2024 Multi-Institutional Prostate Cancer Program Retreat

March 3–5, 2024 • Luskin Center, UCLA, Los Angeles

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2024 Multi-Institutional Prostate Cancer Program Retreat

March 3–5, 2024, Luskin Center, UCLA, Los Angeles, CA

Sunday, March 3, 2024

4:00 PM Check-in/Registration

5:00 PM Welcome Reception
Appetizers and drinks will be served

6:00 PM Opening Remarks
Christopher Barbieri (WCM) & Yu Chen (MSKCC)

6:05 PM Welcome and Introduction
Brad Scroggins (NCI)

6:10 PM Keynote Address
Charles Drake, MD, PhD; Vice President, Immuno-oncology (I-O) at Jassen Research & Development, LLC.

6:40 PM Discussion Session

7:00 PM **Session I—Functional Genomics and Biology**
Moderators and Discussion Leaders: Sarki Abdulkadir (NW) and Timothy Thompson (MD Anderson)

7:00 UCHL1 is a Molecular Indicator and Therapeutic Target for Neuroendocrine Prostate Cancer
Tanya Stoyanova (UCLA)

7:10 Discussion Session
1st question – Varadha Balaji Venkadakrishnan (DFCI)

7:20 Tumor Escape Routes From Effective Targeted Therapies - A Temporal View of Trans-Differentiation
Thomas Graeber (UCLA)

7:30 Discussion Session
1st question – Mohamed Adil (Fred Hutch)

7:40 Multi-Level Functional Genomics Reveals Molecular and Cellular Oncogenicity of Patient-Based 3' Untranslated Region Mutations
Andrew Hsieh (PNW)

7:50 Discussion Session
1st question – Andrew Goldstein (UCLA)

8:00 ERG-Driven Prostate Cancer Emerges From Basal Cells With Luminal Transcriptomic Features
Weiran Feng (MSKCC)

8:10 Discussion Session
1st question – Sarah Cheal (WCM)

8:20 Profiling the Prostate Cancer Epigenome in Patients Using Circulating Nucleosomes in Plasma
David Takeda (NCI)

8:30 Discussion Session
1st question – Beatriz German Falcon (CPDR)

8:40 The Interplay Between EZH2 and m6A RNA Methylation in CRPC
Yang Yi (NW)

8:50 Discussion Session
1st question – Muneeb Alam (MSKCC)

9:00 PM Adjourn

Monday, March 4, 2024

7:00 AM Continental Breakfast

8:00 AM **Session II—New Therapies: Mechanisms, Molecules & Resistance**
Moderators and Discussion Leaders: Ayesha Shafi (CPDR) and Christopher Logothetis (MD Anderson)

8:00 Targeting *de Novo* Lipogenesis Synergizes With Androgen Receptor Inhibitors in Castration-Resistant Prostate Cancer
Pier Vitale Nuzzo (WCM)

8:10 Discussion Session
1st question – Yuanyuan Qiao (U Michigan)

8:20 First-in-Class Dual AR-V7/AR-FL Molecular Glue Degrader for Prostate Cancer Treatment
Evi Giannakakou (WCM)

8:30 Discussion Session
1st question – Weiran Feng (MSKCC)

8:40 Prostate Cancer-Induced Endothelial-to-Osteoblast Transition Generates an Immunosuppressive Bone Tumor Microenvironment
Paul Corn (MD Anderson)

8:50 Discussion Session
1st question – William Ricke (U Wisconsin)

9:00 Ciliogenesis Program Regulated by FOXJ1 is a Mediator of Taxane Resistance
Steven Balk (DF/HCC)

9:10 Discussion Session
1st question – Mindy Graham (NW)

9:20 Novel Antibody Targeting Tumor NKG2D Ligand MIC: Preclinical Studies and Clinical Development Plan for mCRPC
Jennifer Wu (NW)

9:30 Discussion Session
1st question – Hyeong-Reh Kim (Wayne St.)

9:40 AM Break

10:00 AM **Session III—Immunotherapy**
Moderators and Discussion Leaders: Jelani Zarif (JH) and Frank Cackowski (U Michigan)

10:00 Identifying Mechanistic Biomarkers of Response to Novel T Cell Bispecifics in Metastatic Castration-Resistant Prostate Cancer
Sumit Subudhi (MD Anderson)

10:10 Discussion Session
1st question – Frank Cackowski (Wayne St.)

10:20 Understanding the Role of Immune Infiltrate Following Intense Neoadjuvant Androgen Deprivation Therapy in Locally Advanced Prostate Cancer
John Fenimore (NCI)

10:30 Discussion Session
1st question – Zhiyuan Mao (UCLA)

10:40 Androgen Receptor Blockade in Macrophages Primes NLRP3 Inflammasome-Mediated Phagocytosis and Tumor Clearance in Advanced Prostate Cancer
Akash Patnaik (U Chicago)

10:50 Discussion Session
1st question – David Jarrard (U Wisconsin)

11:00 Chimeric Antigen Receptor T Cell Therapies for Subtypes of Metastatic Castration-Resistant Prostate Cancer
John Lee (UCLA)

11:10 Discussion Session
1st question – Yi Bao (U Michigan)

11:20 Evolution of Myeloid-Mediated Mechanisms of Immunotherapy Resistance at Single-Cell Resolution With Prostate Cancer Progression
Aram Lyu (UCSF)

11:30 Discussion Session
1st question – Kayvan Keshari (MSKCC)

11:40 DNA Vaccines in Combination With Androgen Deprivation as Treatment for Prostate Cancer
Douglas McNeel (UW)

11:50 Discussion Session
1st question – David Quigley (UCSF)

12:00 PM Lunch

1:00 PM **Session IV—Imaging and Biomarkers**
Moderators and Discussion Leaders: Ekta Khurana (WCM) and Michael Morris (MSKCC)

1:00 Using Artificial Intelligence Approaches to Personalize Prostate Cancer Therapies
Felix Feng (UCSF)

1:10 Discussion Session
1st question – Kirstin Zettlitz (UCLA)

1:20 Predictive PSMA PET/MRI Imaging and Clinical Biomarkers of Response in High-Risk Prostate Cancer Patients Treated with Neoadjuvant Chemo-hormonal Therapy Prior to Prostatectomy
Steve Cho (UW)

1:30 Discussion Session
1st question – Larry True (Fred Hutch)

1:40 Role of the Gut Microbiome in Androgen Production and Prostate Cancer Treatment Resistance
Angelica Cruz-Lebron (JH)

1:50 Discussion Session
1st question – Isaacson Adelani (JH)

2:00 A Theranostic Program for Imaging and Therapy of Delta-Like Ligand 3 Expressing Neuroendocrine Prostate Cancer
Salomon Tendler (MSKCC)

2:10 Discussion Session
1st question – Masoud Farshbaf (UCLA)

2:20 PSMA-targeted Theranostics : PSMA-targeted Imaging to Improve PSMA-targeted Therapy
Jeremie Calais (UCLA)

2:30 Discussion Session
1st question – Jason Lewis (MSKCC)

2:40 The Role of PSMA in Prostate Cancer and Beyond
Jan Grimm (MSKCC)

2:50 Discussion Session
1st question – Martin Bakht (DFCI/HCC)

3:00 PM Break

3:15 PM Poster Viewing

5:30 PM **Poster Session and Discussion**
Moderators and Discussion Leaders: Misha Beltran (DF/HCC), David Quigley (UCSF), Andrew Goldstein (UCLA)
Seven top posters will each present for 5 minutes and will each include a 10 minute discussion; posters will be selected at the meeting

6:30 PM Poster Award Ceremony for Students, Fellows, and Post-Docs
1st prize $2,000, 2nd prize $1,500, 3rd prize $1,000, 4th prize $500

7:00 PM Dinner

7:30 Patient Advocate Involvement—Help or Hinderance?
SPORE Patient Advocates

8:00 Debates
1. PSMA imaging and metastasis directed therapy
2. Focus on the cell surface markers and antigens vs molecular drivers

9:00 PM Adjourn

Tuesday, March 5, 2019

7:00 AM Continental Breakfast

8:00 AM **Session V—Population Science and Clinical Research**
Moderators and Discussion Leaders: Adam Murphy (NW) and Kevin Kensler (WCM)

8:00 DNA Damage Repair Variants in African Americans Families that have a History of Both Breast and Prostate Cancer
Jennifer Beebe-Dimmer (Wayne St.)

8:10 Discussion Session
1st question – Colm Morrissey (PNW)

8:20 Prostate Cancer: Integrative Multi-Omics Profiling in Patients of African Descent
Isra Elhussin (JH)

8:30 Discussion Session
1st question – Roy Elias (JH)

8:40 The Molecular Landscape of High-Risk Localized Prostate Cancer in the Genomic-Biomarker-Selected Umbrella Neoadjuvant Study (GUNS)
Joshua Scurll (PNW)

8:50 Discussion Session
1st question – Erolcan Sayar (PNW)

9:00 Localized High-Risk Prostate Cancer Harbors an Androgen Receptor Low Subpopulation Susceptible to HER2 Inhibition
Scott Wilkinson (NCI)

9:10 Discussion Session
1st question discussant: Sarah Cheal (WCM)

9:20 Drivers and Barriers to Accessing Prostate Specific Antigen Screening for Early Detection of Prostate Cancer Among Black Men
Jenney Lee (PNW)

9:30 Discussion Session
1st question – Cornelia Ding (UCSF)

9:40 Benefits of PSMA-PET/CT at Biochemical Recurrence after Prostatectomy: A Decision Model Analysis
Kemal Caglar (PNW)

9:50 Discussion Session
1st question – Shiqin Liu (UCLA)

10:00 AM Break and Check out

10:30 AM **Session VI—Androgen Receptor/Plasticity**
Moderators and Discussion Leaders: Amina Zoubeidi (Fred Hutch) and Dave Jarrard (U Wisconsin)

10:30 Androgen Receptor Splice Variant Activity in Castration-Resistant Prostate Cancer is Dependent on Increased FOXA1-mediated Chromatin Accessibility
Larysa Poluben (DFCI/HCC)

10:40 Discussion Session
1st question – Sheng-Yu Ku (DFCI/HCC)

10:50 HOXB13 is Essential to Both AR-Positive and -Negative Prostate Cancer
Nathan Lack (PNW)

11:00 Discussion Session
1st question – Chen Khuan Wong (MSKCC)

11:10 Androgen Receptor Cell-Autonomously Regulates Luminal Cell Lineage Plasticity, Regeneration and Oncogene Residency
Dan Li (MSKCC)

11:20 Discussion Session
1st question discussant: Betul Ersoy-Fazlioglu (DFCI/HCC)

11:30 Understanding Metastatic Tumoral Evolution and Divergence After Castration in a PDX Derived Prostate Cancer Metastasis Model
JuanJuan Yin (NCI)

11:40 Discussion Session
1st question – Arnab Bose (Fred Hutch)

11:50 Detecting Divergent Lineage Plastic Transformations in mCRPC using Circulating Tumor Cell RNA Sequencing
Joshua Lang (UW)

12:00 Discussion Session
1st question – Nicholas Brady (WCM)

12:10 FOXA1 Class-Mutants Distinctly Activate Luminal Neoplastic or Stemness Enhancer Programs in the Mouse Prostate Epithelia
Sanjana Eyunni (UM)

12:20 Discussion Session
1st question – Weiling Li (WCM)

12:30 PM Adjourn/Closing Remarks
Christopher Barbieri (WCM) and Yu Chen (MSKCC)

Boxed Lunch

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Session I

Functional Genomics and Biology

UCHL1 is a Molecular Indicator and Therapeutic Target for Neuroendocrine Prostate Cancer

Shiqin Liu, Timothy Chai, Fernando Garcia-Marques, Qingqing Yin, En-Chi Hsu, Michelle Shen, Angus Martin Shaw Toland, Abel Bermudez, Alifiani B. Hartono, Christopher F. Massey, Chung S. Lee, Liwei Zheng, Maya Baron, Caden J. Denning, Merve Aslan, Holly M. Nguyen, Rosalie Nolley, Amina Zoubeidi, Millie Das, Christian A. Kunder, Brooke E. Howitt, H Tom Soh, Irving L. Weissman, Michael A. Liss, Arnold I. Chin, James D. Brooks, Eva Corey, Sharon J. Pitteri, Jiaoti Huang, and Tanya Stoyanova

Neuroendocrine prostate cancer commonly has a poor prognosis, limited therapeutic options, and resistance to therapies. We report that UCHL1, a deubiquitinating enzyme, is significantly elevated in tissues and plasma from patients with neuroendocrine carcinomas. Loss of UCHL1 decreases tumor growth and inhibits metastasis of these malignancies. UCHL1 maintains neuroendocrine differentiation and promotes cancer progression by regulating nucleoporin, POM121, and p53. UCHL1 binds, deubiquitinates, and stabilizes POM121 to regulate POM121-associated nuclear transport of key transcriptional factors, E2F1 and c-MYC. UCHL1 also ubiquitinates and promotes the degradation of p53. Importantly, treatment with UCHL1 inhibitor, LDN-57444, significantly reduces tumor growth and metastasis of neuroendocrine prostate cancer. The combination of UCHL1 inhibitors with cisplatin, the standard of care used for poorly differentiated neuroendocrine carcinomas, halts tumor growth in pre-clinical settings. Our study reveals new mechanisms of UCHL1 function in regulating the progression of neuroendocrine prostate cancer and identifies UCHL1 as a therapeutic target and potential molecular indicator for diagnosing and monitoring treatment responses in neuroendocrine prostate cancer.

Acknowledgments/Funding: This study was supported by the National Institutes of Health/National Cancer Institute (NCI) R01CA244281 and P50CA09213. T.S. is supported by the National Institutes of Health/National Cancer Institute (NCI) R37CA240822, R01CA244281, R01CA274978, and U.S. Department of Defense Award Number HT9425-23-1-1034. J.D.B. is supported by NIH CA245595 and NIH CA196387. S.J.P is supported by NIH CA196387 and NIH CA196387. S.L. is supported by the Jonsson Comprehensive Cancer Center Fellowship Award. Establishment and characterization for the LuCaP PDX models were funded by Pacific Northwest Prostate Cancer SPORE P50CA97186, and P01CA163227.

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Tumor Escape Routes From Effective Targeted Therapies - a Temporal View of Trans-differentiation

Chia-Chun Chen, Wendy Tran, Kai Song, Tyler Sugimoto, Matthew B. Obusan, Liang Wang, Katherine M. Sheu, Donghui Cheng, Lisa Ta, Grigor Varuzhanyan, Arthur Huang, Runzhe Xu, Yuanhong Zeng, Miyako Noguchi, Zhiyuan Mao, Colm Morrissey, Eva Corey, Peter S. Nelson, Yue Zhao, Jiaoti Huang, Jung Wook Park, Owen N. Witte, and Thomas G. Graeber

Targeted cancer therapy has advanced to reveal new plasticities and escape mechanisms from otherwise effective treatment approaches. An emerging clinical theme involves cancer cells reliant on a therapeutically shutdown oncogene undergoing a change in cell identity to a cell type that is no longer dependent on the oncogene. BRAF inhibition leads to melanoma dedifferentiation, and androgen signaling inhibition and EGFR inhibition can drive adenocarcinomas of the prostate and lung, respectively, to a small cell neuroendocrine state via transdifferentiation. These cancer resistance mechanisms have parallels to transdifferentiation mechanisms present in normal cells during physiological processes such as wound healing and tissue repair.

To gain insight into the molecular events that promote resistance via cancer transdifferentiation, we performed a multi-omics time course analysis of a pan-small cell neuroendocrine cancer model, termed PARCB. The PARCB model is a forward genetic transformation using human prostate basal cells. With integrative analyses of RNA sequencing and ATAC sequencing, a shared developmental trajectory is identified among all transformed patient samples. Further mapping with single cell resolution revealed two distinct lineages defined by mutually exclusive expression of ASCL1 or ASCL2. Pan-cancer analysis of the lineage endpoints revealed additional pan-tissue parallels between prostate and lung cancers, as well as connections to normal neuroendocrine cell states. Cell type and developmental stage analysis revealed that cellular reprogramming precedes the induction of neuronal programs, providing leads on how to therapeutically inhibit the transdifferentiation escape route. The temporal resolution of our study revealed an arc-like plasticity trajectory. Such an arc-like trajectory is commonly observed in unbiased profiling of development and differentiation processes, including in cancer contexts. The arc-like pattern is reminiscent of temporal regulation in development, with the differentiation transition promoted by temporally regulated epigenetic and transcriptomic plasticity programs.

As additional successful targeted therapies come to the clinic, resistance mechanisms involving changes in cell identity stand to further expand.

Acknowledgments/Funding: This project is supported by an NIH UCLA SPORE in Prostate Cancer Grant P50CA092131 (O.N.W. and T.G.G.), the Department of Defense Prostate Cancer Research Program Idea Development Award W81XWH2110806 (O.N.W. and J.W.P.), NIH R01 Grant R01CA222877 (O.N.W. and T.G.G.), W.M. Keck Foundation Award 20182490 (O.N.W. and T.G.G.), UCLA Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research Hal Gaba Director's Fund for Cancer Stem Cell Research (O.N.W. and T.G.G.), Parker Institute for Cancer Immunotherapy (O.N.W.), Pacific Northwest Prostate Cancer SPORE Grant P50CA97186 (C.M., E.C. and P.S.N), NIH R01 Grant R01CA234715 (P.S.N), NIH P01 Grant PO1CA163227 (C.M., E.C. and P.S.N) and the Institute for Prostate Cancer Research (C.M., E.C. and P.S.N). C.C. is supported by the UCLA Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research predoctoral fellowship and UCLA Dissertation Year Fellowship. K.M.S. is supported by the UCLA Medical Scientist Training Program (NIH NIGMS T32 GM008042). G.V. is supported by UCLA Tumor Cell Biology Training Program (USHHS Ruth L. Kirschstein Institutional National Research Service Award T32 CA009056).

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Multi-level Functional Genomics Reveals Molecular and Cellular Oncogenicity of Patient-based 3′ Untranslated Region Mutations

Samantha L. Schuster, Sonali Arora, Cynthia L. Wladyka, Pushpa Itagi, Lukas Corey, Dave Young, Bethany L. Stackhouse, Lori Kollath, Qian Wu, Eva Corey, Lawrence D. True, Gavin Ha, Patrick J. Paddison, Andrew C. Hsieh

3' untranslated region (3'UTR) somatic mutations represent a largely unexplored avenue of alternative oncogenic gene dysregulation. To determine the significance of 3'UTR mutations in disease, we identify 3'UTR somatic variants across 185 advanced prostate tumors, discovering 14,497 single-nucleotide mutations enriched in oncogenic pathways and 3'UTR regulatory elements. By developing two complementary massively parallel reporter assays, we measure how thousands of patient-based mutations affect mRNA translation and stability and identify hundreds of functional variants that allow us to define determinants of mutation significance. We demonstrate the clinical relevance of these mutations, observing that CRISPR-Cas9 endogenous editing of distinct variants increases cellular stress resistance and that patients harboring oncogenic 3'UTR mutations display particularly poor prognosis. This work represents an unprecedented view of the extent to which disease-relevant 3'UTR mutations affect mRNA stability, translation, and cancer progression, uncovering principles of regulatory functionality and potential therapeutic targets in previously unexplored regulatory regions.

Acknowledgments/Funding: This work was supported by NIH award R37 CA230617, R01 CA255526, R01 GM135362, Burroughs Welcome Fund, Career Award for Medicine Scientists (1012314.02), and grants from the Emerson Collective (691630), and the Robert J. Kleberg Jr. and Helen C. Kleberg Foundation to A.C.H. S.L.S. received funding through an NIH F31 NRSA grant (F31 CA260920-03), UW T32 training grant (GM007270), and Fred Hutchinson Cancer Center Co-operative Center for Excellence in Hematology pilot grant (U54 DK106829-P&F). This work was supported by the Department of Defense Prostate Cancer Research Program (W81XWH-14-2-0183), the Pacific Northwest Prostate Cancer SPORE (P50CA97186), the PO1 NIH grant (PO1CA163227), the institute for Prostate Cancer Research, and the Richard M. LUCAS Foundation.

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Revealing and Reprogramming Cell Fate in ERG-driven Prostate Cancer

Weiran Feng, Erik Ladewig, Nazifa Salsabeel, Huiyong Zhao, Young Sun Lee, Anuradha Gopalan, Dan Li, Hanzhi Luo, Wenfei Kang, Ning Fan, Eric Rosiek, Elisa de Stanchina, Brett S. Carver, Yu Chen, Christina S Leslie, Charles L. Sawyers

ERG oncoprotein is activated via TMPRSS2-ERG translocation in 50% of human prostate cancer. However, how ERG translocations cause prostate cancer remains unclear.

Here we performed single cell transcriptional and chromatin accessibility profiling of anautochthonous mouse model at an early stage of disease initiation. Despite broad expression of ERG in prostate epithelial cells, enhanced proliferation primarily occurs in an intermediate subpopulation with a basal identity based on transcriptomics but with luminal morphology and cytokeratin expression.

Lineage tracing and primary prostate epithelial transplantation reveal that tumor initiating activity resides in a subpopulation of basal cells that also express luminal genes such as Tmprss2 and Nkx3.1, but not in the larger population of classical luminal cells. Upon ERG activation, this basal subset expands into a highly proliferative population of intermediate basal-luminal identity.

ERG drives a unique chromatin state in intermediate cells mixed with STAT/NFAT transcriptional network while retaining the ability to rapidly differentiate to luminal cells that reflect the lineage of ERG-positive cancers.

These findings help resolve a preexisting debate about the cell of origin of prostate cancer and narrow the focus for future mechanistic studies to a basal-luminal subpopulation of tumor initiating cells.

Acknowledgments/Funding: We thank the Sawyers lab for valuable critiques and discussions. We appreciate the feedback from Drs. Michael M. Shen and Cory Abate-Shen, and advice from Cory Abate-Shen lab for setting up the intraprostatic adenoviral injection assay. We are grateful to Rona Lester for help in mouse colony management, the Molecular Cytology Core Facility from MSKCC for help with microscopy, and the Flow 5 Cytometry Core Facility from MSKCC for help with FACS experiments.

This work is supported by Howard Hughes Medical Institute (HHMI) (CLS), National Institute of Health grants CA193837 (CLS.), CA092629 (CLS), CA22407910 (CLS), CA155169 (CLS), 1K99CA276888-01 (WF), Department of Defense grant W81XWH-19-1-0323 (WF), Prostate Cancer Foundation 20YOUN22 (WF)

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Profiling the Prostate Cancer Epigenome in Patients Using Circulating Nucleosomes in Plasma

David Y. Takeda, Joonatan Sipola, Aslı D. Munzur, Edmond M. Kwan, Clara C. Y. Seo, Benjamin J. Hauk, Karan Parekh, Yi Jou Liao, Cecily Q. Bernales, Gráinne Donnellan, Ingrid Bloise, Emily Fung, Sarah W. S. Ng, Gang Wang, Gillian Vandekerkhove, Matti Annala, Corinne-Maurice Dror, Kim N. Chi, Cameron Herberts, Alexander W. Wyatt, David Y. Takeda

The prostate cancer epigenome undergoes dramatic reprogramming during tumorigenesis and treatment resistance. Despite the androgen receptor (AR) being expressed in both normal prostate and prostate cancer, the genomic occupancy of AR is altered in cancer to induce oncogenic transcriptional programs. Similarly, reshaping of the enhancer landscape drives progression to castration-resistant disease by re-activating dormant fetal prostate transcriptional programs. These mechanistic insights could not have been elucidated through genomic or transcriptomic approaches alone and relied on profiling primary patient samples. However, performing comprehensive epigenetic profiling in patients with advanced disease has been limited by access to adequate biopsy specimens. Although circulating tumor DNA (ctDNA) provides a noninvasive method to identify genetic alterations, we investigated whether ctDNA could detect epigenetic reprogramming using cell free chromatin immunoprecipitation followed by sequencing (cfChIP-seq). We performed cfChIP-seq in a cohort of metastatic castration-resistant prostate cancer (mCRPC) patients to detect histone post-translational modifications that indicate the epigenetic state of the underlying chromatin. We show that cfChIP-seq captures prostate cancer-specific regulatory elements and successfully identifies subtypes of mCRPC based on the chromatin landscape that may guide treatment decisions. Using patient samples from serial time points, we observe reprogramming of the epigenome associated with treatment emergent neuroendocrine prostate cancer (NEPC). Inferred transcription factor activity based on binding motif enrichment within regulatory elements confirm expected drivers such as AR and FOXA1 in adenocarcinoma and ASCL1 and EZH2 in NEPC. We also provide a framework to analyze cfChIP-seq results accounting for the highly variable tumor fraction that contributes to background signal. Our results demonstrate that cfChIP-seq captures clinically relevant epigenetic alterations in advanced prostate cancers and is a powerful complement to current ctDNA methods to identify dynamic changes in the epigenome during prostate cancer progression.

Acknowledgments/Funding: NIH (ZIABC011973), Terry Fox New Frontiers Program Project Grant, BC Cancer Foundation, Canadian Institutes of Health Research, Prostate Cancer Foundation, Canadian Cancer Society, Jane and Aatos Erkko Foundation, and the Academy of Finland Center of Excellence programme (project no. 312043).

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The Interplay Between EZH2 and m6A RNA Methylation in CRPC

Yang Yi, Joshua Fry, Xuesen Dong, Rendong Yang, Qi Cao

N6-methyladenosine (m6A) is the most prevalent mRNA modification type in mammals which participates in various fundamental bioprocesses as well as cancer development. Emerging evidence suggested that the sophisticated crosstalk between m6A RNA methylation and a series of histone modifications is critical for the precise and synchronous regulation of gene expression, while its underlying mechanism remains understudied. As the catalytic subunit of Polycomb Repressive Complex 2 (PRC2) directing histone H3 lysine 27 trimethylation (H3K27me3), EZH2 is significantly upregulated in castration-resistant prostate cancer (CRPC) and closely correlated with CRPC initiation and progression. By utilizing the cutting-edge Nanopore-seq along with other methods, we revealed that EZH2 could promote the RNA m6A methylome globally in CRPC cells through its lysine methyltransferase activity. Our preliminary data further showed that EZH2 exerts a previously unrecognized role in mRNA splicing regulation via modulating m6A and consequently affects CRPC aggressiveness and drug resistance. Based on these preliminary data, our overall hypotheses are that EZH2 maintains a hyper-m6A state in CRPC cells through activation of an YTHDF1-mediated m6A autoregulation pathway. As a result, EZH2 depletion reshapes the m6A-regulated alternative splicing pattern for a number of transcripts including BCL2L13, an inhibitor of apoptosis which strengthens the chemoresistance of CRPC. Consistently, combinational targeting of EZH2 and m6A could achieve a synergistic effect in advanced prostate cancer treatment. The completion of this project will allow us to dissect how EZH2-mediated Kme3 and m6A RNA methylation are orchestrated to facilitate CRPC tumorigenesis and lay the foundation for the development of novel therapeutic strategies that improve the clinical outcome of CRPC patients.

Acknowledgments/Funding: NIH R01CA256741, R01CA208257 and DoD W81XWH-17–1-0357, W81XWH-19–1-0563, W81XWH-20–1-0504 to Q.C; DoD W81XWH-22-PCRP-IDA to Y.Y.

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Session II

New Therapies: Mechanisms, Molecules & Resistance

Targeting *de Novo* Lipogenesis Synergizes with Androgen Receptor Inhibitors in Castration-Resistant Prostate Cancer

Pier Vitale Nuzzo, Caroline Fidalgo Ribeiro, Silvia Rodrigues, Cynthia Sprenger, Kathryn Epilepsia, Giorgia Zadra, Paolo Chetta, David M Nanus, Stephen Plymate, Massimo Loda

Background: Metabolic rewiring, particularly reprogramming of lipid metabolism, is a hallmark of cancer cells. Prostate cancer (PC) exhibits a significant shift in lipid metabolism, with elevated expression Fatty Acid Synthese (FASN) enzyme ,the rate-limiting step of de novo lipogenesis. FASN overexpression leads to the synthesis of saturated (palmitate) and monounsaturated fatty acids (SFA/MUFA) which play a crucial role in supporting membrane synthesis, energy generation, protection against free radicals, and regulating of major oncogenic pathways. Androgen receptor (AR) in turn activates sterol response element-binding proteins (SREBPs), transcription factors that regulate expression of enzymes responsible for lipid synthesis, including FASN. Preliminary data shown that pharmacologic FASN inhibition decreases AR expression and signaling of AR and of its splice variant AR-V7. We hypothesize that combining FASN inhibition with AR-targeted therapy, specifically Enzalutamide (Enza), could enhance CRPC antitumor activity. Pre-clinical data outlined in the results prompted us to design a Phase I clinical trial of TVB-2640 combined with Enza to evaluate targeting of AR in patients that had become resistant to anti-androgen therapy through inhibition of lipid synthesis, as a new approach to treating mCRPC.

Methods: PC cells (22Rv1 and LNCaP-95 with and without lentiviral-mediated overexpression of AR-V7) and MSK-PCa3 organoids were treated with FASN inhibitor (TVB-2640), Enza, or the combination. Cell growth and AR/AR-V7 expression were measured after 6 days of treatment for cell lines and 25 days for organoids. LuCap 35 castrate-resistant human patient-derived xenografts (PDXs) were implanted into 12 castrated SCID mice and treated with Enza, TVB-2640, or the combination. Tumor growth was measured after 5 weeks of treatment. Immunohistochemistry analysis was performed on tissue microarrays (TMAs) of metastatic sites from 61 mCRPC pts using antibodies against FASN, AR, and AR-V7.

Results: Combining the FASN inhibitor with Enza significantly inhibited cell growth compared to either drug alone in both 22Rv1 and LNCaP-95 cell lines and in a CRPC organoid model. The combination downregulated AR-V7 and FASN. The overexpression of AR-V7 in LNCaP-95 cells partially rescued cell growth inhibition. The drug combination also demonstrated a remarkable antitumor effect in vivo, leading to a significant reduction in tumor growth compared to either drug alone in LuCap 35 PDX tumors.

Multiplexed fluorescent IHC analysis of 55 mCRPC cases showed co-expression of FASN with AR (87% of all mets) and AR-V7 (39% of bone mets) in metastatic PC lesions, further supporting the combination of enzalutamide with lipid synthesis inhibitors in PC pts. A phase I clinical trial [NCT0574362] has commenced aimed at determining the optimal and safest dose of TVB-2640 combined with Enza in mCRPC pts, while also examining the impact of FASN inhibition on metabolism, genetics, and lipid profiles in enrolled patients' blood and tumor tissue.

Conclusion: *De novo* lipid synthesis inhibitors in combination with AR-targeted therapy is a promising new approach to treating mCRPC.

Acknowledgments/Funding: This work was supported in part by National Cancer Institute grants: prostate cancer SPORE P50CA211024 and WCM Prostate Cancer SPORE's Developmental Research Program Award, P01 CA265768, the Prostate Cancer Foundation 2022CHAL05, the DoD W81XWH-19-1-0566.

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First-in-Class Dual AR-V7/AR-fl Molecular Glue Degrader for Prostate Cancer Treatment

CheukMan Cherie Au, Catrina Estrella, Prerna Vatsa, Michelle Naidoo, Michael Miller, Peter T. Meinke, K.C. Nicolaou, David M. Nanus, Paraskevi Giannakakou

Metastatic castration resistance prostate cancer (mCRPC) is a lethal disease due to the development of resistance to standard-of-care treatment such as androgen receptor (AR) signaling inhibitors (ARSi) and taxane chemotherapy. Treatment resistance occurs partly due to the expression of AR splice variants that lack the ligand binding domain (LBD) and are constitutively active in the nucleus. AR-V7 is the most prevalent variant conferring clinical resistance to both ARSi and taxanes. Currently, there is no selective AR-V7 inhibitor leaving patients with limited therapeutic options. Thus, the development of selective AR-V7 inhibitors is a high priority, clinically unmet need.

To identify AR-V7 pharmacologic inhibitors, we performed a high throughput small molecule phenotypic screen using enzymatic complementation and nuclear AR-V7 as the assay endpoint. Our primary screen of ~170K compounds (z score = 0.8), followed by a cell-toxicity counter screen and a secondary GFP screen identified hit compound 7907, as a dual AR-V7/AR-fl protein degrader, with unique chemotype compared to all known AR modulators. Hit to lead optimization by medicinal chemistry/SAR studies identified lead compound 15, with increased potency compared to 7907. Mechanistically, we showed that compound 15 shortened AR-V7 protein half-life by activating the ubiquitin-proteasome pathway and inducing proteasomal degradation of both AR-V7/AR-fl, without affecting their transcription. Importantly, compound 15 induced degradation occurred within 3hr of treatment and was blocked by the clinically approved proteasome inhibitor, bortezomib. TurboID proximity ligation assay identified distinct E3 ligases, uniquely interacting with AR-V7 or AR-fl. Using AR-V7/AR-fl deletion mutants we further showed that compound 15 activity is mediated by the N-terminal domain of AR, present in both proteins. Remarkably, compound 15 sensitized LNCaP95 cells (endogenous AR-V7/AR-fl expression) to enzalutamide suggesting potential therapeutic synergism and ability to reverse enzalutamide resistance. Ongoing studies aim to narrow down the binding site of compound 15 as well as identify the E3 ligases mediating its activity. Together, these data support a molecular glue degrader mechanism of action, consistent with published studies showing that molecular glue degraders are ideal for targeting classically "undruggable" proteins lacking an LBD or containing intrinsically disordered domains, as is the case for AR-V7.

Currently, all AR-directed therapies target the LBD of AR-fl, inhibiting AR signaling. AR-V7 expression is a direct outcome of this inhibition, leading to AR-fl and AR-V7 co-expression in patient tumors. We posit that our drug candidate, by offering dual AR-V7/AR-fl inhibition in a single treatment, has the potential to not only benefit patients with mCRPC but also patients with hormone-sensitive disease, and delay AR-V7 expression.

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Prostate Cancer-Induced Endothelial-to-Osteoblast Transition Generates an Immunosuppressive Bone Tumor Microenvironment

Paul G. Corn, Guoyu Yu, Patricia Troncoso, Christopher J. Logothetis, Guocan Wang, Sue-Hwa Lin

Immune checkpoint therapy has limited efficacy for patients with bone metastatic castrate-resistant prostate cancer (bmCRPC). In this study, we revealed a novel mechanism that may account for the relative resistance of bmCRPC to immune checkpoint therapy. We found that prostate cancer (PCa)-induced bone via endothelial-to-osteoblast (EC-to-OSB) transition causes an ingress of M2-like macrophages, leading to an immunosuppressive bone tumor microenvironment (bone-TME). Analysis of a bmCRPC RNA-seq dataset revealed shorter overall survival in patients with an M2-high versus M2-low signature. Immunohistochemical (IHC) analysis showed CD206+ M2-like macrophages were enriched in bmCRPC specimens compared with primary tumors or lymph node metastasis. In osteogenic PCa xenografts, CD206+ macrophages were enriched adjacent to tumor-induced bone. FACS analysis showed an increase in CD206+ cells in osteogenic tumors compared to non-osteogenic tumors. Genetic, pharmacological or Radium-223 mediated inhibition of the EC-to-OSB transition reduced aberrant bone and M2-like macrophages in osteogenic tumors. RNAseq analysis of tumor-associated macrophages from osteogenic (bone-TAMs) versus non-osteogenic (ctrl-TAMs) tumors showed high expression of an M2-like gene signature, canonical and non-canonical Wnt pathways, and a decrease in an M1-like gene signature. Isolated bone-TAMs suppressed T-cell proliferation while ctrl-TAMs did not. Mechanistically, EC-OSB hybrid cells produced paracrine factors, including Wnts, CXCL14 and LOX, which induced M2 polarization and recruited M2-like TAMs to bone-TME. Our study thus links the unique EC-to-OSB transition, which leads to prostate cancer-induced osteoblastic bone metastases, as an “upstream” event that mechanistically drives “downstream” immunosuppression in the bone-TME. Together, these studies suggest a strategy to improve responses to immunotherapy approaches in patients with bmCRPC by combining them with agents that target PCa-induced bone to reverse immunosuppression in the bone-TME.

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Ciliogenesis Program Regulated by FOXJ1 is a Mediator of Taxane Resistance

Fang Xie, Ada Gjyrezi, Olga Voznesensky, Larysa Poluben, Maryam Labaf, Rupal S. Bhatt, Paraskevi Giannakakou, Steven P. Balk

Despite the central role of taxanes in prostate cancer (PC) therapy, mechanisms of intrinsic or acquired taxane resistance occurring in patients have yet to be established. Taxanes act by binding to the β-subunit of the tubulin αβ heterodimer and preventing microtubule (MT) depolymerization, thus stabilizing microtubules and interfering with microtubule dynamics. One major consequence in cycling cells is mitotic arrest and subsequent apoptotic cell death, but taxanes also disrupt myriad other microtubule-dependent functions in cycling and noncycling cells. Potential resistance mechanisms include those that impair taxane-mediated MT stabilization (such as increased drug efflux or tubulin mutations) or alterations in pathways downstream of MT stabilization that allow cells to survive prolonged mitotic arrest or disruption of MT dynamics during interphase. In PC clinical samples and xenograft models we recently found that taxane resistance was associated with lower taxane-driven MT bundling, but the basis for this decrease was not determined (PMID: 32478682). To determine the molecular basis for this impaired taxane-driven MT bundling, we used castration-resistant PC PDXs to generate docetaxel-resistant tumors. Transcriptome analysis showed increased expression of genes that drive development of multiciliated cells. These included *FOXJ1*, its upstream activator *GMNC*, and multiple FOXJ1 regulated genes whose products are MT associated. While FOXJ1 is a master transcription factor regulating the development of multiciliated cells, where it controls expression of multiple cilia-associated proteins, its functions in non-ciliated cells have not been determined. We then found that overexpression of FOXJ1 in PC cells could decrease sensitivity to docetaxel based on colony formation and G2/M arrest. Mechanistically this was associated with a decrease in docetaxel (and cabazitaxel)-mediated MT aggregation, indicating that FOXJ1 regulated genes act at the level of tubulin to mitigate abnormal MT aggregation. Moreover, xenografts generated from FOXJ1 overexpressing cells were resistant to docetaxel. Overexpression of a MT associated FOXJ1 regulated gene (*TPPP3*) also decreased sensitivity to docetaxel in vitro and in vivo, but to a lesser degree, indicating multiple genes downstream of FOXJ1 contribute to resistance. Conversely, FOXJ1 knockdown enhanced sensitivity to docetaxel-mediated decrease in colony formation and to G2M arrest. Moreover, it decreased basal tubulin acetylation, which was then markedly stimulated by docetaxel. Notably, examination of the SU2C PC database showed that FOXJ1 gene amplification was approximately 2-fold more frequent in patients previously treated with taxane than in taxane naïve patients, supporting increased FOXJ1 as a clinically relevant mechanism. Finally, independent of taxane exposure, increased FOXJ1 expression was broadly associated with gene sets related to increased proliferation and to decreased survival. Further studies are underway to assess FOXJ1 functions and its association with taxane resistance in patients.

