DF/HCC

DANA-FARBER / HARVARD CANCER CENTER

A. David Mazzone Research Awards Program

Final Annual Report of Research Progress and Accounting
Report # 014

Report Period: August 1, 2018 to September 30, 2019

Principal Investigator: Myles Brown, MD
Dana-Farber/Harvard Cancer Center

Co-Principal Investigator: Jonathan Simons, MD
Prostate Cancer Foundation

Respectfully Submitted to
U.S. District Court for the District of Massachusetts

Boston, Massachusetts, March 3, 2020

NCI CCC A Comprehensive Cancer Center Designated by the National Cancer Institute

Beth Israel Deaconess Medical Center
Brigham and Women's Hospital
Boston Children's Hospital
Dana-Farber Cancer Institute
Harvard Medical School
Harvard School of Public Health
Massachusetts General Hospital

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I. Background

The Program is named in memory of the Honorable Judge A. David Mazzone, who provided an indelible contribution to the United States legal system. For nineteen years, Judge Mazzone presided over the federal legal case to clean up the Boston Harbor, a legacy that lives on for generations to enjoy. He demonstrated a life-long commitment to environmental causes and contributed to the organization of local efforts to fundraise for cancer research. Judge Mazzone himself succumbed to Prostate Cancer at a premature age.

The funding agency for the Program is a grant from the U.S. District Court for the District of Massachusetts derived from a pool of unclaimed funds from the 2004 class action suit settlement by TAP Pharmaceuticals. The class action suit was related to marketing and sales practices for the prostate cancer drug Lupron. The Program is administered jointly through Dana-Farber/Harvard Cancer Center (DF/HCC) and the Prostate Cancer Foundation (PCF).

Program Goals: The overarching goal of the program is to leverage the existing institutional infrastructure, funding mechanisms and relationships of DF/HCC and PCF to distribute locally and nationally settlement funds annually on a competitive basis, to support large-scale research collaborations in prostate cancer research; such as cutting-edge pilot projects, development of promising junior investigators, and training talented students.

Specific Program goals:

- To direct leftover Settlement Pool funds from Lupron litigation to research initiatives of merit in prostate cancer and other Lupron-treatable diseases.
- To distribute Settlement Pool funds to researchers in prostate cancer and other Lupron-treatable diseases at the national and local level, and to spur collaborative research between prostate cancer and Lupron-treatable diseases.
- To distribute Settlement Pool funds through existing organizational channels that have an established record of successful grant distributions (i.e., those that have advanced the state of knowledge in the grants' stated areas of research).
- To increase the power and breadth of research in prostate cancer and other Lupron-related diseases, by (i) the strategic administration of new and existing funding mechanisms; (ii) expanding current avenues of investigation; (iii) recruiting new talent into the field; and (iv) ensuring that research is relevant to the primary goals of advancing diagnostic, treatment and quality of life options for patients with prostate cancer and other Lupron-treatable diseases.

Program Mechanisms: Grant applications were solicited annually by DF/HCC and PCF throughout the duration of the program. DF/HCC solicited several categories of grant applications from the faculty of Harvard University and its affiliated hospitals encouraging extramural collaborations. PCF solicited grant applications from interested applicants on a national and international level.

Governance: DF/HCC and PCF convened a high-level scientific advisory board (the "Oversight SAB") to participate in the application review process, and to ensure that Settlement Funds were

distributed fairly, and in accordance with Requests for Proposals guidelines and any other principles associated with such funds.

SAB Members:

- Donald Tindall, PhD, Director and Vice Chair of Urologic Research, Mayo Clinic College of Medicine.
- Howard Soule, PhD, Executive Vice-President and Chief Science Officer at PCF.
- Jonathan Simons, MD, Co-Principal Investigator of the Mazzone Awards Program, President and Chief Executive Officer at PCF.
- Ken Pienta, MD, Director of the Prostate SPORE at the University of Michigan.
- Peter Carroll, MD, Chief of Urology, Director of the Prostate SPORE at University of California, San Francisco.
- Peter Nelson, MD, Director of the Prostate SPORE at the University of Washington.
- Peter Scardino, MD, Chairman of Surgery and Chief of Urology, Director of the Prostate SPORE at Memorial Sloan Kettering Cancer Center.
- William Nelson, MD, PhD, Director of the Cancer Center, Director of the Prostate SPORE at Johns Hopkins University.

- Philip Kantoff, MD, Principal Investigator of the Mazzone Awards Program, Director of the Lank Center for Genitourinary Oncology at DFCI through October 2015.
- Myles Brown, MD, Principal Investigator of the Mazzone Awards Program, effective as of October 2015.

Court Appointed Members:

- Jonathan Tilly, PhD, Director, Vincent Center for Reproductive Biology, Massachusetts General Hospital. –through December 2011.
- Gary Wente, JD, Circuit Executive at United States Courts; US District Court for the District of Massachusetts, Patient Advocate for the Mazzone Awards Program. –through July 2014.

The Court appointed board members and, upon request, Judge Richard G. Stearns, were included in all governance committee and scientific review board correspondence and were invited to face-to-face meetings and conferences related to award governance and/or grantee presentations.

Award Categories: Through the Mazzone Awards mechanism, DF/HCC offered funding opportunities in *High Impact* research grants, High Impact Clinical Trials, *Lupron-Treatable Diseases and Conditions* research grants, *Community Outreach* grants, and *Student Education* grants, as well as *Career Development* grants, *Project Development* grants and *Disparity Research* grants. PCF added Mazzone awards to the number of *Challenge* grants awarded on an annual basis.

The following table provides a description of each category of award made possible through the Program.

Grant Awards Mechanisms (Revised list approved by the Court in 2018)

Award Category	Amount	Duration	Number of Awards
Career Development	100,000	2 years	8
Community Outreach	100,000	2 years	2
Disparities Research	100,000	2 years	7
High Impact Award	500,000	2 years	6
Lupron-treatable	100,000	2 years	3
Project Development	100,000	2 years	9
Student Training	20,000	2 years	10
High Impact Trials	500,000	2 years	1
Seed Fund Community Outreach	10,000		1
PCF Challenge Award	1,000,000	2 years	6
Closing Retreat	23,041		1

Notes of modifications to the original distribution of funding mechanisms:

- In 2012, the Court authorized the DF/HCC contingent of the Mazzone Program to create a new award category for "High Impact Clinical Trials" by reallocating one of the five grants originally approved for "High Impact Awards". This new award was advertised in 2012 and 2013 and an award was issued in 2013.
- Per recommendation of the peer review panel and approval by the Court, a $10,000 - one-time seed funding grant was awarded to Dr. Glenn Bubley in 2012.
- In 2013, two out of four $100K Community Outreach grants remained unfunded. $200K was reallocated to fund a partial High Impact Project
- In 2013, the Court authorized the Prostate Cancer Foundation to fund a Mazzone Program at $500,000 (half of the normal funding amount), which allowed the Foundation to advertise and grant a final Mazzone Challenge Award in 2014 for a total of six PCF awards.
- In 2014, the Court authorized DF/HCC to use previously unallocated funds ($160,000) from the original Lupron grant to support a special RFA on Community Outreach and Disparities Research and additional funding for the Student Training program. Funding in the amount of $140,000 was issued to two grant recipients selected in 2014 for two-year Disparities Research grants. The remainder $20,000 was allocated as additional funding to the DF/HCC CURE Program increasing the total Student Training grant to $180,000.
- In 2018, No Cost Extension was approved, which allowed DF/HCC to fund two additional Career Development awards ($53,000 each), additional funding provided for student training ($20,000) and funding for a final retreat and closing event ($23,041)

II. Program Guidelines

At DF/HCC, applications were reviewed by members of the DF/HCC Prostate Cancer Program and SPORE Governance Committee, and by at least two non-DF/HCC members of the Oversight SAB on a rotating basis. The DF/HCC Prostate Cancer Program and SPORE Governance Committee was comprised of approximately ten Harvard faculty members representing Harvard Medical School and its affiliated institutions. The faculty members were chosen based on their accomplishments, broad vision, impartiality, and diverse expertise. They had expertise and

training in one or more of the following disciplines: medical oncology, urologic oncology, radiation oncology, population science, and basic science.

Grant Disbursements and Program Accounting:
Annual reports on Settlement Pool Account financial activity were submitted to the Court designees and to Judge Stearns. All grant funds were paid to grantees according to contractual obligations; grantees were required to submit quarterly invoices against actual expenditures. Progress and financial reports were required from grantees at the end of the first year with detailed narrative updates and expenditure reports. The issuance of the second year's funding installment was made contingent upon satisfactory progress by grantees. These payments were made with the approval of the respective board chairs and the Oversight SAB. Final progress and financial expenditure reports from grantees were required at the end of the award term.

