumor stroma of AA patients (21% vs 3%).

22 AA and 46 EA prostate tumor tissue samples were similarly compared. 425 upregulated and 514 downregulated genes were identified (corrected p value of 0.05). Although, stroma and tumor have very different transcription patterns, there were 17 genes up-regulated both in tumor-adjacent stroma and tumor epithelium of AA compared to EA and these were enriched in the IL-6 signaling pathway. 21 down-regulated genes were enriched in miRNA targeted genes.

In contrast, to tumor-adjacent stroma, immune response pathways in tumor of AA compared to EA was different, suggesting a distinct immune response in tumor-adjacent stroma compared to tumor in men of different races.