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Novel Antibody Targeting Tumor NKG2D ligand MIC: Preclinical Studies and Clinical Development Plan for mCRPC

Jennifer Wu, Jeff Sosman, Masha Kocherginsky, Anthony Serritella, Maha Hussian, and David James VanderWeele*

In response to genomic insults and DNA double-strand breaks, the stressed epithelial cells express the MHC I-chain related molecule (MIC) on the surface as a byproduct of activating the ATM pathway. The MIC molecule itself has no known impact on tumor survival or growth. In the context of immune microenvironment, tumor cell surface MIC can activate NK cells and co-stimulate CD8 T cells by engaging in the activating receptor NKG2D to hinder the early tumorigenesis. However, in established malignancies, in particular metastatic diseases, tumor cells shed surface MIC to release the soluble form MIC (sMIC). It has been well demonstrated that , in contrary to cell surface MIC, sMIC is highly immune suppressive by disturbing NK cell homeostasis and repolarizing NK cells function, impairing antigen-specific CD8 T cell function by downmodulating NKG2D expression and CD3z expression, and facilitating immune suppression of MDSCs and TAMs in tumor microenvironment. We have demonstrated that MIC is prevalently expressed by prostate tumor cells and significantly upregulated in mCRPC tumors. We have shown significantly elevated levels of serum sMIC in prostate cancer patients with metastatic diseases. We have developed a novel class of clinical candidate monoclonal antibody huB10G5 that can target both sMIC and surface MIC to re-invigorate both NK and CD8 T cell function and inhibit MDSCs and TAMs in preclinical models. The huB10G5 has an acceptable safety profile and is currently completing CMC for IND enabling. We hypothesize that huB10G5 is a novel immune therapeutic reagent for mCRPC patients. To test our hypothesis, we first conducted a serial studies of the clinical candidate huB10G5 in pre-clinical models of prostate cancers. We found that huB10G5 can effectively induce 100% long-term tumor complete regression (CR) as low as 1.0 mg/KG. These animals are fully protected from tumor re-challenge. The anti-tumor effect is mediated by the combined action of NK and CD8 T cells. With the support of our pre-clinical efficacy and safety data, we have planned to initiate a Phase I clinical trial with huB10G5 for mCRPC patients in 2024.

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Session III

Immunotherapy

Identifying Mechanistic Biomarkers of Response to Novel T Cell Bispecifics in Metastatic Castration-Resistant Prostate Cancer

Bilal A. Siddiqui, Jennifer A. Wargo, Padmanee Sharma, James P. Allison, and Sumit K. Subudhi

Our group initially reported that the prostate tumor microenvironment (TME) has a paucity of T cells. We and others have attempted to overcome this with immunotherapeutic strategies (e.g., vaccines, CAR T cells, and immune checkpoint therapies) that drive T cells into the prostate TME but have had limited success, especially in patients with bone-predominant metastases. This has been attributed to primary and adaptive resistance mechanisms, including immunosuppressive myeloid cells and upregulation of inhibitory immune checkpoint molecules. Therefore, novel immunotherapeutic agents and combinations are required to address this issue. Recently, we have observed striking clinical responses with T cell bispecifics ± anti-PD-1 in patients with metastatic castration-resistant prostate cancer (CRPC), even those with bone-predominant disease. We are currently transcriptionally analyzing matched pre- and on-treatment tumor, blood, and stool samples to identify mechanistic biomarkers predictive of responses to this novel therapeutic strategy.

Acknowledgments/Funding: The research work was supported by The University of Texas MD Anderson Cancer Center Prostate Cancer Moon Shot Program; The MD Anderson Cancer Center Prostate Cancer SPORE P50 CA140388; The V Foundation for Cancer Research's Lloyd Family Clinical Oncology Scholar Award D2018-014 (SKS); Prostate Cancer Foundation (PCF) Young Investigator Award (YIA) 22YOUN06 (BAS); and NIH/NCI Award P30CA016672. The Genitourinary Cancers Program of the Cancer Center supports grant shared resources at The University of Texas MD Anderson Cancer Center. JPA and PS are members of the Parker Institute for Cancer Immunotherapy (PICI) at The University of Texas MD Anderson Cancer Center.

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Understanding the Role of Immune Infiltrate Following Intense Neoadjuvant Androgen Deprivation Therapy in Locally Advanced Prostate Cancer

John Fenimore, Anson Ku, Anna Baj, Haydn Kissick and Adam Sowalsky

Background: For patients with locally advanced (high-risk) prostate cancer, neoadjuvant therapies offer early opportunities for systemic therapy combined with the curative potential of definitive surgery. Prostate cancer is typically regarded as immunologically "cold" and the failure of neoadjuvant immune checkpoint therapies to treat prostate cancer has led to a wider discussion of how the immune activity of prostate tumors can be modulated. Because ADT can also promote an immune response, the goal of this project is to assess changes to the local tumor immune microenvironment and determine whether there may be opportunities for combining neoadjuvant ADT and immunotherapy.

Methods: Patients who received six months of neoadjuvant ADT plus enzalutamide prior to surgery were subdivided into responders and non-responders based on the volume of residual tumor in the final surgical specimen. Radical prostatectomy (RP) sections were stained, and immune cell populations were quantified to assess the presence of tumor proximal populations of immune cells. These populations were quantified via HALO software image analysis of each stained cell population. Using spatial techniques, we are characterizing in-depth immune and other cell populations distal and local to the tumor in posttreatment specimens. Our characterization of immune cells includes determinations of T-cell receptor (TCR) diversity as well as the evaluation of differential gene expression associated with activation states of the tumor and proximal immune cell populations across each section of residual tumor.

Results/Conclusion: We observed a dramatic focal infiltration of lymphocytes adjacent to residual tumor foci. We also see a distinct difference between pre and post therapy in the diversity of TCR and BCR populations in patients. By multiplex immunofluorescence, we see evidence of a potentially self-sustaining population of T-cells located in immunological lymph like structures within posttreatment surgical specimens. This population has shown differences between patients based on pathologic outcome; however, it lacks a clear inflammatory profile and expresses genes associated with a reduced level of immune activity. If this is due to an immunosuppressive phenotype by the tumor or its microenvironment, we will aim to determine whether this is due to T cell exhaustion or other inhibition. We will also determine whether immune checkpoint inhibition can augment the antitumor effect of neoadjuvant intense androgen deprivation therapies.

Acknowledgments/Funding: We would like to acknowledge the funding contributions of the iCURE program and was done at facilities of the National Cancer Institute at the National Institutes of Health in Bethesda.

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Androgen Receptor Blockade in Macrophages Primes NLRP3 Inflammasome-Mediated Phagocytosis and Tumor Clearance in Advanced Prostate Cancer

Kiranj Chaudagar, Srikrishnan Rameshbabu* (*Equal contribution), Shenglin Mei, Taghreed Hirz, Ya-Mei Hu, Anna Argulian, Brian Labadie, Kunal Desai, Sam Grimaldo, Doga Kahramangil, Rishikesh Nair, Sabina DSouza, Dylan Zhou, Mingyang Li, Farah Doughan, Raymond Chen, Jordan Shafran, Mayme Loyd, Zheng Xia, David B. Sykes, Amy Moran, Akash Patnaik*

Immune-based therapies induce durable remissions in subsets of patients across multiple malignancies. However, there is limited efficacy of immunotherapy in metastatic castrate-resistant prostate cancer (mCRPC), manifested by an enrichment of immunosuppressive (M2) tumor-associated macrophages (TAM) in the tumor immune microenvironment (TME). Therefore, therapeutic strategies to overcome TAM-mediated immunosuppression are critically needed in mCRPC. Our single-cell RNA sequencing analysis in human tumors revealed that NLR family pyrin domain containing 3 (NLRP3), an innate immune sensing protein, is differentially expressed in TAM from metastatic PC patients treated with standard-of-care androgen deprivation therapy (ADT), relative to other tumor types and untreated primary PC. Furthermore, bulk RNA sequencing analysis in human mCRPC samples revealed an inverse relationship between NLRP3 expression and AR activity, with high NLRP3 expression associated with an M1 signature and a favorable clinical response to ICI in mCRPC. Based on these findings, we hypothesized that androgen axis blockade could enhance NLRP3 expression and potentiate innate immune tumor control in advanced PC. Critically, we discovered that blockade of TAM-intrinsic androgen receptor (AR) activity enhanced NLRP3 expression, but not inflammasome activity in the immunosuppressive (M2) TAM. In contrast, anti-tumor (M1) TAM exhibited high de novo NLRP3 expression, regardless of AR activity. The combination of AR blockade and NLRP3 agonism significantly enhanced phagocytosis of cancer cells by M2 TAM, whereas NLRP3 agonist treatment alone was sufficient to induce phagocytosis in M1 TAM. Following AR blockade/NLRP3 agonist combination treatment, all TAM acquired a distinct phenotype with high PD-L1 and CD86 expression, indicative of phagocytic TAM. Critically, NLRP3 agonism in combination with ADT resulted in significant tumor control in an aggressive c-myc driven advanced PC model, with 55% of mice achieving complete tumor clearance, which was abrogated by concurrent clodronate treatment (which systemically depletes phagocytic macrophages), thus demonstrating TAM-mediated phagocytosis enhancement as a major driver of the observed anti-tumor response. Collectively, our results identify NLRP3 as an AR-regulated "macrophage phagocytic checkpoint" that can be inducibly expressed and activated in TAM following ADT and NLRP3 agonist treatment, respectively, the combination resulting in TAM-mediated phagocytosis and tumor control.

Acknowledgments/Funding: NCI Prostate SPORE Northwestern/University of Chicago; Michael & Lori Milken Family Foundation-Prostate Cancer Foundation Challenge Award

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Chimeric Antigen Receptor T Cell Therapies for Subtypes of Metastatic Castration-Resistant Prostate Cancer

Vipul Bhatia, Nikhil V. Kamat, Tiffany E. Pariva, Li-Ting Wu, Jessica E. Hawley, Michael Schweizer, Vicky Wu, Michael C. Haffner, Peter S. Nelson, Amy E. Moran, Saul J. Priceman, Jun Ishihara, John K. Lee

Chimeric antigen receptor (CAR) T cell therapy is a highly promising approach that has transformed the treatment of several hematologic malignancies. The development of CAR T for solid tumors has been met with challenges but early phase clinical trials are increasingly demonstrating safety and efficacy signals. Prior clinical studies of CAR T directed against prostate-specific membrane antigen (PSMA) and prostate stem cell antigen (PSCA) in metastatic castration-resistant prostate cancer (mCRPC) have shown instances of encouraging biochemical and radiographic responses. However, mCRPC encompasses multiple disease including prostate adenocarcinoma (PRAD) and small cell neuroendocrine prostate cancer (SCNPC) that can be distinguished by differential cell surface protein expression. We therefore focused on developing prostate cancer subtype-specific CAR T.

Six transmembrane epithelial antigen of the prostate 1 (STEAP1) was found to be more broadly expressed than PSMA in lethal cases of PRAD. A second-generation STEAP1 CAR was designed/optimized that exhibited specificity and reactivity in low STEAP1 antigen density conditions. STEAP1 CAR T demonstrated significant antitumor effects and prolonged persistence after systemic administration in multiple human-in-mouse (C4-2B, 22Rv1) and mouse-in-mouse (RM9) models of mCRPC. We also established safety by generating a human STEAP1 knock-in mouse model in which STEAP1 CAR T did not result in apparent toxicities.

L1 cell adhesion molecule (L1CAM) was identified as a cell surface antigen that is highly expressed in neuroendocrine cancers including SCNPC. We repurposed a L1CAM CAR targeting the cancer-dependent glycosylated epitope CE7 that was previously developed and is under active investigation in the phase I Engineered Neuroblastoma Cellular Immunotherapy (ENCIT)-01 trial for relapsed/refractory neuroblastoma. Preliminary findings from this study have indicated safety and tolerability. L1CAM CAR T showed specific activation and cytotoxic killing in co-cultures with SCNPC cell lines (NCI-H660, MSKCC EF1). Further, intravenous administration of L1CAM CAR T significantly inhibited the tumor growth of MSKCC EF1 subcutaneous xenografts established in mice.

These CAR T are being translated to the clinic in early-2024. Both trials will incorporate scientific correlative studies that will allow for a deeper understanding of CAR T persistence/expansion, trafficking, interactions within the tumor microenvironment, and properties that may associate with response or resistance. We are also actively exploring strategies to enhance the potency of CAR T for mCRPC including armored expression of inflammatory cytokines, dual targeting of cell surface antigens, and inhibition of T cell-intrinsic androgen receptor activity.

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Evolution of Myeloid-Mediated Mechanisms of Immunotherapy Resistance at Single-Cell Resolution with Prostate Cancer Progression

Aram Lyu, Zenghua Fan, Diamond Luong, Ali Setayesh, Alec Starzinski, Eliezer M. Van Allen, Lawrence Fong

Background: Patients with metastatic castration-resistant prostate cancer (mCRPC) are generally refractory to immune checkpoint inhibitors (ICIs). This resistance is partly linked to the presence of immunosuppressive myeloid cells residing in tumors. However, broad targeting approaches, such as CSF1R antagonism, have thus far failed clinically largely due to their intricate and heterogeneous nature. Thus, we hypothesized that gaining a deeper insight into distinct immunosuppressive myeloid subsets and their molecular mechanisms at the single-cell level is critical to improve the efficacy of immunotherapy.

Methods: We performed multi-omic single-cell profiling of patient biopsies with either localized disease, metastatic hormone-sensitive prostate cancer, or mCRPC. We then reverse translated these findings in a syngeneic mouse model of CRPC, where we performed multi-omic single-cell assessment and conducted functional assays, tumor efficacy and mechanistic studies.

Results: Using single-cell assessment of patient biopsies, we identified a specific subset of tumor-associated expressing elevated hypoxia signatures, particularly the expression of SPP1 transcripts (referred to as SPP1hi-TAMs). This macrophage population is notably more abundant in mCRPC than earlier disease stages, with elevated levels of immunosuppressive molecular programs and significantly lower CSF1R transcripts relative to other macrophages, potentially explaining the limited effectiveness of CSF1R blockade. To further explore our findings, we performed multi-omic profiling of immune and non-immune cells isolated from a syngeneic mouse model of CRPC. Through systemic transcriptional comparisons and subsequent validation via flow cytometry, we identified a parallel macrophage subset. We demonstrated that these macrophages have the ability to suppress proliferation and activation of CD8+ T cells in co-culture. Consistent with these findings, adoptive transfer of Spp1hi-TAMs into CRPC notably increased resistance to ICIs and worsened survival in vivo. Notably, these macrophages were not effectively ablated by administration of an anti-CSF1R antibody within tumors, pointing to their immunosuppressive signals as potential immunotherapeutic targets. Pathway analysis revealed the enrichment of gene signatures associated with adenosine signaling in SPP1hi-TAMs, both in humans and mice. In line with these findings, blockade of adenosine signaling via antibodies or pharmacologic inhibitors resulted in a significant reduction in suppression of CD8+ T cells mediated by Spp1hi-TAMs in vitro. Furthermore, pharmacologic blockade of adenosine receptors in vivo significantly decreased tumor growth and enhanced the sensitivity of tumor cells to ICI therapies.

Conclusions: Our studies demonstrate a significant increase in the abundance of SPP1hi-TAMs as the disease advances, expressing elevated immunosuppression gene signatures. These macrophages show resistance to CSF1R blockade and play a critical role in driving immunotherapeutic resistance in vivo by activating adenosine signaling. These findings underscore the potential of targeting the associated signals as promising strategies for therapeutic intervention.

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DNA Vaccines in Combination with Androgen Deprivation as Treatment for Prostate Cancer

Anusha Muralidhar and Douglas McNeel

Prostate cancer has been viewed as an immunologically "cold" tumor, devoid of large numbers of tumor-infiltrating lymphocytes. We have been interested in vaccines as T-cell activating therapies to augment tumor-specific cytolytic CD8+ T cells as a treatment for recurrent prostate cancer. Using DNA vaccines, we have focused on mechanisms to improve the immune response to encoded antigens by utilizing agents that affect T cell function during their activation by vaccination. We have also focused on mechanisms of tumor resistance to suggest appropriate combination approaches. In particular, we have explored combinations with androgen deprivation therapy (ADT). ADT has several immunomodulatory effects, including increasing T-cell infiltration into the prostate and enhancing antigen processing and/or presentation. In previous studies we showed that immunizing tumor-bearing mice with a DNA vaccine encoding the androgen receptor significantly slowed tumor growth and prolonged survival. We have recently demonstrated that vaccination prior to ADT significantly improved anti-tumor responses, and increased T-cell tumor infiltration, including vaccine antigen-specific CD8+ T cells. Prolonged ADT, however, led to increased infiltration with myeloid derived suppressor cells (MDSC). Depletion of MDSC led to greater anti-tumor efficacy in combination with ADT and vaccination. These findings, and translation to human trials, will be discussed.

Acknowledgments/Funding: P50 CA269011

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Session IV

Imaging and Biomarkers

Using Artificial Intelligence Approaches to Personalize Prostate Cancer Therapies

Felix Feng

In this talk I will discuss using artificial intelligence approaches to develop biomarkers prognostic of outcome and predictive of treatment response in patients with prostate cancer.

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Predictive PSMA PET/MRI Imaging and Clinical Biomarkers of Response in High-Risk Prostate Cancer Patients Treated with Neoadjuvant Chemo-hormonal Therapy Prior to Prostatectomy

Tsourkas P, Ong I, Mcilwain S, Shin M, Heninger E, Bradshaw TJ, Huang W, Kyriakopoulos C, Lang J, Wells SA, Jarrard DF, Cho SY

Methods: 30 patients with biopsy-proven, locally advanced high-risk prostate cancer (PCa) were enrolled in a prospective Phase II clinical trial at UW-Madison. The patients underwent 3 cycles of neoadjuvant chemo-hormonal therapy (CHT) with docetaxel and ADT, followed by cytoreductive radical prostatectomy. 18F-DCFPyL prostate specific membrane antigen (PSMA) PET with multiparametric prostate MRI (mpMRI) scans were performed on a dedicated PET/MRI scanner (GE Signa, Waukesha WI) before and after neoadjuvant CHT. Fourteen PET and MRI prostate cancer imaging features were collected before and after CHT including PET and MRI visual Likert score, quantitative PET maximum standardized uptake value (SUVmax) and diffusion-weighted imaging (DWI). Additional eight clinical and imaging features were also collected for each patient including pre-CHT prostate tumor volume (TV) (T2W, DynaCAD, Philips), pre- and post-CHT prostate specific antigen (PSA) level. Univariate and multivariate Cox regression analysis was performed with radiology and clinical features as covariates to assess each feature's association with biochemical disease progression. Significance was established using the logrank test and a cutoff of α=0.01.

Results: Univariate analyses of prostate lesion imaging features, post-CHT PET SUVmax (SUV.MAX.Post) was the most significant feature (p=1.2e-6), with post-CHT MRI visual Likert score and DWI (MRI.Post, DWI.Post) the second and third most significant features, respectively. Pre-CHT imaging features were far less significant than post-CHT features. Combining two imaging features in a bivariate analysis did not result in an increase in significance. Of the other imaging and clinical features, TV was the most significant (p=2.5e-6), with the combination of TV and post-CHT PSA more significant than either of these features separately (p=3.5e-7). The combination of SUV.MAX.Post and TV (the two most predictive features in the univariate analyses) emerged as the best predictor of progression (p=5.5e-8).

Conclusion: We have demonstrated PSMA prostate PET/MRI imaging and clinical features that individually, and more superiorly in combination, that are predictive of disease progression in high-risk prostate cancers treated with neoadjuvant CHT prior to prostatectomy. This study provides valuable biomarkers for inclusion in future clinical trials in this emerging prostate cancer therapy approach.

Acknowledgments/Funding: DOD PCRP Impact Award PC15053, NIH/NCI 1P41EB024495-01- Service Project 2 (UW-Madison)

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Role of the Gut Microbiome in Androgen Production and Prostate Cancer Treatment Resistance

Angélica Cruz-Lebrón, Pedro A. Balbuena-Almodóvar, Luke Mummert, Sarah E. Ernst, Mark Markowski, Michelle Rudek, Jason Ridlon, Karen S. Sfanos

Metastatic prostate cancer is often treated via a combination of androgen deprivation therapy (ADT) and the androgen receptor axis-targeted therapies abiraterone acetate + prednisone (AA/P) or enzalutamide. Unfortunately, most individuals undergoing these treatments develop resistance, termed "castration resistance", through an unknown mechanism. Previous studies demonstrate a potential link between the gut microbiota and the treatment efficacy of endocrine therapy in metastatic castration resistant prostate cancer (mCRPC). This may be due in part to gut bacterial communities with the machinery to synthesize androgens using mechanisms distinct from human cells. For example, the bacterial *desAB* genes (e.g., "desmolase") that were first described in the gut commensal bacterial species *Clostridium scindens* convert cortisol and prednisone to the androgenic metabolites 11β-hydroxyandrost-4-ene-3,17-dione (11OHAD) and Δ1-adrenosterone (Δ1-AT), respectively. We hypothesize that androgen synthesis by the gut microbiota promotes treatment resistance to AA/P in advanced prostate cancer. This study aims to determine if androgen-converting gut bacterial species as well as circulating and fecal androgen levels correlate with AA/P treatment response in individuals with mCRPC using a combination of metagenomics and metabolomics. We further aim to demonstrate the relationship between gut bacterial androgen production and prostate cancer treatment response using an in vivo mouse model. Metagenomic sequencing showed an imbalance in microbial communities and identified the presence of androgen-synthesizing bacteria such as C. scindens in fecal samples of individuals undergoing treatment with AA/P. We also demonstrate that the relative abundance of C. scindens, as well as other species reported to generate androgens, are significantly enriched during metastatic disease progression on AA/P. Furthermore, targeted quantitative PCR of the bacterial desmolase (*desA*) gene demonstrated that absolute levels of desmolase are significantly higher in AA/P non-responders relative to AA/P responders, and trended higher in individuals with rising PSA versus stable PSA while on AA/P. Targeted LC/MS/MS analyses of fecal samples demonstrated the presence of testosterone, dihydrotestosterone, 11OHAD, Δ1-AT, and other androgen metabolites in association with rising PSA levels in the AA/P cohort. Furthermore, we demonstrate that *C. scindens* converts hydrocortisone and prednisone into 11OHAD and Δ1-AT, respectively, *in vitro* at 48- and 72h post-treatment, which recapitulates prior published studies. Finally, we have established a prostate cancer mouse model using a VCaP xenograft in which tumor growth was significantly inhibited by abiraterone acetate in comparison to treatment with ADT alone. Additional studies will use this mouse model to study the effects of androgen metabolism by gut bacteria on AA/P efficacy. Overall, this study ascertains the ability of the human gut microbiota to harbor androgen-synthesizing bacteria, which in turn can be an alternative source of androgens that could impact the efficacy of the anti-androgen therapies used to treat metastatic prostate cancer.

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A Theranostic Program for Imaging and Therapy of Delta-Like Ligand 3 Expressing Neuroendocrine Prostate Cancer

Tran T. Hoang, Roberto Degregorio, Alexa Michel, David Bauer, Lukas Carter, Kishore Pillarsetty, Yu Chen, John T. Poirier, Charles M. Rudin, Michael Morris, Jason S. Lewis

Objectives: Neuroendocrine prostate cancer (NEPC) is a highly aggressive subgroup of prostate cancer with selective cell surface expression of the inhibitory Notch ligand Delta-like ligand 3 (DLL3). This facilitates targeted radionuclide therapy accompanied by complementary imaging agents to evaluate and monitor disease progression. The project aims to identify the next generation of DLL3-targeting radioimmunoconjugates to improve the clinical outcome for NEPC patients.

Methods: In collaboration with the Tri-Institutional Therapeutics Discovery Institute, we screened and assessed >100 humanized antibodies for their binding affinities, internalization rates, and other pharmacodynamic properties. Here, we present data for the leading candidate (9-N12) holding highest promise in both imaging and biodistribution studies conducted in DLL3-expressing NEPC tumors. For in vivo imaging, antibodies were radiolabeled with Zirconium-89 (89Zr), which allows for immuno-positron emission tomography (immunoPET). To demonstrate specificity, a blocking cohort co-administered with unconjugated 9-N12 was performed. Human NEPC NCI-H660 (DLL3+) xenografts were implanted in nude athymic male mice. ImmunoPET scans were acquired during multiple time points post-injection of the imaging agent, and a full course biodistribution analysis was performed to determine appropriate doses for an upcoming therapeutic study utilizing Lutetium-177 (177Lu).

Results: The quality control of the 9-N12 in vitro included assessing radiolabeling properties, stability in human serum, and binding affinities to the selected DLL3-expressing human tumor cell lines. The constructs passed the in vitro assays, and was thereafrer tested in vivo. ImmunoPET imaging utilizing 89Zr demonstrated high and specific uptake of 9-N12 conjugate with low background in non-tumor organs. This was highlighted at 72 hours post-injection, with very low tumoral uptake in the blocking cohort (Figure 1A). Dosimetry estimates suggested a high therapeutic index for the 9-N12, with the with the bone marrow being the dose-limiting organ (Figure 1B). Future treatment studies will include administration of 400 and 600 μCi 177Lu-conjugated 9-N12 that will be initiated soon, with appropriate positive and negative controls.

Conclusions: This study highlights the promise held by a novel DLL3-direct radioimmunoconjugate as a diagnostic tracer and therapeutic agent. The lead candidate is promising for translation as both imaging and therapeutic agent for patients with NEPC, the ultimate goal of a first-in-human clinical trial.

Acknowledgments/Funding: These studies were made possible with the generosity from the Prostate Cancer Foundation (PCF) grant (57840/ 20849).

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The Role of PSMA in Prostate Cancer and Beyond

Jan Grimm

Prostate-specific membrane antigen (PSMA) is highly expressed on prostate cancer and currently the premier target for imaging and targeted radiotherapy of prostate cancer. Even though while this protein has been heavily investigated as a target, its biological role in the disease remained elusive. Aberrant activation of the Phosphatidylinositol 3-kinase (PI3K) β-isoform (p110β) is central in the disease, the signaling initiation mechanism that activates p110β remained unknown. We showed that the PI3K/Akt axis is activated through PSMA, also known as folate hydrolase-1. PSMA's carboxypeptidase activity releases free glutamate from folic acid (Vitamin B9) or poly-glutamyl folates. These glutamate molecules in turn activate the G protein-coupled metabotropic Glutamate receptors Group I (mGluR I), which are also expressed on the plasma membrane of prostate cancer cells. The activation of the glutamatergic system by PSMA leads to phosphorylation of p110β with concomitant phosphorylation of Akt and its downstream targets, independent of PTEN loss. Blocking this glutamergic signaling can provide a therapeutic approach. We further found that in prostate cancer patients PSMA expression correlated with upregulated PI3K-Akt signaling, which can be imaged through PSMA-targeting PET agents. Further, PSMA is shed by extracellular vesicles and can be transported to PSMA-negative cells, both tumor cells as well as cells from the tumor microenvironment (TME). This process shapes and alters the TME. One consequence of PSMA transfer to cells is an increased secretion of vascular endothelial growth factor-A (VEGF-A), pro-angiogenic and pro-lymphangiogenic mediators, with increased vascular sprouting and macrophage recruitment.

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Session V

Population Science and Clinical Research

DNA Damage Repair Variants in African Americans Families that have a History of Both Breast and Prostate Cancer

Matt Trendowski, Christine Lusk, Tara Baird, Julie Ruterbusch, Greg Dyson, Kathleen A. Cooney, Jennifer L. Beebe-Dimmer

Background: Prostate and breast cancer are the most common cancers diagnosed among men and women, respectively in the United States. African Americans with these cancers are disproportionately diagnosed with aggressive disease and have a higher mortality compared with survivors of other races and ethnicities. African Americans are also underrepresented in both genome-wide association and sequencing studies so that our understanding of inherited genetic susceptibility for this population is limited.

Methods: The Family First Study is a population-based cohort of African American families diagnosed with both prostate and breast cancer in first-degree family members (parents, siblings, children). Eligible probands were identified using the Metropolitan Detroit Cancer Surveillance System cancer registry with a relative diagnosed with the "discordant" cancer (i.e. breast cancer cases with a family history of prostate cancer or prostate cancer cases with a family history of breast cancer). Enrolled probands assisted with the recruitment of affected family members if possible. Full study participation included completion of a questionnaire and collection of a biospecimen (either saliva or blood) for whole exome sequencing. The current investigation focuses on variants in 35 DNA damage repair (DDR) genes among 128 probands with sequencing data. Pathogenic (P) or likely Pathogenic (LP) variants were identified using a series of bioinformatic criteria including 1) ClinVar classification; 2) REVEL score; 3) Ensembl VEP impact rating; and 4) an expected minor allele frequency (MAF) of less than 1% in African American populations.

Results: We observed 9 unique P/LP variants in 7 DDR genes (*ATM*, *BRCA1/2*, *MLH1*, *MRE11*, *MSH2*, *RAD51B*) among 17 proband cases (13.3%). Most were private (7) missense mutations, however 3 probands had mutations in *RAD51B* (rs534919944). Co-segregation of mutations with disease were observed in two of the 9 families with sequenced first-degree relatives. We did not observe any difference in clinical characteristics between mutation carriers and non-carriers.

Conclusions: Missense mutations in DDR genes are common in African American families with co-clustering of breast and prostate cancer in first-degree relatives. This reinforces the notion that collecting family history of all cancer within families is important in risk prediction and communication of cancer history amongst family members to promote enhanced screening. Further investigation in larger populations will be important in understanding the role of these genes in risk and progression among African Americans diagnosed with breast and prostate cancer.

Acknowledgments/Funding: W81XWH-17-1-0114. The contributions of Rare Variants in Familial Clustering of Prostate and Breast Cancer in African Americans.

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Prostate Cancer: Integrative Multi-Omics Profiling in Patients of African Descent

Isra Elhussin, Ezra Baraban, Tamara L. Lotan, Cathy Handy Marshall, Emmanuel Antonarakis, Moray J. Campbell, Melissa Davis, Michael Dixon, Isaac Kim, Stefan Ambs, Rick Kittles, Adam B Murphy, Clayton Yates

In the United States and globally, prostate cancer (PCa) mortality rates are highest among men of African descent. Previous studies suggested that tumor subtypes or gene expression profiles had the greatest influence on overall racial/ethnic survival, accounting for 24% of the disparities in PCa and remains even when controlled for access to care and stage at presentation. Moreover, the tumor microenvironment plays an essential role in tumor progression, aggressiveness, therapeutic response, and patient outcomes. Additionally, the association of the genomic findings with patient ancestry and other characteristics, such as tumor biology and transcriptomic alterations, remains poorly understood. Here, we performed a multi-Omics approach (N=447) to unravel the complexity of tumor heterogeneity and understand disease progression & distinct tumor biology influenced by genetic ancestry.

We performed Whole Exome (Normal/tumor paired) Sequencing matched with Methyl Seq and Whole transcriptomic Sequencing for three datasets of our African, African American, and European cohorts. Additionally, we ran Spatial NanoString high-plex GeoMx-DSP (Digital Spatial Profiler) at the protein and RNA levels for a total of 118 treatment-naive PCa patients (around 500 ROIs). Simultaneously, we added matched genome-wide Sequencing (whole transcriptome) for these patients. The cohort comprised 87 AAM, 3 unknown, and 28 EAM self-reported individuals. To verify the self-reported race, the genomic ancestry was qualified using genotype and Admixture analysis. To further validate differentially expressed genes at the protein level, we performed multi-plex histological staining of 40 markers to determine the spatial resolution and neighborhood clustering within the tumor and the microenvironment. In parallel, we performed scMultiOmics sequencing (scRNA & scATAC-Seq) at a single-cell resolution (6000 cells/sample at 25K reads per cell) from an additional 12 patients (9 AAM & 3 EAM).

We will use the multi-omics integration method (CoGap) to build a structural, spatial map that reflects tissue/system phenotype and to understand the complementary role of multi-omics (genome, transcriptome, epigenome, and metabolite) interrelation/influence on the disease processes.

Our results demonstrate that patients who self-report as AAM or Nigerian are assigned to high African (> 70%) Ancestry with either Yoruba (Nigeria) and/or Bantu subpopulation in the Sub-Saharan area. Additionally, high African Ancestry patients are diagnosed at a younger age and show advanced pathology stages compared to patients with European Ancestry. Patients with High-African Ancestry express significantly higher immune-inflammatory signatures (IFNG-signaling pathway) compared to patients with Low-African Ancestry. Moreover, African Ancestry is Associated with Immune Suppression (M2 macrophages). Our scRNA-Seq analysis shows that African American men (AAM) have myeloid cells that infiltrate within the tumor cells. The infiltrations of these cells change with age, Gleason Grade, and pathology stage.

Our study provides new insight into how genetic ancestry impacts immune signatures in AAM and contributes to PCa racial disparities. Our findings could lead to new therapeutic strategies using immune modulator drugs to decrease the global disease burden, especially among men at high risk for PCa, such as AAM of African descent.

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The molecular landscape of high-risk localized prostate cancer in the Genomic-biomarker-selected Umbrella Neoadjuvant Study (GUNS)

Joshua M. Scurll, Lucia Nappi, Htoo Zarni Oo, Alexander Wyatt, Himisha Beltran, Neil Fleshner, Peter Black, Amina Zoubeidi, Martin Gleave

Background: Androgen receptor (AR) pathway inhibition (ARPI) is the cornerstone of treatment (Tx) for prostate cancer (PCa), but resistance inevitably develops, often via lineage plasticity. Some genomic alterations (e.g. functional loss of *RB1* or *TP53*) promote lineage plasticity and resistance to ARPI, but some potentially offer opportunities for therapeutic exploitation. The Genomic-biomarker-selected Umbrella Neoadjuvant Study (GUNS, NCT04812366) is a multi-center adaptive phase-II clinical trial evaluating combination treatments in high-risk localized PCa, prior to radical prostatectomy, based on screening for genomic alterations. GUNS has four active sub-protocols that combine ARPI with one of the following: chemotherapy for *RB1*, *PTEN*, or *TP53* functional loss (SP2); PARP inhibition for DNA damage repair deficiency (SP3); anti-PD-L1 for mismatch repair (MMR) or CDK12 deficiency (SP4); or additional ARPI otherwise (SP1). This neoadjuvant trial offers a novel platform to investigate lineage plasticity in PCa with genomic context.

Methods: Tumor specimens undergo 648-gene panel DNA sequencing (DNA-seq, Tempus xT), whole-transcriptome (WT) RNA sequencing (RNA-seq, Tempus RNA), and immunohistochemistry (IHC). Pre- and post-Tx tumor tissue from Patient 01-001 was additionally analyzed by NanoString GeoMx WT Digital Spatial Profiling (DSP); based on pan-cytokeratin immunofluorescence staining and automated segmentation, epithelial and non-epithelial areas were profiled separately. Single-sample gene set enrichment analysis (ssGSEA), unsupervised hierarchical clustering (UHC), and PAM50 classification were applied to RNA-seq data. UHC and differential gene expression analysis were applied to DSP data.

Results: We report the molecular landscape of tumors in GUNS from pre-Tx DNA-seq data from ≥70 patients, matching pre-Tx RNA-seq data from ≥38 patients, and post-Tx data from ≥3 patients. The pre-Tx genomic landscape is dominated by *ETS* gene fusions (~33% cases, predominantly *TMPRSS2-ERG*) and *FOXA1* alterations (~30% cases). Functional loss of *TP53*, *PTEN*, or *RB1* was reported in ~25% of cases, deleterious germline and/or somatic *BRCA2* alterations in ~9% of cases, and SP4-qualifying genomic profiles in 4 patients. IHC implied more frequent PTEN loss than reported by Tempus xT. UHC of RNA-seq data largely aligned with *ETS*-fusion, *SPOP*, and *FOXA1* genomic statuses and luminal/basal subtypes. Enrichment scores for neuroendocrine (NE) signatures correlated closely with *ETS*-fusion status in pre-Tx tumors. Post- vs pre-Tx comparisons suggested lineage plasticity. Patient 01-001 (SP4) had a genomically and phenotypically aggressive tumor that dramatically increased NE signatures after Tx. DSP analysis of Patient 01-001 showed elevated neuronal gene expression across profiled residual tumor regions despite molecular heterogeneity, but also an abundance of immune cells in both the pre- and post-Tx specimens and presence of CD8+ T cells following anti-PD-L1 immunotherapy.