Both DF/HCC and PCF required that no award funds were directed to overhead expenses at grantee institutions. Therefore, the Settlement Pool funds were subject to IDC at only one point in the overall award process, i.e., upon receipt of funds by DF/HCC. Appendix 1 provides the grant Disbursement Structure and this period's financial report issued by DFCI. The funding structure was updated annually to reflect revisions in funding categories and to reflect the no cost extension for the period of this report.

Program Reporting Schedule: The Program's effective start date was October 1, 2010. The Program issued and sustained grants for nine years. The original reporting schedule ran from October 1, 2010 through September 30, 2017 (seven years). Appendix 2 provides the full reporting schedule as approved by the Court with update for the no cost extensions granted to awardees and a no cost extension to support two special Career Development awards through September 30, 2019.

III. Report of activities and Progress

Communications: To support high quality, innovative, and collaborative translational research, the program utilized effective methods to communicate and publicize funding opportunities to the research community broadly. For example, to reach members of the Harvard-affiliated research community, DF/HCC utilized its weekly bulletin, targeted mailings, and website postings to inform its 1,000 cancer center members and colleagues of the opportunity. In addition, DF/HCC provided information on the program to other cancer centers across the country and advertised the RFA on the Prostate Cancer Foundation national newsletter and website.

DF/HCC and PCF were successful in generating both local and national interest as evidenced by the 120 combined applications received in 2011 and 150 applications in 2012. Building upon this interest and enthusiasm, DF/HCC and PCF made strategic efforts to publicize the 2013 RFA. The program generated considerable interest as evidenced by 112 applications received and reviewed in 2013. The Program established a national and international presence as a source of funding for prostate cancer research and inter-institutional collaborations. This is reflective of the unprecedented number of letters of intent the program received for the 2013 funding round. 90 letters of intent were received through the DF/HCC funding mechanisms, involving 212 researchers in 87 institutions. In 2014, DF/HCC received three applications in response to its special RFA on Community Outreach and Disparities Research. DF/HCC funded two Disparities Research Grants for the period 2014 - 2016. The Prostate Cancer Foundation received 55 applications for its 2014 RFA to fund one Mazzone Grant for the period 2014 – 2016. Using grantee's unspent balances, the Program to funded two special Career development awards in the period of 2018 to 2019.

As of its ninth year, the Program supported a total of 142 investigators working on 43 Prostate Cancer research projects throughout United States institutions. These projects include 37 projects funded by DF/HCC through six award mechanisms and six projects funded through the PCF Challenge Awards mechanism. A full list of Program grantees is provided below under Appendix 3.

On a no cost extension over the original Program end date, the Program funded two Career Development projects in the period of 2018 - 2019 and sponsored a closing retreat in the fall of 2019. This report summarizes the scientific progress and accounting reports for the no cost extension period. During the period 2018 – 2019, the Program did not issue new requests for applications. During this period, the Program continued to monitor progress on active awards.

Report of Progress for Program Period August 2018 – July 2019: As of July 2019, two special Career Development awards funded in 2018 by DF/HCC completed their single-year of project activities. Progress reports were reviewed by grantees' mentors and DF/HCC Cancer Center program directors, Drs. Steven Balk (BIDMC) and Dr. Massimo Loda (DFCI). Investigators demonstrated success toward the goals of their projects. Annual progress and accounting reports guidelines and requirements were stipulated to each grantee institution in grant contractual agreements at the time of issuing each grant.

This report incorporates full text of progress and accounting reports collected from grantee institutions and the DF/HCC CURE Student Training Program under Appendix 4.

IV. Highlights of Scientific Progress DF/HCC Sponsored Investigators

Career Development 2018-2019
The role of TMPRSS2_ERG fusions in modulating tumor microenvironment in prostate cancer
PI: Hubert Pakula (DFCI)
Mentor: Massimo Loda (DFCI)
Most if not all work that has been done on prostate cancer in the last 20 years was focused on genetic changes in the epithelium. This, however, does not provide a full picture of prostate cancer development. In fact, still little is known about the precise mechanism regulating the initiation of PCa at both genetic and cellular levels. The researchers thoroughly characterized the role of T-ERG fusion in modeling the TME as an early event of PCa development. By single cell RNAseq they identified 4 clusters corresponding to stromal cell populations in T-ERG mice that are not present in WT. Interestingly, immunohistochemical analysis showed that T-ERG+ epithelium favors expression of Pdgfrβ+ over Pdgfrα in the stromal compartment and recruits Postn+ stromal cells. Moreover, they observed an increased expression of Wnt pathway components such as Lgr5 and Porcn in these population. This suggests that Wnt pathway in stromal cell is induced by ERG-positive epithelium. These findings will help to elucidate the role of the microenvironment in tumor promotion and progression, thereby aiding the development of stroma-targeted therapies. Therapeutic targeting of the T-ERG- induced Wnt+ stromal cells may benefit patients that harbor the TMPRSS2- ERG fusion, including those with concomitant PTEN loss.

Future Plans:
In order to reproduce in the mouse, the stages assessed in humans, the researchers will determine the stromal composition in T2E/PTEN+/- (PIN but no invasion). They will also use a model with a different genetic background, the Hi-Myc model that develops PIN, invasive cancer {Ellwood-Yen, 2003 #17}. The combinatorial approach of using Cre recombinase fused to a modified estrogen receptor ligand binding domains (ERT2) permits the temporal induction by delivery of a tamoxifen and tissue specificity by directing the Cre expression with a lineage- specific promoter (e.g., PDGFRαCreERt2, Pdgfr-β-CreERT2, Col1a1-CreERT2, αSMACre- ERT2). When crossed with mice recapitulating Tmprss2-ERG fusions in Pten-loss [80], or over-expression of MYC with Ai65D reporter mice, recombination can be detected by expression of red fluorescence (TdTomato). The researchers will trace the dTomato+ lineage progenies of the labeled stromal cells for a prolonged period after the tamoxifen treatment has been terminated. The ultimate goal of this study will be: i) to get a spatial distribution of stromal cells in different genetic backgrounds and stages of PCa development and ii) to characterize these cells molecularly and 3) to interrupt the stromal feedback to the epithelium and halt tumorigenesis.

Career Development 2018-2019
Downregulation of DPP4 mediates resistance to androgen deprivation therapy in castration-resistant prostate cancer (CRPC)
PI: Joshua W. Russo (BIDMC)
Mentor: Steven P. Balk (BIDMC)
Downregulation of DPP4 is tightly associated with PCa progression and the development of resistance to ADT both in preclinical models and in patient samples. (Russo et al, 2018). DPP4 is an AR stimulated gene, yet remains downregulated even when AR signaling is restored in castration resistance. As DPP4 is known to degrade a number of pro-survival growth factors and cytokines, these findings suggest that DPP4 downregulation might have a functional role in ADT resistance.

Inhibitors of DPP4 enzymatic activity decrease the effectiveness of ADT in xenograft and PDX models of PCa (Russo et al, 2018). This result has clear implications for men with Type II diabetes on a DPP4 inhibitor that are newly diagnosed with metastatic prostate cancer and about to start ADT. The combination of DPP4 inhibitor and ADT should be used with caution, as the DPP4 inhibitor might decrease the effectiveness of ADT.

NPY, a pro-survival growth factor and known target of DPP4 degradation, has an inversely correlated expression pattern compared to DPP4. DPP4 downregulation might cause increased local concentrations of NPY, allowing PCa tumors to resist ADT.

Future Plans:
Experiments to define the growth factor/kinase cascade target of DPP4 are already underway for Aim 2 using the VCaP cell lines stably expression shRNA and cDNA to DPP4. Once a candidate growth factor/kinase cascade is identified, future studies will focus on using inhibitors of the signaling cascade in the VCaP xenograft and PDX animal model setting to counteract the effects of DPP4 downregulation/inhibition on tumor growth. As this signaling cascade might generally contribute to ADT resistance, any targeted therapy could be used to treat patients whose tumors have become resistant to ADT.

As a prostate cancer early investigator, the PI aspires to become an independent academic research scientist with a focus on determining the causes of advanced metastatic prostate cancer resistance to ADT and developing therapies to combat this resistance. Downregulation of DPP4 protein expression in castration-resistant prostate cancer represents one of these mechanisms. Publications resulting from the Mazzone Program award will form the basis of future grant applications.

DF/HCC CURE Program

The overall goal of Dana-Farber/Harvard Cancer Center student's training program is to engage the scientific curiosity and promote the academic success and future research careers of promising young scientists from underrepresented communities. We are grateful to the A. David Mazzone Research Awards for helping to support our program and advance our mission.

Alongside their summer research experiences, trainees participated in regular journal clubs, seminars, and career development workshops. This summer, trainees experienced a journal club that was focused on immunology.