Conclusions: The genomic landscape of high-risk localized PCa in GUNS is dominated by *ETS* gene fusions and *FOXA1* alterations. Transcriptomes largely cluster in alignment with these alterations and with luminal/basal subtypes, and NE ssGSEA scores correlate with *ETS*-fusion status. A case study of one MMR-deficient tumor with a truncal *RB1* mutation indicated a positive response to immunotherapy but also demonstrated severe lineage plasticity.

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Localized High-Risk Prostate Cancer Harbors an Androgen Receptor Low Subpopulation Susceptible to HER2 Inhibition

Scott Wilkinson, Anson T. Ku, Rosina T. Lis, Isaiah M. King, Shana Y. Trostel, John R. Bright, Nicholas T. Terrigino, Anna Baj, John M. Fenimore, Chennan Li, BaoHan Vo, Caroline Jansen, Huihui Ye, Nichelle C. Whitlock, Stephanie A. Harmon, Nicole V. Carrabba, Rayann Atway, Ross Lake, Haydn T. Kissick, Peter Pinto, Peter L. Choyke, Baris Turkbey, William L. Dahut, Fatima Karzai, Adam G. Sowalsky

Background: Emerging neoadjuvant therapies for localized high-risk prostate cancer (PC) introduce systemic therapies effective in metastatic disease earlier in the disease course. We recently completed a phase 2 clinical trial in which 37 patients received six months of androgen deprivation plus enzalutamide prior to radical prostatectomy. However, only 15 patients exhibited exceptional responses as measured by complete pathologic response or minimal residual disease. Therefore, we sought to identify molecular determinants at baseline that drove resistance to therapy.

Methods: Tumors from 37 patients with locally advanced PC were subjected to immunohistochemistry (IHC) and laser capture microdissection to obtain 143 distinct foci for generating RNA-seq libraries. Differentially-expressed genes were determined per-unit of residual tumor volume using linear mixedeffect models. Ingenuity Pathway Analysis (IPA) was used to identify upstream regulators of differential gene expression lists from tissue and single-cell RNA-seq. Semiquantitative IHC was performed on posttreatment tumors. PC cell lines were treated with 8 different EGFR-family inhibitors with or without ARaxis inhibitors and cell viability, RNA and protein were measured. Four patient-derived PC organoids were treated with neratinib to examine the sensitivity of HER2 inhibition. A multiplex immunofluorescence panel against AR, PSA, and HER2 was used on 64 additional untreated cases.

Results: Prior to ADT and enzalutamide, RNA-seq of baseline biopsies showed an inverse correlation between AR activity and posttreatment tumor volume using IPA ($z = -5.2$). Conversely, incomplete/nonresponding tumors displayed an increase in HER2 activity ($z = 3.7$, $p = 2.6\times10\text{-}37$). Between EGFR, HER2 and HER3, HER2 was the most abundantly expressed protein by IHC in posttreatment tumors. Baseline HER2 protein expression by IHC H-score positively correlated with posttreatment HER2 expression ($rho = 0.39$; $p = 0.024$), and baseline HER2 H-scores were significantly greater in poor responders ($p = 0.017$). Single cell analysis of LNCaP cells treated with antiandrogen showed HER2 and AR activities in pre-existing resistant and sensitive cells, respectively. Afatinib and neratinib demonstrated effective cell killing with a rapid time-dependent increase in apparent AR transcriptomic activity in three PC cell lines, which we confirmed at the protein level. In four different organoid models, treatment with neratinib for 48h showed >80% cell death in two models, and >65% in a third. We observed additive effects on cell viability and proliferation by treating cell lines with combination therapy of antiandrogen therapy and these inhibitors. Via multiplex IF, prostate tumors from patient tissue exhibited heterogeneous but mutually exclusive high expression of HER2 and PSA in tumor cells.

Conclusions: Overall, our data suggest de novo prostate tumors harbor a variable proportion of cells that are intrinsically HER2-elevated, and this increase in HER2 activity defines an AR-low subtype of PC that is resistant to AR-targeted therapies. Our observations suggest that some PCs exhibit a divergent path for tumorigenesis that is less dependent on AR. Heterogeneous responses in the organoid models suggest that the proportion of HER2-dependent cells may vary between individuals due to pre-existing subpopulations. These findings represent new opportunities for combination therapy in newly-diagnosed high-risk disease.

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Drivers and barriers to accessing prostate specific antigen screening for early detection of prostate cancer among Black men

Jenney R Lee, Dante Morehead, Ben Young, Victor Tolbert, John Masembe, Garrett Britt, Lars Neuenschwander, Kyle Schuppe, Richard Pelman, Debi Johnson; Sung Min Kim, Erika M Wolff, John L Gore, Yaw A Nyame

Introduction: Black individuals have a two-fold higher rate of prostate cancer (PCa) death in the US compared to the average PCa population. Yet few guidelines support race conscious screening practices among at-risk Black individuals. Our objective was to examine structural factors that facilitate or impede access to PCa screening among Black Americans.

Methods: This prospective, mixed methods study included semi-structured interviews with Black individuals in the Pacific Northwest, and a survey of primary care providers (PCPs) and urologists in the WWAMIO region regarding prostate cancer screening. Consensus coding and thematic analysis were used to analyze interviews; anonymous online surveys were distributed to physicians via email and analyzed using REDCap.

Results: 29 men participated in interviews; a primary finding was that PCPs, as gatekeepers in accessing PSA testing, lack knowledge specific to Black American's risk for PCa and may hold attitudes about PSA testing that do not support its use. Interviewees also reported a lack of trusted relationships with PCPs to support shared decision-making. 32 urologists and 31 PCPs completed the survey. While both groups reported high awareness of USPSTF guidelines, PCPs (6.5%) were significantly less likely than urologists (70.0%) to believe in the value of PSA testing or the role of early detection to prevent PCa-related mortality (Table 1).

Conclusion: Findings from the survey support interviewee perception that PCPs may not value PSA testing for prostate cancer early detection, nor appreciate its role in reducing the risk of prostate cancer-related mortality. Results also demonstrate that PCPs are significantly more likely to be influenced by USPSTF guidelines, which currently do not provide guideline recommendations for screening high-risk groups including Black Americans. Our findings further demonstrate that (1) Black individuals have unique needs around early detection of PCa and (2) patient partnerships can identify these areas of need and best inform patient-centered design of practice-changing research studies.

Table 1. Provider Survey Responses (%)

	Urologists (n=32)	PCPs (n=31)
Awareness of current screening guidelines		
US Preventive Services Task Force	93.8	96.7
American Urologic Association	93.7	45.2
Guidelines that influence use of PSA testing for screening		
US Preventive Services Task Force	31.2	96.8
Other guidelines	90.6	51.6
Early detection reduces cancer-related mortality		
For all cancers	84.3	83.9
For prostate cancer	81.3	41.9
Beliefs about PSA testing		
PSA is valuable	96.9	48.4
PSA significantly reduces prostate cancer mortality	70.0	6.5
Often/always discuss PSA testing		
Patient with family history of prostate cancer	90.6	80.6
Patient who is Black/African American	87.5	61.3

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Benefits of PSMA-PET/CT at Biochemical Recurrence after Prostatectomy: A Decision Model Analysis

Kemal Caglar Gogebakan, Felipe Montano-Campos*, Zizi Elsisi, Yibai Zhao, Lukas Owens, Amir Irvani, Justin Ferdinandus, Wolfgang P. Fendler, Jeremie Calais, Thomas A. Hope, Roman Gulati, Ruth Etzioni*
**The first 2 authors contributed equally to this article.*

Background: Prostate-specific membrane antigen (PSMA) positron emission tomography (PET)/computed tomography (CT) is reshaping treatment options at multiple decision points. In this decision analysis, we examined the expected long-term benefits of PSMA-PET/CT at biochemical recurrence (BCR) after prostatectomy.

Design: Patients were classified into three risk groups based on prostate-specific antigen (PSA) levels and PSMA-PET/CT status (PSMA-positive, PSMA-negative). Proportions of patients by PSMA-PET/CT result, treatment distributions with and without knowledge of PSMA-PET/CT status, and overall survival by treatment and risk were sourced from published studies. We integrated these data using a mathematical framework that projects life years gained due to knowledge of PSMA-PET/CT status given assumptions relating this status to overall survival by risk and treatment.

Results: In a management scenario derived from a prospective single-arm clinical trial, knowledge of PSMA-PET/CT status increases receipt of systemic therapy for the PSMA-negative and decreases receipt of systemic therapy for the PSMA-positive group. Under this counter-intuitive treatment change, over 12 years, PSMA-PET/CT at BCR does not yield a benefit, resulting in a loss of 3 life years per 100 patients on average. In a second hypothetical scenario, where knowledge of PSMA-PET/CT status decreases receipt of systemic therapy for the PSMA-negative group and increases receipt of systemic therapy for the PSMA-positive group, 4 life years per 100 patients are gained on average over 12 years. In this treatment scenario, the benefit of PSMA-PET/CT is driven by the prognostic import of a PSMA-positive result in patients receiving localized versus systemic or combination therapy.

Conclusion: Precision oncology tools such as PSMA-PET/CT must be accompanied by treatment changes that improve survival in high-risk patients enough to offset any loss in life expectancy in survival among low-risk patients. While we await long-term results of PSMA-tailored treatment trials, modeling can inform expectations about likely outcomes and conditions necessary for benefit.

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Session VI

Androgen Receptor/Plasticity

Androgen Receptor Splice Variant Activity in Castration-Resistant Prostate Cancer is Dependent on Increased FOXA1-mediated Chromatin Accessibility

Larysa Poluben, Mannan Nouri, Betul Ersoy Fazlioglu, Olga Voznesensky, Henry W. Long, Joshua W. Russo, Steven P. Balk

Castration-resistant prostate cancer (CRPC) recurring is in most cases still dependent on androgen receptor (AR) activity. AR activity in these tumors can be suppressed by agents such as abiraterone or AR antagonists such as enzalutamide (ENZ), but patients treated with these androgen signaling inhibitor (ASI) drugs invariably progress. The majority of tumors that become resistant to ASI drugs continue to have high levels of AR and AR transcriptional activity. Increasing evidence indicates that expression of AR splice variants that are constitutively active due to deletion of the ligand binding domain (LBD), of which AR variant-7 (ARv7) is the most common, may contribute to this persistent AR activity. However, the extent to which these variants drive AR activity, and their dependence on the full length AR (ARfl), remain unclear. To address this, we treated VCaP cells with ENZ to generate a subline that was resistant to 16μM ENZ (VCaP16 cells). ENZ treatment initially suppressed AR activity, despite an increase in ARv7, while the ENZ-resistant VCaP16 cells had restoration of AR signaling. This restored AR activity was not associated with further increases in ARv7 expression. However, it was linked to increased ARv7 chromatin binding that occurred at a subset of strong ARfl occupied sites, but not at any unique sites. Further approaches using RNAi and an AR degrader targeting ARfl showed that ARv7 was the major driver of AR transcriptional activity in the VCaP16 cells, despite much higher levels of ARfl expression, and was not dependent on ARfl.

We next found that VCaP16 cells had a global increase in chromatin accessibility by bulk ATAC-seq, with the greatest increase being at ARv7 sites. This suggested these sites had decreased nucleosome occupancy, which was confirmed by MNase-seq. Furthermore, a notable rise in AR-motif activity was globally observed in VCaP16 cells, with scATAC-seq showing a particularly significant difference in a specific cell cluster responsible for establishing the ENZ-resistant state, confirming our findings by bulk ATAC-seq. We next examined FOXA1 cistromes in VCaP16 and parental VCaP cells. This showed enriched FOXA1 binding across all FOXA1 sites in the VCaP16 cells, with the greatest increases being at ARv7 binding sites and at sites that showed the greatest increases in accessibility by ATAC-seq. This apparent increase in FOXA1 activity was not associated with increased FOXA1 expression or mutations. However, by mass spectrometry we found increased phosphorylation adjacent to a site that mediates histone binding. Finally, silencing of FOXA1 using RNAi resulted in striking downregulation of gene sets including Androgen Response, E2F Targets, and G2M Checkpoint in the VCaP16 cells, but not in parental VCaP, indicating increased dependence on FOXA1 in the VCaP16 cells. Together these findings show ARv7 can drive the AR program independently of ARfl, but that this ARv7 activity is dependent on adaptations that enhance chromatin accessibility, which may be driven by FOXA1. Studies are underway to further identify these adaptations and determine whether they are vulnerabilities that may be targeted therapeutically.

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HOXB13 is Essential to Both AR-Positive and -Negative Prostate Cancer

Shreyas Lingadahalli, Betul Ersoy Fazlioglu*, Umut Berkay Altintas, Ahmet Cingoz, Emirhan Tekoglu, Ivan Pak Lok Yu, Ugur Meric Dikbas, Hans Andomat, Ibrahim Kulac, Tunc Morova, Kevin Xiao, Martin Gleave, Ladan Fazli, Paloma Cejas, Artem Cherkasov, Wilbert Zwart, Henry Long, Colin Collins, Tugba Bagci-Onder, Nathan A. Lack*

Androgen receptor (AR) signaling is essential to the growth of prostate cancer (PCa) and represents the main pharmacological target to treat late-stage or aggressive tumours. The strong selective pressure from potent AR pathways inhibitors can drive a subset of tumours to transdifferentiate into an AR-negative cancer. While most research has focused on the role of HOXB13 on AR activity, we demonstrate that HOXB13 is expressed in many AR-negative tumours and is essential to the proliferation of both AR-positive and -negative PCa. This dependency is highly tissue selective and loss of HOXB13 has almost no effect on nearly all non-prostatic tissue. Interestingly, despite the common essentiality in PCa, HOXB13 activity is markedly different in AR-negative PCa and the chromatin binding sites change through interactions with AP-1. The resulting HOXB13 cistrome phenocopies the euchromatin observed in stem-cell like castration-resistant prostate cancer. Once bound to chromatin we demonstrate that in both AR-positive and -negative PCa HOXB13 commonly interacts with SMARCD2, a component of the mSWI/SNF chromatin remodling complex. The HOXB13/SMARCD2 interaction alters chromatin accessibility at HOXB13 binding sites and increase proliferation in PCa models. Overall, this work demonstrates a novel mechanism of action for HOXB13 and highlights its critical role in AR-negative castration-resistant prostate cancer.

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Androgen receptor cell-autonomously regulates luminal cell lineage plasticity, regeneration and oncogene residency

Dan Li, Naitao Wang, Vaishnavi Reeya Callychurn, Cindy J. Lee, Makhzuna N. Khudoynazarova, Nicholas A. Teri, Woo Hyun Cho, Weiran Feng, Anuradha Gopalan, Wenfei Kang, Ning Fan, Ping Chi, Yu Chen

Androgen deprivation (castration) results in involution of the normal gland to ~90% of its original size because of the loss of luminal and basal cells. The prostate regenerates when androgen is restored. Interestingly, the luminal cells of distal prostate can change to proximal luminal cell like and survive after castration and can change back to distal prostate luminal cells after androgen restored. Oncogenic pathways (Pten loss) can also induce luminal cell distal-like to proximal-like change. The mechanism of AR in regulating the luminal cell castration/regeneration process, castration or oncogene-induced distal-like/proximal-like cell state shift remains elusive. Using luminal cell specific AR knockout mice and luminal cell specific ETV4 overexpression mice, ATAC-seq, ChIP-seq and single cell RNA-seq, we found 1. AR luminal cell-autonomously regulates androgen deprivation (castration) induced-luminal cells death; 2. AR regulates luminal cell distal-like to proximal-like change by directly regulating AP-1 transcription factors. 3. AR regulates luminal cell regeneration when androgen restoration. 4. AR promotes or inhibits oncogene's function via regulating distal-like/proximal-like luminal cell state shift.

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Understanding metastatic tumoral evolution and divergence after castration in a PDX derived prostate cancer metastasis model

JuanJuan Yin, Anson Ku, Asha Daryanani, Jack Bright, Neil Alilin, Brian Capaldo, Adam Sowalsky, Kathy Kelly

Despite advances in diagnosis and treatment, metastatic castration-resistant prostate cancer (mCRPC) is still a major clinical challenge. Treatment failures are mainly due to tumor cellular heterogeneity and plasticity. The development of novel therapies is hampered by the lack of clinically relevant metastasis models reflecting intra-and inter-tumoral heterogeneity. Using a patient-derived AR positive (AR+) xenograft (PDX), LuCaP136, we have established a PDX-derived metastasis model (PDM136) which develops metastases in bone and soft tissues. With concurrent loss of TP53 and RB1 function, PDM136 represents a subgroup of difficult-to-treat metastatic prostate cancers. In untreated mice, metastatic growth often leads to paraplegia and/or cachexia 6 weeks after tumor cell inoculation. Although surgical castration initially inhibits metastatic progression; all mice eventually die from tumor progression. Importantly, tumor cells retain high AR expression in all metastatic sites, such that PDM136 is the first AR+ in vivo model that recapitulates the disease progress of human mCRPC. To define the molecular mechanisms of castration resistance in PDM136, we performed gene expression profiling on tumor cells that metastasized to the bone, adrenal gland, and liver, collected from intact and castrated mice. Gene set enrichment analyses show decreased AR signaling and increased neuroepithelium transformation in the castrated group. SOX2 and ASCL1, two neuroendocrine differentiation drivers, are among the top up-regulated genes after castration, further confirming a transition from prostate adenocarcinoma (PRAD) phenotype to a more neuroendocrine prostate cancer (NEPC)-like phenotype. Immunohistochemistry staining was used to compare the expression of PRAD marker AR, NEPC markers SOX2, ASCL1 and SYP, as well as glucocorticoid receptor (GR). Histomorphometry analysis revealed organ-specific and inter-/intra-tumor phenotypical heterogeneity in which metastases of the same model in the same mouse displayed different phenotypes depending on organ tropism. In most bone lesions, AR and GR levels were undetectable or weak. By contrast, substantial numbers of adrenal lesions expressed moderate but mutually exclusive pattern of AR and GR. SOX2 and ASCL1 were expressed in a subpopulation of AR negative lesions, while SYP was detected only in a subpopulation of SOX2 positive foci. Notably, liver lesions expressed the highest proportion of SYP when compared to lesions from other organs, suggesting that the liver microenvironment promotes neuroendocrine differentiation. In summary, PDM136 recapitulated the transition to NEPC and identified mechanisms driving treatment-induced tumor heterogeneity. Our finding of multiple coexisting phenotypes indicates that combination therapies targeting multiple pathways are essential for effective control of mCRPC.

Acknowledgments/Funding: This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research, and the Department of Defense Prostate Cancer Research Program.

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Detecting divergent lineage plastic transformations in mCRPC using Circulating Tumor Cell RNA sequencing

Marina Sharifi, Jamie Sperger, George Zhao, Joshua Lang

Lineage plasticity has emerged as the dominant hypothesis to understand molecular alterations that drive treatment resistance in prostate adenocarcinoma. These transformations include the development of neuroendocrine prostate cancer (NEPC) in a subset of patients with prostate adenocarcinomas that has been treated with androgen receptor signaling inhibitors (ARSIs). Other lineage states have been identified in translational models, including luminal, basal, amphicrine and double negative prostate cancers. We have previously reported development of a targeted RNA RT-PCR assay to identify mCRPC with neuroendocrine transformation with single cell sensitivity. This assay is now CLIA certified and is the subject of the upcoming ARCTIC trial for clinical validation. We have now developed a method for high purity circulating tumor cell (CTC) RNA sequencing that allows unbiased analysis for lineage plastic transformations and molecular associations in mCRPC. We have confirmed small cell/NEPC transcriptional signatures in a cohort of patients with biopsy proven NEPC transformations. We identify expression of therapeutically targetable proteins in these patients and a potential screening biomarker for such studies. We further identified luminal to basal transformations that associate with elevated proliferation indices and RB loss that suggest drivers of ARSI treatment resistance beyond NEPC. These findings, and translation to human trials, will be discussed.

Acknowledgments/Funding: P50 CA269011, PCF Challenge award, NCI UH2CA260389, NCI R01CA276269

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FOXA1 class-mutants distinctly activate luminal neoplastic or stemness enhancer programs in the mouse prostate epithelia

Sanjana Eyunni, Abhijit Parolia*, Eleanor Young, James George, Rahul Mannan, Sandra E. Carson, Yuping Zhang, Jean Tien, Mustapha Jaber, Jie Luo, Matthew Pang, Rohit Mehra, Xuhong Cao, Fengyun Su, Rui Wang, Marcin Cieslik, Dong-Kee Lee, Jianming Xu, and Arul Chinnaiyan*

Androgen receptor (AR) signaling is critical for the initiation and proliferation of prostate cancer (PCa) cells, thus establishing androgen deprivation therapy (ADT) as the mainstay for PCa treatment. Notably, oncogenic transcriptional functions of AR are dependent on a host of chromatin-binding regulatory proteins, which includes FOXA1, a pioneer transcription factor. FOXA1 binds to nucleosomal DNA and de-compacts it to enable AR's DNA binding, thereby dictating its downstream gene expression program. Recently, our lab classified FOXA1 alterations into three distinct structural classes that recur in over 35% of metastatic PCa cases in Caucasian men. Subsequent studies have reported FOXA1 mutations to be detected in over 40% of primary prostatic tumors in Chinese patients, which positions FOXA1 as a principal oncogene in this disease. However, hitherto, no studies have defined the causal pathobiology of FOXA1 alterations in prostate tumorigenesis. Here, we have developed the first-in-field transgenic mouse models that conditionally overexpress FOXA1 mutants spanning all three alteration classes in the prostate luminal epithelia. We found the truncal class1 FOXA1$^{R265\text{-}71del}$ mutant to drive luminal hyperplasia in a monogenic context, or multifocal, hyperproliferative adenocarcinoma in a compound Trp53-deficient background with complete penetrance by 40-52 weeks of age. Mechanistically, the class1 mutants concurrently upregulate the AR and mTORC1/2 pathways, the latter being independent of Akt or its upstream effectors. Overexpression of class1-mutants in prostate organoids phenocopy these transcriptomic changes with a dramatic increase in lumen size and cystic morphology, as well as enhance growth in media lacking EGF and/or DHT. Notably, even in organoid models, class1-mutants in conjunction with CRISPR-inactivation of Trp53 trigger hyper-proliferation and increased stratification of the luminal epithelia. These findings credential FOXA1 class1 mutants as a bonafide oncogene with this being the first report of FOXA1-driven prostate adenocarcinoma in mice. In contrast, the FOXA1^{P358fs} class2 mutant—acquired in the castration-resistant disease—did not drive prostate luminal transformation in mice. Instead, single-cell 10X Multiomics (RNA + ATAC) profiling of class2-mutant prostate glands uncovered extensive chromatin and transcriptional remodeling of epithelial cells, leading to a 15-20-fold expansion of the Tasctd2+/Ck4+ stem-like luminal progenitors. These are akin to the Hillock or Club luminal cells detected in the human prostates – also implicated in driving resistance to ADT. Consistently, class2-mutant-expressing prostate glands show minimal regression upon castration in mice, with improved survival and proliferation (Ki67) of luminal epithelial cells relative to the wild type and class1-mutant tissues. We also found class2-mutant-expressing prostate organoids to have increased grafting ability when implanted subcutaneously in mice. Mechanistically, we found the cistromically-dominant class2 mutants to pioneer over 40,000 neo-enhancer elements containing motifs of stemness-associated transcription factors like the Klf and Sox family, which activate stemness genes. This suggests that FOXA1 class2 mutants alter the luminal cell fate to drive resistance to ADT – consistent with their acquisition in metastatic castration-resistant tumors. Altogether, findings from our mouse models uncover the versatility of the FOXA1 oncogene that, depending on the type of mutation, either activates enhancer-wired luminal tumorigenesis (class1–initiating event) or therapy resistance-associated stemness (class2–promoting alteration) gene programs in the mouse prostate tissue.

Acknowledgments/Funding: This work was performed at the Michigan Center for Translational Pathology (MCTP), University of Michigan, Ann Arbor USA and was funded through shared MCTP research grants including Prostate Cancer Foundation awards (PCF), DoD idea development award, NIH F99/K00 fellowship, Molecular and Cellular Pathology Research grant and Rackham Research grant.

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1. Genomic and Proteomic Approaches to Elucidate HOXB13 Function in Prostate Cancer Epigenetic Reprogramming

Aleksandra I. Adamovich and David Y. Takeda

The transcription factor HOXB13 has a significant role in prostate cancer through processes such as regulating AR expression and redirecting AR binding to tumor-associated sites in the genome. To better understand the mechanisms necessary for HOXB13-mediated prostate cancer function, in-situ mutagenesis with CRISPR-Cas9 base editor libraries was used to identify essential mutations in a proliferation-based screen. These screens identified several mutants in the N-terminus of HOXB13, outside of the DNA binding domain, that resulted in a loss of function phenotype. These mutants upregulated various genes, including those involved in proinflammatory pathways, that were not upregulated by loss of HOXB13 expression. Mutants were also characterized by loss of HOXB13 and AR binding to DNA at shared sites, and by loss of AR binding at AR-specific sites. In contrast, mutations that affect HOXB13 DNA binding were further accompanied by near-complete loss of HOXB13 binding at HOXB13-specific sites and increased HOXB13 recruitment to AR-specific sites, two traits that were not seen in the N-terminus mutants. Mass spectrometry analysis also indicated that HOXB13 N-terminus mutants had diminished interactions with AR and members of SWI/SNF chromatin remodeling complexes. This data suggests that these HOXB13 mutants are mediating changes to chromatin remodeling and subsequently inhibiting the DNA-binding capabilities of AR. These results will ideally help elucidate HOXB13 function and identify methods to target HOXB13 activity in prostate cancers.

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2. Elevated miR-196a-2 and novel HOXC variant gene expression in Castration-Resistant Prostate Cancer

Isaacson B. Adelani,† Akira Kurozumi, † Eddie Imada, Michael Haffner, Su Mi Choi, Luigi Marchionni, Vasan Yegnasubramanian, and Shawn E. Lupold
†co-first authors

Background: Androgen deprivation therapy or Androgen Receptor targeted therapies are used to treat metastatic Prostate Cancer (PCa). However, nearly all cases eventually progress to Castration-Resistant Prostate Cancer (CRPC). The molecular pathways associated with CRPC development and progression remain incompletely defined. This study aims to identify CRPC drivers and delineate the underlying mechanisms.

Methods: Three isogenic castration-sensitive and castration-resistant prostate cancer cell lines were analyzed by RNA sequencing and small RNA sequencing. Gene transcripts associated with castration resistance were further characterized by RT-PCR, Rapid Amplification of cDNA Ends (RACE), and bioinformatic analyses.

Results: We discovered a significant miR-196a upregulation in castration-resistant cell lines. To further identify the origin of miR-196a expression, we explored the transcriptional profiles of the cell lines using the sequencing data and detected an activity upstream of miR-196a-2 on chromosome 12. miR-196a expression in CRPC patients was further defined using clinical samples and secondary datasets. In addition, we performed 5'-RACE and 3'-RACE to identify the transcription start site of pri-miR-196a-2 and define splicing isoforms. Additional transcripts from the HOXC gene cluster were found to be associated with castration resistance and metastasis.

Conclusions: A subset of castration-resistant prostate cancers activate MIR196A2 and HOXC gene transcription.

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3. Advance prostate cancer detection through epigenomic profiling of cfDNA

Mohamed Adil,† Brian Hanratty,† Pallabi Mustafi, Chitvan Mittal, Ilsa Coleman, Radhika A. Patel, Helen M Richards, Anna-Lisa Doebley, Robert Patton, A Eden Cruikshank, Patricia Galipeau, Ruth Dumpit, Martine P. Roudier, Jin-Yih Low, Navonil De Sarkar, Robert B. Montgomery, Eva Corey, Colm Morrissey, Peter S. Nelson, Gavin Ha, Michael C. Haffner
†co-first authors

Introduction: Metastatic castration-resistant prostate cancer (mCRPC) is a heterogeneous disease which can be classified into clinically relevant subtypes based on the expression of transcription factors (TF), such as the androgen receptor (AR) and neuroendocrine markers. Neuroendocrine prostate cancer (NEPC), characterized by gain of stem-like and neuroendocrine features and lack of AR expression is a clinically aggressive variant. Due to the absence of adequate biomarkers, NEPC is usually detected at a very advanced stage. There is mounting evidence that molecular subtype changes seen in NEPC are enforced by widespread epigenetic alterations, in particular DNA methylation changes. In this study, we aim to devise a novel DNA methylation-based assay for molecular subtyping and disease monitoring from cell-free DNA (cfDNA).

Methods: We analyzed genome wide methylation patterns in 56 prostate cancer patient-derived xenograft (PDX) and 128 mCRPC tumors using array- and sequencing-based assays. We integrated DNA methylation at promoters, gene bodies and TF cistrome to determine the landscape of methylation alterations at key lineage specific genes. Using low-pass Enzymatic Methyl-Seq (EM-seq) cfDNA data derived from PDXs with matching transcriptomics we developed a deep learning framework to predict expression of all genes. We additionally developed a probabilistic likelihood estimation model to quantify molecular subtype specific DNA methylation changes at TFBS. These models were then used to discern tumor molecular phenotypes from tissue and cfDNA in three independent cohorts of mCRPC patients using whole genome bisulfite sequencing and low-pass EM-seq.

Results: We observed a tight association between promoter, gene body and TFBS methylation with gene expression status. Inferring gene expression from methylation for lineage specific markers such as AR, STEAP1, ASCL1, SRRM4 and DLL3 we classified molecular subtypes from both tissue and plasma cfDNA. For AR and ASCL1, we identified core sets of TFBSs whose differential methylation allowed for accurate assay-independent molecular subtype quantification. Applying the optimized quantitative model to mCRPC patients who underwent comprehensive tissue sampling by rapid autopsy we observed perfect subtype prediction from both tissue samples and cfDNA (AUC=1). A similar analytical performance was observed in additional clinical mCRPC cohorts with cfDNA.

Conclusions: We show that methylation patterns at promoters, gene body and TFBSs can determine gene activity and be used to classify molecular subtypes from both tumor tissue and cfDNA. For prostate cancer, we demonstrate that this approach can accurately detect NEPC by cost-effective low-pass EM-seq. More broadly, this study provides a novel analysis framework for robustly assessing molecular tumor phenotypes in cfDNA with applications in solid and liquid tumor diagnostics.

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4. Modulating the pharmacokinetics of antibody fragments targeting prostate stem cell antigen (PSCA) for optimized radioimmunotherapy (RIT)

Saad N Ahmed, Bao Y Chen, Felix B Salazar, Jennifer Chean, Tove Olafsen, Anna M Wu and Kirstin A Zettlitz

Background: Radiopharmaceutical therapies for prostate cancer have shown efficacy but are limited by tumor heterogeneity and therapeutic resistance, making the development of alternative therapies targeting complementary tumor antigens a pressing need. Prostate stem cell antigen (PSCA) is a promising target for antibody-based theranostics that is overexpressed in prostate cancer with minimal expression in normal tissues. We have previously engineered anti-PSCA A2 scFv-Fc2 antibody fragments (A2Fc2, t½ ~80 h) with two mutations in the FcRn binding site (double mutant, A2DM, $t_{½}$ ~11 h) to accelerate blood clearance and reduce bone marrow toxicity. Here, we evaluate novel variants and the impact of PK-modulation on the therapeutic index for RIT.

Methods: Novel anti-PSCA scFv-Fc2 antibody fragments with one mutation (single mutant, A2SM) or with two mutations and genetically aglycosylated (A2DMaG) were produced. Purity and integrity of purified proteins were confirmed by SDS-PAGE and size exclusion chromatography (SEC). Specific binding to recombinant antigen (ELISA) and PSCA-positive cells (flow cytometry) was evaluated. A2SM and A2DMaG were conjugated with p-SCN-DFO and radiolabeled with zirconium-89 (^{89}Zr). The plasma half-life of ^{89}Zr-A2SM, and ^{89}Zr-A2DMaG was calculated from blood curves after injection of the tracers (10 µg/0.2 MBq) into human PSCA knock-in mice (hPSCA KI). For immunoPET imaging, ^{89}Zr-A2SM or ^{89}Zr-A2DMaG (10 µg/1.7 – 2.0 MBq) were injected into hPSCA KI mice bearing syngeneic prostate cancer tumors (RM9 or RM9-hPSCA) and PET/CT scans (Molecubes) were acquired at 4, 24, 44, 96 h p.i. After the last scan, ex vivo biodistribution was conducted and %ID/g was calculated.

Results: Two novel anti-PSCA scFv-Fc variants (A2SM, A2DMaG) were designed, produced, and analyzed in comparison to previous variants (A2Fc2, A2DM). SDS-PAGE and SEC analysis confirmed purity and dimeric self-assembly. Saturation binding studies showed both A2SM and A2DMaG retained antigen specific binding and affinity to both recombinant hPSCA and PSCA-expressing cells with a low nanomolar affinity. A2SM and A2DMaG were successfully radiolabeled with ^{89}Zr (labeling efficiency: >90%, radiochemical purity >99%, n≥2) resulting in a specific activity of ~0.2 MBq/µg. Blood curves confirmed modulated PKs from slowest to fastest: A2Fc2 (~80 h) > A2SM (~20 h) > A2DM (~11 h) > A2DMaG (~6 h).

ImmunoPET showed specific uptake in RM9-hPSCA s.c. tumors for both ^{89}Zr-A2SM and ^{89}Zr-A2DMaG (14.6 ± 0.5 and 3.0 ± 0.5 %ID/g at 96 h p.i., n≥4) which was significantly higher than in RM9 tumors (6.7 ± 0.5 and 2.1 ± 0.4 %ID/g). The longer half-life of A2SM resulted in higher tumor uptake but lower tumor-to-blood ratio compared with A2DM (12.3 ± 0.9 vs 38.2 ± 3.4).

Conclusion: Surrogate ^{89}Zr-immunoPET successfully profiled the in vivo tumor targeting, biodistribution, and clearance of the novel PK-modulated anti-PSCA scFv-Fc variants in hPSCA KI mice. ^{89}Zr-A2SM with an intermediate blood half-live reached higher tumor uptake while ^{89}Zr-A2DM resulted in the highest tumor-to-blood ratio. The lead candidate A2DM will be further developed for lutetium-177 radioimmunotherapy in prostate cancer.

Acknowledgments/Funding: Research reported in this abstract includes work performed in City of Hope's Small Animal Imaging Core (NIH NCI P30CA33572).

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5. Microsatellite instability and genomic predictors of response to immune checkpoint blockade in prostate adenocarcinoma

Muneeb Alam, Andrew T. Lenis, Sunny Nalavenkata, Jacob Tallman, Vignesh Ravichandran, Samantha Brown, Samantha Vasselman, Emily Harrold, Alicia Latham, Barbara Nweji, Karen A. Autio, Michael J. Morris, Rona Yaeger, Susan F. Slovin, Dana Rathkopf, Daniel Danila, Howard Scher, Sungmin Woo, Hebert Alberto Vargas, Oguz Akin, Vincent P. Laudone, Behfar Ehdaie, Brett Carver, Maria Arcila, Michael F. Berger, Agnes Viale, Nikolaus Schultz, Anuradha Gopalan, Hikmat Al-Ahmadie, Mark T.A. Donoghue, Irina Ostrovnaya, Konrad H. Stopsack, Zsofia Stadler, Wassim Abida, David B. Solit

Immune checkpoint blockade (ICB) demonstrates only modest response in unselected patients with prostate cancer, but exceptional responses can be observed in patients with microsatellite instability-high (MSI-H) and mismatch repair deficient (dMMR) tumors. Less is known about the response of microsatellite stable (MSS) prostate cancers with high tumor mutational burden (TMB-H) despite tissue agnostic approval for ICB in this setting. Our group has initiated a comprehensive clinal and genomic evaluation of MSI-H/dMMR and MSS/TMB-H prostate cancer treated with ICB.

Patients were prospectively enrolled in a genomic profiling protocol (MSKCC IRB 12-245) and/or an institutional Lynch Syndrome registry (MSKCC IRB 21-315). Targeted exome sequencing was performed using the MSK-IMPACT assay, and whole exome sequencing (WES) was performed for a subset. MSI status was determined using the MSIsensor score to define tumors as MSI-H (score ≥10), MSI-indeterminate (MSI-I, score 3-10), and MSI-stable (MSS, score <3). MSI-I tumors with a deleterious MMR gene alteration were considered MSI-H. We also explored the utility of the MiMSI machine learning algorithm to adjudicate MSI-I status. TMB was defined by the number of non-synonymous mutations, and TMB-H was defined as ≥10 muts/Mb. Clinical outcomes were evaluated with a focus on response to ICB.

MSI-H/dMMR prostate cancer represented 2.2% of cases, of which 13.2% were associated with a pathogenic/likely pathogenic germline mutation in a Lynch Syndrome-associated gene. Only 46.2% of prostate cancers diagnosed in patients with Lynch Syndrome had an MSI-H/dMMR phenotype. Somatic and germline mutations in MSH2 and MSH6 were most common, and 41.8% of MSI-H/dMMR tumors had a somatic and/or germline mutation in more than one MMR gene. MiMSI was able to adjudicate the majority of MSI-I cases (103/105) to either MSS (82/103) or MSI-H (21/103). Only 1.5% of cases were MSS and TMB-H. Median TMB in this group was lower than in the MSI-H/dMMR group (41 vs. 12 mut/mB, $p<0.001$). Half of the MSS/TMB-H tumors exhibited a >50% decline in PSA following ICB, but no radiographic responses were observed. Based on WES, neoantigen count (11.1 vs. 6.8, $p=0.01$) and indel count (8.7 vs. 2.1, $p<0.01$) were higher in MSI-H/dMMR than MSS/TMB-H tumors. Mutational signatures were dMMR-predominant in MSI-H/dMMR tumors but were more heterogenous across MSS/TMB-H tumors. No significant genomic markers of response to ICB were observed in MSI-H/dMMR tumors, potentially due to limited sample size. Complete and durable responses to ICB in MSI-H/dMMR prostate cancer were observed in the localized and metastatic setting.

MSI-H/dMMR prostate cancer is a rare subtype that demonstrates exquisite sensitivity to ICB, potentially through the generation of numerous immunogenic neoantigens. Prospective identification of these tumors represents an exciting opportunity to select patients for ICB, particularly in the localized and early metastatic settings.

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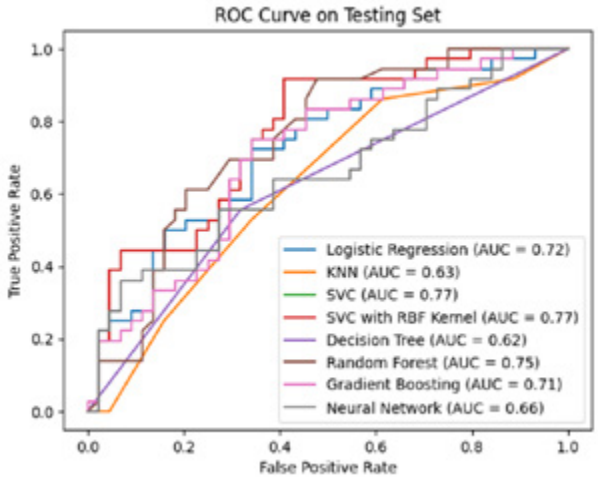

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6. Timely Prediction of Metastatic Castration-Resistant Prostate Cancer: Multimodal Fusion of Clinicopathological and Genomic Features with Machine Learning

Mohammad K. Alexanderani, Francesca Khani, Matthew Greenblatt, Brian Robinson, Massimo Loda, Luigi Marchionni

Background: The transition from metastatic castration-sensitive prostate cancer (mCSPC) to its resistant variant presents significant challenges, including the need for escalated medication regimens, potential toxic side effects, and substantial financial burdens for men undergoing treatment. Moreover, this transition is accompanied by a marked decline in overall survival rates. Our study proposes a multimodal approach that integrates clinical, pathological, and genomic features to optimize prediction of metastatic castration-resistant prostate cancer (mCRPC) events. By applying machine learning (MML) techniques to multimodal data, we aim to stratify these patients based on the risk of developing mCRPC, contributing to the development of safer and personalized treatment strategies.

Methods: We analyzed a large cohort of patients with mCSPC (n=399) with a minimum of 36-month follow-up, annotated with clinical, pathological, and genomic features, encompassing relevant variables such as disease volume, PSA, metastases timing, variant classification, Grade Group, fraction of genome altered, MSI score, mutation counts, karyotypes, tumor mutational burden, and purity. Using a multimodal machine learning framework, we extracted informative features and employed eight distinct machine learning algorithms: K-Nearest Neighbor, Support Vector Machine, Decision Tree, and Logistic Regression, Support Vector Machine with RBF Kernel, Random Forest, and Gradient Boosting, in addition to a Deep Neural Networks architecture for building predictive models in supervised binary classification experiments. The models' performance was evaluated on separate training (80%) and testing sets (20%), and standard evaluation metrics, such as the area under the receiver operating characteristic curve (AUROC), and accuracy, were computed. Correlations were utilized to explore the relationships between features, and permutation analysis was employed to determine the importance of predictors. All raw data included in the analysis are available on cBioPortal database.

Results: Of the 399 mCSPC patients, 56% developed castration resistance, 50% had Grade Group 5 disease, and 69% exhibited missense mutations. In a series of 8 experiments, the Support Vector Machine with RBF Kernel ensemble model and SVC exhibited the highest performance with an AUROC score of 0.77, while the remaining models tested in the study demonstrated AUROC scores ranging from 0.62 to 0.75. Notably, the fraction of altered genome emerged as the top-ranked predictor, displaying the highest importance score.

Conclusions: Our study demonstrates the strong predictive performance of MML models in accurately identifying mCRPC events by leveraging routine clinical, pathological, and genomic data, offering valuable guidance for optimizing patient stratification and tailoring personalized therapeutic interventions.

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7. Targeted Autophagic Degradation of Androgen Receptor Mutants and AR-v7 by AR-AUTOTAC in Castration-Resistant Prostate Cancer

Tae Hyun Bae,† Ki Woon Sung,† Tri Pham,† Abdo J. Najy, Alaleh Zamiri, Hyejeong Jang, Su Ran Mun, Seongho Kim, Dongping Shi, Steven Kregel, Elisabeth I. Heath, Michael L. Cher, Yong Tae Kwon, Hyeong-Reh Choi Kim**
†co-first authors

Traditional drug development often focuses on inhibiting the activity of disease-causing proteins using small molecules. However, the effectiveness of functional inhibition is often sub-optimal, and their effects are temporary. As a result, protein degradation technology (PDT) has emerged as an innovative approach to drug development. Recently, we developed a groundbreaking PDT called AUTOphagy-TArgeting Chimera (AUTOTAC) for targeted protein degradation. A bivalent AUTOTAC molecule comprises a target-binding ligand (TBL) linked to an autophagy targeting ligand (ATL) of p62/SQSTM1. AUTOTAC brings the target to the autophagic receptor p62 via its TBL and ATL, forming a p62-cargo complex that leads to autophagy-lysosomal degradation. Notably, AUTOTAC-mediated target degradation does not require interactions between the target protein and p62. This is in contrast to the most well-known PDT platform, PROTAC (PROteolysis TArgeting Chimera), which depends on the structure-function relationship between a specific E3 ligase and its target protein to facilitate target protein ubiquitination necessary for degradation. Based on this unique feature of the AUTOTAC platform, we hypothesize that AUTOTAC effectively degrades structurally altered, mutated target proteins and/or their binding partners critical in their pathogenic activities.

Genetic alterations play a pivotal role in various human diseases, particularly cancer. The androgen receptor (AR) is a crucial transcription factor driving prostate cancer (PCa) progression across all stages. Current AR-targeting therapies utilize competitive AR antagonists or pathway suppressors. However, therapy resistance often emerges due to AR mutations and AR splice variants, such as AR-v7. In the present study, we developed ATC-324, an AR degrader, using the AUTOTAC platform. ATC-324 comprises enzalutamide as a TBL and YT 6-2 (a ligand of the autophagy receptor p62/SQSTM1) as an ATL. ATC-324 induces the formation of the AR/p62 complex, leading to autophagy-lysosomal degradation of AR. Remarkably, ATC-324 exhibited significant efficacy in degrading all tested AR mutants such as L702H, H874Y, F877L, T878A, and M896V, while treatments with enzalutamide (TBL) or YT 6-2 (ATL) displayed minimal impact on their expression levels. Importantly, ATC-324 co-degrades AR-v7 as a heterodimer with full-length AR. ATC-324 reduces nuclear AR levels and downregulates the target gene expression of AR and AR-v7, leading to cytotoxicity in AR-positive PCa cells. RNAseq analysis further confirmed that ATC-324 reverses the gene expression signatures associated with both wild-type AR and AR-v7. We also provide evidence of the therapeutic potential of ATC-324 *in vivo* as well as in *ex vivo* bone organ culture. Moreover, ATC-324 remains potent in enzalutamide-resistant PCa cells. These results demonstrate the potential of the AUTOTAC platform to target previously "undruggable" proteins, such as AR mutants and AR-v7, and to overcome certain drug resistance mechanisms.

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8. Profiling PSMA Heterogeneity in Pre-Clinical Models Using Multi-Omics Approaches

Martin K. Bakht, Yasutaka Yamada, Sheng-Yu Ku, Varadha Balaji Venkadakrishnan, Kei Mizuno, Adam Presser, Henry W. Long, Matthew Freedman, Anthony P. Belanger, Quang-De Nguyen, Himisha Beltran

Prostate-specific membrane antigen (PSMA) is a metabolic enzyme involved in glutamate and folate pathways. Being a cell surface protein, PSMA serves as a theranostic target in prostate cancer. PSMA-radioligand therapy (PSMA-RLT) has been approved for men with PSMA-PET positive metastatic castration-resistant prostate cancer (CRPC). However, not all patients with PSMA-PET positive tumors respond to PSMA-RLT, partially due to the heterogeneity in tumor expression of PSMA. We previously developed a series of orthotopic CRPC models using the PSMA-positive 22Rv1 cell line and demonstrated PSMA-suppression and heterogeneity in liver metastases. In this study, we profiled these models using mass spectrometry (MS), H3K27ac ChIP-seq, and spatial transcriptomics to understand the metabolomic alterations associated with PSMA-suppression and heterogeneity. The spatial transcriptomics maps indicated enrichment of amino acid transport and biosynthesis pathways in PSMA-suppressed liver metastases. Similarly, MS metabolomics profiling revealed elevated levels of neutral amino acids, such as L-Leucine, in liver metastases. H3K27ac ChIP-seq showed that only liver metastatic tumors in the 22Rv1 models exhibited an H3K27ac peak at the SLC7A5 (LAT1) gene locus, correlating with low PSMA expression. Importantly, a PSMA-suppressed 22Rv1 liver metastasis-derived cell line showed increased sensitivity to LAT1 inhibition, suggesting its potential as an alternative therapeutic target. This multi-omics profiling of 22Rv1 orthotopic models suggests that PSMA-suppression and heterogeneity are associated with targetable epigenetic and metabolomic alterations, warranting further validation in clinical samples.

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9. Dual Inhibitory Domain iCARs Improve Efficiency of the AND-NOT Gate CAR T Strategy

Nathanael J. Bangayan, Liang Wang, Giselle Burton Sojo, Miyako Noguchi, Donghui Cheng, Lisa Ta, Donny Gunn, Zhiyuan Mao, Shiqin Liu, Qingqing Yin, Mireille Riedinger, Keyu Li, Anna M. Wu, Tanya Stoyanova, Owen N. Witte

CAR T cell therapy has shown clinical success in treating hematological malignancies, but its treatment of solid tumors has been limited. One major challenge is on-target, off-tumor toxicity, where CAR T cells also damage normal tissues that express the targeted antigen. To reduce this detrimental side-effect, Boolean-logic gates like AND-NOT gates have utilized an inhibitory CAR (iCAR) to specifically curb CAR T cell activity at selected non-malignant tissue sites. However, the strategy seems inefficient, requiring high levels of iCAR and its target antigen for inhibition. Using a TROP2-targeting iCAR with a single PD1 inhibitory domain to inhibit a CEACAM5-targeting CAR (CEACAR), we observed that the inefficiency was due to a kinetic delay in iCAR inhibition of cytotoxicity. To improve iCAR efficiency, we modified three features of the iCAR – the avidity, the affinity, and the intracellular signaling domains. Increasing the avidity but not the affinity of the iCAR led to significant reductions in the delay. iCARs containing twelve different inhibitory signaling domains were screened for improved inhibition, and three domains (BTLA, LAIR-1, SIGLEC-9) each suppressed CAR T function but did not enhance inhibitory kinetics. When inhibitory domains of LAIR-1 or SIGLEC-9 were combined with PD-1 into a single dual-inhibitory domain iCAR (DiCARs) and tested with the CEACAR, inhibition efficiency improved as evidenced by a significant reduction in the inhibitory delay. These data indicate that a delicate balance between CAR and iCAR signaling strength and kinetics must be achieved to regulate AND-NOT gate CAR T cell selectivity.

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10. A Ubiquitin-Activating Enzyme Mediates Cancer Immune Escape via Suppressing Interferon Signaling

Yi Bao, Gabriel Cruz, Yuping Zhang, Rahul Mannan, Jing Hu, Mahnoor Gondal, Yuanyuan Qiao, Jae Eun Choi, Jiali Yu, Heng Lin, Xuhong Cao, Fengyun Su, Rui Wang, Marcin Cieslik, Weiping Zou, and Arul M. Chinnaiyan

How cancer cells escape immune surveillance and resist immunotherapies remain to be elucidated. Out of 614 candidate genes frequently amplified in cancer, we identified expression of a gene encoding a ubiquitin-activating enzyme as being the most negatively correlated with signatures related to effector CD8+ T cells. High expression of this gene was also strongly predictive of resistance to immune checkpoint blockade (ICB) and poor survival in ICB cohorts. In immunocompetent models, but not in the immunodeficient, we found that overexpression of this gene in cancer cells promoted tumor growth and reduced intratumoral functional CD8+ T cells. Conversely, depletion of this gene impaired tumor progression, accompanied with increase of intratumoral functional CD8+ T cells. Importantly, CD8+ T cell depletion rescued tumor growth impairment mediated by the gene depletion. Additionally, inhibition of this ubiquitin-activating enzyme by a selective inhibitor delayed tumor growth and augmented anti-PD1 efficacy in various syngeneic models, in a manner dependent of CD8+ T cells. Treatment of this inhibitor also provided systemic and prolonged protection against cancer, accompanied with elevation of memory CD8+ T cells. Mechanistically, depletion or inactivation of this ubiquitin-activating enzyme stabilized JAK1 in cancer cells, facilitated response to both type-I and -II interferons, resulting in elevated expression of key immune modulators, including CXCL9, CXCL10, and MHC-I. Collectively, our data supports that this ubiquitin-activating enzyme mediates cancer immune escape via suppressing interferon signalling.

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11. Racial and Ethnic Variation in Receipt and Intensity of Active Surveillance for Older Patients with Localized Prostate Cancer

Spyridon P. Basourakos, Anjile An, Meenakshi Davuluri, Laura C. Pinheiro, Bashir Al Hussein Al Awamlh, Leonardo D. Borregales, Danny Luan, Rulla M. Tamimi, Jim C. Hu, Kevin H. Kensler

Introduction: The use of active surveillance (AS) for prostate cancer is increasing, however racial disparities in its implementation have emerged. We investigated differences by race and ethnicity in the utilization and intensity of AS by race and ethnicity among older men with low- and favorable intermediate-risk prostate cancer, with particular focus on the integration of multiparametric MRI (mpMRI) into AS protocols.

Methods: Using the Surveillance, Epidemiology, and End Results (SEER) and Medicare fee-for-service linked database, we identified a cohort of men diagnosed between 2010-2017 with low- or favorable intermediate-risk prostate cancer. The odds of receiving AS were compared by patient race and ethnicity using multivariable logistic regression models, while the rates of usage of PSA tests, biopsy, and mpMRI within two years of diagnosis among men on AS were assessed using multivariable Poisson regression models.

Results: Our cohort included 33,542 men. The proportion of men with low-risk disease who underwent AS increased from 29.5% in 2010 to 51.7% in 2017, while the proportion among men with favorable-intermediate disease grew from 11.4% to 17.2%. Hispanic (OR=0.68, 95% CI 0.58-0.79) and non-Hispanic Black men (OR=0.78, 95% CI 0.68-0.89) were less likely to receive AS than non-Hispanic White men for low-risk disease, while non-Hispanic Black men were more likely to receive AS for favorable-intermediate disease (OR=1.21, 95% CI 1.04-1.39). Non-Hispanic Black men receiving AS underwent prostate MRI at a lower rate compared to non-Hispanic White men, regardless of whether they had low-risk (IRR=0.77, 95% CI 0.61-0.97) or favorable-intermediate (IRR=0.61, 95% CI 0.44-0.83) risk disease, respectively.

Conclusion: The overall adoption of AS for low-risk prostate cancer increased among Medicare fee-for-service beneficiaries. However, a significant disparity exists for non-Hispanic Black men, as they exhibit lower rates of AS utilization. Moreover, non-Hispanic Black men are less likely to have access to novel technologies, such as mpMRI, as part of their AS protocols.

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12. Chimeric Antigen Receptor T Cell Therapies for Subtypes of Metastatic Castration-Resistant Prostate Cancer

Vipul Bhatia, Nikhil V. Kamat, Tiffany E. Pariva, Li-Ting Wu, Jessica E. Hawley, Michael Schweizer, Vicky Wu, Michael C. Haffner, Peter S. Nelson, Amy E. Moran, Saul J. Priceman, Jun Ishihara, John K. Lee

Chimeric antigen receptor (CAR) T cell therapy is a highly promising approach that has transformed the treatment of several hematologic malignancies. The development of CAR T for solid tumors has been met with challenges but early phase clinical trials are increasingly demonstrating safety and efficacy signals. Prior clinical studies of CAR T directed against prostate-specific membrane antigen (PSMA) and prostate stem cell antigen (PSCA) in metastatic castration-resistant prostate cancer (mCRPC) have shown instances of encouraging biochemical and radiographic responses. However, mCRPC encompasses multiple disease including prostate adenocarcinoma (PRAD) and small cell neuroendocrine prostate cancer (SCNPC) that can be distinguished by differential cell surface protein expression. We therefore focused on developing prostate cancer subtype-specific CAR T.

Six transmembrane epithelial antigen of the prostate 1 (STEAP1) was found to be more broadly expressed than PSMA in lethal cases of PRAD. A second-generation STEAP1 CAR was designed/optimized that exhibited specificity and reactivity in low STEAP1 antigen density conditions. STEAP1 CAR T demonstrated significant antitumor effects and prolonged persistence after systemic administration in multiple human-in-mouse (C4-2B, 22Rv1) and mouse-in-mouse (RM9) models of mCRPC. We also established safety by generating a human STEAP1 knock-in mouse model in which STEAP1 CAR T did not result in apparent toxicities.

L1 cell adhesion molecule (L1CAM) was identified as a cell surface antigen that is highly expressed in neuroendocrine cancers including SCNPC. We repurposed a L1CAM CAR targeting the cancer-dependent glycosylated epitope CE7 that was previously developed and is under active investigation in the phase I Engineered Neuroblastoma Cellular Immunotherapy (ENCIT)-01 trial for relapsed/refractory neuroblastoma. Preliminary findings from this study have indicated safety and tolerability. L1CAM CAR T showed specific activation and cytotoxic killing in co-cultures with SCNPC cell lines (NCI-H660, MSKCC EF1). Further, intravenous administration of L1CAM CAR T significantly inhibited the tumor growth of MSKCC EF1 subcutaneous xenografts established in mice.

These CAR T are being translated to the clinic in early-2024. Both trials will incorporate scientific correlative studies that will allow for a deeper understanding of CAR T persistence/expansion, trafficking, interactions within the tumor microenvironment, and properties that may associate with response or resistance. We are also actively exploring strategies to enhance the potency of CAR T for mCRPC including armored expression of inflammatory cytokines, dual targeting of cell surface antigens, and inhibition of T cell-intrinsic androgen receptor activity.

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13. Understanding Androgen Receptor-Addiction in Prostate Cancer

Arnab Bose, Brian Hanratty, Marc Martinez, Samuel Ritmeester-Loy, Dapei Li, Yong Tao, Ilsa Coleman, Michael Nyquist, Jared Lucas, Peter Nelson

Background: Prostate cancer (PC) is a leading cause of cancer-associated deaths in men. It is notable for a unique dependence on androgen receptor (AR) signaling, a fact that has been exploited for therapeutic benefit. However, the precise mechanisms by which PC cell survival and growth is regulated by the AR is unclear. The AR cistrome comprises of thousands of genomic regions, and several hundred genes are transcriptionally regulated by AR activation. The present study aims to identify the direct AR targets and downstream cellular network(s) regulated by the AR that mediate PC cell survival, and that could highlight therapeutically vulnerable nodes for new treatment strategies.

Methods: We identified genes which display androgen-dependent upregulation through RNA sequencing analysis of AR-positive prostate cancer (ARPC) cells and integrated these results previously published nascent-RNA sequencing data along with AR variant (ARv7)-associated genes to generate a comprehensive "AR-regulated" gene list of over 1200 cellular genes. A pooled CRISPR_Cas9 deletion library was then generated which contained 10 guide RNAs (gRNA) against each gene. We leveraged ARPC models of diverse genomic backgrounds and subjected publicly available ARPC lines, namely LNCaP_FGC, LNCaP_C4-2B, 22Rv1, VCaP and LAPC4 cells to pooled CRISPR screens. Additionally, we established cell line models of 2 LuCaP-Patient derived xenografts (PDX), LuCaP-35CR_CL and LuCaP-189.4_CL and evaluated them for AR-regulated gene dependencies by CRISPR screens . Each of the cell lines were harvested at a final population doubling of 6 (PD6), the gRNAs identified by next-generation sequencing and data analyzed for dropouts with respect to the reference PD0 time point using the Model-based Analysis of Genome-wide CRISPR/Cas9 Knockout (MAGeCK) algorithm.

Results: We identified several genes which display common dependencies across all ARPC models. These genes encompass a wide range of cellular networks and processes including transcriptional regulation, translational control, organelle homeostasis, stress response, cholesterol or lipid metabolism, and a few genes of underexplored functionalities. Validatory efforts using cell competition assays highlighted differential temporal effects on an ARPC cell proliferation, where the knockout of a subset of genes strongly impaired growth while the deletion of others displayed a modest, albeit gradual proliferative decline. Interestingly, the former class exhibit varied cellular functions and may suggest multiple dominant cellular nodes of dependence for survival.

Conclusions: We screened 7 ARPC cell lines and identified a common or "core" androgen regulated gene set, the continued expression of which seem necessary for the survival of all ARPC models evaluated. We noted that although the MAGeCK analysis suggested genetic dropouts within the temporal confines of the primary screen, namely PD6, the individual dependencies are relative in nature and the genes can further be sub-categorized based on the strength of their respective dependencies. We conjecture that this strength is rooted in the cellular function of the corresponding gene product. Current efforts are directed toward mechanistic dissection of these cellular functions to better understand the roles of AR target genes in mediating prostate cancer survival and growth.

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14. CHD7 Suppresses Androgen Signaling in Neuroendocrine Prostate Cancer

Nicholas J. Brady, Caden N. McQuillen, Kate Dunmore, Richard Garner, Richa Singh, Alyssa M. Bagadion, Brian D. Robinson, Olivier Elemento, Himisha Beltran, David S. Rickman

Background: The progression from castration-resistant prostate cancer (CRPC) to neuroendocrine (NE) prostate cancer (NEPC) is driven by several molecular events, including the acquisition of epigenetic changes. In a cohort of advanced prostate cancer patients, we found that the chromatin modifier CHD7 was overexpressed in NEPC patients. Moreover, using a genetically engineered mouse model (GEMM) of prostate cancer we revealed that CHD7 is hypomethylated and overexpressed in NE foci compared to adenocarcinoma. While these findings suggest that CHD7 may be linked with NEPC, the direct mechanism by which CHD7 is involved in disease progression remains unknown.

Methods: We used single-cell based approaches, such as scRNA-seq and scATAC-seq, in conjunction with bulk RNA-seq and RRBS, to fully characterize the development of NEPC in a novel GEMM driven by N-Myc in the context of Pten and Rb1 loss. We modeled androgen deprivation in LNCaP cells and assessed transcriptomic changes by RNA-seq. We utilized CRISPR-mediated gene editing to delete CHD7 in human NEPC patient-derived models. In these newly created models, we determined transcriptional changes by RNA-seq and assessed epigenetic changes to chromatin accessibility and enhancer activation by ATAC-seq and H3K27ac ChIP-seq.

Results: We performed scRNA-seq and scATAC-seq on GEMM prostates collected at 6 and 8 weeks of age which revealed that increased Chd7 expression correlated with the expression of NE markers. Analysis of the methylome demonstrated concordant hypomethylation and overexpression of CHD7 in both human and mouse models of NEPC. Using an in vitro model of long-term androgen withdrawal in LNCaP cells, we showed that chronic androgen deprivation synergizes with known genetic drivers of NEPC to promote the expression of CHD7 and NE markers while reducing responsiveness to androgen stimulation. Genomic deletion of CHD7 in human NEPC samples led to the increased expression of AR target genes and genes containing ARE motifs. These changes induced by CHD7-loss also correlated with increased chromatin accessibility around ARE motifs and the increased deposition of H3K27ac at the enhancers of AR target genes.

Conclusions: We have shown that CHD7 is hypomethylated and upregulated during the progression to NEPC. Using NEPC patient-derived models we have revealed that CHD7 normally functions to suppress androgen signaling and may help to maintain NE differentiation. Future studies will determine the potential to target CHD7 therapeutically to prevent or reverse the transition to NEPC.

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15. Uncovering the Role of TEADs in Treatment Induced Small Cell or Neuroendocrine Prostate Cancer

Lisha G. Brown, Ilsa M. Coleman, Tony L.H. Chu, Daniel W. Lin, John K Lee, Erolcan Sayar, Lawrence D. True, Ruth Dumpit, Eva Corey, Peter S. Nelson Michael C. Haffner, and Colm Morrissey

Study Purpose: Determine the role of the transcription factor TEAD1 in promoting the neuroendocrine phenotype in castration-resistant prostate cancer (CRPC).

Experimental Procedures: We quantitated YAP-pathway associated transcripts, protein abundance, and splicing events in androgen receptor (AR) positive prostate adenocarcinoma (ARPC) and small cell or neuroendocrine prostate cancer (SCNPC) using RNAseq, scRNAseq, qPCR, immunohistochemistry (IHC), CpG methylation, and western blot analyses on patient samples, patient-derived xenograft (PDX) models, and cell lines. In addition, we tested the impact of the pharmacological inhibition and knockdown of proteins and genes associated with TEAD1 activity in ARPC and SCNPC cell lines on TEAD1 expression, cell number, and tumor cell differentiation.

Results: Transcriptomic analyses revealed a decrease in YAP1, TAZ, LATS2, and TEAD2 and an increase in LATS1, TEAD1 and RBFOX2 transcript levels in SCNPCs compared to ARPCs in SU2C, rapid autopsy metastases, and PDX models. Similar results were observed in ARPC and SCNPC cancer cell lines, in the transplantable LTL331 prostate cancer transdifferentiation model, and scRNAseq of liver CRPC metastases from patients. The decrease in YAP1, TEAD2 and LATS2 expression correlates with increased methylation and the increase in TEAD1 correlates with decreased methylation in SCNPC PDX models, suggesting epigenetic control. The changes in YAP and YAP-associated proteins (most importantly TEAD1) at the epigenetic and transcriptional level were confirmed by IHC analysis comparing ARPC to SCNPC PDX models. Of the four TEAD proteins, TEAD1 expression was increased, with a concomitant decrease in TEAD2 and TEAD3, whereas low levels of TEAD4 transcript are maintained in the SCNPC phenotype. Notably, RBFOX2 was increased in the SCNPC datasets. RBFOX2 is a pre-RNA splicing regulator that promotes the inclusion of exon 6 in TEAD1 mRNAs associated with increased TEAD1 activity. The RBFOX2 spliced TEAD1 was observed in the SCNPC PDX models, while not detected in the ARPC PDX models.

Conclusions: The conversion of ARPC to SCNPC involves the loss of transcriptional regulators: AR, YAP, and REST inactivation in SCNPC. It also involves the expression of ASCL1 or NEUROD1, well described master regulators that are implicated in the conversion to and maintenance of the SCNPC phenotype. Our work suggests TEAD1 is another transcriptional regulator in SCNPC that warrants further investigation.

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16. The DEAD-box Helicase 55 (DDX55) is an Oncogenic and Survival Factor in Prostate Cancer

Ziwei Cai and Syam Somasekharan

Castration-resistant prostate cancer (CRPC) is a subtype of prostate cancer (PCa) that develops as hormone-refractory or hormone-resistant PCa after androgen deprivation therapy (ADT), with a poor survival of less than 2 years. In CRPC, the oncogene androgen receptor (AR) can be activated independently of ligand binding. We conducted an RNA translatome profiling study to identify therapeutic targets for CRPC. We found that an RNA-binding protein, D-E-A-D Box Helicase 55 (DDX55), is highly expressed in CRPC tissue, potentially serving as a survival factor for PCa. We hypothesize that DDX55 is a proto-oncogene and a potential therapeutic target. Our recent study focuses on two aspects: (1) studying the survival functions of DDX55 in prostate cancer cells, and (2) identifying the effects of DDX55 on AR. The survival functions of DDX55 were investigated by silencing this gene in PCa cells. Two major observations were made. First, DDX55 knockdown reduced cell proliferation by inhibiting the expression of oncogenes and transcriptional promoters, while upregulating tumor suppressor genes. Oncogenes such as EGFR, NRAS, BCL6, FKBP5, and ABL2, promoting cell survival and enhancing PCa progression, were down-regulated by more than two folds upon DDX55 knockdown. Moreover, DDX55 silencing induced G1/S phase cell cycle arrest by inhibiting the gene expression of FOXM1 and CDK1. However, tumor suppressors like PTEN and ALDH1L1, inhibiting cell proliferation and promoting apoptosis, were upregulated following DDX55 knockdown. Secondly, DDX55 knockdown induced apoptosis of cancer cells via activation of the TNFR and TRADD death receptor signaling pathway and disruption of mitochondrial protein synthesis, resulting in the activation of BAX and impaired mitochondrial respiration. The gene expression of the NDUFAF family proteins, required for the assembly of mitochondrial complex I, was significantly reduced, disrupting mitochondrial respiration, stimulating mitochondrial ROS production, and release of cytochrome C. Because DDX55 is more expressed in CRPC, it is important to study its interactions with AR. Interestingly, although the protein level of AR is not affected by DDX55 knockdown, AR activity is reduced by 80%. The expression of downstream genes of AR and biomarkers of PCa, such as PSA (KLK3), KLKP1, KLK4, and KLK15, is reduced by more than 50%. DDX55 can play a critical role in promoting AR-responsive element activation. Our results demonstrate that DDX55 is an oncogenic and survival factor for cancer cells. Interestingly, similar observations were not made in normal prostate cells, suggesting that DDX55 can be a therapeutic target for PCa. Our proposed research will advance the understanding of DDX55's functions and the mechanism of cancer progression.

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17. Canonical AREs are Tumor Suppressive Regulatory Elements in the Prostate

Xuanrong Chen, Michael A. Augello, Deli Liu, Kevin Lin, Alex Hakansson, Martin Sjöström, Francesca Khani, Lesa D. Deonarine, Yang Liu, Jaida Travascio-Green, Jiansheng Wu, Massimo Loda, Felix Y. Feng, Brian D. Robinson, Elai Davicioni, Andrea Sboner, Christopher E. Barbieri

The androgen receptor (AR) is the central determinant of prostate tissue identity and differentiation, controlling normal, growth-suppressive prostate-specific gene expression. It is also a key driver of prostate tumorigenesis, becoming "hijacked" to drive oncogenic transcription. However, the regulatory elements determining the execution of the growth-suppressive AR transcriptional program, and whether this can be reactivated in prostate cancer cells, remain unclear. Canonical androgen response element (ARE) motifs are the classic DNA binding element for the AR. Here, we used a genome-wide strategy to modulate regulatory elements containing AREs to define distinct AR transcriptional programs. We find that activation of AREs is specifically associated with differentiation and growth suppressive transcription, and this can be reactivated to cause death in prostate cancer cells. In contrast, repression of AREs is well tolerated by prostate cancer cells, but deleterious to normal prostate cells. Finally, gene expression signatures associated with AREs are associated with improved prognosis and luminal phenotypes in human prostate cancer patients. This study shows that canonical AREs are responsible for a normal, growth-suppressive, lineage-specific transcriptional program, that this can be reengaged in prostate cancer cells for potential therapeutic benefit, and genes controlled by this mechanism are clinically relevant in human prostate cancer patients.

Acknowledgments/Funding: We are indebted to prostate cancer patients and families who contributed to this research. We thank Dr. Dawid Nowak, Dr. Pengbo Zhou, and Dr. Jonathan E. Shoag (WCM) for helpful discussion. We thank the Weill Cornell Medicine (WCM) Genomics Core Facility, the Biospecimen and Pathology Core and the Computational and Biostatistics Core of the WCM SPORE in Prostate Cancer and Memorial Sloan Kettering Cancer Center cBioPortal. This work was funded by: the NCI, US (WCM SPORE in Prostate Cancer, P50CA211024, R37CA215040, and R01CA233650, C.E.B.), Damon Runyon Cancer Research Foundation, MetLife Foundation Family Clinical Investigator Award, US (to C.E.B.), and the Prostate Cancer Foundation, US (M.A.A. and D.L.).

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18. AR Inhibition Increases MHC Class I Expression and Improves Immune Response in Prostate Cancer

Lisa N. Chesner, Julie N. Graff, Fanny Polesso, Alexis Smith, Arian Lundberg, Rajdeep Das, Tanushree Shenoy, Zheng Xia, Ya-Mei Hu, Martin Sjöström, Simon Linder, William S. Chen, Adam Foye, Haolong Li, Lisa Kim, Megha Bhalla, Thomas O'loughlin, Duygu Kuzuoglu Ozturk, Tony Hua, Raunak Shrestha, Scott Wilkinson, Shana Y. Trostel, Andries M. Bergman, Davide Ruggero, Charles G. Drake, Adam G. Sowalsky, Lawrence Fong, Matthew Cooperberg, Alan Ashworth, Wilbert Zwart, David A. Quigley, Luke A. Gilbert, Amy E. Moran, Felix Y. Feng

Immunotherapy is a treatment option that has had limited success in prostate cancer patients. The major histocompatibility complex (MHC) Class I plays a pivotal role in the adaptive immune response by presenting neo-antigens on the surface of cancer cells to CD8+ T cells. Prostate cancer cells have markedly lower expression of MHC Class I genes compared to immunoresponsive cancers. Loss of MHC Class I is also associated with more aggressive disease and immune evasion in prostate cancer. However, the mechanisms that control MHC Class I downregulation in prostate cancer are still unknown. We hypothesize that increasing MHC Class I expression in prostate cancer cells will increase antigen presentation and improve immunotherapy efficacy.

To investigate the mechanism of MHC Class I regulation in prostate cancer cells, we conducted a whole-genome CRISPRi flow cytometry screen. In this screen, C42B cells containing a non-catalytic Cas9 (dCas9) were infected with a lentivirus that contained a sgRNA library of 100,000 guides targeting 20,000 genes. Infected cells were selected and stained with a pan-Class I MHC antibody. Cells were then sorted, and the highest and lowest 25-30% of MHC Class I expressing cells were collected for analysis.

Surprisingly, knockdown of AR and AR co-factors GRHL2 and FOXA1 dramatically increased MHC Class I surface expression. AR inhibition using enzalutamide, the AR degrader ARD-61, or charcoal-stripped serum increased MHC Class I expression over time. Additionally, elimination of androgen response elements upstream of MHC Class I processing and presentation gene transcriptional start sites increased MHC Class I expression, indicating a role for transcriptional repression in AR's regulation of MHC Class I. Increased MHC Class I expression due to AR inhibition also increased antigen presentation and T cell response in co-culture in vitro models. These results were replicated in vivo using an AR-KO TrampC1 model which showed increased MHC Class I expression, increased CD8 T cell tumor infiltration and TCR engagement, and decreased tumor growth compared to wild-type tumors. These observations have also been validated in patient cohorts. RNA expression analyses of patient biopsy samples taken before or after neoadjuvant enzalutamide treatment in two clinical trials showed significantly increased MHC Class I expression post-treatment. Importantly, RNA expression analyses comparing responders and non-responders to anti-PD1 therapy in enzalutamide treated patients showed decreased expression of AR and increased expression of MHC Class I in patients who responded to immunotherapy.

Overall, our data show that AR suppresses MHC Class I expression in prostate cancer, and that androgen-targeted therapies can increase expression of these antigen presenting proteins to improve immune cell recognition. By understanding how AR regulates MHC Class I expression, we can identify new combination treatments utilizing AR signaling inhibition and immunotherapy that lead to an improved anti-cancer response.

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19. Ancestry Informed Molecular Determinates of Gleason Grade

Roy Elias, Levent Trabzonlu, Ajay Vaghasia, Busra Ozbek, Tracy Jones, Jessica Hicks, Qizhi Zheng, Anuj Gupta, Jennifer Meyers, Sarah Wheelan, Angelo M De Marzo, Srinivasan Yegnasubramanian

Introduction: Prostate Cancer (PCa) is the most diagnosed cancer among U.S. men, with variable outcomes ranging from indolence to rapid systemic metastasis. Clinical outcomes are influenced by Gleason grade and racial disparities, with Black men experiencing higher risk levels. Developing a better understanding of ancestry informed biological determinants of aggressive prostate cancer and Gleason grade may aid development of better risk stratification tools for prostate cancer.

Methods: We conducted WGS and RNAseq on laser-microdissected matched tumor/normal samples from 116 patients (n = 244 samples) post-radical prostatectomy. Genetic ancestry was inferred from the WGS data. We carried out integrative molecular analysis to identify associations with Gleason grade and ancestry.

Results: Our cohort included 44 patients (37.9%) of African (AFR) descent and 72 patients (62.1%) of European (EUR) descent. Clinical characteristics were comparable across cohorts. ERG fusion events were notably higher in the EUR group (48.6% vs. 27.3%; p = 0.032), whereas retention of GSTP1 was higher in the AFR cohort ($p < 0.05$). No significant differences were observed in the frequency of somatic alterations (SNVs, indels, or homozygous deletions) in key driver genes (FOXA1, SPOP, KMT2C, PTEN, and IDH1) between ancestral groups. When comparing high risk (Gleason Grade Group [GGG] 9 and 10; n = 41) to low risk (GGG 6 and 7; n = 45) lesions, we found a higher prevalence of deletions in chr16q24.3 (48.8% vs. 15.6%; adj. p = 0.054) and chr5q21.1 (43.9% vs. 6.7%; adj. p = 0.022), and gains in 8q24.21 (39% vs. 4.4%; adj. p = 0.012). Next, we explored the interaction of Ancestry and GGG, with particular focus on high-risk tumors. Differential expression analysis revealed 272 genes with significant differential expression (adj. $p < 0.05$; $|\log2 \text{ fold change}| > 1$) between AFR and EUR groups in GGG high risk tumors. Gene Set Enrichment Analysis (GSEA) of the Hallmark gene sets showed 32 out of 50 sets significantly enriched (adj. $p < 0.05$) in the AFR group, particularly in immune-related pathways (Interferon Gamma, Normalized Enrichment Score [NES] = 2.51, adj. $p = 2.31 \times 10^{-15}$; Interferon Alpha, NES = 2.53, $p = 5.09 \times 10^{-11}$) and mTOR signaling (NES = 2.24 ,adj. $p = 1.37 \times 10^{-10}$). Next, we investigated whether increasing global percentage of AFR ancestry was associated with gene expression in the AFR subset of patients (n = 44). Controlling for Gleason grade, we identified 104 differentially expressed genes (adj. $p < 0.05$; $|\log \text{fold change}| > 1$) associated with AFR ancestry. Among Hallmark gene sets, only Protein Secretion (NES = 1.86, adj. p = 0.0005) was positively correlated with AFR ancestry, whereas 22 gene sets were negatively correlated. These included gene sets involved in metabolism (Oxidative phosphorylation, NES = -2.51, adj. $p = 8.92 \times 10^{-14}$), Myc signaling (NES = -2.47, adj. $p = 6.67 \times 10^{-8}$), and epithelial to mesenchymal transition (NES = -2.19, adj. $p = 8.72 \times 10^{-9}$).