The program hosted the fourth annual Beyond Academia: Conversations on Health and Life Science Careers event at Dana-Farber. Nearly 30 representatives from a number of local biotech and pharmaceutical companies as well as public health, government agencies and academic presses participated in small group informational interviews with attendees.

Funded Students in 2018

Destiny Porte – Destiny completed her senior year at Kipp Academy and was accepted in the class of 2022 at Tufts University. Destiny returned to the lab of Dr. Keisha McCall to focus on the reproducibility of molecular imaging of glucose metabolism.

Graciella Ortega – A rising Freshman at Simmons College, Graciella completed her second-year ins the CURE program. Her focus this summer was on surviving, a highly expressed protein in many cancer malignancies.

Robert Pepen – Robert returned for a second summer in the research environment of Larissa Nekhlyudov. He continued his research on establishing worldwide cancer survivorship guidelines.

Edmilson (Ianic) Pires – Ianic is a rising sophomore at Boston College. For the past two summers he investigated the association between probiotic intake and microbiome composition under the guidance of Kerry Ivey, PhD.

Funded Students in 2019

David Bamgbowu – David is a rising sophomore at UMass Amherst majoring in biology. This past summer, he worked in the lab of Dr. Alejandro Gutierrez, at Boston Children's Hospital. His research focuses analyzing the localization of CHKA in relation to treatment with nitrogen mustard, the active metabolite of cyclophosphamide.

Arlin Arias – Arlin is a rising sophomore at Boston College and majors in chemistry. Under the direction of Othon Iliopoulos, MD his research focus included testing therapeutic agents to treat patients with hemangioblastoma.

V. Final Retreat and Closing

On September 13, 2019, the program held a scientific retreat to celebrate the Program accomplishments and to share the wealth of groundbreaking accomplishments made by Program grantees. Following is an outline of the retreat program. The full content of the retreat program and presentations is included below under Appendix 5.

Session 1: High Impact Awards

Steven Balk, Beth Israel Deaconess Medical Center: "*Prostate Cancer Molecular Heterogeneity and Response to Intensive Androgen Deprivation Therapy*"

- Prostate cancer (PCa) is identified based on loss of normal basal cell layer and nuclear atypia, and graded by Gleason pattern (Gleason score is sum of two major patterns).
  - Gp3 - well-formed and separated glands
  - Gp4 - glands fusing, cribiform or intraductal growth
  - Gp5 - single cells and sheets of cells invading stroma
- Gleason pattern matters: men who are confirmed to have only Gp3 (Gleason score 3+3=6) after radical prostatectomy almost never relapse.
- Can we identify Gp3 tumors that can just be monitored (Active Surveillance) versus those that are likely to progress?

Matthew Freedman, **Dana-Farber Cancer Institute**: "*Charting the Prostate Epigenome*"

- AR undergoes extensive reprogramming during progression of prostate adenocarcinoma
- Metastatic prostate adenocarcinoma programs do not arise de novo
- Reprogramming appears to (re)activate fetal programs
- Integration of genetic and epigenetic datasets can identify state-specific, functionally relevant, non-coding regulatory loci
- Epigenomic profiling identifies FOXA1 as a critical mediator of prostate cancer lineage plasticity

Session 2: Career Development, Disparities Research

David Miyamoto, Massachusetts General Hospital: "*Molecular Characterization of Circulating Tumor Cells in Prostate Cancer*"

- High quality Circulating Tumor Cell (CTC) RNA can be isolated via CTC-iChip microfluidics
- Inter and intra-patient heterogeneity in CTCs
- Pre-treatment CTC digital RNA score predicts response to therapy in metastatic prostate cancer
- Pre-treatment CTC digital RNA score in localized prostate cancer predicts microscopic dissemination and pathologic stage
- Advantages of RNA-based analyses:
- Snapshot of the cell: real-time biological states (e.g. EMT, signaling, etc.)
- RNA assays do not require prior knowledge or sequencing of mutations
- CTC RNA assays can provide universal non-invasive cancer biomarkers that are mutation agnostic

Lorelei Mucci, Harvard School of Public Health: "*Prostate Cancer Epidemiology Studies of Racial Disparities*"

- Even after adjusting for risk factors, screening patterns, and socioeconomic status

- Excess risk of prostate cancer overall (and fatal) in black men and men of Scandinavian descent
- Differences in prevalence of obesity, smoking, and vitamin D status account for part of disparity in risk of prostate cancer
- Prostate cancer clinical trials to date have lacked racial/ethnic diversity

Session 3: Project Development

Zhe Li, Brigham and Women's Hospital: "*LY6D Links Castration-Resistant Prostate Luminal Cells to Prostate Progenitors and Cancer*"

- Single cell analysis identifies a highly heterogenous luminal compartment in the prostate
- we found that a novel marker Ly6d marks luminal cells that are intrinsically resistant to castration
- LY6D marks luminal cells that are resistant to castration with bi-lineage capacity.
- LY6D correlates with prostate cancer development from the luminal lineage.
- LY6D expression in human prostate cancer correlates with early disease progression.

Robert Cormack, Brigham and Women's Hospital: "*Localized Chemo Radiation Therapy for Prostate Cancer*"

- This project aimed to develop a drug eluting spacer for sustained release of olaparib in the setting of permanent prostate brachytherapy.
- Olaparib nanoformulation increases effect of radiation in cells
- Olaparib nanoformulation and radiation delay tumor growth compared to individual application
- Dose enhancing effect not seen when released from polymer spacer
- Efforts have evolved to
- different sites and PARP inhibitors
- different sensitizer in spacers for prostate

Session 4: Prostate Cancer Foundation Challenge Award

Jennifer Wu, Northwestern University: "*First-in-class Approach to Sensitize Prostate Cancer to Immunotherapy*"

- This project studies Highly Immune Suppressive Tumor Microenvironment in Prostate Cancer (sMIC).
- Induction of MIC at early tumorigenesis provides immune protection
- Is sMIC a therapeutic target for metastatic PCa?
- Targeting sMIC debulks primary tumor and eliminates metastasis
- Who are the best patients for target?
  define selection criteria for serum sMIC level.
- When is the best window for therapy?
  define disease stages?
- How to integrate with Standard Care of Pca?

Glenn Liu, University of Wisconsin: "*Precision Imaging in Prostate Cancer: reIMAGinING Patient Care*"

Dr. Liu's presentation reports on innovative and groundbreaking technology for imaging of bone response or progression upon drug therapy.

- 99mTc-MDP
  2D, planar-based imaging
- SPECT
  3D, single photon emission CT
  Can see smaller lesions (e.g. in spine) due to anatomic localization
- NaF PET/CT
  Captures physiologic activity with attenuation correction and anatomic localization
- The study met its primary objective; 22 of 22 (100%) evaluable men had ≥ 1 responding bone lesion on QTBI at PSA progression
- The proportion of progressive lesions increased from a mean 7.8% (range, 0-29) at PET2 to 9.4% (range, 0-32) at PSA progression

Appendix 1. Grant Funding Plan and Accounting Report by Dana-Farber Cancer Institute

A. David Mazzone Awards Program
Funding Structure Plan- nine Years (2010 - 2019)

Revised by Juan Carlos Hincapie on September 30, 2019

Court Disbursements	Tranche 1 (2011-2013)	Tranche 2 (2012-2014)	Tranche 3 (2013-2015)	Tranche 4 (2015-2016)	Tranche 5 (2016-2017)	Tranche 6 (2017-2019)	Total
Payment Issue Date	Nov-10	Nov-12	Jan-15	Nov-15	May-18	Expected February 2020	
Expected Disbursement	4,000,000	4,000,000	2,000,000	1,585,000	50,728	101,000	11,736,728
IDC	400,000	400,000	200,000	158,500	5,073	10,100	1,173,673
PCF	1,800,000	1,800,000	1,200,000	200,000	0	0	5,000,000
DF/HCC	**1,800,000**	**1,800,000**	**600,000**	**1,226,500**	**45,655**	**90,900**	**5,563,055**

Estimated Awards Funding Distribution by DF/HCC for 2011 - 2017 Based on Original Grant Proposal Approved by the Court

The following table shows the expected funding distribution per the proposal approved by the Court and expected adjustments for distribution changes to award mechanisms.

Award funding will run from August to July and it will be distributed to grant recipients based on a cost reimbursement method.

Program funding received November 2010 will support 75% of award payments for Round 1 from August 2011 to July 2012. Funding received in November 2012 will support final award payments (25%) for round 1 and 75% of Round 2 awards from August 2012 to July 2013. Funding received in November 2014 will support final award payments (25%) for Round 2 and new awards from August 2013 to July 2015 and additional allocation for 2014 - 2019.