Conclusions: Our findings reveal distinct molecular profiles between EUR and AFR cohorts and identify gene expression associated with degree of AFR ancestry within the AFR ancestry cohort, underscoring the need to consider genetic ancestry in PCa research and molecular risk stratification.

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20. Increased ErbB2 Signaling is an Early Adaptation to Androgen Signaling Inhibition and Persists in Castration Resistant Prostate Cancer

Betul Ersoy-Fazlioglu, Jude Owiredu, Anastasia-Maria Stavridi, Liyang Wang, Carla Calagua, Olga Voznesensky, Fang Xie, Huihui Ye, Yue Sun, David J. Einstein, Xin Gao, Charlene Mantia, William J. Muller, Steven P. Balk, Joshua W. Russo

Androgen signaling inhibition (ASI) is the standard of care for metastatic prostate cancer, but tumors invariably progress to become metastatic castration-resistant prostate cancer (CRPC). Increases in ErbB2 signaling have previously been implicated as a mechanism of resistance to ASI in prostate cancer (PCa), but clinical trials targeting ErbB2 in unselected CRPC patients have shown little benefit. Here, we demonstrate that ErbB2 signaling increases early after ASI and persists in ~26% of advanced CRPC. Furthermore, the expression of the ErbB3/ErbB2 activating ligand NRG1 occurs in ~75% of residual tumor after neoadjuvant ASI, suggesting that some degree of ErbB2 activity may be broadly contributing to tumor survival. Additionally, we identify upregulation of an active ERBB2 splice variant (d16ERBB2) as an alternative mechanism of increased ErbB2 signaling in the post-ASI setting. Using covalent inhibitors of ErbB2 we block the increased ErbB2 signaling mediated by both mechanisms in CRPC models and enhance responses to ASI in castration-sensitive xenograft tumors. The early increases in NRG1 and d16ERBB2 we observed suggest that potent covalent ErbB2 inhibitors may broadly enhance responses to ASI in castration-sensitive PCa. Moreover, IHC for phosphorylated ErbB2 may be a biomarker of CRPC that will respond to this ErbB2 inhibition. We hypothesize that the failures of targeting the ErbB2 pathway in past CRPC clinical trials are due to a combination of factors including the use of antibodies (which may be less effective in tumors like PCa that do not overexpress ErbB2), the use of 1st generation non-covalent ErbB2 inhibitors such as lapatinib (which we show are ineffective against those PCa expressing d16ERBB2), and to the lack of selection for patients with advanced tumors with ErbB2 pathway activation. The findings from our study provided support for a biomarker driven investigator-initiated phase II study of the covalent ErbB2 inhibitor neratinib in CRPC (NCT04781374). While the study was terminated prior to any patients being treated, we established the feasibility of using p-ERBB2 on tumor biopsies as a biomarker. Overall, our data indicate that ErbB2 activation is an acute adaptation to AR inhibition and provides support for a clinical trial of ErbB2 inhibition combined with intensive ASI in castration-sensitive PCa, meeting the current trend in metastatic PCa treatment of moving therapies forward.

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21. Enhancing Real-Time Visualization of Prostate Cancer Using a Near-Infrared Fluorescent Anti-PSCA Antibody Fragment

Masoud Farshbaf, Bao Ying Chen, Felix B. Salazar, Saad N. Ahmed, Anna M. Wu, Kirstin A. Zettlitz

Introduction: There is an urgent need for intraoperative visualization of malignant prostate tumor, as the positive surgical margins and the cancer cells that spread to nearby lymph nodes can impede curative prostate cancer resection. In this study, a tumor-specific near-infrared fluorescent (NIRF) probe was developed based on an antibody fragment targeting human prostate stem cell antigen (hPSCA) for real-time fluorescence-guided prostate cancer surgery.

Methods: The anti-hPSCA antibody fragment, A2scFv-Fc2 double mutant (A2DM) was conjugated with IRDye800CW (a NIR fluorophore, Exmax: 775 nm, Emmax: 794 nm) using random (NHS ester, A2DM-NHS800) or site-specific (maleimide, A2DM-mal800) chemistry. Gel electrophoresis and size exclusion chromatography (SEC) were conducted to confirm the conjugation and integrity of A2DM-NHS800 and A2DM-mal800. The specific binding of A2DM-NHS800 and A2DM-mal800 to recombinant antigen (hPSCA-mFc) and hPSCA+/- RM9 cells was evaluated by ELISA and flow cytometry, respectively. Both fluorescent probes were further conjugated with p-SCN-Bn-Deferoxamine and radiolabeled with zirconium-89 (89Zr-A2DM-NHS800, 89Zr-A2DM-mal800) for surrogate immunoPET to profile pharmacokinetics and biodistribution in hPSCA knock-in C57BL/6 mice (hPSCA KI) implanted with subcutaneous syngeneic hPSCA+/- RM9 tumors. A2DM-NHS800 was evaluated for NIRF imaging in the same tumor-bearing mouse model at 24 and 48 h p.i.

Results: A2DM was successfully conjugated with IRDye800CW through both random and site-specific conjugation methods, and SEC results indicated that A2DM retained its dimeric conformation after conjugation. Conjugation with IRDye800 did not impact the subnanomolar binding affinity to recombinant hPSCA: A2DM: 0.12 ± 0.01 nM, A2DM-NHS800: 0.08 ± 0.01 nM, and A2DM-mal800: 0.26 ± 0.06 nM. Furthermore, A2DM, A2DM-NHS800 and A2DM-mal800 demonstrated antigen-specific binding to hPSCA+ prostate cancer cells, but no binding to hPSCA- cells. In vivo surrogate immunoPET imaging showed hPSCA+-specific tumor uptake (vs. hPSCA- tumor) of 89Zr-A2DM-NHS800 (7.3 ± 0.1 vs. 4.7 ± 0.1 %ID/g), 89Zr-A2DM-mal800 (6.7 ± 0.5 vs. 3.6 ± 0.1 %ID/g) and 89Zr-A2DM (17.1 ± 0.6 vs. 9.4 ± 0.8 %ID/g) at 48 h p.i. Furthermore, the presence of IRDye800 increased liver accumulation and accelerated blood clearance, resulting in a higher tumor-to-blood ratio of 89Zr-A2DM-NHS800 (20.3 ± 0.8) and 89Zr-A2DM-mal800 (14.6 ± 0.6) compared to 89Zr-A2DM (11.3 ± 2) at 48 h p.i. NIRF imaging demonstrated antigen-specific uptake of A2DM-NHS800 in hPSCA+ RM9 tumors and no visible uptake in hPSCA- tumors or in the surrounding tissue, resulting in high-contrast images at 24 and 48 h p.i. Though fluorescent signal was also visible in the liver (confirming the immunoPET imaging results), no signal was detected in the kidneys or bladder resulting in an unobstructed view of the pelvic region, crucial for prostate cancer detection.

Conclusion and future directions: A2DM-NHS800 and A2DM-mal800 showed specific in vivo tumor targeting and rapid blood clearance resulting in a high tumor-to-blood ratio at early time points, which would facilitate next-day image-guided surgery. A2DM-NHS800 resulted in high NIRF signal in hPSCA+ tumors. Ongoing experiments will evaluate A2DM-mal800 for NIRF imaging and determine the lead candidate to be further evaluated by NIRF imaging in intramuscular, orthotopic and metastasized lymph node models of prostate cancer.

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22. A Phase II Study of Lutetium-177 DOTATATE in Metastatic Prostate Cancer with Neuroendocrine Differentiation

John Floberg, Robert Jeraj, Glenn Liu, Bryan Bednarz, Joshua Lang, Steve Cho

Neuroendocrine differentiated (NED) prostate cancer is a particularly aggressive form of prostate cancer. Treatment options for this cancer are limited, typically multi-agent chemotherapy with few options beyond this, and prognosis is poor. The somatostatin receptor is a potential target for NED prostate cancers. NED prostate cancers, and even advanced metastatic castration resistant prostate cancers (mCRPCs) showing neuroendocrine differentiation, demonstrate expression of the somatostatin receptor. A drug targeting the somatostatin receptor is the radiopharmaceutical therapy (RPT) lutetium-177 (Lu-177) DOTATATE. Lu-177 DOTATATE has proven to be very effective therapy for neuroendocrine gastrointenstinal tumors. We have opened a phase II trial investigating this agent. The primary objective of this trial is to evaluate the objective response rate (ORR) at 6 months for patients with NED prostate cancer treated with Lu-177 DOTATATE. Six-month radiographic progression-free survival is a secondary objective.

A number of biomarkers will also be investigated in this trial, including an imaging response biomarker, radiation dosimetry, and circulating biomarkers (namely mRNA extracted from circulating tumor cells (CTCs)). These biomarkers may ultimately aid in identifying which patients with NED prostate cancer might benefit from this therapeutic approach.

RPTs are particularly suited to imaging biomarkers, as many of these agents can be used for imaging as well as therapy. We will investigate an image analysis tool developed at the University of Wisconsin, Quantitative Total eXtensible Imaging (QTXI). This provides an automated means of assessing imaging response based upon multiple PET images. We will evaluate if change in FDG-PET signal as determined by QTXI correlates with the ORR as determined by conventional means (i.e. RECIST).

Radiation dosimetry is another imaging-based marker that we will investigate. Lu-177 DOTATATE can be imaged after it has been administered using single-photon emission computerized tomography (SPECT), and these images can be converted into a radiation dose delivered, either on an organ or a voxel level. Us of radiation dosimetry has been investigated in the context of Lu-177 DOTATATE therapy for neuroendocrine gastrointestinal cancers, and has been shown as a means of potentially safely delivering additional cycles of Lu-177 DOTATATE. We will investigate Lu-177 DOTATE dosimetry for the first time in patients with NED prostate cancer, and determine if dosimetry is associated with ORR as well as renal toxicity.

Finally, we will investigate a circulating biomarker using RNA extracted from CTCs. We have previously demonstrated that incorporating information from multiple serial liquid biopsies can detect NED prostate cancer with high accuracy, including in patients who were initially diagnosed with androgen receptor-driven prostate cancers. We will evaluate this marker over the course of Lu-177 DOTATATE therapy in these patients with NED prostate cancer, and its relationship to response to the therapy. We will also use this platform to identify gene expression signatures of resistance to treatment at the time of progression.

This clinical trial will be an opportunity to investigate a novel treatment approach in a disease with a poor prognosis and few treatment options, and to investigate several biomarkers that could help maximize this therapy's impact.

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23. A Somatic Mutation in a Novel Mitochondrial Encoded Microprotein is Implicated in Prostate Cancer Biology

Melanie Flores, Thalida Arpawong, Susanne Henning, Pei Liang, Brendan Miller, Kelvin Yen, Hemal Mehta, Hiroshi Kumagai, Junxiang Wan, Ricardo Ramirez, Ana Silverstein, William J. Aronson, Pinchas Cohen

Mutations in mitochondrial DNA (mtDNA) have been linked to increased prostate cancer aggressiveness and disease progression. Here, using aggregated data from The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) we have identified a single nucleotide variant (SNV) in the D-loop region of the mtDNA, representing a somatic mutation in prostate cancer tumors, found in 1.3% of cancers but in no germline mtDNA. This somatic mutation alters a small open reading frame and results in an amino acid change in a mitochondrial microprotein we have named Small Cancer-Related Effector d-loop-Associated Microprotein (SCREAM). Multiple publicly available RNA-Seq datasets confirm the presence of the SCREAM SNV in prostate tumors. Analysis of tumor RNA-Sequencing data reveals that SCREAM is differentially expressed in prostate cancer tumors compared to adjacent normal tissue. In vitro assays in multiple prostate cancer cell lines, show that the mutant form of this microprotein, named Y18H, leads to increased proliferation and decreased apoptosis and necrosis in prostate cancer cell lines. Mouse MycCaP allograft tumor models were treated for 30 days with 5-mg/kg/day with either vehicle control, WT-SCREAM or Y18H. Mice treated with Y18H showed a significant increase in tumor growth compared to vehicle controls or WT-SCREAM peptide. The identification of this novel somatic mutation and its resulting oncogenic change in the corresponding mitochondrial microprotein may provide a novel precision-based target for prostate cancer therapeutics.

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24. Excess Death and Potential Life Years Lost Among Black Prostate Cancer Patients (1970-2020)

Nana A. Frimpong, Sarah K. Holt, Daniel Carson, Yohali Burrola-Mendez, Jenney Lee, Erika Wolff, John L Gore, Yaw A. Nyame

Introduction: Prostate Cancer demonstrates a wide and sustained disparity in mortality among Black Americans compared with the average US population. These disparities have persisted over 50 years despite significant diagnostic and therapeutic advances in prostate cancer care. We evaluated trends in excess prostate cancer mortality and excess years of potential life lost between Black and White Americans between 1970-2020.

Methods: This is a serial cross-sectional study using US national level data from CDC WONDER linked with annual life expectancy by 10-year age groups from the National Center for Health Statistics life tables for individuals aged 25-84 years. Data from non-Hispanic White and non-Hispanic Black Americans with a cause of death of prostate cancer across all age groups were included. We measured excess age-adjusted prostate cancer mortality, age-specific prostate cancer mortality, excess deaths, and excess years of potential life lost among Black vs. White Americans.

Results: The age-adjusted excess mortality rate increased from 21.1 to 44.8 deaths per 100,000 from 1970-1993. From 1993-2020, this rate declined from 44.8 to 18.8 excess deaths per 100,000. The annual age-adjusted mortality ratio between Black and White Americans has ranged from 2.0 to 2.5 (1980-2020) with its highest peak in 2001. The excess deaths per 100,000 Black males ranged from 1,959 to 2,522 (1980-2020) with its highest peak in 1996 (Figure 1). The trend in excess years of potential life lost per 100,000 black males had been steadily decreasing from 39,416 years lost in 1996 to 31,746 years lost in 2013. From 2013-2020 the excess years of potential life lost per 100,000 black males increased from 31,746 to 39,782. The excess potential years of life lost increases with increasing age and peaks in the 65-74 years age group.

Conclusion: Over a 50-year period, Black Americans have experienced more than 117,348 excess deaths due to prostate cancer resulting in 1,509,384 excess years of potential life lost in the US. Although we have seen a dramatic decrease in prostate cancer deaths since the introduction of PSA testing, Black prostate cancer patients continue to experience excess deaths which result in a loss of productivity and support (i.e., social, economic, interpersonal, etc.) for communities and families across the country.

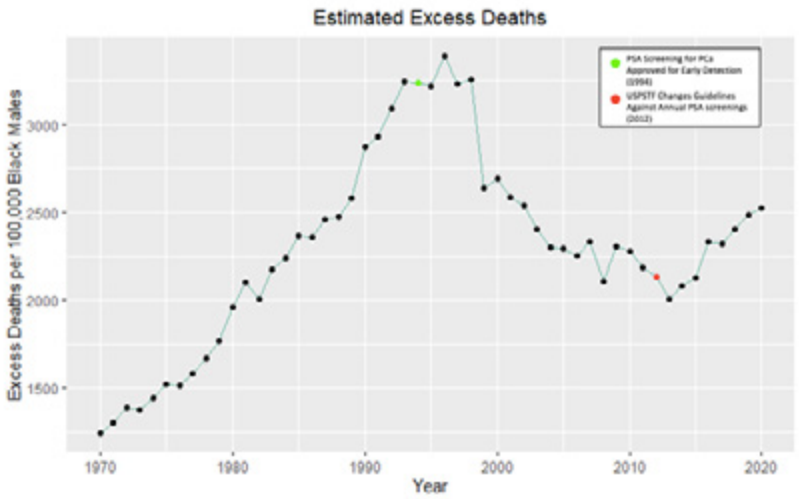

Figure 1: Excess death prostate cancer deaths per 100,000 Black patients compared with White patients.

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25. Mechanism of ONECUT2 Inhibition: A Potential Treatment for Lethal Prostate Cancer

Brad Gallent, Chen Qian, Avradip Chatterjee, Yanpeng Xing, Madhusudhanarao Katiki, Salma Kaochar, Michael Jung, Isla P. Garraway, Ramachandran Murali, Michael R. Freeman

Prostate cancer (PC) is one of the most common cancers and the second most lethal cancer in men, claiming over 30,000 lives each year in the US alone. These PC patient deaths are typically due to castration-resistant PC (CRPC), which is resistant to androgen deprivation therapy, androgen receptor signaling inhibitors (ARSI), and chemotherapies. Our group and others have shown that the transcription factor ONECUT2 (OC2) is a critical driver of lineage plasticity and drug resistance in CRPC and is upregulated following ARSI treatment. We discovered a class of small molecule inhibitors of OC2 (OC2i) that binds to OC2 (KD 3.5-4.5 uM), inhibit growth and promote cell death of metastatic PC in cell culture (IC50 of 2.0-5.0 uM) and suppress metastatic CRPC tumors in xenograft mouse models. A mechanistic understanding of how these OC2i bind to and inhibit OC2 function is imperative to developing them into a clinically viable treatment option for patients. Our recent work shows OC2i bind Fe with high affinity (KD <1.0 uM) and the Fe:OC2i combination is necessary to inhibit OC2 binding of DNA. We have solved the crystal structure of OC2 bound to its conjugate DNA response element, visualizing their atomic-level interactions. We are working on a crystal structure of the OC2i bound to OC2 to understand their molecular interactions and mechanism of OC2i inhibition. Using the specific interactions between OC2i and OC2, we can tailor modifications to the inhibitor chemical scaffold for improvements in binding affinity. These findings demonstrate that OC2 can be directly inhibited with small molecules that show substantial in vivo suppression of CRPC.

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26. Investigating the Role of FOXA2 During the Transition to Neuroendocrine Prostate Cancer

Richard Garner, Nicholas J. Brady, Kate Dunmore, Richa Singh, Alyssa Bagadion, Rohan Bareja, Andrea Sboner, Olivier Elemento, Brian Robinson, Himisha Beltran, and David S. Rickman

Background: Acquired resistance to androgen receptor (AR)-targeted therapies and the progression to metastatic-castrate resistant prostate cancer (CRPC) remains a significant clinical problem. Mechanisms of acquired resistance include lineage plasticity, by which CRPC tumors can become AR-negative and acquire neuroendocrine features (neuroendocrine prostate cancer [NEPC]). Clinical prognoses for patients with NEPC are very poor and we lack a comprehensive understanding of the molecular mechanisms underlying lineage plasticity and NEPC progression. Single cell analyses from genetically engineered mouse models and patient tumor data show that FOXA2, a key lineage-determining pioneer transcription factor (TF), is significantly upregulated in subsets of CRPC and NEPC patient tumors. However, little is known about the role of FOXA2 in regulating lineage plasticity and progression from CRPC to NEPC. Elucidation of this mechanism is essential to identify novel biomarkers and potential therapeutic targets for NEPC treatment.

Methods: In this study, we first examined the role of FOXA2 in suppressing androgen signaling and promoting progression towards an NEPC phenotype in vitro. We then conducted ATAC-seq, FOXA2 ChIP-seq, and RNA-seq in FOXA2-engineered prostate cancer cell lines and patient-derived NEPC models to characterize how FOXA2 alters the chromatin accessibility and transcriptional activity, and to identify potential FOXA2 interactors. We further characterized the FOXA2-interactome by performing rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME) to identify FOXA2-protein interactors on chromatin and validated these interactions using biochemical approaches.

Results: We found that FOXA2 overexpression suppressed androgen signaling and promoted progression to a NEPC phenotype under short- and long-term androgen deprivation conditions. Further, FOXA2 redirected the chromatin accessibility landscape to be consistent with an NEPC gene expression program, including increased chromatin accessibility for key NEPC TFs. FOXA2 ChIP-seq showed FOXA2 to be bound at known NEPC driver genes and epigenetic modifiers. Lastly, we discovered that FOXA2 physically interacts with key NEPC TFs and epigenetic regulators, suggesting that these FOXA2 physical interactions are required for NEPC progression.

Conclusions: Overall, our data from murine and human indicate that FOXA2 functions to suppress androgen signaling and function as a pioneer TF to redirect chromatin accessibility towards an NEPC molecular program. Further, we characterized the FOXA2 cistrome in patient-derived NEPC models and found that FOXA2 physically interacts with critical NEPC TFs and epigenetic regulators. These findings provide novel mechanistic insights underlying how FOXA2 regulates lineage plasticity and NEPC progression.

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27. EZH2 Regulates Cell Identity by Inducing Alternate Transcriptional Programs Dependent on Activation of Translation and Regulation of RNA Maturation

Beatriz German, Katherine L. Morel, Deborah L. Burkhart, Teia Noel, Nadia Boufaied, Felipe Dezem, Henry W. Long, Sylvan Baca, Matthew L. Freedman, Himisha Beltran, Christopher J. Sweeney, Jasmine T. Plummer, Simon R.V. Knott, Sunjun Che, David P. Labbé, Leigh Ellis

Background: Second-generation androgen deprivation therapies (ADT) have provided significant life-extension for patients with metastatic castration resistant prostate cancer (mCRPC), but unfortunately tumors will eventually progress via therapy resistance and currently no therapies provide durable response. Approximately between 15-20% of these mCRPC tumors are independent of AR activity (CRPC-AI) via a mechanism termed phenotypic plasticity. The development of phenotypic plasticity acquire resistance to ADT through distinct epigenetic and alternate transcriptome programs associated with loss of function of the tumor suppressor genes retinoblastoma *RB1* and/or *TP53*. *RB1* is critical for normal development, lineage specification, and chromatin reorganization and its genomic loss is predictor of poor survival in mCRPC, whereas alterations in *RB1* and TP53 were associated with shorter response to ADT. These tumors often display altered kinase signaling, chronic inflammation, and multilineage states involving divergent cell identities including neuroendocrine-like, stem-like, and basal-like gene signatures. Using genetically engineered mouse models (GEMMs) devoid of *Pten* and *Rb1*, it has been previously demonstrated that the chromatin reprogramming factor Enhancer of zeste homolog 2 (EZH2) is an important regulator of alternative transcription programs promoting phenotypic plasticity. Moreover, the loss of Tristetraprolin (TTP, gene ZFP36), an RNA binding protein that regulates mRNA stability, increases NF-κB activation and the related inflammatory response, has been associated with higher rates of aggressive prostate cancer in human patients and phenotypic plasticity development in *Pten* GEMMs. Here, our overall goal was to better understand EZH2 role in reprogramming and how this could be exploited therapeutically.

Methods: To investigate the cell identity control by EZH2, prostate cancer GEMMs devoid of *Pten* and *Rb1* were treated with the EZH2 inhibitor – EPZ011989. Analysis was performed using a multi-omics approach involving CRISPR/Cas9 functional genomics screen, rapid immunoprecipitation mass spectrometry of endogenous protein (RIME) and snRNASeq. To validate these data, genetic and chemical tools were used with in vitro and in vivo prostate cancer models.

Results: EZH2 regulates alternate transcription programs leading to a multilineage cell states downstream of RB1 loss that is associated with activation of the mammalian target of rapamycin complex 1 (mTORC1). These data are further supported through RIME and functional genomic data validating cross talk between EZH2 and activation of translation. Combined chemical inhibition of EZH2 and PI3K/mTORC1 resulted in superior anti-tumor activity in murine and human phenotypic plastic models and was most significant when this combination was used with castration. Moreover, we elucidated that the regulation of cellular state transition by EZH2 inhibition requires activation of the RNA degrader, TTP.

Conclusions: Together, these data indicate phenotypic plasticity dependence on coordination between EZH2, TTP and mTORC1 signaling and represent a novel therapeutic approach for this lethal prostate cancer phenotype.

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28. Prostate Lineage-specific Metabolism Governs Luminal Differentiation and Response to Antiandrogen Treatment

Jenna M. Giafaglione, Preston D. Crowell, Amelie M.L. Delcourt, Takao Hashimoto, Sung Min Ha, Aishwarya Atmakuri, Nicholas M. Nunley, Rachel M.A. Dang, Mao Tian, Johnny A. Diaz, Elisavet Tika, Marie C. Payne, Deborah L. Burkhart, Dapei Li, Nora M. Navone, Eva Corey, Peter S. Nelson, Neil Y.C. Lin, Cedric Blanpain, Leigh Ellis, Paul C. Boutros, Andrew S. Goldstein

Lineage transitions are a central feature of prostate development, tumourigenesis and treatment resistance. While epigenetic changes are well-known to drive prostate lineage transitions, it remains unclear how upstream metabolic signaling contributes to the regulation of prostate epithelial identity. To fill this gap, we developed an approach to perform metabolomics on primary prostate epithelial cells. Using this approach, we discovered that the basal and luminal cells of the prostate exhibit distinct metabolomes and nutrient utilization patterns. Furthermore, basal to luminal differentiation is accompanied by increased pyruvate oxidation. We establish the mitochondrial pyruvate carrier (MPC) and subsequent lactate accumulation as regulators of prostate luminal identity. Inhibition of the MPC or supplementation with exogenous lactate results in large-scale chromatin remodeling, influencing both lineage-specific transcription factors and response to antiandrogen treatment. These results establish reciprocal regulation of metabolism and prostate epithelial lineage identity.

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29. Convergent Alterations in the Tumor Microenvironment of MYC-driven Human and Murine Prostate Cancer

Mindy K Graham, Rulin Wang, Roshan Chikarmane, Bulouere Wodu, Ajay Vaghasia, Anuj Gupta, Qizhi Zheng, Jessica Hicks, Polina Sysa-Shah, Xin Pan, Nicole Castagna, Jianyong Liu, Jennifer Meyers, Alyza Skaist, Yan Zhang, Kornel Schuebel, Brian W Simons, Charles J. Bieberich, William G Nelson, Shawn E. Lupold, Theodore L DeWeese, Angelo M De Marzo, Srinivasan Yegnasubramanian

Background: The tissue microenvironment in prostate cancer is profoundly altered. While such alterations have been implicated in driving prostate cancer initiation and progression to aggressive disease, how prostate cancer cells and their precursors mediate those changes is unclear, in part due to the inability to longitudinally study the disease evolution in human tissues.

Methods: To overcome this limitation, we performed extensive single-cell RNA-sequencing (scRNA-seq) and rigorous molecular pathology of the comparative biology between human prostate cancer and key time points in the disease evolution of a genetically engineered mouse model (GEMM) of prostate cancer.

Results: Our studies of human tissues, with validation in a large external data set, revealed that cancer cell-intrinsic activation of MYC signaling was the top up-regulated pathway in human cancers, representing a common denominator across the well-known molecular and pathological heterogeneity of human prostate cancer. Likewise, numerous non-malignant cell states in the tumor microenvironment (TME), including non-cancerous epithelial, immune, and fibroblast cell compartments, were conserved across individuals, raising the possibility that these cell types may be a sequelae of the convergent MYC activation in the cancer cells. To test this hypothesis, we employed a GEMM of prostate epithelial cell-specific MYC activation in two mouse strains. Cell communication network and pathway analyses suggested that MYC oncogene-expressing neoplastic cells, directly and indirectly, reprogrammed the TME during carcinogenesis, leading to the emergence of cascading cell state alterations in neighboring epithelial, immune, and fibroblast cell types that paralleled key findings in human prostate cancer. Importantly, among these changes, the progression from a precursor-enriched to invasive-cancer-enriched state was accompanied by a cell-intrinsic switch from pro-immunogenic to immunosuppressive transcriptional programs with coinciding enrichment of immunosuppressive myeloid and Treg cells in the immune microenvironment.

Conclusions: In summary, these findings implicate the activation of MYC signaling in reshaping convergent aspects of the TME of prostate cancer as a common denominator across the otherwise well-documented molecular heterogeneity of human prostate cancer.

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30. Large Cribriform Glands (>0.25 mm diameter) as a Predictor of Adverse Pathology in Men with Grade Group 2 Prostate Cancer

Nancy Y Greenland, Janet E Cowan, Bradley A Stohr, Jeffry P Simko, Peter R Carroll, Emily Chan

Introduction: A recent outcome-based, radical prostatectomy study defined >0.25 mm diameter to distinguish large versus small cribriform glands, with >0.25 mm associated with worse recurrence-free survival. This study evaluates whether identification of >0.25 mm cribriform glands in Grade Group 2 patients at biopsy is associated with adverse pathology at radical prostatectomy.

Methods: Tumor containing biopsy slides for 133 patients with Grade Group 2 prostate cancer with subsequent radical prostatectomy were re-reviewed for large cribriform glands (diameter >0.25 mm). The primary outcome was adverse pathology (Grade Group 3-5; stage pT3a or greater, or pN1). The secondary outcome was recurrence-free survival.

Results: Cribriform pattern was present in 52/133 (39%) patients; of these, 36/52 (69%) had large cribriform glands and 16/52 (31%) had only small cribriform glands. Patients with large cribriform glands had significantly more adverse pathology at radical prostatectomy compared to patients with small cribriform glands and no cribriform glands (large 11/16, 69%; small 12/36, 33%; no cribriform 25/81, 31%; Chisq p-value 0.01). On multivariate analysis, large cribriform glands were also associated with adverse pathology, independent of age, PSA/PSA density at diagnosis, year of diagnosis, and biopsy cores % positive (global p-value 0.02). Large cribriform glands were associated with increased CAPRA-S surgical risk score (Kruskal-Wallis p-value 0.02).

Conclusions: Large cribriform glands using a diameter >0.25 mm definition in Grade Group 2 patients on biopsy are associated with increased risk of adverse pathology at radical prostatectomy. The presence of large cribriform histology should be considered when offering active surveillance for those with Grade Group 2 disease.

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31. Morphologic Patterns Observed in Prostate Biopsy Cases with Discrepant Grade Group and Molecular Risk Classification

Nancy Y. Greenland, Matthew R. Cooperberg, Peter R. Carroll, Janet E. Cowan, Jeffry P. Simko, Bradley A. Stohr, Emily Chan

Background: Molecular-based risk classifier tests are increasingly being utilized by urologists and radiation oncologists to guide clinical decision making. The Decipher prostate biopsy test is designed to predict likelihood of high-grade disease at radical prostatectomy (RP) and risk of metastasis and mortality. The test provides a risk category of low, intermediate, or high. We investigated histologic features of biopsies in which the Grade Group (GG) and Decipher risk category were discrepant.

Methods: We included patients who had the Decipher molecular assay performed from 2016 to 2020 and were either GG3+ with low Decipher risk category or GG1-2 with high Decipher risk category. The biopsy slide on which Decipher testing was performed was re-reviewed for Gleason score and various histologic patterns.

Results: From 2016 to 2020, of 234 men who underwent prostate biopsy and had Decipher testing performed, 51 (22%) had discrepant GG and molecular risk, 33 of which slides were available for review. Of these 33 cases, 30% had GG3+ with low Decipher risk (n=10) and 70% had GG1-2 with high Decipher risk (GG1 n=5, GG2 n=18) (Table 1). The GG on the original pathology report and on blinded review were concordant in 29/33 (88%) cases. Of the 5 GG1 cases, 3 had atrophic carcinoma, 1 had mucinous fibroplasia, 1 had carcinoma with mucinous features, and 1 had carcinoma with basal cell marker expression. Of the 10 GG>3 low Decipher risk cases, 6 had known aggressive histologic patterns, including 4 with cribriform, 1 with intraductal carcinoma (IDC), and 1 with Gleason pattern 5.

Conclusions: In the GG>3 low Decipher risk cases, aggressive histologic patterns such as large cribriform and IDC were occasionally observed. Therefore, the molecular classifier may not capture all high-risk histologic patterns. In all GG1 high Decipher risk cases, at least one difficult to grade or classify pattern was seen. Whether these constitute higher risk patterns requires further study.

Table 1. Morphologic patterns observed on re-review.

	Large cribriform (no small cribriform)	Small cribriform (no large cribriform)	Large and small cribriform	Glomerulations	Fused glands pattern 4	Poorly formed glands pattern 4	Complex papillary	Single cell/single file pattern 5	IDC or suspicious for IDC	Ductal carcinoma	Carcinoma with prominent vacuoles	Carcinoma with mucinous features	Atrophic carcinoma	Foamy gland carcinoma	Pseudohyperplastic carcinoma	Mucinous fibroplasia	Carcinoma with basal cell marker expression	Mild stromal reaction
GG>3 and Low Decipher (n=10)	1	2	1	2	7	5	1	1	1	1	1	0	0	0	0	0	0	1
GG1 and High Decipher (n=5)	0	0	0	0	0	1	0	0	0	0	0	1	3	0	0	1	1	1
GG2 and High Decipher (n=18)	0	4	2	5	17	12	2	0	1	3	0	5	2	1	2	3	1	1

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32. Multi-Ancestry Germline Drivers of Prostate Cancer Clinical & Molecular Evolution

Roni Haas, Nicole Zeltser, Adam Kinnaird, Alexandre Zlotta, Amar Kishan, Bogdan Pasaniuc, Paul Boutros

Prostate cancer is the most heritable solid cancer type. Some of the genetic features that make individuals more likely to be diagnosed are well-known: BRCA2 and other DNA Damage Repair genes, and common germline polymorphisms aggregated into polygenic risk scores (PRSs). But these studies have left three key gaps in the field. First, why do existing genetic factors only explain a fraction of prostate cancer heredity? What are the missing genetic factors? Second, are there genetic factors associated with the clinical course of prostate cancer (beyond BRCA2)? Third, there are large observed tumour differences between prostate cancers arising in individuals of different ancestry – what causes these?

We will discuss these three key questions in prostate cancer genetics. First, we outline how different classes of previously unstudied aberrations can explain missing heritability. Second, we outline data demonstrating that ancestry-sensitive polygenic risk scores, especially those leveraging tumour somatic information in their design, can predict specific clinical features such as grade at diagnosis and biochemical relapse. Third, we outline how specific germline genetic features underlie the differential somatic features of prostate cancers arising in men of differing ancestries, particular those of African and Asian ancestry.

Taken together, we outline the latest results extending our understanding of how prostate cancer germline genetics influence tumour clinical and molecular evolution.

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33. The Immunosuppressive Impact of Androgen Receptor on CD4 T Cells in the TME

Zachary Hay, Fanny Polesso, Aaron Ko, Xiangnan Guan, Zheng Xia, Amy Moran

Androgen deprivation therapy (ADT) is standard of care treatment for prostate cancer patients, where reducing androgen receptor (AR) activity, through reduction of androgens or inhibition of AR, decreases cancer cell proliferation. However, AR is also expressed by immune cells, including T cells, where its function is not fully understood. Previous work from our group and others has demonstrated that androgens have a suppressive impact on T cells, impairing their ability to respond to antigenic challenge and/or targeted immunotherapy. To date, the studies performed have focused on how androgens and/or AR impact CD8 T cell function. However, there is mounting evidence that cytolytic CD4 T cells participate in anti-tumor immunity and contribute to immunotherapy outcomes. In fact, we provide evidence that MHC class II genes (HLA- DP/DQ/DR) are some of the most significantly upregulated genes in metastatic castration resistant prostate cancer patients that respond to ADT and anti-PD-1 combination therapy (NCT02312557). Increased expression of MHC class II in responders suggests CD4 T cells contribute to a successful anti-tumor immune response. Thus, further research into androgen modulation of CD4 T cell biology is needed to improve immunotherapy outcomes in prostate cancer. To investigate the consequence of AR-inhibition on CD4 T cell differentiation and function, we generated a novel mouse model with a CD4 T cell specific deletion of AR, allowing us to probe with specificity the influence AR has on tumor infiltrating CD4 T cells. These animals have normal thymic and peripheral T cell development suggesting AR does not impair CD4 T cell lineage selection or differentiation. Using a competitive mixed bone marrow chimera model, our preliminary experiments demonstrate that AR-KO CD4 T cells persist at a higher frequency in the TME compared to wild-type CD4 T cells. In addition, we observe fewer regulatory T cells (CD4+Foxp3+) in ARKO CD4 tumor bearing animals. We have further evidence that AR-KO CD4 T cells confer superior tumor control relative to WT CD4 T cells. These results indicate that AR has a role in CD4 T cell function in the prostate TME that results in suboptimal tumor control.

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34. Development of an Orally Bioavailable SWI/SNF ATPase Degrader and Anticipation of Potential Resistance Mechanisms

Tongchen He, Caleb Cheng, Abhijit Parolia, Alex Hopkins, Yuanyuan Qiao, Lanbo Xiao, Arul M. Chinnaiyan

Background: The Switch/Sucrose Non-Fermentable (SWI/SNF) complex is a critical player in chromatin remodeling, and its dysregulation is a hallmark of over 20% of cancer cases. In this study, we have successfully developed a second-generation orally bioavailable proteolysis-targeting chimera (PROTAC) degrader named AU-24118. This PROTAC was designed to target the SWI/SNF ATPase subunits SMARCA2, SMARCA4, and PBRM1, in comparison to its predecessor, AU-15330. AU-24118, much like its first-generation counterpart, exhibits high specificity, selectively targeting SMARCA2, SMARCA4, and PBRM1, while also demonstrating superior therapeutic efficacy in vivo. However, an inherent challenge in the use of PROTAC degraders is the potential development of resistance in cancer cells over time. Hence, understanding the underlying mechanisms of resistance and devising strategies to overcome this acquired resistance are of paramount significance. In this investigation, we classified the resistance mechanisms in prostate cancer cells to PROTAC degraders into two distinct categories and subsequently engineered an innovative, orally bioavailable SWI/SNF PROTAC degrader, AU-24118, designed to address one resistance mechanism.