Award Category	Number of Awards Approved	Total Funding Approved	Number of Awards funded 18% of total Proposals received for (2011-2013)	Total Funding Round 1 (75% of total award will fall in this period)	Number of Awards funded 22% of total Proposals received for (2012-2014)	Total Funding Round 2 (75% of awards plus 25% from Rnd 1)	Number of Awards funded 18% of total Proposals received for (2013-2015)	Total Funding Round 3 (100% of awards plus 25% from Round 2)	Special RFA 2014, 67% of total Proposals received for (2014-2016)	Total Funding Round 4 (100% of 2 final awards)	No Cost Extension from Sep 2018 to Sep 2019	Number of Awards Funding in No Cost Extension	Grand Total Projected Direct Cost
Career Development	6	600,000	3	225,000	2	225,000	1	150,000					600,000
CD Additional Allocation 2019	2	106,000									106,000	2	106,000
Community Outreach	4	400,000			1	75,000	1	125,000					200,000
Disparities Research	5	500,000	2	150,000	2	200,000	1	150,000					500,000
DR Additional Allocation 2014	2	140,000							2	140,000			140,000
High Impact Award	4	2,000,000	1	375,000	2	500,000	3	1,325,000					2,200,000
Lupron-treatable	3	300,000	1	75,000	2	175,000		50,000					300,000
Project Development	9	900,000	4	300,000	3	325,000	2	275,000					900,000
Student Training	8	160,000		12,793		17,013		130,194					160,000
ST Additional Allocation 2014	2	20,000								20,000			20,000
ST Additional Allocation 2019	2	20,000									20,000		20,000
New Awards Approved in 2012													
High Impact Trials	*1*	500,000					1	500,000					500,000
Seed Fund Community Outreach	*1*	10,000			*1*	*10,000*							10,000
Closing Retreat		23,041									*23,041*		23,041
Total DF/HCC		**5,679,041**	**11**	**1,137,793**	**12**	**1,527,013**	**9**	**2,705,194**	**2**	**160,000**	**149,041**	**2**	**5,679,041**
											DF/HCC Variance from original Approved Total Disbursement:		**115,986**
PCF	5	5,000,000	2	2,000,000	2	2,000,000	1	500,000	1	500,000			**5,000,000**

Total Approved Direct Expense	10,679,041
Indirect Cost Assessment	1,173,673
Total Estimated in Grant Proposal	**11,852,714**
Actual Projected Disbursement	11,736,728

Notes:

In 2012, two one-time seed funding Community Outreach awards for $10,000 each were approved . Only one seed project was funded in 2012

In 2012 one High Impact award was divided evenly between two projects.

In 2013, two out of four $100K Community Outreach grants remained unfunded. $200K was reallocated to fund a partial High Impact Project

In 2013, PCF funded one partial Mazzone Challenge Award. The remainder ($500K) to be awarded through a 2014 RFA

In 2014, DF/HCC funded 2 additional Disparities Research Grants for $140K total, plus $20K additional Funding for Student training , PCF funded one partial Mazzone Challenge Award with the remainder ($500K) from 2013

In 2018, No Cost Extension was approved, which allowed DF/HCC to fund two additional Career Development awards ($53,000 each), additional funding provided for student training ($20,000) and funding for a final retreat and closing event ($23,041)

Dana-Farber Cancer Institute

ANNUAL REPORT OF EXPENDITURES
FOR THE AWARD PERIOD 08/01/16 THROUGH 09/30/17

LUPRON SETTLEMENT POOL (A. DAVID MAZZONE AWARDS PROGRAM)
UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS
ONE COURTHOUSEWAY, SUITE 2300
BOSTON, MA 02210

DFCI 6192900

AWARD PERIOD: 08/06/10 - 09/30/19

REPORTING PERIOD: 08/01/16 - 09/30/19

INVESTIGATOR: Brown, Myles A.

		Year 7	Cumulative
TOTAL AWARD		0.00	11,700,000.00
UNEXPENDED BALANCE FROM PREVIOUS PERIOD		163,041.19	0.00
NET AWARD		163,041.19	163,070.19
CASH RECEIVED TO DATE		50,728.29	11,635,728.29
Compounded summary of DFCI grants			
EXPENDITURES			
	SPECIAL FUND PERSONNEL	23,617.87	1,382,575.07
	FRINGE BENEFITS	1,297.29	358,895.87
	SUPPLIES	0.00	173,038.35
	OTHER	20,539.01	373,854.89
	SERVICE CENTERS/CORE CHARGES	27,804.51	188,269.99
	OTHER RESEARCH SERVICE CHARGES	0.00	561.50
	AFFILIATED INSTITUTIONS	52,338.94	1,825,107.95
	AFFILIATED INSTITUTIONS CARRY-FORWARI	0.00	546,268.35
	ANIMAL FACILITY BOARDING	0.00	8,269.08
	DOMESTIC TRAVEL	2,842.41	17,493.18
	INCENTIVES	0.00	14,190.74
	CONSULTANTS	0.00	10,000.00
	CLOSEOUT/REALLOC	0.00	(11,892.23)
	ANIMAL PRUCHASE	10,408.61	15,855.57
SUBTOTAL OF DIRECT EXPENSES		**138,848.64**	**4,902,488.31**
Grants to Institutions outside DFCI			
	Prostate Cancer Foundation	0.00	5,000,000.00
	Nancy Keating/HMS	0.00	100,000.00
	Kathryn Wilson/HSPH	0.00	100,000.00
	David Miyamot/MGH	0.00	100,000.00
	Kathryn Penny/BWH	0.00	100,000.00
	Stacey Misser/BWH	0.00	100,000.00
	Pier Paolo Pandolfi/BIDMC	0.00	103,319.14
SUBTOTAL OF DIRECT EXPENSES		**0.00**	**5,603,319.14**
TOTAL DIRECT EXPENSES		138,848.64	10,505,807.45
TOTAL INDIRECT EXPENSES @ 10%		39,776.90	1,209,776.90
TOTAL EXPENSES		178,625.54	11,715,584.35
CASH BALANCE *as of 9/30/17*			(79,856.06)

Leslie Y. Colon, Manager of Research Accounting

12/23/19
Date

Appendix 2. Original Plan for Program Reporting to the Court

A. David Mazzone Awards Program
Reporting Schedule

Awards Program Timeframes

Overall program start and end date	October 1, 2010 through September 30th, 2017 (seven years)
Overall program reporting period	October 1, 2010 through September 30th, 2017 (seven years)
All program grants issued between	July 1, 2011 and July 1, 2015 (five years)

Reporting Period	Report Number	Report due by	Notes
October 1, 2010- June 30, 2011	One	July 31, 2011	This report will describe programmatic progress
July 1, 2011 - December 31, 2011	Two	March 31, 2012	This report and all subsequent March reports will describe programmatic progress
July 1, 2011 - June 30, 2012 ***annual report***	Three	September 30, 2012	This report and all subsequent September reports will rep on annual research progress and accounting for all grants
July 1, 2012 - December 31, 2012	Four	March 30, 2013	Programmatic report
July 1, 2012 - June 30, 2013 ***annual report***	Five	September 30, 2013	Research progress and accounting for all grants
July 1, 2013 - December 31, 2013	Six	March 30, 2014	Programmatic report
July 1, 2013 - June 30, 2014 ***annual report***	Seven	September 30, 2014	Research progress and accounting for all grants
July 1, 2014 - December 31, 2014	Eight	March 30, 2015	Programmatic report
July 1, 2014 - June 30, 2015 ***annual report***	Nine	September 30, 2015	Research progress and accounting for all grants
July 1, 2015** - December 31, 2015	Ten	March 30, 2016	Programmatic report
July 1, 2015** - June 30, 2016 ***annual report***	Eleven	September 30, 2016	Research progress and accounting for all grants
July 1, 2016 - December 31, 2016	Twelve	March 30, 2017	Programmatic report
July 1, 2016 - September 30, 2017 ***final annual report***	Thirteen	December 31, 2017	Final reports on extended grants and final reconciliation reports

** Last scheduled 2 year grants issued by July 31, 2015, for closure by June 30, 2017

Notes:
As per later agreement with the Court, Research Progress and Accounting reports are for annual research activities covering the period August 1 to July 31 each year, as opposed to the period July 1 to June 30.

After no cost extension through September 2019, a final progress in accounting report was scheduled to describe activities during the period of the extension.