Methods and Results: To assess the oral bioavailability of AU-24118, we conducted Tandem Mass Tag Mass Spectrometry (TMT MS) analysis to validate its specificity, comparing it to AU-15330. Remarkably, AU-24118 displayed comparable efficacy in inhibiting cell growth as AU-15330. Furthermore, in a Castration-Resistant Prostate Cancer (CRPC) VCaP xenograft model, combining AU-24118 with enzalutamide resulted in complete tumor regression, with no observable toxicity. In the context of PROTAC resistance, we employed AU-15330, specifically designed to degrade SWI/SNF subunits (BRG1, PBRM1, BRM). We exposed the prostate cancer cell line 22rv1 to varying concentrations of AU-15330 over a month, generating AU-15330-resistant cell lines. Whole exome sequencing (WES) analysis revealed that those cells developed under high-dose (1uM) AU-15330 treatment acquired multiple point mutations within or near the AU-15330-targeting bromodomain of BRG1. Conversely, resistant cell lines developed under lower AU-15330 concentrations (100nM) did not exhibit BRG1 point mutations. Instead, RNA-seq analysis demonstrated elevated expression of ABCB1 (also known as Multidrug Resistance Protein 1) in cells that developed resistance to 100nM AU-15330. This upregulation was further confirmed through Western blot and qPCR. Importantly, this resistance extended to several potent PROTAC degraders (e.g., ARD-61, ZBC-260) when ABCB1 was expressed in AU-15330-resistant cells. We found that Zosuquidar, an ABCB1 inhibitor, effectively overcame ABCB1-mediated resistance to AU-15330 and other PROTAC degraders. This suggests that inhibiting ABCB1 may serve as a direct and efficacious strategy for restoring PROTAC degrader potency when ABCB1-mediated drug resistance emerges. At the same time, AU-24118 also effectively overcame resistance in ABCB1-overexpressed cell lines, inducing growth inhibition and target protein degradation.

Conclusion: We have introduced AU-24118, a groundbreaking orally bioavailable SWI/SNF PROTAC degrader. This compound not only exhibits substantial therapeutic efficacy but also effectively addresses resistance mechanisms. Our study underscores the diverse nature of resistance mechanisms to PROTAC degraders, which is contingent upon drug concentrations, and emphasizes the potential of ABCB1 inhibition as a strategy to restore PROTAC degrader effectiveness in the face of drug resistance.

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35. Next-generation PSMA Radioligands for Targeted Alpha Therapy of CRPC

Reinier Hernandez, Carolina Ferreira, Liudmila Lambert, Anatoly Pinchuk, Amanda Carston, Hansel Comas Rojas

Although the potential of PSMA-based targeted alpha therapy in castration-resistant prostate cancer (CRPC) is undeniable, PSMA-617 as a delivery vehicle for ^{225}Ac has several disadvantages such as fast kinetics, poor tumor retention, and significant off-target accumulation in the salivary glands. Therefore, novel agents designed with optimal pharmacokinetics and tumor retention for long-lived ^{225}Ac, and with reduced salivary gland uptake are needed to realize the full curative potential of targeted alpha therapy in mCRPC. To improve upon the pharmacokinetics of PSMA-617 we synthesized a next-generation low-molecular weight PSMA-targeted PET radiopharmaceutical ((1-carboxy-5-(18-(4-(2-(4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclodo-decan-1-yl) acetamido)phenyl)octa-decanamido)-pentyl)carbamoyl)glutamic acid (ART-101). The pharmacokinetic and tumor-targeting properties of ART-101 radiolabeled with the positron-emitting radionuclide ^{86}Y were tested and compared to those of ^{86}Y-PSMA-617. In longitudinal PET/CT imaging studies using the immunocompromised mouse model of prostate cancer, PC3-PIP, ^{86}Y-PSMA-617 showed rapid tumor uptake peaking at 4.0 ± 0.2 %IA/g at 4 hours post-injection (p.i.). However, tumor retention of ^{86}Y-PSMA-617 was poor, with only 0.4 ± 0.3 %IA/g remaining 24 h post-injection of the radioligand. On the other hand, ^{86}Y-ART-101 clearly delineated PSMA-expressing tumor xenografts, with tumor uptake peaking at 8.9 ± 1.7 percent injected activity per gram of tissue (%IA/g), 24 h p.i.. of the tracer and remaining elevated up to 72 h p.i. with 7.9 ± 1.1 %IA/g at this timepoint. This resulted in a significantly enhanced area under the curve of the tumor time-activity curves for ^{86}Y-ART-101 compared to ^{86}Y-PSMA-617. Given its prolonged tumor retention, ART-101 can leverage the extended half-life of the alpha emitter Ac-225 to deliver significant absorbed doses to prostate tumors. Consequently, we tested the efficacy of ^{225}Ac-ART-101 in mice bearing LNCaP tumor grafts. Groups of mice bearing PC3-PIP or LNCaP xenografts (n=5) received a single IV injection of saline, ^{225}Ac-ART-101 20 kBq (~0.5 μCi), or 20 kBq (~1 μCi), or ^{225}Ac-ART-101 40 kBq (~1 μCi). Consistent with the reported literature, treatment with ^{225}Ac-PSMA-617 40 kBq resulted in marked inhibition of tumor growth and significant ($P < 0.0001$) survival advantage compared to controls. Similarly, treatment with ^{225}Ac-ART-101 at either injected activity, 20 or 40 kBq, led to significant tumor regression and prolongation in mouse survival. Median overall survival was 43, 84, 92, and 92 days for control, ^{225}Ac-PSMA-617 (40 kBq), ^{225}Ac-ART-101 (20 kBq), and ^{225}Ac-ART-101 (40 kBq), respectively. Importantly, toxicology data in the form of health assessments, CBC, and CMP indicated a favorable safety profile of ^{225}Ac-ART-101 at relevant human equivalent injected activity levels. In conclusion, ART-101 showed significantly improved circulation half-life, enhanced tumor uptake, prolonged retention, and primarily hepatic clearance, which translated into an enhanced therapeutic efficacy of ^{225}Ac-ART-101 in murine models of mCRPC.

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36. Targeting Mechanisms of mRNA Translation Initiation in Castration Resistant Prostate Cancer

Patrick Hoang, Phillip Corrin, Sujata Jana, and Andrew Hsieh

Castration resistant prostate cancer (CRPC) is increasing in occurrence and is uniformly fatal with over 30,000 deaths yearly in the US alone. We uncovered a new functional link between androgen receptor (AR) signaling and the process of mRNA translation initiation. We found that decreased AR activity commonly seen in CRPC patients leads to a compensatory increase in translation initiation specifically through a dynamic interaction between eIF4E and eIF4G. Using genetic models and tool compounds, we demonstrated that the eIF4E:eIF4G interaction is critical to CRPC. One major impediment in the field has been a lack of small molecules with drug-like properties that can effectively target this interaction. We used machine learning to query drug-like small molecules that can target this protein-protein interface. After screening 2.7 million compounds in silico and conducting a phenotypic proliferation screen followed by molecular studies, we have identified a lead compound with an IC50 of 406 nM which can inhibit the oncogenic eIF4E:eIF4G interaction in CRPC. Notably, the screen demonstrated the compound's remarkable selectivity, as it did not affect “normal” prostate epithelial cells. This molecule also increases the tumor suppressive eIF4E:4EBP1 interaction which suggests that it may function as a molecular glue that decreases protein synthesis. Our findings reveal a novel clinically relevant small molecule that effectively targets the oncogenic eIF4E:eIF4G interaction, showcasing a potential breakthrough for CRPC treatment.

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37. Single Cell Analysis Reveals Upregulation of Immune Stimulatory Pathways in Quiescent Metastatic Castration Resistant Prostate Cancer Cells

Kristina G. Ibrahim, Cullen Hudson, Greg Shelley, Seeya A. Munj, Yating Wang, Elisabeth I. Heath, Julie L. Boerner, Russell S. Taichman, Evan T. Keller, Suzan Arslanturk, Laura A. Buttitta, and Frank C. Cackowski

Quiescent, or G_0 cell cycle phase, cells are common in most prostate cancer (PCa) cases, yet are relatively resistant to many treatments, especially cytotoxic chemotherapy. They also play key roles in tumorigenesis, and dormancy and recurrence after curative intent therapy, but are impossible to identify when viable. To identify characteristics of quiescent prostate cancer cells, we used previously published FACS methods to isolate cancer cells from bone or bone marrow of metastatic castration resistant prostate cancer (mCRPC) patients and generated single cell RNA sequencing (scRNA-seq) gene expression data of individually sorted cells. In order to develop a neural network mathematical model to identify G_0 cells in scRNA-seq datasets, we generated scRNA-seq data of PC3, C42B, and MycCaP cell lines grown in low and high serum conditions. Before sequencing, these cells were sorted by FACS into G_0 vs. G_1/S/G_2/M (not_G_0) populations using fluorescent markers for p27 and Cdt1, which are both positive in G_0. In the patient sample data, we first created a UMAP plot of putative cancer cells from five patients, PCa cell lines, and normal bone marrow cells as a negative control. We eliminated a priori any potentially contaminating cells based on cluster location, lack of expression of PCa markers (*NKX3.1*, *KLK3* / PSA, *AR*) and expression of bone marrow markers (*PTPRC* / CD45, and *GYPA* / CD235a), leaving 221 high confidence PCa cells. To identify characteristics of G_0 cells, we separated the patient cells into quiescent and cycling groups based on expression of MKI67 (Ki67). GSEA analysis of KEGG pathways revealed enrichment of primarily immune related pathways in quiescent cells with the top 3 pathways; "allograft rejection," "autoimmune thyroid disease," and "type I diabetes mellitus." Type 1 HLA gene, *HLA-A*, and type 2 HLA gene, *HLA-DR*, were common among multiple pathway groups suggesting potential differences in antigen presentation. Functional validation of these results and improvement of the neural network model for further G_0 vs. not_G_0 analyses are ongoing.

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38. Aneuploidy-associated SQLE Gain Promotes Prostate Cancer Aggressiveness by Altering Lipid Metabolism

Thomas Janas, Konrad H. Stopsack, Daniel R. Schmidt, Duanduan Ma, Zhe Li, Matthew G Vander Heiden, Kathryn L. Penny, Paul A. Scheet, Tamara L. Lotan, Angelika Amon, Lorelei A. Mucci, Xiaofeng A. Su

Prostate cancer (PCa) is the second leading cause of cancer-related death in men in the US. Epidemiology studies on primary PCa cohorts in Physicians' Health Study and Health Professionals Follow-up Study (PHS and HPFS) have shown that high levels of whole-genome aneuploidy, featured by imbalanced chromosome numbers, correlate with lethal progression in PCa. However, details of the mechanisms of how aneuploidy drives PCa aggressiveness are still unclear. Here, we used the case of chromosome 8q (chr 8q, the long arm of chr 8) gain to study aneuploidy-associated prostatic malignancies. Chr 8q gains are the most frequent gain events that occur in approximately 23% of PCa cases. By using the PHS and HPFS cohorts, we modeled the increased expression of each gene located on chr 8q, for predicting the risks for lethal progression, and obtained each corresponding gene's odds ratio (OR). By ranking the ORs, we revealed that a cholesterol biosynthesis gene, squalene monooxygenase (SQLE), is one of the top associators with lethal progression, amongst all chr 8q genes. SQLE plays a pivotal role in cholesterol synthesis. Previous lymphoma studies have shown that loss of SQLE as a cause of cholesterol auxotrophy, and when SQLE is present, squalene alters the lipid profile and protects against oxidative cell death. In our experimental study, we have used normal and cancerous TMPRSS2-ERG-driven organoid models and found that over-expression of SQLE promotes formation of invasive structures and proliferation in cancer organoids. Interestingly, overexpression of SQLE decreased the protein levels of TMPRSS2-ERG, which appeared to be independent of androgen receptor levels. We also utilized the TMPRSS2-ERG positive VCaP cell line, which harbors gains of SQLE gene copies. We found that knocking down SQLE expression significantly upregulated ERG protein levels. Our recent study has shown that ERG (or other ETS) positive prostate cancers have a strong correlation with downregulation of fatty acid metabolism signature. We speculate that gain of SQLE can drive aggressiveness of prostate cancer by modulating lipid metabolism for growth and migration. Inhibition of SQLE could translate to better clinical outcomes regarding prostate cancer lethality.

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39. LSD1 Inhibition Transcriptionally Reprograms NEPC Exposing Unique Therapeutic Vulnerabilities

Sumer M. Jasmine, Timothy E. G. Kreuger, Susan L. Dalrymple, Lizamma Antony, Samuel R. Denmeade, W. Nathaniel Brennen

Therapeutics have been developed to target castration-resistant prostate cancer (CRPC) classified as adenocarcinoma (CRPC-Adeno). However, an increasing frequency of CRPC-Adeno tumors progress to the highly aggressive and lethal neuroendocrine prostate cancer (NEPC) by acquiring a neuroendocrine (NE) phenotype as a mechanism of therapeutic resistance. This transformation from CRPC-Adeno to NEPC requires distinct epigenetic reprogramming by lysine-specific demethylase 1 (LSD1), which can be exploited for development of novel therapies to target NEPC. LSD1 is upregulated in NEPC and promotes a stem-like transition state through lineage plasticity which enables the cancer cell to undergo NE differentiation. We have shown that LSD1 inhibition reduces the NE program, driving NEPC towards a non-neuroendocrine state through ASCL1 suppression and YAP1 re-expression. This less aggressive non-neuroendocrine state reveals unique vulnerabilities for the development of novel combination therapies to treat this lethal disease.

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40. Clinical and Functional Analyses of an African-ancestry Gain-of-Function HOXB13 Variant Implicated in Aggressive Prostate Cancer

Mayuko Kanayama, Yidong Chen, Daniel Rabizadeh, Lauren Vera, Changxue Lu, Sarah M. Nielsen, Emily M. Russell, Edward D. Esplin, Hao Wang, William B. Isaacs, Emmanuel S. Antonarakis, Jun Luo

Background: Heritable genetic risk factors play a significant role in prostate cancer etiology and clinical practice. Recent reports have uncovered a stop-loss *HOXB13* variant c.853delT (referred to as X285K) predisposing men of West African ancestry to prostate cancer. The clinical relevance and protein function associated with this inherited variant is unknown. Our study aims to determine the clinical relevance of *HOXB13* (X285K) in comparison with *HOXB13* (G84E) and *BRCA2* pathogenic/likely pathogenic (P/LP) variants, and to elucidate the oncogenic mechanisms of the X285K protein.

Methods: To determine the clinical relevance of *HOXB13* (X285K) in comparison with *HOXB13* (G84E) and *BRCA2* P/LP variants, real-world data from 21,393 men with prostate cancer undergoing the Detect Hereditary Prostate Cancer (DHPC) sponsored testing program from 2019-2022 (Invitae Corporation, San Francisco, CA) was analyzed. Genetic testing results were compared among patient groups according to self-reported race/ethnicity, Gleason scores, and AJCC stages using exact test. For evaluation of oncogenic functions associated with the X285K protein, cell-line models were subjected to RNA sequencing, ChIP sequencing, ATAC sequencing, and Western blot analyses.

Results: *HOXB13* (X285K) was significantly enriched in self-reported Black (1.01%) versus White (0.01%) patients. We observed a trend of more aggressive disease in the *HOXB13* (X285K) and *BRCA2* P/LP carriers than in the *HOXB13* (G84E) carriers. In in vitro functional analysis, replacement of the wild-type (WT) *HOXB13* protein with the X285K protein resulted in a gain of an E2F/MYC signature, validated by the elevated expression of Cyclin B1 and c-Myc, without affecting the androgen response signature. Elevated expression of Cyclin B1 and c-Myc was explained by enhanced binding of the X285K protein to the promoters and enhancers of these genes with resultant increased chromatin accessibility. The limitations of the study are the lack of complete clinical outcome data for all patients studied and the lack of a control population.

Conclusions: *HOXB13* X285K is significantly enriched in self-reported Black patients and X285K carriers detected in the real-world clinical setting have aggressive prostate cancer features similar to the *BRCA2* carriers. Functional studies revealed a unique gain-of-function oncogenic mechanism of the X285K protein in regulating E2F/MYC signatures. Our studies lend evidence to the pathogenicity of the X285K variant, which may factor in the variant classification framework to move the clinical classification of this variant from 'likely benign" to "likely pathogenic". Our findings underscore the clinical utility of germline genetic testing in patients with prostate cancer and provide new information to facilitate clinical interpretation of genetic testing findings in a population disproportionately affected by prostate cancer.

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41. Towards Identifying Genetic Determinants of Radioresistant Prostate Cancer

Sumeyra Kartal, Shana Trostel, Rosina Lis, Adam Sowalsky and Deborah Citrin

Background: Radiotherapy (RT) is a commonly used treatment modality for treating localized prostate cancer with curative intent. For patients with high-risk disease that is still organ-confined, androgen deprivation therapy (ADT) is often given concurrently with definitive RT to improve survival and reduce rates of recurrence. Beyond its impact on the transcriptional activity of the androgen receptor (AR), ADT synergizes with RT by interfering with AR-mediated DNA double strand break repair. Overall, ionizing radiation causes immunologic cell death by releasing tumor associated antigens, affecting the tumor immunophenotype, tumor microenvironment and stroma. In the current study, we aim to investigate the genetic and phenotypic changes associated with RT and radioresistance by comparing benign epithelial cells, tumor cells and stroma before and after RT ± ADT.

Methods: Tissue samples were prospectively collected before treatment and at time of clinical or biochemical recurrence in an observational study of patients with intermediate or high-risk prostate cancer treated with RT ± ADT. Out of a planned enrollment of 220 patients, we have selected the first 30 patients with recurrent prostate cancer for inclusion of our analysis. These cases have paired pre-treatment and post-treatment biopsies (both templated and US/MRI-fusion targeted). All H&E-stained slides were scanned and whole-slide images were annotated by a board-certified anatomic pathologist. Informative immunostains, including PTEN, ERG, and PIN-4 cocktail, were used to identify benign glands, malignant glands and stroma. Laser capture microdissection was used to capture regions for whole-transcriptome sequencing.

Results and conclusions: To date, we have completed annotation of 27 matched pairs of patient samples and performed laser capture microdissection on two pairs of pre- and post-treatment tissues. At the conference, we will provide an update on the patterns of gene expression we have observed by RNA-seq of these tissues. Our analysis will include differentially enriched pathways in radiated stroma and radiated benign glands relative to the corresponding pre-treatment tissue. Similarly, differentially enriched pathways in recurrent will be compared to baseline tumor samples to identify potential mechanisms of radiorecurrence in terms of genetic changes will aid in the identification of pre-existing resistance tumor clones and opportunities for precision systemic therapy.

Clinical trial: NCT01834001

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42. Notch Signaling Suppresses Neuroendocrine Differentiation and Alters the Tumor Immune Microenvironment in Advanced Prostate Cancer

Sheng-Yu Ku, Yanqing Wang, Maria Mica Garcia, Yasutaka Yamada, Kei Mizuno, Mark D. Long, Spencer Rosario, Loredana Puca, Martin K. Bakht, Varadha Balaji Venkadakrishnan, Brian D. Robinson, Andrés M. Acosta, Kristine M. Wadosky, David W. Goodrich, Himisha Beltran

Background: The underlying mechanisms driving prostate cancer lineage plasticity and trans-differentiation from adenocarcinoma to a neuroendocrine lineage are not fully understood. This lineage plasticity is associated with loss of *RB1* and *TP53* and expression of neuroendocrine and neuronal lineage markers including Achaete-scute homolog 1 (ASCL1) and Delta-like ligand 3 (DLL3), which are negative regulators in Notch signaling. Therefore, we hypothesize that downregulation of Notch signaling leads to neuroendocrine differentiation.

Methods: We evaluated the expression of Notch signaling genes by RNA-seq in public cohorts of prostate cancer. We generated GEMMs with constitutively expressing Nicd1 in both DKO (*Pten*/*Rb1*)-/- and TKO (*Pten*/*Rb1*/*Trp53*)-/- GEMMs. We introduced the active form of NOTCH2 (NICD2) in human NEPC prostate cancer organoids and generated in vivo xenograft models. We knockout NOTCH2 in 22Rv1 cells combing *RB1* loss. Nanostring Digital Spatial Profiling (DSP) and IHC were used to examine tumor lineages. Single-cell RNA sequencing was performed to profile immune cell composition in GEMMs.

Results: Gene expression profiles of human samples and mouse models of NEPC show significant downregulation of Notch signaling and which negatively correlates with NEPC signature. Overexpression of Nicd1 in AR low/negative DKO and TKO (Nicd1-DKO/TKO) GEMMs result in a transition from a predominantly AR-low NEPC phenotype towards an AR-positive luminal phenotype. Ectopic expression of NICD2 in human NEPC organoids suppresses neuroendocrine gene expression and significantly impaired tumor growth *in vivo*. NICD2-expressing NEPC tumor displays areas of adenocarcinoma phenotype with glandular differentiation, abundant cytoplasm, and prominent nuclei. IHC shows that NICD2-expressing PDXs exhibited three distinct lineages: luminal lineage (KRT8+/SYP-/INSM1-), NE lineage (KRT8-/SYP+/INSM1+), and transition (KRT8+/SYP+/INSM1-). DSP analysis revealed that areas of luminal lineage lose neuronal developmental genes and upregulate genes enriched in human benign prostate. AR expression and activity, however, is not rescued. *ASCL1* knockout also restores Notch signaling and suppresses neuroendocrine differentiation. Inhibition of Notch signaling in CRPC cells, however, is not efficient to induce neuroendocrine differentiation. Interestingly, Notch expression in both GEMMs and human NEPC organoids upregulate MHC class I/II genes and enrich for type I interferon response, suggesting that activation of Notch signaling in NEPC suppresses neuroendocrine differentiation and might influence immunogenicity

Conclusions: Notch signaling is suppressed in neuroendocrine prostate cancer resulting. We demonstrate that Notch signaling inhibits NEPC growth, suppresses neuroendocrine differentiation, and alters tumor immune microenvironment. Our study provides new insights of Notch signaling in influencing tumor lineages of prostate cancer.

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43. BET Bromodomain Inhibition Blocks Survival of Prostate Cancer Models of Lineage Plasticity

Joshua A. Kuleape, Diana Flores, Chao Zhang, Canping Chen, Eva Rodansky, Anbarasu Kumaraswamy, Raymond Cavalcante, Zhi Duan, Zheng Xia, Joel A. Yates, Joshi J. Alumkal

Background: At diagnosis, prostate cancer adenocarcinomas are dependent on the androgen receptor (AR) for proliferation and maintaining luminal differentiation. AR signaling inhibition is the principal treatment for this disease, but resistance is nearly universal. An increasingly appreciated resistance mechanism is lineage plasticity, exemplified by loss of AR signaling and a switch from a luminal to an alternate differentiation program. Neuroendocrine prostate cancer (NEPC) is the most virulent form of prostate cancer lineage plasticity, for which there are limited treatment options. Loss of the tumor suppressors *TP53* and *RB1* is ubiquitous in NEPC, and genetically engineered mouse models deficient in *TP53*/*RB1* have shown that loss of these factors promotes NEPC lineage plasticity and tumor aggressiveness, recapitulating human disease. However, the molecular mechanisms that explain how *TP53*/*RB1* loss promotes NEPC are largely unknown. Our prior work demonstrated that BET bromodomain inhibition (BETi) is a promising approach to block a neuronal survival program in NEPC tumors. Importantly, a subset of NEPC patients experienced prolonged tumor control in our prior BETi clinical trial. However, mechanisms of BETi anti-tumor activity in NEPC and putative resistance mechanisms are unclear.

Methods: Using *TP53*/*RB1*-knockout vs. intact mouse cell line models, we performed RNA-seq and ATAC-seq to identify chromatin accessibility and transcriptional changes induced by *TP53*/*RB1* loss. We treated *TP53*/*RB1*-knockout or -loss cell lines in vitro to determine sensitivity to BETi. To understand pathways modulated by BETi treatment of *TP53*/*RB1*-loss cells, we performed RNA-seq pathway analysis. Finally, we treated a *TP53*/*RB1*-loss NEPC patient-derived xenograft model and measured anti-tumor activity.

Results: *TP53*/*RB1* loss leads to global chromatin accessibility changes associated with gene activation, including in epithelial to mesenchymal, neuronal differentiation, and neuronal developmental pathways. Importantly, RNA-seq pathway analysis demonstrates that BETi treatment blocks induction of many of these pathways induced by *TP53*/*RB1* loss. Finally, BETi treatment suppresses growth of NEPC models both in vitro and in vivo and a subset of NEPC tumors in patients, suggesting these tumors may be particularly susceptible to BETi.

Conclusions: Our results provide insights on how *TP53*/*RB1* loss promotes lineage plasticity. Further, our work suggests BETi is a promising approach to block induction of the program induced by *TP53*/*RB1* loss and to block NEPC tumor growth. Currently, we are clarifying factors activated by *TP53*/*RB1* loss that cooperate with BET bromodomain proteins and attempting to identify rational combination strategies to augment the anti-tumor activity of BETi.

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44. Identifying LSD1 Co-Targeting Approaches by CRISPR Screen to Target Neuroendocrine Prostate Cancer

Anbarasu Kumaraswamy, Zhi Duan, Olivia A. Swaim, Chao Zhang, Karan Bedi, Asmita Bhattacharya, Michael Bassik, Joel A. Yates, Joshi J. Alumkal

Lysine-specific demethylase 1 (LSD1) is a histone demethylase that promotes stemness and cell survival in cancers such as prostate cancer. Most prostate tumors are adenocarcinomas that express a luminal differentiation program. However, a subset undergoes cellular reprogramming, through lineage plasticity (LP) to an even more lethal phenotype with neuronal differentiation called neuroendocrine prostate cancer (NEPC). Importantly, the incidence of LP is increasing since the more widespread use of potent androgen receptor signaling inhibitors, and there are no effective treatments for such tumors. Loss of *TP53* and *RB1* through genomic or non-genomic mechanisms is nearly universal in NEPC. However, there are no effective therapies to treat NEPC or overcome the reprogramming induced by *TP53*/*RB1* loss.

We previously determined that LSD1 suppresses *TP53* function post-translationally to promote prostate cancer cellular reprogramming and survival of NEPC cells that have undergone LP. However, co-targeting approaches to enhance the anti-tumor activity of LSD1 inhibition in *TP53*/*RB1*-null tumors are unknown.

To identify combinatorial strategies, we performed a high throughput CRISPR screen focused on targets of FDA approved drugs, kinases, and phosphatases that can be readily targeted with small molecule inhibitors in *TP53*/*RB1*-null NEPC cells. Importantly, cells in the screen were treated with vehicle or LSD1 inhibition. Guide RNAs (gRNAs) targeting pan-essential genes or those linked to *TP53*/*RB1*-loss induced reprogramming were depleted in both conditions, However, we also identified unique gRNAs only depleted in the LSD1 inhibitor condition, suggesting these may by rational genes to target along with LSD1 inhibition. Work validating the importance of specific genes is ongoing.

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45. Functional Interrogation of the AR Enhancer Using Cas9 Base Editors

Shin-Ai Lee, Rachel Xiang, Matt Kipp, David Takeda

The most frequent genomic alteration in castration resistant prostate cancer is amplification of AR locus. It was recently found that an enhancer of the AR is co-amplified with the AR gene body in the majority of metastatic CRPC tumors, resulting in AR overexpression and treatment resistance. However, the mechanism underlying the activation of AR enhancer is largely unknown. Here, we performed high-resolution base editor screens to map the AR enhancer at the single nucleotide level. 5 regions within the enhancer were identified to be potentially essential for the function of the enhancer, and we demonstrated that each region contributes to the cell proliferation and AR transcription to a different extent in metastatic prostate cancer cell. Notably, single cell-derived clones harboring point mutations within the one of these regions showed dramatically impaired AR transcription. By performing H3K27Ac ChIP-seq and ATAC-seq, we observed a significant loss of histone acetylation and chromatin accessibility across the entire AR enhancer in these clones. Collectively, our work identifies functionally critical regions within the AR enhancer and provides evidence that a change in the single nucleotide of a noncoding regulatory element can affect the overall chromatin features of the regulatory element.

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46. Predicting Gene Expression from Cell-free DNA Whole-Genome Sequencing

Weiling Li, Marjorie Roskes, Alexander Martinez-Fundichely, Sandra Cohen, Ekta Khurana

When a cell dies, it releases cell free DNA (cfDNA) into the bloodstream. Nucleosome protected regions survive in plasma, while nucleosome depleted regions are degraded. Genome-wide studies have shown that nucleosome-depleted regions (NDRs) are present at the transcription start sites of active genes and enhancers. This results in lower depth of sequencing coverage near transcription start sites (TSSs) and diversity of cfDNA fragment size values observed from cfDNA whole-genome sequencing (WGS). Thus, we incorporated six cfDNA features in a support vector regression model for gene expression prediction. For training, we used cfDNA WGS and RNA-Seq profiling of peripheral blood mononuclear cells (PBMC) from a healthy participant. We then tested our model on 14 prostate cancer patients with RNA-seq and cfDNA WGS at matched time point. The concordance was evaluated using Pearson correlation between predicted gene expression and gene expression observed from RNA-seq of tissue site/s. 12 out of 14 test samples show good correlations ranging from 0.5 to 0.65 (random expected would be zero). We also compared our results with the most recent gene expression prediction paper (EPIC-Seq [Esfahani et al, 2022]). Every sample shows a higher correlation with our model than EPIC-Seq (when without grouping), and most of correlations in our model are higher than EPIC-Seq (when grouped by 10). Our method can be used for metastatic patients of different cancer types to predict tumor gene expression..

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47. Shed Trop2 Drives Prostate Cancer Progression and Trop2 is a Novel Tissue Prognostic Biomarker and a Candidate Urinary Marker for Prostate Cancer

Shiqin Liu, Sarah J. Hawley, En-Chi Hsu, Christian A. Kunder, Michelle Shen, Merve Aslan, Fernando Jose Garcia Marques, Chung S. Lee, Abel Bermudez, Lennart Westphalen, Heidi Auman, Lisa F. Newcomb, Donna Peehl, Daniel W. Lin, Peter S. Nelson, Ziding Feng, Maria S. Tretiakova, Lawrence D. True, Funda Vakar-Lopez, Peter R. Carroll, Jeffry Simko, Martin E. Gleave, Dean A. Troyer, Jesse K. McKenney, Sharon J. Pitteri, James D. Brooks, Michael A. Liss, Tanya Stoyanova

Distinguishing indolent from clinically significant localized prostate cancer and treatment of metastatic prostate cancer are two major clinical challenges in prostate cancer. The development of novel predictive biomarkers will help with risk stratification, and influence clinical decision-making between treatment and active surveillance, leading to a decrease in over or under-treatment of patients with prostate cancer. Here, we report that Trop2, an oncogenic transmembrane surface protein, is a prognostic tissue biomarker for clinically significant prostate cancer by utilizing the Canary Prostate Cancer Tissue Microarray (CPCTA) cohort composed of over 1100 patients from a multi-institutional study. We demonstrate that higher Trop2 expression is correlated with worse clinical features and elevated Trop2 expression at radical prostatectomy predicts worse overall survival in men undergoing radical prostatectomy. Additionally, we detected shed Trop2 in urine from men with clinically significant prostate cancer. We further define the functional role of shed Trop2 on metastasis in prostate cancer and identify that shed Trop2 increases cell migration, invasion, metastatic colonization, and spontaneous metastasis *in vitro* and *in vivo*. Proteomic profiling reveals that shed Trop2 modulates a set of proteins associated with invasion, migration, mTOR signaling, and epithelial-to-mesenchymal transition. Shed Trop2 binds to EGFR and results in the activation of the EGFR-PI3K-AKT-mTOR pathway in prostate cancer. Our study reveals the new function of shed Trop2 in driving prostate cancer progression and identifies Trop2 as a novel tissue prognostic biomarker and a candidate non-invasive marker for prostate cancer that could be used to optimize treatment decision-making.

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48. Developing TCR Immunotherapy Targeting Late-Stage Prostate Cancer

Zhiyuan Mao, Xiaojing Chen, Doyeon Koo, Weixian Deng, Jami McLaughlin, Miyako Noguchi, Lisa Ta, Pavlo Nesterenko, James A. Wohlschlegel, Dino Di Carlo, K. Christopher Garcia, Owen N. Witte

Late-stage prostate cancer is an incurable disease with no effective therapy currently available. 20-30% of patients received local therapy will experience disease relapse. The rise in serum prostate-specific antigen (PSA) level in these patients is often described as biochemical recurrence. This stage of prostate cancer, when micro-metastasis has occurred and overall tumor burden is low, can be a critical time window for cell-mediated immunotherapy. We aim to develop T cell receptor (TCR) immunotherapy targeting prostatic acid phosphatase (PAP) to treat patients with chemically recurrent prostate cancer. Elevated expression of PAP is commonly observed in early and late stages of prostate cancer. PAP was previously used to develop the first FDA-approved cancer vaccine, Provenge, but the specific epitopes and cognate TCRs were not clearly defined. Our group has profiled the immunopeptidome of PAP on HLA-A*02:01 using a secreted MHC-based platform (ARTEMIS), and successfully isolated multiple TCRs reactive with PAP. Recent results have also demonstrated that further engineering with “catchbonds” on those candidate TCRs lead to dramatically improved cytotoxicity. This work demonstrated the feasibility of developing TCRs targeting PAP. Epitopes and TCRs on multiple high-frequency HLA alleles need to be defined to cover a larger cohort of patients from diverse ethnic groups, especially African American patients, who tend to have worse prognoses. Six HLA types are needed to cover >90% of patients worldwide. One of the major bottlenecks in isolating TCRs is the efficiency of currently available techniques. An improved workflow is needed to rapidly define TCRs and their cognate epitopes with high specificity and sensitivity. We recently developed a platform to discover new TCRs using hydrogel nanovials decorated with pMHC monomers and effector cytokine capture antibodies. This nanovial workflow has enabled multiparametric definition of TCRs by incorporating measurement of a combination of key parameters required to define high quality TCRs: surface markers, secreted effector molecules, pMHC-TCR affinity/avidity, and epitope information. With this nanovial platform we have been able to isolate reactive T cells with high specificity, sensitivity, and accuracy. We aim to apply this newly developed nanovial technique to isolate and define TCR candidates targeting PAP epitopes on high-frequency HLA alleles.

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49. Convergent Evolution of Extrachromosomal DNA Drives Therapy Resistance in DNA Repair-deficient mCRPC

Thaidy Moreno, Meng Zhang, Arian Lundberg, Raunak Shrestha, Martin Sjöström, Anupama Pasam, Joanna Chan, Adam Foye, Alana S. Weinstein, Anna Trigos, Alexander W. Wyatt, Joshi J. Alumkal, Eric J. Small, Rahul Aggarwal, Felix Feng, Shahneen Sandhu, David A. Quigley

Targeted cancer therapies prolong the lives of men with metastatic castration resistant prostate cancer (mCRPC). However, these treatments also selectively favor the growth of tumor cells that can resist targeted therapy, and mCRPC is currently lethal. It has been challenging to study the development of therapy resistance in this setting because few autopsy studies have been performed in the settings of DNA-repair deficient mCRPC. Here, we assessed how resistance to targeted cancer therapies evolved in an autopsy cohort of 53 mCRPC tumors from six such men using deep whole genome and transcriptome analysis, validating our observations in an independent cohort of 135 mCRPC tumors. We identified intra-patient heterogeneity in clinically actionable DNA repair deficiencies and transcriptionally-defined tumor subtypes. Identical polygenic DNA repair resistance mutations were present in physically distinct tumors within the same individual, suggesting that these mutations pre-exist selection by later targeted therapy. Extra-chromosomal DNA (ecDNA) was present in more than half of mCRPC biopsies and frequently amplified the androgen receptor (*AR*) and enhancers of *AR* and *MYC*. Individual ecDNA amplicons included multiple driver genes on different chromosomes, and arose multiple times within distinct tumors in a single patient. The presence of ecDNA was significantly associated with whole genome doubling, chromothripsis, and with inactivating *TP53* alterations. We conclude that ecDNA amplification is a major contributor to therapy resistance in mCRPC and that late-stage mCRPC develops intra-patient heterogeneity in response to targeted therapy.

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50. Alterations in Glycolytic Pathway Enzymes and Metabolic Plasticity in Prostate Cancer Progression

Mika Munari, Cynthia Sprenger, Shihua Sun, Kathryn Soriano Epilepsia, Stephen R. Plymate

Background: Although oxidative phosphorylation (OXPHOS) is the main ATP source in primary prostate cancer, as the tumor progresses to castration resistance and/or metastatic disease, glycolysis becomes increasingly prevalent. Dependence on glycolysis can further increase under hypoxic conditions found in advanced cancers. We previously demonstrated that a novel small molecule, BKIDC-1553, inhibits proliferation and tumor growth of prostate cancer cell lines and xenografts, through a hexokinase-2 (HK2) dependent inhibition of glycolysis. HK2 is a glucose 6-phosphorylating enzyme that works in conjunction with glucose transporter-1 (GLUT1) to drive glycolysis in cells. While our previous work suggests that BKIDC-1553 works primarily through a HK2 dependent mechanism, the objective of this current project is to further examine how expression and function of HK2 and GLUT1 are altered in response to BKIDC-1553 treatment.