Appendix 3. List of 2011- 2019 Grantees

List of All Mazzone Program Grantees 2011 - 2014

Total: 39 Research Projects, One Seed Funding Project, One Student Training (CURE) Program grant

DF/HCC Roster 1 (August 2011 – July 2013) -11 Projects

Principal Investigator	Project Title	Institution	Grant amount
High Impact			
Matthew Freedman	Functional annotation of prostate cancer risk loci discovered through GWAS	DFCI	500,000.00
Project Development			
Myles Brown	Epigenetic reprogramming of AR function in CRPC	DFCI	100,000.00
Nathaniel Gray	Pharmacological validation of Etk/BMX as a target for the treatment of prostate cancer	DFCI	100,000.00
Xiaole Liu	DNase-seq for cost-effective identification of functional mutations in prostate cancers	DFCI	100,000.00
Pier P. Pandolfi	*Cancer stem cells targeting in CRPC*	BIDMC	100,000.00
Lupron Treatable Diseases and Conditions			
Stacey. Missmer	*Cancer and endometriosis*	BWH	100,000.00
Career Development			
David Miyamoto	*Analysis of AR signaling in circulating tumor cells in prostate cancer*	MGH	100,000.00
Kathryn Penney	*Prostate cancer genetic variants, molecular alterations and mRNA expression*	BWH	100,000.00
Kathryn Wilson	*Phosphorus and calcium intake, tumor microenvironment and prostate cancer progression*	HSPH	100,000.00
Disparities Research			
Donna Berry	Enhancing usability of the Personal Patient Profile-Prostate (P3P) for black and Hispanic men	DFCI	100,000.00
Nancy Keating	Understanding racial differences in prostate cancer mortality	HMS	100,000.00

DF/HCC Roster 2 (August 2012 – July 2014) -12 Projects

Principal Investigator	Project Title	Institution	Grant amount
High Impact			
Levi Garraway	Defining the spectrum of resistance to androgen ablation therapy in prostate cancer	DFCI	250,000.00
Steven Balk	Molecular Characterization of Gleason 3 Tumors That Progress to Gleason 4	BIDMC	250,000.00
Project Development			
Zhe Li	Castration-Resistant Luminal Cells in the Prostate	BWH	100,000.00
Massimo Loda	Developing a Blood-based Metabolomic Signature of Gleason Score	DFCI	100,000.00
Robert Cormack	Nanoplatforms for Localized Chemo Radiation Therapy for Prostate Cancer	DFCI	100,000.00
Lupron Treatable Diseases and Conditions			
Aaron Styer for Jose Teixeir	Pre-clinical in vivo studies investigating the efficacy of mTOR inhibitors for uterine fibroids	MGH	100,000.00
Elizabeth Henske	Targeting estrogen-dependent mechanisms in lymphangioleiomyomatosis LAM	BWH	100,000.00
Career Development			
Julie Batista (Formerly Kasp	Within-Person Molecular Differences in Primary Versus Metastatic Prostate Cancer	BWH	100,000.00
Jennifer Rider	Inflammation and tissue microenvironment as predictors of prostate cancer risk, mortality and therapy response among men with an initially benign TURP	HSPH	100,000.00
Disparities Research			
Lorelei Mucci	Estimating the prostate cancer burden attributed to lifestyle and genetic factors among African-American and White men	HSPH	100,000.00
Karen Emmons	Factors Influencing Willingness to Participate in Biobanking Among Black Men With and At-Risk for Prostate Cancer	DFCI	100,000.00
Community Outreach			
Jennifer Allen	Engaging African American Faith Communities in Prostate Cancer Education	DFCI	100,000.00
Glenn Bubley	*One Time seed funding for grant application preparation*	BIDMC	10,000.00

DF/HCC Roster 3 (August 2013 – July 2015) - 8 Projects			
Principal Investigator	**Project Title**	**Institution**	**Grant amount**
High Impact			
Karen E. Knudsen	Co-Targeting AR and ERG to Treat Advanced Prostate Cancer	Thomas Jefferson University	500,000.00
Felix Feng		University of Michigan	
Myles Brown		DFCI	
Mark Pomerantz	Genome-Wide analysis of response to androgen deprivation therapy	DFCI	500,000.00
Peter Nelson	Targeting Androgen receptor bypass pathways	University of Washington	200,000.00
Marc Vidal		DFCI	
High Impact Trials			
Mary-Ellen Taplin	Clinical Trials Assessing Mechanisms Mediating Sensitivity and Resistance to Enzalutamide	DFCI	500,000.00
Bruce Montgomery		University of Washington	
Xin Yuan		BIDMC	
Elahe Mostaghel		Fred Hutchinson Cancer Research Center	
Project Development			
Gregory Verdine	Targeting the co-activator of the Androgen Receptor	Harvard University	100,000.00
Karen Cichowski	Developing Novel Targeted Therapies for Advanced Prostate Cancer	BWH	100,000.00
Career Development			
Jennifer Sinnott	Impact on Prognosis of Inter- and Intratumor Heterogeneity in Prostate Cancer	Harvard School of Public Health	100,000.00
Disparities Research			
Lisa Signorello	Chronic Stress and Racial Disparities in Prostate Cancer	Harvard School of Public Health	100,000.00
Community Outreach			
Larissa Nekhlyudov	Shared Medical Appointments: An Innovative Approach to Prostate Cancer Survivorship Care	HVMA/Atrius Health	100,000.00
Student Training	Continuing Umbrella of Research Experiences (CURE) Program	DFCI	180,000.00

DF/HCC Roster 4 (August 2014 – July 2016) - 2 Projects			
Principal Investigator	**Project Title**	**Institution**	**Grant amount**
Zoltan Szallasi	Whole Genome Sequencing Based Identification of Genomic aberrations Specific to Prostate cancer Cases in African Americans	BCH	$50,000
Mark Preston	Do Baseline Prostate Specific Antigen (PSA) Levels Predict Advanced Prostate Cancer in African-American Men?	BWH	$90,000

DF/HCC Roster 5 (July 2018 - June 2019) - 2 Projects			
Principal Investigator	**Project Title**	**Institution**	**Grant amount**
Hubert Pakula	The role of TMPRSS2_ERG fusions in modulating tumor microenvironment in prostate cancer	DFCI	$53,000
Joshua W. Russo	Tumor Suppressor Function of Dipeptidyl Peptidase 4 in Castration-Resistant Prostate Cancer	BIDMC	$53,000

Prostate Cancer Foundation			
Principal Investigator	**Project Title**	**Institution**	**Grant amount**
2011			
Glenn Liu	Imaging biomarkers of treatment response using NaF PET/CT imaging: a prostate cancer clinical trials consortium effort	University of Wisconsin	1,000,000.00
William Nelson	Induction of synthetic lethality with epigenetic therapy (ISLET) for systemic treatment of prostate cancer	Johns Hopkins University	1,000,000.00
2012			
Bert O'Malley	Targeting the p160 Steroid Receptor Coactivators (SRCs) in Castration Resistant Prostate Cancer (CRPC)	Baylor College of Medicine	1,000,000.00
Martin Pomper	Promoter-Driven Molecular Radiotherapy for Prostate Cancer	Johns Hopkins University;	1,000,000.00
2013			
Jennifer Wu	Synergistic Immune and Lipid Metabolism Targeting for Metastatic Prostate Cancer	Medical university of South Carolina	500,000.00
2014			
David Baltimore	T Cell Receptor Gene Therapy for Treatment of Lethal Prostate Cancer	California Institute of Technology	500,000.00

Appendix 4. Progress and Financial Reports by 2018-2019 Grantees

Please use this form to submit your progress report. Submit financial report and additional documents as separate attachments

Research Grant Progress Report
DF/HCC Mazzone Awards Program

☐ Prelimary Report ☒ 1st Annual Report ☐ 18-month Report
☐ 2nd Annual Report ☐ 30-month Report ☒ Final Report

Grant Name:
DF/HCC Mazzone Awards Program Young Investigator Award

Grant Number:
6192900

Grant Term:

Principal Investigator:
PI: Hubert Pakula, Mentor: Massimo Loda

E-Mail:
hubert_pakula@dfci.harvard.edu

Phone Number:
617-640-7478

Institution: (Address, City, State, ZIP)
Dana-Farber Cancer Institute
450 Brookline Ave, 02115 Boston, MA

Institutional Official: (Title, Address, E-Mail, Phone)
Department of Oncologic Pathology

E-Mail:
BryanA_Hickey@DFCI.HARVARD.EDU

Phone Number:
617-582-9527

Human Subjects: ☐ Yes ☒ No

Human Protocol Number:

Research Exempt? ☐ Yes ☐ No

IRB Approval Date:

Animal Subjects: ☒ Yes ☐ No

Animal Protocol Number: 17-034

IACUC Approval Date:

Biospecimens? ☐ Yes ☐ No
If yes, please check the type(s) used:
☐ Tissue
☐ Cell Lines
☐ Plasma
☐ Serum

PRINCIPAL INVESTIGATOR ASSURANCE

By submitting this completed report, I certify that the statements herein are true and accurate to the best of my knowledge:

PI Signature Massimo Loda, MD Digitally signed by Massimo Loda, MD DN: cn=Massimo Loda, MD, o, ou, email=mloda@med.cornell.edu, c=US Date: 2019.11.22 12:19:32 -05'00'

Date Nov 22, 2019

TITLE: The role of TMPRSS2_ERG fusions in modulating tumor microenvironment in prostate cancer

Overview:

Prostate cancer (PCa) is a clinically heterogeneous disease with marked variability in progression. Gene fusions of the 5'-untranslated region of TMPRSS2 with the ETS transcription factor family members, most frequently ERG, results in its enhanced activity in ~50% of human PCa [1]. Despite the fact that this the most frequent genetic alteration in prostate cancer, murine transgenic models were devoid of an epithelial phenotype [2]. During PCa progression, ERG induces other signaling pathways such as WNT, MYC and PI3K/AKT/PTEN [3]. While the molecular consequences of TMPRSS2-ERG fusions have been dissected in the epithelial compartment, little is known on their impact on the tumor microenvironment (TME).

We previously found that stroma, but not epithelium, distant from tumor in radical prostatectomies is very different from stroma in prostates without tumor, and that gene expression in the tumor adjacent stroma is strongly associated with Gleason grade, mimicking in many ways the bone microenvironment [4]. The overall objective of this proposal was therefore to ask whether genetic changes in the epithelium induces molecular gene expression changes in the stromal microenvironment that would in turn promote tumorigenesis. In order to accomplish this, we utilized genetically engineered mouse models (GEMMs) that phenocopy disease in initiation and progression (T-ERG and T-ERG; Pten+/-), and obtained gene expression from stromal cells by single cell RNASeq. We also studied human samples from prostates free of tumor, radical prostatectomies with localized disease, and biopsies from distant metastatic sites to histologically validate stromal markers.

The Mazzone award has enabled us to achieve our overall goal. Here we provide a comprehensive understanding of the role of T-ERG fusion in modeling the TME and its impacts on PCa development. By single cell RNAseq we identified stromal cell populations in T-ERG mice that differed from wild type mice these populations showed an increased expression of Wnt receptors and ligands.

Since TMPRSS2/ERG fusions represent an early event in prostate tumorigenesis, we provide here a mechanism whereby induction of Wnt signaling in the stroma by epithelial cells driven by the translocation, increases the stem cell compartment in both epithelial and stromal cells. These data suggest that ERG positive epithelial cells activate stromal cells to increase Wnt signaling, possibly enhancing prostate carcinogenesis. This will inform strategies toward the identification of targeted therapeutics in patients harboring ERG+ tumors.

Scientific Accomplishments:

Below is a detailed summary of our research activity addressing both Aims: 1) To characterize cell composition of stroma in Tmprss2-ERG mice and 2) To determine whether T-ERG cooperates with Pten-loss to drive carcinogenesis by modulating stroma through ERG-induced upregulation of Wnt-signaling.

To define the roles of ETS fusions in modulating prostatic stroma at the early stages of carcinogenesis, we took advantage of the ETS knock-in mouse model in which an ectopic expression of human ERG cDNA is under the transcriptional regulation of the androgen/estrogen-responsive mouse Tmprss2 promoter/enhancer. Thus, the fusion transcript recapitulates the TMPRSS2-ERG (T-ERG or T2E) fusion in patients [13]. Whereas T-ERG mice did not show alterations in the epithelium by the age of 3 months, we noticed an increase in the number of stromal cells adjacent to the epithelial glandular structures. Herovici histochemistry [5] showed a pronounced light blue stroma surrounding prostatic acini in T-ERG mice indicating the presence of immature collagen fibrils and ECM remodeling (FIG1a). To identify lineages from the heterogenous stroma of T T-ERG, we used a single-cell barcoding and sequencing platform that utilizes droplet microfluidics (inDrop). We sequenced mRNA of thousands of mouse prostate cells representing all lineages (epithelial and stromal) of Dorsolateral (DLP), Ventral (VP) and Anterior (AP) prostate lobes from FVB/N wild type (WT) and T2E fusion

mutant (MT) mice [6]. Following library preparation and whole-transcriptome sequencing, we implemented the Seurat single cell pipeline to i) compute the expression level of each gene across all cells and ii) identify distinct cell populations by semisupervised clustering. We identified 13 cell clusters with distinct gene expression signatures (FIG1b). Expression of some candidate stromal and myofibroblast genes showed that clusters 0, 3, 6 and 8 contained the majority of Platelet-derived growth factor receptor β (Pdgfrβ) and actin alpha 2 (αSma)+ cells whereas clusters 0, 3 and 6 contained the majority of Platelet-derived growth factor receptor α (Pdgfrα), collagen, type I alpha 1 (Col1a1) and Periostin (Postn) (Figure1c). Interestingly, immunofluorescence (IF) analysis of clarified mouse prostate tissues have shown that stroma of T-ERG mice overexpresses Pdgfrβ rather than Pdgfrα that is highly expressed in the WT stroma. In addition, IF volumetric analysis from the DLP lobes showed nearly 5 times more Pdgfrβ in T-ERG stroma than in WT stroma (Fig 2a). IF volumetric analysis of Postn in the VP lobes showed over 4 times more of this marker in the stroma of T-ERG than in WT (Fig 2b). Finally, we validated these findings in prostate TCGA cohort, choosing the subsets of two cohorts ERG positive and ERG negative patients. We observed statistically significant upregulation of both POSTN and PDGFRB in ERG-fusion positive patients in comparison with no fusion patients, suggesting that ERG+ epithelium puts a selective pressure on these stroma specific populations in TME (Fig 2c).

More interestingly, scRNAseq analysis of murine stromal populations showed a two fold increased expression of Wnt receptors Leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5) and Frizzled Class Receptor 7 (Fzd7), and Wnt ligands such as Secreted Frizzled Related Proteins 2 and 4 (sFRP2 and sFRP4), Wnt6 and Rspo2 (Fig. 3A). Of note, WNT signaling SFRP2 and SFRP4 were present in the human stroma signature [4] and both are critical for bone remodeling during prostate cancer development [7]. Interestingly, COL1A1 and Periostin were found in human stroma adjacent to high Gleason [4] [8]. While COL1A1 is an osteoblastic differentiation marker and its expression is modulated by WNT, POSTN is produced by stromal αSMA+VIM+ (αSMA) fibroblasts recruiting Wnt ligands and thereby increases Wnt signaling in cancer stem cells [9[. Wnt-related genes such as Lgr5 or Porcn were significantly upregulated in the DLP and VP lobes of T-ERG mutants compared to WT mice, corroborating the importance of Wnt genes found in scRNAseq (Fig. 3B). Since TMPRSS2-ERG fusions represent an early event in prostate tumorigenesis [10][11][12] additional oncogenic events such as PTEN loss drive PCa progression. By using i) T-ERG, ii) T-ERG; Pten-loss mice and iii) Pten-loss mice, we showed that stroma adjacent only to the T-ERG+ but not Pten-loss alone+ epithelium overexpressed WNT-secretion regulator-enzyme Porcn suggesting that ERG triggers Wnt secretion in the stromal cells (Fig. 4). We showed that T-ERG+ epithelium induces stroma to supply Wnt ligands that bind to Wnt receptors such as Lgr5, ultimately activating Wnt pathway cascade in epithelial cells. In fact, FACS analysis showed more Lgr5+cells in the stromal compartment of T-ERG prostate than in WT (Fig. 5A-D). Furthermore, sorted Lgr5-stromal cells upregulate PdgfrB suggesting that this stromal lineage is the essential source of Wnt for ERG fusion positive epithelium. It has been shown in mouse models that T-ERG fusions leads to either a minor phenotype or almost no observable abnormalities in epithelium [13]. However, ectopic ERG expression can cooperate with Pten loss to drive prostate cancer development. In order to show the activation of Wnt+ epithelial cells in the T-ERG; Pten-/- PINs, performed a lineage-tracing experiment in the knock-in Lgr5-eGFPcre line [14]. While the Wnt+ GFP/Lgr5+ epithelial cells fueled PINs, unexpectedly, Lgr5-expressing cells were also found throughout the stroma (Fig. 5E). This suggests that T-ERG cooperates with Pten-loss to drive cancer development by modulating stroma through ERG-induced upregulation of Wnt-signaling.

To test whether T-ERG in cooperation with Pten-loss modulates the bidirectional communication between stroma and epithelium, we isolated epithelial and stromal tissue from T-ERG, T-ERG; Pten+/-, Pten+/- alone and WT, and expanded them by 3D organoid culture. The ultimate goal was to perform renal grafts where both the mesenchymal and the epithelial compartment were infected with GFP or RFP-lenti-virus carrying certain Wnt mutations. This system would allow us to visualize the distribution of both mesenchymal and epithelial cells, trace them over time and characterize their role.