Methods: Hypoxic conditions are common in late-stage prostate cancer. Effects of hypoxia on cell proliferation and expression levels of HK1, HK2, and GLUT1 were examined with and without BKIDC-1553 treatment. To examine role of HK1, HK2, and GLUT1 in response to BKIDC-1553 treatment, we created CRISPR knockouts and shRNA knockdowns of HK1 and HK2 in the LNCaP prostate cancer cell line. GLUT1 was inhibited using a commercially available inhibitor, BAY-876. PCR and Western blots were used to confirm expression levels of HK1, HK2, and GLUT1. MTS proliferation assays were used to examine effect of KO and KD on response to BKIDC-1553 and BAY-876.

Results: GLUT1 levels, but not HK1 or 2, increased at 72 hrs under hypoxic conditions. HK1, HK2, and Glut 1 levels all increased in cells in response to 144 hrs of BKIDC-1553 compared with 72 hrs treatment in both normoxic and hypoxic conditions. The KO and KD studies were all conducted in normoxic conditions. Westerns blots demonstrated that HK2 KO, but not HK1 KO, results in increased levels of GLUT1. HK1 or HK2 KD, however, did not alter GLUT1 levels. HK2 KD cells, with GLUT1 levels remaining the same, displayed less growth inhibition than control cells in response to BKIDC-1553 treatment, while HK2 KO cells, with increased levels of GLUT1, remained sensitive to BKIDC-1553. Because GLUT1 levels increase in response to HK2 KO, we wanted to see if inhibiting GLUT1 in these cells could decrease their proliferation. We found that KO cells were indeed more sensitive to growth inhibition of BAY-876.

Conclusion/Summary: These studies suggest that KO of HK2 increases levels of GLUT1. These cells remain sensitive to BKIDC-1553 treatment, which could in part be due to the cells continued reliance on glycolysis through increased GLUT1. A continued reliance on glycolysis allows BKIDC-1553 to continue to inhibit cell growth. Future studies examining how combination treatment with BKIDC-1553 and BAY-876 affects growth of advanced prostate cancer cells is underway.

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51. Proteolytically Activated Membrane Binding Probes as Targeted Cancer Theranostics

Apurva Pandey, Garima Arvikar, Conner Bardine, Ningjing Zhang, Fiona Quimby, Charles Craik, Michael Evans

The recent FDA approvals (Lutathera, Azedra, Pluvicto) and the swell of promising experimental agents in clinical trials underscore the surging enthusiasm to investigate molecularly targeted radiotherapy (TRT) as a treatment modality for cancers. However, tumor responses to TRTs are often transient and/or variable among patients. Thus, there is an urgent unmet need to develop new strategies to maximize the therapeutic benefit of TRT for cancer patients. For the past several years, the nuclear medicine field has prioritized developing low MW small molecule or peptide radioligands (RLTs) that rapidly exit the bloodstream to minimize host toxicity. However, tumoral responses to RLTs are limited by several factors, including heterogeneous target expression among tumors, dissociation or degradation of ligand/receptor complexes, and incomplete target saturation due to low mass doses and infrequent repeat dosing. Thus, exploring new strategies beyond RLTs for the tumoral delivery of radioisotopes is a worthwhile goal.

We have approached this challenge by developing a new class of radiopharmaceuticals termed "restricted interaction peptides" (RIPs) which are linear and unstructured low MW peptides that are internally cleaved by a tumor endoprotease of interest to unmask a radiolabeled, helical membrane binding peptide. Once liberated, the radiolabeled helical peptide immediately and irreversibly attaches to a nearby phospholipid membrane in the tumor. Using PET, we have found that RIPs may have several properties advantageous for TRT, including catalytic amplification of tumor uptake and long persistence of the radioisotope in tumors due to the stability of the peptide/lipid membrane interaction. Thus, RIPs offer an unusual combination of the desirable safety profile characteristic of a low MW RLT with a high tumoral uptake more typical of a large MW TRT. Collectively, these findings provide a strong scientific rationale to test for the first time if radiolabeled RIPs can be effectively leveraged to treat tumors. We have synthesized and evaluated the preclinical pharmacology of several RIPs as targeted radiotherapies. The early preclinical data suggest unique advantages for RIPs compared to RLTs.

Acknowledgments/Funding: ACS Mission Boost Grant, PCF Challenge Award, R01CA258297, UCSF Benioff initiative for Prostate Cancer Research

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52. Integrative Analyses Implicate Cooperation of ONECUT2 and Kaiso in Prostate Cancer Lineage Plasticity

Lillian M. Perez, Qian Yang, Chen Qian, Huixian Lin, Isra El-hussin, Sungyong You, Leigh Ellis, Stephen Freedland, Salma Kaochar, Isla P. Garraway, Moray J. Campbell, Clayton C. Yates, Michael R. Freeman

Prostate cancer (PC) health disparities among African American (AA) and European American (EA) men persist in screening, incidence, disease aggressiveness, and mortality. To address these disparities and improve outcomes for AA men, it is crucial to understand underlying mechanisms. Our lab identified the HOX/CUT transcription factor ONECUT2 (OC2) as a master regulator that drives PC metastasis and lineage plasticity. OC2 is expressed at highest levels in advanced disease, however it can also be active in primary PC, suggesting potential therapeutic opportunities for targeting this protein prior to the emergence of AR-independent lineage variants in castration resistance prostate cancer (CRPC). OC2 can be directly suppressed *in vivo* with novel small molecules developed by our group. ChIP-seq performed in 22Rv1 CRPC cells with enforced OC2 (OC2 OE) identified a high frequency of OC2 binding at sequence-specific (SS) motifs for the methyl-binding protein Kaiso (ZBTB33). Kaiso expression and activation are disproportionately linked to AA PC. Kaiso SS motifs were also highly ranked in ATAC-seq data derived from OC2-OE LNCaP cells. Integration of Kaiso LNCaP ChIP-seq data with OC2 LNCaP CUT&RUN data showed that Kaiso and OC2 co-binding was enriched in narrowly defined promoter regions of over 2,000 genes, suggesting the two proteins coregulate a large gene expression network. OC2 and Kaiso OE and knockdown RNA-seq data were computationally integrated to produce OC2 and Kaiso activity signatures. Application of these signatures to multiple datasets showed a high correlation between Kaiso and OC2 activities in CRPC and NEPC patient datasets, lineage plasticity genetically engineered mouse models, and human PC xenografts. Analysis of single-cell RNA-seq data from human PC indicates a high correlation between Kaiso and prostate specific antigen expression (PSA/KLK3) in primary PC but not CRPC. Collectively, these findings suggest an AR-independent coordinate role for Kaiso and OC2 in advanced disease. These results may be relevant to novel therapeutic opportunities relevant to PC in AA men.

Acknowledgments/Funding: NCI Prostate Cancer T32 training grant awarded to Cedars-Sinai

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53. Targeting Therapy Resistant Phenotypic Plastic Prostate Cancer with Ataxia Telangiectasia and Rad3-Related (ATR) and DNA Methyltransferase Inhibitors

Daniel A. Petkovich, Praveen Koganti, Beatriz Germán, Deborah L. Burkhart, Peter Wu, Atish D. Choudhury, Leigh Ellis

Prostate cancer (PCa) is the second leading cause of cancer-related deaths in men in the US. PCa initiation and progression is largely dependent on androgen receptor (AR) expression and function. Androgen-deprivation therapy (ADT) which can target both AR and the androgen biosynthesis pathways is the first-line therapy for PCa. However, ADT is not curative of metastatic disease and approximately 15 to 20% of patients exhibit therapy resistance involving independence of AR signaling (CRPC-AI). This phenotype referred to as phenotypic plasticity is associated with tumors displaying neuroendocrine-like features, stem or basal cell-like phenotype, altered kinase signaling, and characteristic epigenetic alterations. Preclinical and clinical data demonstrate that CRPC-AI is predominantly driven by the combinatorial loss-of-function (LOF) mutations of *PTEN*, *TP53*, and *RB* tumor suppressor genes. About 70% to 90% of CRPC-AI/small cell prostate cancer tumors with phenotypic plasticity acquire *RB* loss of function mutations and is currently the strongest prognostic marker for worst overall survival in patients; however, there are no therapeutic options to provide durable response in patients.

To discover genetic vulnerabilities that can be translated to therapeutics, we performed a genome-wide CRISPR/Cas9 knockout screen to identify genes essential for proliferation or survival using a Rb-deficient prostate cancer cell line derived from the DKO GEMM (PbCre;Pten$^{lox/lox}$;Rb$^{lox/lox}$).

We found that Rb-deficiency induced a genetic dependency on DNA damage repair (DDR) kinases including ATM, ATR, CHK1 (HR signaling). ATR inhibition in Rb-deficient cells was associated with prolonged DNA damage, increased double-strand DNA structures, and an interferon gene response indictive of viral mimicry. We validated the expression of two well-known interferon response genes by flow cytometry (PD-L1 and MHC-I) and demonstrated that their expression was dependent on Stimulator of Interferon Genes (STING). In addition, superior response to ATR inhibition was dependent on expression and function of wild-type TP53. Suspecting epigenetic reprograming, we focused on two major epigenetic programs mediating chromatin remodeling in phenotypic plasticity in prostate cancer that were highlighted from our CRISPR/Cas9 screen – polycomb gene repression and DNA methylation. Inhibition of EZH2 (polycomb) failed to rescue the viral mimicry phenotype (data not shown), whereas inhibition of DNA methyltransferase-1 (DNMT1) successfully rescued the interferon gene response associated with viral mimicry. Further, combination of ATRi and DNMT1i in models with TP53 LOF demonstrated drug synergy.

Currently, our data implies that targeting prostate cancers with RB1 LOF could be responsive to DDR targeted therapies, however this sensitivity is lost with prostate cancer progression involving TP53 LOF. Loss of TP53 promotes further tumor evolution via chromatin remodeling involving DNA methylation. Inhibition of DDR pathways and DNMT1 induces viral mimicry interferon response genes and provides proof of concept that this combination approach can promote potential tumor response to immunotherapy.

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54. Co-Targeting Androgen Receptor Signaling and DNA Damage Response in Castrate Sensitive Prostate Cancer

Patrick G. Pilié, Chuandong Geng, Ganiraju Manyam, Man-Chao Zhang, Shakuntala Kondraganti, Sanghee Park, Paul G. Corn, Timothy C. Thompson

Background: Though next generation androgen receptor signaling inhibitors (ARSi) have significantly improved outcomes for many men with prostate cancer, resistance to ARSi is inevitable; and this castrate resistant (CRPC), androgen indifferent disease is lethal. PARP inhibition (PARPi) has shown benefit for CRPC with certain DNA damage response (DDR) defects (DDR-D) via synthetic lethality. In addition, AR signaling regulates key DDR pathways, and PARP1 can be upregulated at time of progression to CRPC and neuroendocrine disease. Recent preclinical and clinical data has shown a subgroup of DDR-wildtype prostate cancer may benefit from combination ARSi and PARPi; however, there is significant heterogeneity in response to ARSi and/or PARPi, even within DDR-D molecular subgroups. Speckle-type POZ protein (SPOP) is important in DDR and maintenance of genomic stability, and missense mutations in SPOP are frequent in advanced prostate cancer. Our studies revealed that SPOPmut CRPC demonstrates upregulation of DNA damage and immunosuppressive non-canonical (NC) STING signaling in patients. We propose that the heterogeneity in outcomes for ARSi +/- PARPi, across and within molecular subgroups, is in part due to differences in compensatory DDR and replication stress pathways after the application of ARSi, as well as contribution from the balance of pro-tumor immunosuppression NC-STING signaling versus anti-tumor canonical STING signaling.

Methods: Using genomic and transcriptomic preclinical and patient tumor data, proteomics analysis and genetically modified cell line models, we demonstrate mechanistic links between DDR mutations, STING signaling alterations and sensitivity to ARSi plus PARPi combination therapy.

Results: Human and mouse stably transduced SPOPmut-expressing castrate-sensitive prostate cancer (CSPC) and CRPC models treated with ARSi alone and post-ARSi DDRmut patient tissues with retained tumor cells demonstrated increased DNA damage (yH2AX), replication stress (p-ATM, p-ATR, PARP1), and activation of immunosuppressive NF-κB signaling due to ARSi treatment. Specifically, ARSi treatment led to increased NC-STING signaling (p-p65 and IL-6) selectively (relative to vector control [VC] cells) in CSPC SPOPmut models compared to vehicle control. Interestingly, PARPi selectively reduced NC-STING signaling and IL-6 expression and increased canonical STING markers (p-STING and IFN-β) in CSPC. CSPC and CRPC SPOPF133V models demonstrated significantly increased cell growth inhibition in response to ARSi + PARPi combination treatment compared to ARSi or PARPi alone. Importantly, ARSi + PARPi treatment further suppressed NC-STING and markedly increased canonical STING markers in CSPC and CRPC SPOPmut models relative to PARPi alone, and alterations in the tumor microenvironment, including in myeloid cells, compared to vehicle control.

Conclusions: Combination therapy with ARSi and PARPi therapy can be effective for CSPC primarily through enhanced DNA damage and a shift in innate immune pathway toward anti-tumor canonical STING signaling. Biomarkers based on this mechanism are being assessed in prospective clinical trials underway of ARSi and PARPi in patients with hormone naïve advanced prostate cancer (NCT04947254 and NCT05367440).

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55. Glutamine Antagonist Prodrug JHU083 Reprograms Immunosuppressive Tumor-Associated Macrophages (TAMs) to Drive Tumor Immunity in Urologic Cancers

Monali Praharaj, Fan Shen, Alex J. Lee, Liang Zhao, Thomas R. Nirschl, Debebe Theodros, Alok K. Singh, Xiaoxu Wang, Kenneth M Adusei, Kara Lombardo, Raekwon A. Williams, Laura A. Sena, Elizabeth A. Thompson, Ada Tam, Srinivasan Yegnasubramanian, Edward J. Pearce, Robert D. Leone, Jesse Alt, Rana Rais, Barbara S. Slusher, Drew M. Pardoll, Jonathan D. Powell, Jelani C. Zarif

Glutamine metabolism in the tumor microenvironment is emerging as a critical regulator of immune-mediated anti-tumor responses. Using publicly available dataset of human bone metastatic prostate cancer, we establish that gene expression of key enzymes in glutamine metabolism are upregulated in tumor-associated macrophages (TAMs). JHU083, a novel pro-drug of glutamine antagonist 6-Diazon-5-oxo-L-norleucine (DON), led to potent tumor growth inhibition in both prostate and bladder cancer murine models. Further investigation found that JHU083 induced TNF, inflammatory, and mTORC1 signaling in TAMs. Functionally, reprogrammed TAMs enhanced tumor cell phagocytosis and reduced angiogenesis. Adoptive transfer of JHU083-reprogrammed TAMs or tumor infiltrated monocytes (TIMs) resulted in significant tumor growth inhibition. From a metabolism perspective, TAMs exposed to JHU083 exhibited features like increased glycolysis, broken TCA cycle and disruption in purine metabolism. Although the anti-tumor effect of glutamine antagonism was less dependent on T cell as demonstrated by T cell depletion, it promoted a stem cell-like phenotype in CD8+ T cells and decreased the Treg population. Lastly, JHU083 impaired glutamine metabolism in tumor cells, leading to downregulation of HIF-1α, c-MYC phosphorylation and ultimately, induction of tumor cell apoptosis.

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56. Identifying and Targeting Upregulated Histone Modifying Enzymes Involved in Prostate Cancer Cell Survival After Androgen Deprivation Therapy

Tanaya A. Purohit, Emily Schmitt, Marcelo Bigarella, Kayla Bahr, Sean Sardeson, Diana Garcia, Karla Esbona, Peter W. Lewis, John M. Denu, Wei Huang, Bing Yang, David F. Jarrard

Objective: The cornerstone of advanced prostate cancer (PC) is androgen deprivation therapy (ADT) which induces quiescence, senescence, but rarely apoptosis. Persistent cancer cells lead to castration resistance. Aberrant expression of specific histone modifying enzymes (HME) that regulate histone post-translational modifications and gene pathways may be important in cancer cell survival after ADT. We seek to identify upregulated HMEs in PC patients during early ADT, which are hypothesized to be essential for tumor cell survival and potential targets for synergistic inhibition.

Methods: RNA sequencing was used to evaluate the altered expression of 95 HMEs in 10 ADT-treated neoadjuvant(3mos) and 26 untreated high-risk PC samples. Upregulated HMEs in ADT-treated PCs were validated using two publicly available expression datasets (n=11; n=9) comparing ADT-treated and untreated PC tumors. Candidate HME protein expression was examined in ADT-exposed LNCaP cells by Western. A tissue microarray (TMA) was constructed from 32 ADT-treated and 29 untreated post-prostatectomy samples to evaluate EZH1/EZH2 expression with immunohistochemistry and automated imaging. LNCaP cells were exposed to the EZH1/2 FDA-approved inhibitor Valmetostat ± ADT and synergy calculated (Calcusyn™).

Results: Compared to untreated tumors, ADT-treated samples upregulate 18 candidate HMEs in RNA seq data. Analysis of post ADT-treated PC samples in two public datasets validated the upregulation of four HMEs: EZH1, MECOM, HDAC5, and PRDM16 ($p<0.1$). EZH1, a histone methyltransferase within the PRC2 complex, increases in LNCaP cells up to 2 weeks after ADT exposure. Comparing ADT-treated and untreated PC tumors reveal a persistence of EZH1 expression at 3mo after ADT (24 % vs 26%, respectively). Combination therapy with Valmetostat (a dual EZH1/EZH2 inhibitor) ± AST did not demonstrate a synergistic effect in LNCaP. Days to PSA recurrence negatively correlated with EZH1 expression in the ADT-treated PC tumors ($r=-0.22$; $p=0.04$) suggesting that high expression of EZH1 might predict worse patient outcomes.

Conclusions: We identified EZH1 as a HME with persistent/upregulated expression in PC after ADT treatment. EZH1 is a histone methyltransferase involved in preventing senescence that represents a potential enzyme target (when specifically targeted) when combined with ADT to improve tumor responses.

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57. ONECUT2 Activates Diverse Resistance Drivers of Androgen Receptor-Independent Heterogeneity in Prostate Cancer

Chen Qian, Qian Yang,* Mirja Rotinen, Rongrong Huang, Hyoyoung Kim, Brad Gallent, Yiwu Yan, Radu M. Cadaneanu, Baohui Zhang, Salma Kaochar, Stephen J. Freedland, Edwin M. Posadas, Leigh Ellis, Dolores Di Vizio, Colm Morrissey, Peter S. Nelson, Lauren Brady, Ramachandran Murali, Moray J. Campbell, Wei Yang, Beatrice S. Knudsen, Elahe A. Mostaghel, Huihui Ye, Isla P. Garraway, Sungyong You, Michael R. Freeman*

Androgen receptor- (AR-) indifference is a mechanism of resistance to hormonal therapy in prostate cancer (PC). Here we demonstrate that ONECUT2 (OC2) activates resistance through multiple drivers associated with adenocarcinoma, stem-like and neuroendocrine (NE) variants. Direct OC2 targets include the glucocorticoid receptor (GR) and the NE splicing factor *SRRM4*, which are key drivers of lineage plasticity. Thus, OC2, despite its previously described NEPC driver function, can indirectly activate a portion of the AR cistrome through epigenetic activation of GR. Mechanisms by which OC2 regulates gene expression include promoter binding, enhancement of genome-wide chromatin accessibility, and super-enhancer reprogramming. Pharmacologic inhibition of OC2 suppresses lineage plasticity reprogramming induced by the AR signaling inhibitor enzalutamide. These results demonstrate that OC2 activation promotes a range of drug resistance mechanisms associated with treatment-emergent lineage variation in PC and support enhanced efforts to therapeutically target OC2 as a means of suppressing treatment-resistant disease.

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58. Unleashing The Power of PIKfyve Inhibition Against Neuroendocrine Prostate Cancer

Yuanyuan Qiao, Yang Zheng, Xia Jiang, Sarah N. Yee, Caleb Cheng, Rahul Mannan, Yuping Zhang, Yuzhuo Wang, Arul Chinnaiyan

Therapies targeting the androgen receptor (AR) as the main driver of prostate cancer (PCa) can lead to various mechanisms of resistance and promote progression to castration-resistant PCa (CRPC), which has a median survival of only 13-23 months. Amongst recurrent CRPC, 17%-30% of patients develop neuroendocrine prostate cancer (NEPC), which is a PCa subtype characterized by a unique histology. NEPC exhibits a loss of AR signaling during neuroendocrine transdifferentiation which results in resistance to AR-targeted therapies and gain of cell characteristics resembling poorly differentiated neuroendocrine tumors. Despite advances in the understanding of NEPC development, treatment options remain limited, with platinum-based chemotherapy as the first-line treatment for both de novo and treatment-induced NEPC. However, response to first-line chemotherapy in NEPC is short, with a median survival of only seven months. The poor prognosis of NEPC is attributed in part to late diagnosis and a lack of effective therapeutic agents. Thus, development of new therapies targeting the emergent vulnerabilities of NEPC or AR-negative forms of PCa is an urgent need.

Our previous work demonstrated that inhibition of the lipid kinase PIKfyve via a small molecule ESK981 led to preferential tumor inhibition in NEPC and AR-negative PCa than AR-positive CRPC. Here, we intensively examined the dependency on PIKfyve in multiple preclinical NEPC CDX and PDX models including NCI-H660, LTL-352, LTL-545, LTL-610, LTL-331R. Results indicate that NEPC and AR-negative PCa indeed exhibit higher dependency on PIKfyve than AR-positive CRPC. Mechanistically, we hypothesize that the highly hypoxic tumor microenvironment of NEPC require PIKfyve for nutrient supply through functional lysosome. The therapeutic strategy of targeting PIKfyve in NEPC is confirmed by using a first-in-class PIKfyve specific PROTAC degrader that we have developed in house. Additionally, in AR-positive PCa, we discovered that AR antagonist can prime the AR-positive tumor to a state that is highly dependent on PIKfyve for survival. Here, we have demonstrated that the combined approach of AR and PIKfyve inhibition led to tumor regression in AR-positive PCa.

By translating our finding to clinical application, we propose PIKfyve inhibition as a monotherapy for NEPC and AR-negative PCa, and dual blockade of AR and PIKfyve in AR-positive CRPC. Given the heterogeneous AR status and tumor types in advanced prostate cancer patients, the combined therapeutic approach of dual targeting AR and PIKfyve holds great promise for enhancing patient outcomes.

Acknowledgments/Funding: Department of Defense

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59. Stearoyl-CoA Desaturase: A Key Modulator of Adipocyte-Driven Stress Pathways Supporting Survival of Metastatic Prostate Cancer in Bone

Shrila Rajendran, Shane Mecca, Mackenzie Herroon, Laimar Garmo, and Izabela Podgorski

Bone is the preferable site for metastatic prostate cancer (PCa). Despite advancements in chemotherapy and detection, the current treatment options for metastatic PCa are mainly palliative, and most patients succumb to skeletal disease. Bone metastatic niche is a uniquely harsh and complex microenvironment that contributes to chemoresistance and enhanced survival of tumor cells. Studies are needed to uncover the molecular mechanisms that drive tumor cell adaption in the bone marrow niche and identify new therapeutic targets for this devasting disease, and this research project aims to address this need.

Our previous studies have highlighted that adipocyte, an abundant cell type within the bone marrow, induce oxidative and ER stress pathways during adipocyte-tumor cell crosstalk as potential adaptation mechanisms to allow for survival in bone. My preliminary data have shown that high lipid peroxidation levels are initially induced in PCa cells during adipocyte exposure, while prolonged exposure to adipocytes decreases peroxidation levels in PCa cells, which coincides with the augmented expression of stearoyl-CoA desaturase (SCD). This ER-resident enzyme balances lipid-induced stress and has been shown to regulate the ER stress response. Comprehensive data analysis of the Oncomine database indicated that SCD and its transcriptional regulators SREPB1 and SREPB2, along with several genes in the desaturase pathways, are upregulated in patients with metastatic PCa. We have now gathered results showing that SCD inhibition in PCa cells exposed to adipocytes induces cytotoxic levels of ER stress and augments lipid peroxidation levels, indicating SCD is a key mediator of lipid-induced stress involved in tumor adaptation in the harsh bone marrow microenvironment. Interestingly, RNAseq analyses from PCa cell lines exposed to adipocytes and then subjected to SCD inhibition indicated changes in DNA damage sensing and repair pathways, hinting that SCD inhibition induces DNA damage, which we have confirmed by comet assays and p-H2AX immunofluorescence staining. Additionally, our data show that siRNA-mediated or pharmacological inhibition of SCD reduces the spheroid size of PCa cells in 3D culture when exposed to adipocytes. Our data also reveal that SCD expression levels correlate with the expression of ER stress gene, ATF4 in PCa cells, and SCD inhibition augments the ATF4-regulated enzyme asparagine synthetase (ASNS), which plays a role in glutamine metabolism. This suggests that metastatic PCa cells engage the desaturase and ER stress pathways to rewire tumor metabolism in support of adaptation and progression in bone. Together, our results indicate that SCD is a key enzyme in regulating lipid-mediated oxidative damage and stress while interacting with the ATF4 pathway to reprogram tumor metabolism. Our studies will identify new molecular targets for metastatic PCa and significantly increase the understanding of the role of bone marrow adiposity in cancer progression.

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60. Targeting DDX3 in AR Low/Double Negative Prostate Cancer

William A. Ricke, Teresa Liu, Len MacGillivray

Prostate cancer (CaP) driven by androgen receptor (AR) is treated with androgen deprivation; however, therapy failure results in lethal castration-resistant prostate cancer (CRPC). AR-low/negative (ARL/−) CRPC subtypes have recently been characterized and cannot be targeted by hormonal therapies, resulting in poor prognosis. RNA-binding protein (RBP)/helicase DDX3 (DEAD-box helicase 3 X-linked) is a key component of stress granules (SG) and positively and negatively affects protein translation. Here, we investigated DDX3-mediated posttranscriptional regulation of AR mRNA (messenger RNA) in CRPC.

Using patient samples and preclinical models, we quantified DDX3 and AR expression in ARL/− CRPC. We utilized CRPC models to identify DDX3:AR mRNA complexes by RNA immunoprecipitation, assess the effects of DDX3 gain/loss-of-function on AR expression and signaling, and address clinical implications of targeting DDX3 by assessing sensitivity to AR-signaling inhibitors (ARSI) in CRPC xenografts *in vitro* and *in vivo*. To assess the effects of pharmacologically targeting DDX3 in vivo, we administered RK33 (IP), a DDX3 inhibitor to adult male C57Bl/6 mice.

We observed in ARL/− CRPC AR mRNA positivity despite diminished levels of AR protein. DDX3 protein was highly expressed in ARL/− CRPC, where it bound to AR mRNA. Inhibiting DDX3 was sufficient to restore 1) AR protein expression, 2) AR signaling, and 3) sensitivity to ARSI in vitro and in vivo. Moreover, combination treatment with DDX3 inhibitors (RK33) with antiandrogens significantly ($P<0.05$) increased cell death (cCaspase) and decreased proliferation (Ki67). Mice treated RK33 had no frank changes in overall appearance or health and little change in AR protein within the prostate.

Our findings implicate the RBP protein, DDX3, as a mechanism of posttranscriptional regulation for AR in CRPC. Clinically, DDX3 may be targetable for sensitizing ARL/− CRPC to AR-directed therapies.

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61. Targeting the CBP/p300 Axis in Castration Resistant Prostate Cancer Impacts DNA Damage Repair Function

Sumaira Sardar, Lakshmi Ravindranath, Christopher McNair, Saswati N. Chand, Wei Yuan, Denisa Bogdan, Jon Welti, Adam Sharp, Matthew J. Schiewer, Lisa Butler, Johann de Bono, Kris Frese, Nigel Brooks, Neil Pegg, Karen E. Knudsen, Ayesha A. Shafi

Prostate cancer (PCa) is the second leading cause of cancer-related deaths in men in the US. The androgen receptor (AR), a hormone-activated transcription factor, plays vital roles in the development and progression of PCa. Thus, androgen-deprivation therapy (ADT) is a standard-of-care first-line therapy for metastatic PCa. Resistance to ADT leads almost uniformly to lethal disease, termed castration-resistant prostate cancer (CRPC). As such, there is a largely unmet clinical need to identify and develop novel strategies, that work either alone or in concert with AR-directed therapeutics, to combat CRPC. The highly conserved histone acetyltransferases CBP/p300 are potent co-activators for AR, and high p300 expression is associated with locally advanced disease and castration-resistant AR function. This study shows that CBP and p300 are highly expressed and correlate closely with AR gene expression and AR activity score in primary PCa and CRPC. Thus, it will be critical to determine the role of CBP/p300 in PCa in order to potentially develop novel therapeutic targets for precision medicine to enhance patient outcome. By employing clinically relevant PCa models, the clinical significance of CBP/p300 expression in PCa patients as well as mechanistic evaluation of CBP/p300 transcriptional reprogramming and DNA damage response pathways have been undertaken. Lastly, the molecular response to CBP/p300 inhibition will be assessed to discern novel metrics for precision medicine for PCa patients to improve therapeutic efficacy.

Previous studies have relied on non-specific compounds and genetic silencing to target CBP/p300 and its associated transcriptional machinery. In this study, CBP/p300 mediated bromodomain activity is targeted by CCS1477 (Inobrodib), a first-in-class bromodomain inhibitor developed by Cell Centric. CCS1477 was shown to demonstrate effective inhibition in growth and clonogenicity assays. Inhibition of the CBP/p300 bromodomain with CCS1477 resulted in significant downregulation of AR-FL, AR-V7, and its targets' mRNA expression in addition to inhibition of associated factors such as c-MYC and its downstream targets in PCa cell lines as well as patient derived xenograft (PDX) models. Transcriptional mapping identified CBP/p300 as regulators of cell proliferation and DNA repair processes, which were functionally confirmed across several PCa model systems. To assess relevance, exogenous challenge with radiation revealed that CBP/p300 bromodomain is required for AR-mediated DNA repair, and CBP/p300 expression is linked to DNA repair capacity in the clinical setting. Molecular analyses revealed that CBP/p300 facilitate double-strand break (DSB) repair efficiency via homologous recombination (HR) mediated DNA damage repair (DDR). Congruently, CBP/p300 strongly correlated with HR gene expression in PCa patient tissue. These collective findings reveal that CBP/p300 govern repair of DNA DSBs by regulating HR, thus modulating genome integrity and promoting CRPC growth. In sum, CBP/p300 inhibition mediates HR repair and impacts patient outcome.

In conclusion, these studies identify CBP/p300 as a driver of PCa tumorigenesis through coordinated control of critical transcriptional events and lay the groundwork to optimize therapeutic strategies for advanced PCa via CBP/p300 inhibition, potentially in combination with AR-directed therapies. Combined, these studies have the capacity for significant near-term impact in the prevention and/or management of metastatic disease.

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62. Assessment Of Cell Surface Targets In Metastatic Prostate Cancer: Expression Landscape And Molecular Correlates

Erolcan Sayar, Azra Ajkunic, Martine P. Roudier, Radhika A. Patel, Ilsa M. Coleman, Navonil De. Sarkar, Brian Hanratty, Mohamed Adil, Jimmy Zhao, Samir Zaidi, Lawrence D. True, Jamie M. Sperger, Heather H. Cheng, Evan Y. Yu, Robert B. Montgomery, Jessica E. Hawley, Gavin Ha, John K. Lee, Stephanie A. Harmon, Eva Corey, Joshua M. Lang, Charles Sawyers, Colm Morrissey, Michael T. Schweizer, Roman Gulati, Peter S. Nelson, Michael C. Haffner

Background: Cell surface protein targeting approaches have emerged as an important strategy for precision oncology. To understand the potential therapeutic benefit of drugs targeting cell surface proteins, an in depth understanding of the expression patterns of the target proteins in tumor tissues is required. While PSMA is currently the most frequently utilized cell surface antigen in prostate cancer, several emerging agents are undergoing pre-clinical and clinical investigation. Here, we investigate the expression patterns of trophoblast cell-surface antigen 2 (TROP2), delta-like ligand 3 (DLL3), and carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) in CRPC samples from a rapid autopsy cohort as part of the University of Washington Tissue Acquisition Necropsy (UW-TAN) cohort.

Methods: We determined the immunohistochemical expression of TROP2, DLL3 and CEACAM5 on a well-annotated tissue microarray (TMA) set consisting of over 800 samples from 52 rapid autopsy patients (with up to 20 metastases per case). In addition, somatic genomic and epigenetic alterations were correlated with cell surface marker expression.

Results: We show that DLL3 and CEACAM5 exhibit the highest expression in neuroendocrine prostate cancer (NEPC), while TROP2 is expressed across different CRPC molecular subtypes, except for NEPC. We observed variable intra-tumoral and inter-tumoral heterogeneity and no dominant metastatic site predilections for TROP2, DLL3, and CEACAM5. Co-expression analyses demonstrates frequent association of PSMA with TROP2 in AR+/NE- tumors and DLL3 with CEACAM5 in AR-/NE+ tumors. We further show that AR amplifications were associated with higher expression of PSMA and TROP2 but lower DLL3 and CEACAM5 levels. Conversely, PSMA and TROP2 expression was lower in *RB1*-altered tumors. In addition to genomic alterations, we demonstrate a tight correlation between epigenetic states, particularly histone H3 lysine 27 methylation (H3K27me3) at the transcriptional start site and gene body of TACSTD2 (encoding TROP2), DLL3, and CEACAM5, and their respective protein expression in CRPC patient-derived xenografts.

Conclusion: Collectively, these findings provide novel insights into the patterns and determinants of expression of TROP2, DLL3, and CEACAM5 with important implications for the clinical development of cell surface targeting agents in CRPC.

Acknowledgments/Funding: CPDR CORE Funds (Shafi), PCF Challenge Award (Knudsen, do Bono), Support from CellCentric (Frese, Brooks, Pegg).

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63. Tracking the Evolutionary Progression of Metastatic Prostate Cancer Using Recordable Barcodes

Serio, Ryan N.; Scheben, Armin; Lu, Billy; Gargiulo, Domenic V.; Patruno, Lucrezia; Buckholtz, Caroline L.; Jibilian, Megan C.; Persaud, Steven G.; Staklinski, Stephen J.; Ramazzotti, Daniele; Barbieri, Christopher E.; Siepel, Adam C.; Nowak, Dawid G.

Prostate cancer (PC) causes approximately 30,000 deaths in the United States each year, almost exclusively resulting from metastases. The substantial decrease in survival rate of metastatic patients underscores the urgency to delineate the changes that drive the transition between primary and specific metastatic sites to ensure optimal therapy for patients before the cancer advances to an incurable stage. We have designed a mouse model (EvoCaP) to aid in defining specific genetic vulnerabilities in primary tumors that may enable the interruption of metastases development, and potentially target already existing metastases. Our model is based on combined double knockout of *Pten* and *Trp53*. Loss of *Pten* and *Trp53* causes a robust Myc-dependent cancer phenotype that results in metastasis in approximately one-half of injected mice. We follow tumorigenesis and metastatic dissemination using bioluminescence and tumor clones are identified and dissected after 60 weeks using fluorescence. We have routinely detected metastases to liver, lymph nodes, lungs, and bone, recapitulating human disease. Our EvoCaP model contains a synthetic target array (Barcode) with diminishing gene editing potential that is amenable to alteration by CRISPR/Cas9 technology. Using analysis of edited Barcode by amplicon sequencing, we traced the lineages of metastatic lesions, generated phylogenetic trees, and used ecological diversity parameters to define the clonal landscape in primary tumors and metastases. These include intra-organ alpha diversity measured by Shannon Index to ascertain heterogeneity within a tumor and inter-organ beta diversity measured by Bray-Curtis Dissemination to determine mono- vs. polyclonality, timing of dissemination, and the site of clonal origin. We have found using Barcode analysis that prostate tumor metastases show polyclonal seeding and widespread intratumoral heterogeneity from the primary tumor. Migration paths revealed mainly seeding from the primary tumor to metastases with metastasis-to-metastasis seeding to a lesser extent. Detailed exploration of the molecular mechanisms underlying metastatic organotropism through the use of our model will lead to the identification of metastatic driver genes previously missed by conventional modeling systems.

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64. A Prostate Cancer Gastrointestinal Transcriptional Phenotype is Found in Clinical mCRPC Samples and may be Associated with Diminished Response to AR-targeted Therapy

Aishwarya Subramanian, Meng Zhang, Marina Sharifi, Thaidy Moreno-Rodriguez, Eric Feng, Nicholas R. Rydzewski, Raunak Shrestha, Xiaolin Zhu, Shuang G. Zhao, Rahul Aggarwal, Eric J. Small, Cornelia Ding , David A. Quigley, Martin Sjöström, Felix Feng

Background: Metastatic prostate cancer eventually becomes resistant to all therapies and is uniformly lethal; one mechanism of resistance is lineage plasticity, where the tumor undergoes a transformation to an AR-indifferent phenotype, most widely studied in the context of neuroendocrine prostate cancer (NEPC). However, activation of additional de-differentiation programs such a gastrointestinal (GI) gene expression circuit has been suggested as alternative methods of resistance. In this study, we aimed to explore the GI prostate cancer phenotype (PCa GI) in a large cohort of clinical metastatic castration-resistant prostate cancer (mCRPC) biopsy samples.

Methods: We interrogated a dataset of 634 mCRPC samples with batch effect corrected gene expression data from the West Coast Dream Team (WCDT), the East Coast Dream Team (ECDT), the Fred Hutchinson Cancer Research Center (FHCRC) and the Weill Cornell Medical center (WCM). The WCDT and ECDT had survival data annotated. A gene expression GI score was calculated using the sum of z-scores of genes from a published set of PCa GI defining genes (N=38), and survival analysis was performed using the Kaplan-Meier method and Cox proportional hazards regression.