Next, we developed 3D organoid culture and GFP-Lenti virus-based plasmids with either loss-or-gain-of-function-mutations for Wnt signaling. After optimizing a spin-infection protocol, we obtained both epithelial and mesenchymal compartments carrying an edited Wnt pathway. As an alternative to in vivo studies, we have established a novel model of tumor-stroma interaction that will enable us to track and quantify morphological changes in 3D co-cultures, in real-time live-cell settings. To this end, we used VCAP cells known to carry ERG overexpression and combined them with 3T3 fibroblast cells that were infected with either Red Fluorescence Protein (RFP) empty vector or with RFP-b-catenin GOF mutation vector. Activation of the Wnt pathway in the mesenchymal compartment leads to PORCN overexpression in organoid co-culture (Fig. 6). Next, we will profile the surfaceomes, perform secretome analysis of sorted cells, and identify Wnt ligands with clustered Wnt-receptor. These will be targeted to validate mechanistically that genetic alterations in the epithelium drive

canonical wnt signaling in the mesenchymal compartment which, in turn, drives tumorigenesis in the transformed epithelial cells. Next steps for this aim include extending of this methodology to human primary organoid co-cultures deriving from radical prostatectomies and performing secretome/surfaceome analysis.

List of key accomplishments:

1) Invited Speaker: Stromal Regulation of Prostate Cancer Progression - Microenvironment: The Cancer Swamps- 7th Annual Coffey Holden Prostate Cancer Academy of Prostate Cancer Foundation (PCF) June 21st, 2019, Los Angeles, CA

2) Invited Speaker: The role of TMPRSS2_ERG fusions in modulating tumor microenvironment in prostate cancer (Minisymposium on Signalling in the Tumor Microenvironment during AACR Annual Meeting 2019, Apr 1, 2019, Atlanta, Georgia)

3) Poster Session: The role of TMPRSS2-ERG fusions in modulating tumor microenvironment in prostate cancer (March 3-5, 2019 at the W Hotel in Fort Lauderdale, Florida

4) Poster Session: The role of TMPRSS2-ERG fusions in modulating tumor microenvironment in prostate cancer (September 10-13, 2018; EMBO EMBL Symposium: Organoids: Modelling Organ Development and Disease in 3D Culture, Heidelberg, Germany)

Conclusions:

Most if not all work that has been done on prostate cancer in the last 20 years was focused on genetic changes in the epithelium. This, however, does not provide a full picture of prostate cancer development. In fact, still little is known about the precise mechanism regulating the initiation of PCa at both genetic and cellular levels. Here we have thoroughly characterized the role of T-ERG fusion in modeling the TME as an early event of PCa development. By single cell RNAseq we identified 4 clusters corresponding to stromal cell populations in T-ERG mice that are not present in WT. Interestingly, immunohistochemical analysis showed that T-ERG+ epithelium favors expression of Pdgfrβ+ over Pdgfrα in the stromal compartment and recruits Postn+ stromal cells. Moreover, we observed an increased expression of Wnt pathway components such as Lgr5 and Porcn in these population. This suggests that Wnt pathway in stromal cell is induced by ERG-positive epithelium. Our findings will help to elucidate the role of the microenvironment in tumor promotion and progression, thereby aiding the development of stroma-targeted therapies. Therapeutic targeting of the T-ERG- induced Wnt+ stromal cells may benefit patients that harbor the TMPRSS2- ERG fusion, including those with concomitant PTEN loss.

Future plans:

In order to reproduce in the mouse the stages assessed in humans, we will determine the stromal composition in T2E/PTEN+/- (PIN but no invasion). We will also use a model with a different genetic background, the Hi-Myc model that develops PIN, invasive cancer{ Ellwood-Yen, 2003 #17}. We have recently established cre-lines representing stromal components to perform in vivo lineage tracing experiments. The combinatorial approach of using Cre recombinase fused to a modified estrogen receptor ligand binding domains (ERT2) permits the temporal induction by delivery of a tamoxifen and tissue specificity by directing the Cre expression with a lineage- specific promoter (e.g., PDGFRαCreERt2, Pdgfr-β-CreERT2, Col1a1-CreERT2, αSMACre-

ERT2). When crossed with mice recapitulating Tmprss2-ERG fusions in Pten-loss [80], or over-expression of MYC with Ai65D reporter mice, recombination can be detected by expression of red fluorescence (TdTomato). This system allows for the spatial and temporal activation of Cre labeling and the identification of stromal cell populations such as fibroblasts, myofibroblasts (i.e. Pdgfrα +; pdgfrβ +; αSMA+) and ECM components (i.e. Col1a). We will trace the dTomato+ lineage progenies of the labeled stromal cells for a prolonged period after the tamoxifen treatment has been terminated. The ultimate goal of this study will be: i) to get a spatial distribution of stromal cells in different genetic backgrounds and stages of PCa development and ii) characterize these cells molecularly and 3) interrupt the stromal feedback to the epithelium and halt tumorigenesis.

FIGURES: attached

Literature.

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12. Linn DE, Penney KL, Bronson RT, Mucci LA, Li Z. Deletion of Interstitial Genes between TMPRSS2 and ERG Promotes Prostate Cancer Progression. Cancer Res 2016;76(7):1869-81.

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Description of Report Period Activities

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Dana-Farber Cancer Institute

FINANCIAL REPORT OF EXPENDITURES

LUPRON SETTLEMENT POOL (A. DAVID MAZZONE AWARDS PROGRAM)
UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS
ONE COURTHOUSEWAY, SUITE 2300
BOSTON, MA 02210

AWARD: **6192900**

DFCI NO. **6317301**

AWARD PERIOD: **10/01/10 - 09/30/19**

REPORTING PERIOD: **07/01/18 - 06/30/19**

INVESTIGATOR: **Pakula, Hubert T.**

	Year 1	Cumulative
TOTAL AWARD	53,000.00	53,000.00
UNEXPENDED BALANCE FROM PREVIOUS PERIOD	0.00	0.00
NET AWARD	53,000.00	53,000.00
CASH RECEIVED TO DATE	0.00	0.00
EXPENDITURES		
SALARY & WAGE EXPENSE	4,633.09	4,633.09
FRINGE BENEFITS	1,297.29	1,297.29
ANIMAL PURCHASE	10,408.61	10,408.61
SERVICE CENTERS/CORE CHARGES	27,804.51	27,804.51
TRAVEL	2,842.41	2,842.41
OTHER	6,014.09	6,014.09
TOTAL DIRECT EXPENSES	53,000.00	53,000.00
TOTAL INDIRECT EXPENSES @ 0.00%	0.00	0.00
TOTAL EXPENSES	53,000.00	53,000.00

Leslie Y. Colon, Manager of Reseach Accounting

12/23/19
Date

Please use this form to submit your progress report. Submit financial report and additional documents as separate attachments

Research Grant Progress Report
DF/HCC Mazzone Awards Program

☐ Prelimary Report ☐ 1st Annual Report ☐ 18-month Report
☐ 2nd Annual Report ☐ 30-month Report ☒ Final Report

Grant Name:

Downregulation of DPP4 mediates resistance to androgen deprivation therapy in castration-resistant prostate cancer (

Grant Number:

6192971

Grant Term:

1 yr/$53,000

Principal Investigator:

Joshua W. Russo

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Phone Number:

617-735-2111

Institution: (Address, City, State, ZIP)

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Randy Mason
Vice President of Research Operations

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rmason@bidmc.harvard.edu

Phone Number:

617-667-1803

Human Subjects: ☒ Yes ☒ No

Human Protocol Number: 18-406

Research Exempt? ☒ Yes ☐ No

IRB Approval Date: Nov 8, 2018

Animal Subjects: ☒ Yes ☐ No

Animal Protocol Number: 086-2016

IACUC Approval Date: Nov 1, 2018

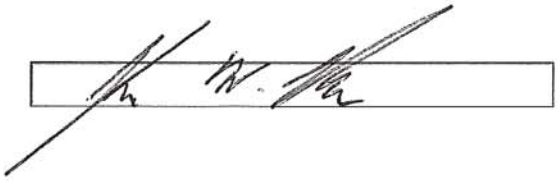
Biospecimens? ☒ Yes ☐ No

If yes, please check the type(s) used:

☒ Tissue
☒ Cell Lines
☐ Plasma
☐ Serum

PRINCIPAL INVESTIGATOR ASSURANCE

By submitting this completed report, I certify that the statements herein are true and accurate to the best of my knowledge:

PI Signature  **Date** 9/12/19

Grant Name: Downregulation of DPP4 mediates resistance to androgen deprivation therapy in castration-resistant prostate cancer (CRPC)
Grant Number: 6192971
PI: Joshua W. Russo

1. LAY SUMMARY

Using experiments on mouse models of prostate cancer (PCa) treated with castration and the androgen deprivation therapies (ADTs), abiraterone and enzalutamide, I have identified the gene *DPP4* as being significantly downregulated in castration-resistant prostate cancer (CRPC). The *DPP4* gene product is an enzyme that targets numerous pro-survival growth factors for degradation and prostate cancer cells would benefit greatly from downregulating DPP4 expression in order to increase the local concentrations of these pro-survival factors. Significantly, chemical inhibitors of DPP4 activity, (which would mimic DPP4 downregulation) are widely used to treat Type II diabetes and studies funded by this award show that DPP4 inhibitor treatment makes prostate cancer more resistant to ADT. As there is likely significant overlap between men with Type II diabetes and men with metastatic prostate cancer, this raises important questions about the interaction of DPP4 inhibitors with ADT.