Results: The GI score had a bimodal distribution, indicating the existence of a distinct set of tumors with an activated GI expression pattern. Approximately 35% of samples were classified as PCa GI which is concordant with prior reports. No correlation was observed between GI score and proliferation, AR signaling, or a NEPC score. However, MYC amplified tumors showed higher GI scores (p=0.0001). Patients with PCa GI tumors had a shorter survival (HR=1.5 [1.1-2.1], p=0.02), but not after adjusting for liver as metastatic site (HR=1.2 [0.82-1.7], p=0.35)., Patients with PCa GI low samples had a better outcome after androgen receptor signaling inhibitors (ARSI, abiraterone or enzalutamide) than other therapies (HR=0.2 [0.07-0.54, p=0.002) while there was no difference for PCa GI high samples (HR=1.1 [0.13-8.7], p=0.95, p interaction = 0.049).

Conclusions: The PCa GI phenotype is found in clinical mCRPC samples and may represent a distinct biological entity. PCa GI tumors may respond less to ARSI and could offer a strategy to study novel therapeutic targets.

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65. CDK12 Loss Promotes Prostate Cancer Development

Jean Ching-Yi Tien, Yunhui Cheng, Yuping Zhang, Rahul Mannan, Mindy Xiaoming Wang, Palak Shah, Sanjana Eyunni, Abhijit Parolia, Yu Chang, George Wang, Shuqin Li, Xuhong Cao, Fengyun Su, Rui Wang, Ke Ding, Arul M Chinnaiyan

Metastatic castration-resistant prostate cancer (mCRPC) is a uniformly fatal disease for which new treatment options are urgently needed. Current pharmacotherapies confer only limited lifespan extension, and do not specifically address mutational profiles of individual cancer sub-types. Our group previously defined biallelic inactivating mutations of the gene encoding cyclin-dependent kinase 12 (CDK12) in 7% of metastatic castration resistant prostate cancer (mCRPC). *CDK12* phosphorylates RNA polymerase II to transcriptionally regulate mRNAs involved in cell proliferation and DNA damage repair. *CDK12* mutant tumors comprise a new mCRPC subset that is genetically distinct from those defined by primary genetic drivers such as ETS fusions, *SPOP* mutations, homologous repair deficiency (HRD), and mismatch repair deficiency (MMRD). Nonetheless, *CDK12* inactivation overlaps with other mCRPC-related mutations, including inactivation of *P53*. We hypothesized that *CDK12* loss drives prostate cancer formation, while enhancing progression of tumors lacking functional P53.

To test the hypothesis, we first intercrossed mice harboring floxed *Cdk12* loci with animals expressing the androgen responsive probasin Cre recombinase—therein conditionally ablating *Cdk12* in the prostate epithelium. These $Cdk12^{-/-}$ animals had phenotypically normal prostates in young adulthood, but, by 1 year of age, developed pre-neoplastic lesions characterized by basal cell hyperplasia, increased DNA damage (gH2AX staining) and T-cell-predominant immune infiltrates similar to those seen in human tumors with CDK12 inactivation. We next generated basal cell-derived organoids from the $Cdk12^{-/-}$ prostate. Compared to organoids with intact *Cdk12*, *Cdk12-/-* organoids displayed a dramatic phenotype: lacking lumens and exhibiting basal-luminal disorganization. Compared with wild type organoids, $Cdk12^{-/-}$ organoids showed increased proliferation in androgen replete and depleted settings. We next used CRISPR-Cas9 to ablate the *p53* gene in the $Cdk12^{-/-}$ organoid system. Compared with *p53*-null organoids, *p53*/ *Cdk12* double knockout organoids showed increased proliferation in vitro. Moreover, double knockout organoids formed adenocarcinoma-like tumors in an allograft system, while organoids lacking *p53* alone were unable to do so.

Acknowledgments/Funding: Department of Defense, UM Prostate SPORE, Prostate Cancer Foundation

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66. Use of a Novel, 3D Microscope with AI Improves Performance of Prostate Needle Biopsies

Lawrence D. True, Lab of Jonathan Liu, Lab of Anant Madabushi, 3D GU pathologists (see full author list in acknowledgments/ funding seciton)

Background: The pathological diagnosis and grading of prostate cancer is based on a several 4 um thick sections of 1 mm thick core biopsies. Since these sections sample <!% of the biopsy tissue, important features may be missed. And, since the images are 2D, 3D structures of clinical importance cannot be seen.

Methods: Tissue (fresh or fixed) is stained with fluorescence analogues of H&E, optically cleared and digitally imaged with a two laser microscope using refractive index-matched optics. Virtual immunostains for low and high MW keratin made by AI algorithms enable segmentation of images to distinguish glands from stroma and, potentially (work in progress) cancer from benign glands.

Results:

- A set of 12 needle core biopsies can be diagnosed and graded within an hour.
- AI algorithms enable creation of virtual immunohistochemical stains for keratins 5 and 8 to make more efficient viewing >200 virtual levels of core biopsies.
- The extent of heterogeneity of grade in each biopsy is extensive (kappa 0.34 – 0.43).
- Of histological elements not seen on routine levels:
    - 13% of biopsies had the cribriform variant of Gleason pattern 4
    - 4% of biopsies had cancer
- Novel 3D features associated with biochemical recurrence after radical prostatectomy:
    - Gland to convex hull ratio
    - Lumen boundary curvature
    - Cancer & stromal cell nuclear volume

Additional results:

- No tissue is consumed, enabling macrodissection of cancer-rich areas for sequencing.
- Histological findings can be stored forever as large (terabytes in size) digital images.

Acknowledgments/Funding: ***GU pathologists*** –Richard Colling, Michelle Downes, Xavier Farre, Pedro Fernandez, Peter A. Humphrey, Andrew Janowczyk, Priti Lal, Tuomas Mirtti, Nicholas Reder, Lawrence True, Funda Vakar-Lopez, Clare Verrill.

Research group of Jonathan Liu – Kevin Bishop, Gan Gao, Adam Glaser, Sarah Chow, Vanessa Roybal, Rob Serafin, Weisi Xie, et. al.

Research group of Anant Madabhushi – Hisham Abdeltawab, Germán Corredor, Rohan Dhamdhere, Jennifer Salguero, Kamal Hammouda, Can Koyuncu, Sebastian Medina, Pushkar Mutha, Tilak Pathak, et al.

Canary Foundation – Sarah Hawley

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67. Nuclear Export Inhibitor Cooperates with PARP Inhibitor to Suppress the Growth of Metastatic Castration Resistant Prostate Cancer In Vivo

Md. Hafiz Uddin, Amro Aboukameel, Husain Y. Khan, Sahar F. Bannoura, Laiba N. Monir, Sarah Motorwala, Khaled Keffri, Medha Jasti, Rachael Virga, Frank Cackowski, Rafic Beydoun, Gregory Dyson, Seongho Kim, Julie Boerner, Vinod Shidham, Amr Mohammed, Bassel El-Rayes, Herbert Chen, Anthony Shields, Khalil Choucair, Eliza W. Beal, Miguel Tobon, Donald Weaver, Steve Kim, Mohammad Najeeb Al-Hallak, Philip A. Philip, Ramzi M. Mohammad, Boris C. Pasche, Asfar S. Azmi, Elisabeth I. Heath

Aberrant nuclear protein transport, often observed in cancer, causes mislocalization-dependent inactivation of critical cellular proteins. Earlier we showed that overexpression of nuclear export protein exportin 1 (XPO1) is linked to higher grade and Gleason score in metastatic castration resistant prostate cancer (mCRPC). The network topology computational approach (NTCP) determined a higher synthetic lethal score between XPO1 and poly (ADP-ribose) polymerase (PARP1). We showed that selective inhibitor of nuclear export (SINE) could synergized with PARP inhibitors in mCRPC cell lines. Here we evaluated the efficacy of SINE and PARP inhibitor (PARPi) combination in cell line derived as well as patient derived xenograft (CDX and PDX) models and deciphered the mechanism of synergy.

For CDX model, the 22rv1 mCRPC cells were grown as subcutaneous xenografts in ICR-SCID male mice. For PDX model, tissue was collected from Champions Oncology (CTG-3581) and grown subcutaneously in CEIA/NOG male mice. SINE dosed orally at 10-15 mg/kg twice a week and PARPi dosed orally at 50 mg/kg daily. For in vitro mechanistic study, 22rv1 cells were subjected to RNAseq and proteomic analysis after treatment. Data analysis was performed using iPathwayGuide (advaitabio.com).

The CDX and PDX showed pronounced anti-cancer efficacy by this combination compared to single agents without any significant weight loss. Survival analysis in CDX model demonstrated enhanced benefits to the mice of combination group. Immunohistochemistry (IHC) revealed apoptotic cell death in the combination group which is evident from cleaved caspase 3 staining and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). To decipher the mechanism of synergy we performed transcriptomic (RNAseq) and proteomic analysis in vitro. We observed downregulation of DNA replication related gene minichromosome maintenance complex component 6 (MCM6) and cell division cycle 6 (CDC6) in the combination treatment. Gene set enrichment analysis (GSEA) also showed low enrichment scores for DNA replication. Proteomic analysis revealed a down regulation of DNA replication modulators such as HMGB2 and DNAJC9 which work on DNA coiling and histone respectively.

Taken together, this study revealed the therapeutic potential of SINE-PARPi combination via targeting DNA damage response pathway in mCRPC. Further evaluation of molecular synergy in the xenograft models are underway through RNA interference (RNAi) technique and Digital Spatial Profiling (DSP).

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68. Genomic and Transcriptomic Relationships in Pre- and Post-Invasive Prostate Neoplasia

Ajay Vaghasia, Levent Trabzonlu, Anuj Gupta, Ibrahim Kulac, Jessica L. Hicks, Alyza Skaist, Jennifer Meyers, Busra Ozbeck, Qizhi Zheng, Michael Haffner, Christopher Heaphy, Alan Meeker, William Nelson, Angelo De Marzo, Srinivasan Yegnasubramanian

Background: The initiation and progression of primary prostate cancer is thought to involve transitions from benign and inflammatory lesions of the prostate to prostatic intraepithelial neoplasia (PIN) to invasive adenocarcinoma. However, recent evidence from our group has suggested that it is possible for post-invasive cancer cells to show "retrograde" invasion into otherwise normal ducts to masquerade as PIN from a morphological standpoint. Here, we sought to more thoroughly understand the precise clonal evolutionary and transcriptomic properties of pre- and post-invasive neoplastic lesions in the prostate.

Methods: We implemented a rigorous molecular pathology and laser capture microdissection (LCM) enrichment of multiple benign, PIN, and cancerous regions from 55 subjects (n=322 samples total), and carried out whole genome and whole transcriptome sequencing.

Results: We detected SNVs, CNVs, and SVs across all patients and found known drivers of prostate cancer to be mutated at similar frequencies in our cohort as seen with previous prostate cancer genomic studies. Unsupervised analysis of the transcriptomic data revealed that PIN lesions clustered in a continuum between benign and cancer lesions, with some PIN clustering entirely within the cancer group. In further preliminary analyses of whole genome sequencing data, we have carried out clonal evolutionary reconstructions with examples of lesions that were initially characterized to be PIN, but showed evidence of further progression from nearby invasive cancer regions. These examples provide further illustration of the concept that invasive cancer can show retrograde invasion of normal glands to mimic PIN morphologically; we term this type of lesion as post-invasive intraepithelial carcinoma (PIC). Additional analyses with these data are aimed at developing molecular markers that can be used to distinguish true PIN from PIC lesions.

Conclusions: Such PIC lesions have important implications for prostate cancer diagnosis and prevention/interception.

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69. Lineage-specific PRC2 Activity Contributes to Terminal Differentiation of Neuroendocrine Prostate Cancer

Varadha Balaji Venkadakrishnan, Matthew Booker, Richa Singh, Yasutaka Yamada, Kei Mizuno, Adam G. Presser, Sheng-Yu Ku, Henry Long, Michael Tolsturukov, Myles Brown, David Rickman, Himisha Beltran

Lineage plasticity is an emerging mechanism of treatment-resistance in prostate cancer, which can manifest clinically as histologic transformation from prostate adenocarcinoma to small-cell neuroendocrine prostate cancer (NEPC). NEPC is associated with poor prognosis, and new treatment strategies are urgently needed. Epigenetic dysregulation has been implicated as a key driver of prostate cancer lineage plasticity, mediated in part by EZH2. EZH2 is a component of the polycomb repressive complex (PRC2) regulating the transcriptional repressive mark H3K27me3. PRC2 also controls lineage determination during normal development by differential cell-type specific distribution of H3K27me3 marks. EZH2 is targetable, and several EZH2 inhibitors (EZH2i) are in clinical development; these trials are currently in biomarker-unselected prostate cancer populations. A thorough understanding of the mechanism of action of EZH2 across histological subtypes is imperative for predicting optimal response to EZH2i and for the development of robust combination treatment strategies.

To investigate the differential action of PRC2 in NEPC vs castration-resistant prostate adenocarcinoma (CRPC), we performed H3K27me3 CUT&Tag on 18 clinical samples (9 CRPC and 9 NEPC) obtained from rapid autopsy. Unsupervised hierarchical clustering and principal component analysis of H3K27me3 distribution genome-wide resulted in a distinct separation of NEPC from CRPC samples. We hypothesized that preclinical models of prostate adenocarcinoma and NEPC may exhibit variable response to EZH2i based on differential H3K27me3 distribution. As expected, cell viability studies across a diverse panel of adenocarcinoma/NEPC prostate cancer models indicated variable responses to tazemetostat (EZH2i) treatment. The CRPC cell line LNCaP-abl showed a greater than 50% reduction in cell viability upon EZH2i (5μM) while LNCaP and 22Rv1 showed moderate response with 15-25% reduction in cell viability. However, none of our 5 NEPC patient-derived models showed significant response to EZH2i which was also validated in vivo. Hierarchical clustering of RNA-seq profiles of responder and non-responder models revealed distinct cell-type specific EZH2 targets and clusters of genes that may be associated with response to EZH2i. EZH2 inhibition resulted in further upregulation of neuronal lineage determining genes such as ASCL1, LHX2, and PROX1. Further upregulation of neuronallineage PRC2 targets in NEPC models upon EZH2i may provide justification for lack of lineage reversal and maintenance of terminal differentiation. These results were also validated using models of EZH2-knockout and rescue using recombinant dTAG-EZH2.

Based on the differential canonical activity of EZH2 observed in prostate adenocarcinoma as compared to NEPC, we posited that the non-canonical activity of EZH2 may also be divergent. Interrogation using a previously reported gene-signature confirmed that EZH2 co-activator function is lineage-specific as EZH2i did not downregulate majority of the genes in NEPC as compared to CRPC. EZH2i in prostate adenocarcinoma mainly impacts the E2F activity including downregulation of cyclinA. We performed pilot studies in NEPC models with novel cyclin-CDK inhibitor currently under clinical development. Cyclin-CDK inhibition significantly impacted cell proliferation of majority of NEPC models suggesting that lack of response to EZH2i can be overcome with cyclin-CDK inhibition.

Our data shows that EZH2i was associated with modest response in NEPC models and did not revert lineage plasticity. Understanding lineage-specific action of EZH2i may inform biomarkers in ongoing clinical trials. Genes and pathways dysregulated in lineage-specific manner by EZH2i might also be exploited as potential candidates for co-targeting.

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70. The HOX/CUT Transcription Factor ONECUT2 is a Driver of Metabolic Plasticity in Lethal Prostate Cancer

Smrruthi V. Venugopal, Jagpreet S. Nanda, Chen Qian, Qian Yang, Lillian Perez, Jenna Giafaglione, Andrew Goldstein, Isla P. Garraway, Michael R. Freeman

The ONECUT2 (OC2) transcription factor acts to promote lineage plasticity in prostate cancer by attenuating androgen receptor (AR) activity and upregulating expression of multiple neuroendocrine drivers and other pathways. OC2 can be directly inhibited with a novel class of small molecules our group has developed. We have shown that OC2 can modify the epigenome, which is tightly coupled to metabolic state, suggesting that OC2 may trigger reprogramming of tumor metabolism. In this study, RNA-seq analysis indicated that overexpression of OC2 in human prostate cancer cells under conditions of lipoprotein deficiency activated both glycolysis and oxidative phosphorylation. RNA and protein expression measurements demonstrated that enforced OC2 causes robust over-expression of PDK4, an enzyme that promotes aerobic glycolysis, suppresses mitochondrial reactive oxygen species, and stimulates acidification of the tumor microenvironment from secretion of lactate. Enforced OC2 promoted a euchromatin state at the PDK4 promoter, as shown by ATAC-seq and ChIP-seq. CUT&RUN-qPCR demonstrated an increase in OC2 binding at the PDK4 promoter in OC2-enforced cells under nutrient stress. Seahorse Mito-stress test, glycolytic stress test and lactate quantification assays demonstrated higher mitochondrial respiration, glycolytic capacity and lactate secretion with enforced OC2. This extensive metabolic shift was suppressed by pharmacologic OC2 inhibition. A gene expression signature associated with these metabolic changes was found to be upregulated in human castration-resistant and neuroendocrine prostate cancers with high OC2 expression. Our findings reveal that OC2 promotes simultaneous activation of both aerobic glycolysis and oxidative phosphorylation, suggesting these metabolic effects of OC2 activation underlie some of its oncogenic effects on cell proliferation, malignant growth, metastasis, and drug resistance. We conclude that OC2 activates a “hybrid” metabolic phenotype and, consequently, is a targetable master regulator of tumor metabolism in prostate cancer.

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71. Whole-slide Multiplexed Tissue Imaging of Prostate Adenocarcinoma Reveals Distinct Immune Niches in High- Versus Low-Grade Tumors

Jeremiah Wala, Jia-Ren Lin, Brian Labadie, Daniel Peiffer, Andreánne Gagne, Kiranj Chaudagar, Yu-An Chen, Eliezer Van Allen, Peter Sorger

Objectives: Tumor-immune interactions are a critical determinant of tumor evolution and are increasingly targeted by novel therapeutics (e.g. checkpoint blockade). However, their significance in treatment-naive localized prostate cancer remains largely unknown. Here, we employ multiplexed tissue imaging on radical prostatectomy specimens across Gleason grades to create a single-cell spatial atlas of primary prostate tumors and characterize their innate and adaptive immune landscape.

Methods: Whole-slide radical prostatectomy (RP) specimens were obtained from 28 patients: 14 with Gleason Grade Group ≤ 3 (Low-grade; LG) and 14 with Gleason Grade Group ≥ 4 (High-grade; HG). Tumors were imaged with our cyclic immunofluorescence (CyCIF) platform using a 27-marker panel encompassing epithelial, T-cell, innate immune and stromal markers. Tumor and stromal compartments were manually annotated by a pathologist. Cell densities were compared between tumor and stromal compartments of LG and HG tumors.

Results: Across the 28 RP specimens, we identified and spatially resolved over 27 million individual cells. Overall, higher Gleason grade was associated with a significantly increased innate and adaptive immune reaction. CD8+ cytotoxic T-cell density was significantly higher in HG vs LG ($p = 0.003$) specimens, although this effect was largely due to enrichment in adjacent stroma rather than direct tumor infiltration. By contrast, FOXP3+ T-cells were significantly increased throughout the tumor and stromal compartments of HG compared with LG tumors ($p = 0.001$). Tertiary lymphoid structures (TLS) and CD163+ myeloid cells were also significantly enriched in HG tumors relative to LG tumors ($p = 0.029$ and 0.039, respectively). PD-1+ cells were specifically enriched in TLS structures in HG tumors ($p < 0.001$). There were no significant differences in CD11c+ and CD103+ cell densities between LG and HG tumors.

Conclusions: This spatially resolved single-cell atlas of localized prostate cancer reveals that high-grade tumors exhibit significant inflammation, particularly of TLSs and stromal CD8+, and are enriched in markers of T-cell anergy and myeloid-derived suppression. These findings raise the hypothesis that immunotherapy may have activity in treatment-naïve high-grade prostate cancer, despite the failure of checkpoint blockade in mCRPC.

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72. Synergistic CDK12/CDK13 and Akt Inhibition for Advanced Prostate Cancer Treatment

Xiaoju Wang, Yu Chang, Jean Tien, Rahul Mannan, Somnath Mahapatra, Jianzhang Yang, Zhen Wang, Xuhong Cao, Cynthia Wang, Ke Ding, Arul M. Chinnaiyan

Cyclin-dependent kinases 12 and 13 (CDK12 and CDK13) play pivotal roles in orchestrating transcription coordination, elongation, and mRNA processing. Notably, aberrant CDK12 expression and mutations have been documented in various malignancies, encompassing breast cancer, prostate cancer, and ovarian cancer. A growing body of evidence underscores CDK12's significance as both a cancer biomarker and a promising therapeutic target.

Previously, we designed and characterized a dual CDK12/13 degrader, denoted as 7f, employing the proteolysis-targeting chimera (PROTAC) technology (J Med Chem. 2022; 65(16): 11066–11083). In vitro, compound 7f exhibited remarkable selectivity for CDK12/13, as assessed by global proteomics, and a profound inhibitory effect the proliferation of multiple triple negative breast cancer cell lines. Importantly, in vivo PD studies demonstrated the efficacy of CDK12/13 degradation, positioning it as a lead candidate for the continued development of CDK12/13 degraders as a novel and targeted therapeutic modality for cancer patients.

In this study, we further refined compound 7f into a potent and highly selective CDK12/13 degrader, known as YJ9069. In vitro investigations with prostate cancer cells affirm the remarkable effectiveness of YJ9069 in inhibiting proliferation, while benign and normal prostate cells remain unaffected. The degradation of CDK12/13 leads to a gene-length dependent elongation defect, notably downregulating DNA damage response genes. This event triggers cell cycle arrest at the sub-G1 phase and induces apoptotic cell death. Interestingly, CDK12/13 degradation sensitized prostate cells to Akt inhibitor by suppressing the acquired activity of Akt phosphorylation at Ser 473. In further in vivo experiments, YJ9069 demonstrated significant tumor suppression in multiple xenografted models and patient-derived xenograft (PDX) models.

Furthermore, we developed an orally bioavailable CDK12/13 degrader, YJ1206, which maintains a consistent potency in degrading the target CDK12/13 proteins. Significantly, YJ1206 has demonstrated a dramatic reduction in toxicity levels, as rigorously tested in immunocompetent CD-1 mice. The juxtaposition of efficacy and safety renders YJ1206 highly promising for translational purposes. Notably, our study has unveiled a remarkable therapeutic potential in the domain of combination therapy. The co-administration of YJ1206 and the Akt inhibitor uprosertib yields a striking synergy, resulting in the profound suppression of tumor growth in both xenografted and PDX models.

These findings highlight the inherent potential of CDK12/13 as an instrumental drug target and emphasize the compelling prospects of a synergistic approach involving YJ1206 and the Akt inhibitor, underscoring its viability as a prospective combination therapy for the treatment of advanced prostate cancer.

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73. Leveraging a Neoadjuvant Clinical Trial in High Risk Localized Prostate Cancer to Unveil Mechanisms of Androgen Mediated Immune Suppression

Benjamin R. Weeder, Reed M. Hawkins, Sushil Kumar, Ryan Kopp, Mark Garzotto, Reid F. Thompson, Amy Moran

Aggressive prostate cancers frequently exhibit an initial response to androgen axis inhibition. However, in a subset of patients, androgen ablation therapy fails resulting in castration resistant disease. The emergence of checkpoint blockade immunotherapy has revolutionized the clinical management of many solid tumors, however, in prostate cancer they have largely failed. One mechanism of resistance is the abundance of immunosuppressive androgens in the prostate tumor microenvironment. Having recently demonstrated that the androgen receptor directly limits CD8 T cell function and responsiveness to immunotherapy in metastatic castration resistant prostate cancer, herein we explore how simultaneous androgen axis inhibition with checkpoint blockade reshapes the tumor immune landscape in the primary neoadjuvant setting (NCT03753243). In this study we perform single-cell transcriptomic profiling of paired temporal samples from subjects receiving androgen axis inhibition in combination with PD-1 blockade. By leveraging single cell approaches, we demonstrate how treatment leads to a significant reduction in malignant cells and results in concurrent reduction in AR activity and upregulation of antigen presentation machinery within the malignant population, even in the absence of IFN-gamma response. We also highlight how mast cells and other components in the tumor microenvironment contribute to tissue dysregulation through angiogenic and immune-suppressive signaling.

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74. Correlating Prostate-Membrane Antigen with Molecular Pathways in Treatment Naïve Prostate Cancer

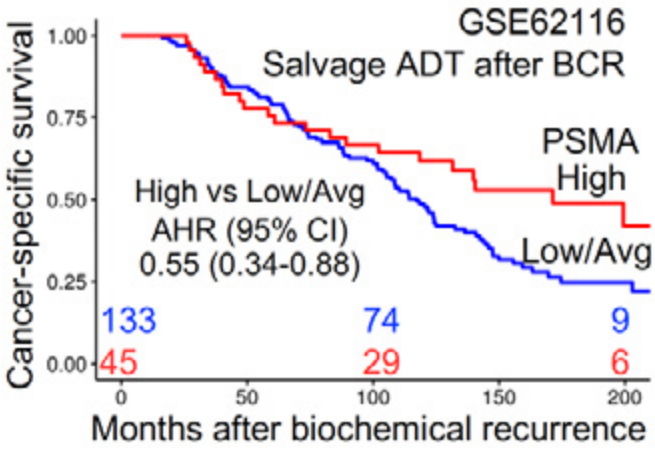
Adam B. Weiner, Eric V. Li, JJ H. Zhang, Elai Davicioni, Edward M. Schaeffer, Ashley E. Ross, Luca F. Valle, Paul Boutros, Jeremie Calais, Amar U. Kishan, Robert E. Reiter

Introduction: Prostate cancer (PC) is extremely common and biologically diverse - necessitating avenues to further precision care. The advent of PSMA PET has helped augment staging for PC but not all PC is seen on PSMA PET. We hypothesize heterogeneity in PSMA expression might reflect differential tumor biology for treatment naïve PC and could thus be leveraged to help individualize targeted molecular tumor testing and treatment selection.

Methods: First, we correlated PSMA RNA abundance (*FOLH1*) with SUVmax in a prospective cohort who underwent surgery (NCT03392181; n=55). Using RNA abundance as a proxy for uptake on PET, we then compared differential molecular pathway enrichment using multivariable linear regressions in primary, treatment naïve PC from The Cancer Genome Atlas (TCGA; n=491) with validation in the GRID database (NCT02609269; n=2612). Independent datasets were then assessed to validate correlated pathways and treatment susceptibilities.

Results: In NCT03392181, PSMA RNA expression was moderately correlated with SUVmax (Spearman ρ = 0.41). A total of 25 hallmark pathways correlated with PSMA expression in the TCGA and GRID cohorts. Importantly, PSMA high tumors tended to be enriched in the androgen response pathway. Accordingly, in a cohort of 178 patients with biochemical recurrence after prostatectomy managed with salvage androgen deprivation therapy alone, patients with PSMA high tumors noted a longer cancer-specific survival (HR 0.55, 95% CI 0.34-0.88; **Figure**). PSMA low tumors were notable for markers of stemness including epithelial mesenchymal transition, angiogenesis, hypoxia, and inflammation. These pathways have been previously associated with resistance to radiotherapy and in a cohort of 248 patients who received primary radiotherapy, those with PSMA low tumors tended to recur sooner (HR 0.50, 95% CI 0.28-0.90). Notably, in a cohort of patients who underwent radical prostatectomy, quartile of PSMA was not associated with time to metastatic recurrence.

Conclusion: Treatment naïve PC with low PSMA may be relatively resistant to hormonal therapy and radiotherapy compared to tumors with high PSMA. Due to these associations, primary surgery should be considered for patients with low PSMA tumors.



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75. Meitner Auger Radiotherapeutic Targeting of the Androgen Receptor

Chen Khuan Wong, Darren Veach, Zhongke Yao, Ouathek Ouerfelli, Yu Chen

Prostate cancer depends on androgen receptor (AR) signaling for growth and survival. While the disease initially responds to androgen deprivation therapy, it inevitably progresses to a lethal, castration-resistant stage where reactivation of AR signaling through AR amplification or mutations represents the most common driver of resistance and persistent growth. Here, we seek to develop a nonsteroidal AR agonist with Meitner Auger electron emission that delivers high linear energy transfer to the nuclei of AR-positive prostate cancer. We synthesized and characterized iodo-S1 as a nontoxic candidate that induces AR translocation to the nucleus and upregulates AR transcriptional activity in LNCaP-AR cells. Radiolabeling of a tributyl tin S1 precursor resulted in a high yield of 125I-S1 that is stable in cell culture medium and plasma. We will assess the ability of 125I-S1 to induce DNA damage and selective cytotoxicity against AR-positive prostate cancer cells. We will also perform a high-throughput screen to identify novel compounds with high AR agonistic activity and assess their ability to form stable, lethal AR-targeting Meitner Auger radiotherapeutics.

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76. The Novel MYC Inhibitor, MYCi975, Uncovers Novel Therapeutic Avenues by Exploiting Tumor Mitochondrial Vulnerabilities via Synthetic Lethality

William Yang, Qianyu Guo, Zachary Chalmers, Songhua Quan, Mihai Truica, Tiffany Mays, Yara Rodriguez, Gary E Schiltz, Navdeep Chandel, Sarki A. Abdulkadir

Leveraging the inherent mitochondrial vulnerabilities in tumors provides transformative clinical potential and unveils novel biological insights, especially when exploiting their pronounced sensitivity to the MYC inhibitor, MYCi975, for therapeutic intervention. We conducted a comprehensive genome-wide CRISPR knockout (KO) screen to identify genetic susceptibilities and synthetic lethality interactions with MYCi975. Central to our findings, mitochondrial perturbation and oxidative stress emerged as synergistic pathways under MYCi975 treatment. While many genes played roles in these pathways from our screen, NDUFA3, integral for Complex I assembly (representing mitochondrial perturbation), and SOD2, vital for ROS detoxification (highlighting oxidative stress), were illustrative of these synergistic mechanisms. Treatment with metformin, a Complex I inhibitor, in conjunction with MYCi displayed synergy across multiple cell line models both in vitro and in vivo, underscoring a therapeutic strategy reminiscent of NDUFA3 KO effects. Furthermore, combining radiation, a standard of care, with MYCi amplified its potency by escalating ROS or oxidative stress, paralleling findings with SOD2 KO. Intriguingly, the radiation and MYCi combo also intensified immunogenic cell death and led to a surge in CD3+ cell infiltration. Mechanistic studies showed MYCi975's pronounced effect on mitochondrial morphology, oxidative phosphorylation, glycolysis, and an increase in ROS levels Thus, targeting mitochondrial vulnerabilities with MYCi975 could offer a potent therapeutic strategy against cancer cells, with synthetic lethality interactions highlighting potential intervention points.

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77. Analysis of Super-Enhancer-Associated lncRNA Landscape Identifies IGF1R-AS1 that Interacts with Chromatin Remodeling Complexes and Promotes Prostate Cancer Progression

Yongyong Yang, Ting-You Wang, Yingming Li, Yanan Ren, Qingshu Meng, Yanan Ren, Qingxiang Guo, Adam B Weiner, Tamara L Lotan, Douglas Yee, Edward M Schaeffer, Scott M Dehm, Qi Cao, Rendong Yang

Background: Transcriptional dysregulation of oncogenic and tumor-suppressor signaling pathways is frequently associated with tumorigenesis and tumor progression. Among the most important epigenetic alternations, enhancers and super-enhancers (SE) are considered as critical drivers of oncogenic gene expression in cancer cells. The enhancer is a class of regulatory DNA sequence bound by epigenetic modifications (such as H3K4me3 and H3K27ac) which enhances the promoter activity to increase target gene expression through specific transcription factors, while SE is large clusters of enhancers with a high density of transcription factor binding sites spanning across a long-range region of genomic DNA. LncRNAs, which are classified as transcripts longer than 200 nucleotides without protein coding potential, can regulate gene expression at both the transcriptional and translational levels and play critical roles in different cellular processes and disease progression, including prostate cancer (PCa).

Methods: To discover novel SE-related lncRNAs in prostate cancer, we first comprehensively analyzed the expression of genes and lncRNAs in RNA sequencing data from metastatic prostate tumor tissues and identified more than 4000 novel lncRNAs, among which 132 novel lncRNAs are differentially expressed in prostate tumor tissues.

Results: We comprehensively analyzed expression of genes and lncRNAs related with SEs in RNA sequencing data from metastatic prostate tumor tissues and identified lncRNA IGF1R-AS1 as a novel lncRNA associated with the strongest super-enhancer signal in our analysis. We revealed the SWI/SNF complex regulated super-enhancer-promoter interactions activated IGF1R-AS1 expression, which in turn fuels oncogenic processes in PCa by bolstering the E2F and Myc pathways. Mechanistically, IGF1R-AS1 interacts with components of SWI/SNF and ISWI complexes and enhances their bindings at the promoters of E2F1 and Myc and thus promote the downstream gene expresson. Clinically, IGF1R-AS1 is correlated with PCa progression and is a strong prognostic predictor of poor survival. Taken together, our findings demonstrated a model in which the chromatin complexes and lncRNA crosstalk promotes PCa progression, suggesting a new therapeutic direction in PCa.

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78. Integrative Analysis of Ultra-Deep RNA-seq Reveals that Alternative Promoter Usage Activates Oncogenic Programs During Prostate Cancer Progression

Meng Zhang, Martin Sjöström, Adam Foye, Kyle Farth, Raunak Shrestha, Arian Lundberg, Ha X. Dang, Phillip G. Febbo, Rahul Aggarwal, Joshi J. Alumkal, Eric J. Small, The SU2C/PCF West Coast Prostate Cancer Dream Team, Christopher A. Maher, Felix Y. Feng, David A. Quigley

Background: Prostate tumor progression is driven by transcription factors (TFs), most prominently the Androgen Receptor, that drive proliferation and therapy resistance. Many genes have several promoters that can be bound by distinct TFs. It is unclear what role alternative promoter selection plays in tumor progression. We hypothesized that driver TFs target novel gene promoters during prostate tumor progression, and that understanding how this targeting occurs would reveal novel aspects of prostate tumor biology.

Methods: We performed ultra-deep RNA sequencing on 104 metastatic Castration Resistant Prostate Cancer (mCRPC) biopsies, generating > 450M reads/sample (10X the typical depth), in samples with matched whole genome DNA and methylation sequencing. We combined these data with published deep transcriptomes in normal prostate and localized prostate cancer for a total of 274 samples. Promoter activity was evaluated in all 274 biopsies using the proActiv tool. Differential promoter activity levels were integrated with transcription factor motif enrichment, chromatin immunoprecipitation sequencing, and previously published somatic and methylation sequencing data in the mCRPC samples.

Results: Using normal prostate tissue as a baseline, we identified 463 and 3,237 APs with differential activity in localized prostate cancer and mCRPC, respectively. Increased alternative promoter (AP) usage was associated with upregulation of prostate tumor driver genes. Elevated androgen signaling in both localized and mCRPC was correlated with increased AP use. In localized prostate tumors, APs preferentially switch to promoters harboring binding sites for the AR pioneer factor FOXA1. In mCPRC, APs were further enriched for binding of MYC, E2F1, and HIF1A. Furthermore, AP usage reflects transcriptional dysregulation related to lineage plasticity in response to therapy. APs in neuroendocrine prostate cancer (NEPC) were bound by neuroendocrine TFs such as ASCL1 and HAND2. While canonical promoters are usually unmethylated, methylation levels at APs were strongly linked to AP activity.

Conclusions: Our data show that transcription initiated at APs is a key contributor to increased gene activity in prostate tumors. APs that are upregulated in prostate tumors are linked to transcription factors activated by recurrent somatic alterations and lineage plasticity. Methylation at APs is an important and underappreciated driver of promoter choice in mCRPC. Understanding how somatic alterations and epigenetic reprogramming of TFs affect APs is essential to understanding how these processes impact therapy resistance.

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79. Enzalutamide-mediated Metastasis in Immunocompromised Castration-resistant Prostate Cancer Mouse Model via MET Signaling Pathway

Yang Zheng, Yuanyuan Qiao, Stephanie Simko, Jean Tien, Andrew Delekta, Nathan Hodge, Parth Desai, Arul Chinnaiyan

Enzalutamide, a potent androgen-receptor inhibitor, has demonstrated significant clinical benefits in patients with metastatic castration-resistant prostate cancer (mCRPC) and nonmetastatic castration-resistant prostate cancer (nmCRPC). Despite its success, recent preclinical studies in immunocompromised mice have unveiled unexpected complexities regarding its metastatic effects. In this study, we sought to assess Enzalutamide's impact on metastasis in a controlled experimental setting.

Using a VCaP-CRPC subcutaneous model in SCID mice, we investigated the consequences of Enzalutamide treatment (10 or 20 mg/kg) after castration, a standard protocol mimicking clinical practices. Intriguingly, while Enzalutamide effectively curtailed primary tumor growth, it led to a substantial increase in metastatic frequency. Notably, the femur emerged as a prevalent site of metastasis, followed by lung and kidney. Histological analysis confirmed micro-metastases, with immunohistochemical markers AR and ERG validating metastatic tumor identity.

Delving into the molecular underpinnings, our study revealed a dynamic interplay between Enzalutamide, androgen receptor (AR) signaling, and the MET pathway. Enzalutamide treatment elicited a marked upregulation of both AR and MET expression. ChIP-seq analysis indicated that the AR binding to the MET promoter region was enriched post-Enzalutamide exposure. Notably, CRISPR SAM-mediated MET overexpression in an intracardial injection model substantiated the role of MET in driving Enzalutamide-induced metastasis, offering a mechanistic insight into this phenomenon.

Our findings underscore the imperative for a nuanced understanding of Enzalutamide's metastatic effects, especially within immunocompromised contexts. Unraveling the intricacies of the Enzalutamide-AR-MET axis provides invaluable insights for refining therapeutic strategies in nmCRPC. This study not only contributes to the evolving landscape of prostate cancer research but also holds promise for tailored interventions, paving the way for improved patient outcomes in the face of prostate cancer metastasis.

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