Extending my preliminary results, I conclusively showed downregulation of DPP4 expression in a mouse xenograft model of castration-resistant prostate cancer **(FIGURE 1)**. I then validated these results in several clinical RNA sequencing data sets produced by our lab and in primary and metastatic CRPC tissue sections. In nearly all cases, DPP4 RNA and protein expression is high in the primary PCa setting and nearly absent in the castration-resistant setting **(FIGURE 2)**. These results were followed by a series of three mouse xenograft experiments demonstrating that inhibition of DPP4 enzyme activity with a DPP4 inhibitor used to treat Type II diabetes caused the tumors in these mice to relapse faster. This work suggests that drugs which inhibit DPP4 enzyme activity could interfere with the ADT used to treat metastatic prostate cancer. These results have been published in manuscript form (Russo et al, 2018).

In addition I have also undertaken work to developed cell lines that stably express constructs with inducible downregulation and overexpression of DPP4. These cell lines will be used to test the effects of altering DPP4 protein levels directly and help to identify the important pro-survival growth factor DPP4 targets for degradation. Once the pro-survival growth factor responsible for promoting ADT resistance is identified, then it can be targeted directly to block resistance. The cell line with inducible downregulation of DPP4 has already been established and is currently growing in mice as xenografts. The inducible DPP4 overexpression vector is under construction.

2. PROGRESS REPORT

Overview

When men develop prostate cancer that spreads or metastasizes to other parts of their body, the first and second line treatments used by doctors attempt to block the effects of the androgen hormones testosterone and dihydrotestosterone (DHT) on prostate cancer cells. This type of therapy is called androgen deprivation therapy or ADT because it deprives the prostate cancer cells of these important androgen hormones. Testosterone and DHT bind to the androgen receptor (AR) within cancer cells and stimulate the growth and progression of prostate cancer. Gonadotropin-releasing hormone agonists (GnRH-agonists), abiraterone, and enzalutamide are three drugs commonly used to block the pro-cancer hormone signaling that occurs through testosterone and DHT. GnRH agonists inhibit the testicular production of androgen hormones. Abiraterone inhibits an enzyme called CYP17A1, which decreases the levels of testosterone and DHT. Enzalutamide blocks the ability of testosterone and DHT to bind to androgen receptor (AR) and stimulate prostate cancer cells. These drugs are initially effective at stopping prostate cancer progression, but in nearly all men the cancer eventually becomes resistant. The subject of this research proposal was determining how downregulation of the gene *DPP4* and its protein product helps prostate cancer to become resistant to ADT and how DPP4 inhibitors used to treat Type II diabetes influence prostate cancer progression. Over the past year I have shown that DPP4 downregulation is tightly associated with PCa progression in preclinical models and in clinical biopsy materials. In the VCaP xenograft model and in the majority of clinical cases, as PCa becomes resistant to ADT, AR signaling is restored. DPP4 is an AR stimulated gene similar to PSA. However, in the resistant setting, while PSA expression is restored, DPP4 expression is not.

This suggests that the continued downregulation of DPP4 might have functional significance in PCa survival, especially since DPP4 is known to degrade various pro-survival growth factors and cytokines. Of greater significance, I have also shown that inhibitors of DPP4 enzyme activity decrease the effectiveness of ADT. As it will be difficult to identify therapies capable of increasing DPP4 protein expression within PCa cells, the next important step in this work will be to identify the pro-survival growth factor that is degraded by DPP4 and the kinase signaling cascade the growth factor activates to promote ADT resistance. This will allow us to target the growth factor and its associated receptor/kinase cascade directly to block ADT resistance.

As a prostate cancer researcher exploring the mechanisms of resistance to ADT in PCa, I have several projects underway. The support of the Mazzone award allowed me to focus my efforts over the past year on the DPP4 project, resulting in a first author publication and extension of my DPP4 research efforts.

Scientific Accomplishments

Aim 1. Assess the functional significance of DPP4 downregulation in the PCa xenograft setting.

Given the hypothesis that DPP4 targets PCa pro-survival growth factors/signaling peptides for degradation, I expected that inhibition of DPP4 would increase the local concentrations of pro-survival growth factors/signaling peptides and thereby decrease the sensitivity of PCa xenografts to castration resulting in faster time to relapse and increased tumor growth. Mice with VCaP xenograft tumors were treated with the DPP4 inhibitor sitagliptin or vehicle and there was a significant difference in terminal tumor volume by 42 days of treatment with tumors in sitagliptin treated animals measuring, on average, ~50% larger than controls (1659 mm3 vs 829 mm3) **(FIGURE 3)**. Sitagliptin-treated tumors also exhibited increased levels of DPP4 protein, consistent with the tumor cells no longer being driven to downregulate DPP4 expression as drug treatment with the inhibitor serves the same function. These studies were extended using the LNCaP xenograft and the BRCA2-deficient BID-PC-1 patient derived xenograft (PDX), where sitagliptin treatment had a similar detrimental effect on the effectiveness of ADT in these tumors. These results show that the effects of DPP4 inhibition are penetrant across PCa with different genomic backgrounds, VCaP (AR amplification, TMPRSS2:ERG fusion), LNCaP (PTEN deficient), and BID-PC-1 (BRCA2 deficient). As we obtain additional castration-sensitive PDX models, I will continue to test the effects of DPP4 inhibition. This work was published in a first author manuscript (Russo et al, 2018).

While these studies clearly indicate that DPP4 inhibitors reduce the effectiveness of ADT in in vivo models, it is important to demonstrate that this effect is mediated through direct effects on DPP4. In order to address this, I have undertaken the development of VCaP cell lines expressing inducible shRNA or cDNA to DPP4. I have established a VCaP cell lines that stably expresses either an inducible nonsense control shRNA or an shRNA against DPP4. Knockdown of DPP4 will mimic the downregulation of DPP4 protein seen in castration resistance and should also mimic the inhibition of enzyme activity caused by DPP4 inhibitors. Using these cells lines, I will grow up xenograft tumors in mice, then induce knockdown of DPP4. I predict that tumors with knockdown of DPP4 expression will exhibit similar resistance to castration as observed for DPP4 inhibitors. Xenograft tumors derived from this cell line are currently growing in intact mice. I expect to have a VCaP cell line stably expressing an inducible cDNA to DPP4 shortly and will use these cell to conduct similar experiments in the overexpression setting. In this case, DPP4 overexpression should increase the effectiveness of castration at inhibiting tumor growth. These cells lines will also aid in the identification of the growth factor/cytokine targeted by DPP4 as described below.

Aim 2. Determine the signaling cascades effected by DPP4 downregulation/inhibition and the corresponding growth factors/cytokines targeted by DPP4 that are responsible for ADT resistance.

The studies proposed in Aim 2 are moving forward with the development of the VCaP cell lines with the stably incorporated inducible shRNA to DPP4 and the planned inducible DPP4 cDNA described above. Briefly, xenografts of these shRNA cells will be grown up intact mice, followed by doxycycline induction. Tumor samples from the control shRNA expressing tumors and the DPP4 shRNA expressing tumors will be taken 7 days after induction and compared for activity of important kinase signaling cascades (PI3K/AKT/mTOR, MAPK/ERK, p38MAPK, etc.) using western blot, reverse phase proteome array (RPPA), and mass-spec phosphoproteome analysis. In parallel, I will also assay DPP4's effects on signaling in a more physiological setting by conducting a similar experiment in the castrate setting that compares unaltered VCaP tumors treated with sitagliptin with

matched tumors that have sitagliptin treatment withdrawn for 5 days. Molecular studies (IHC, western, RT-PCR) will be performed on the resulting tumor sets to validate those signaling cascades identified by analysis of the western blot, RPPA, and phosphoproteome data as well as additional signaling cascades know to be important in PCa, including AR and Wnt signaling. Signaling pathways that are similarly altered in both mouse experiments will have a high likelihood of being the important pathway effected by DPP4 downregulation. Signal transduction pathways activated by known targets of DPP4 degradation, including NPY, bFGF, and SDF-1α will also be interrogated. Once a candidate signaling peptide/cascade is identified, future studies will focus on using inhibitors of the signaling cascade in the VCaP xenograft and PDX animal model setting to counteract the effects of DPP4 downregulation/inhibition on tumor growth.

In an effort to interrogate likely growth/factor cytokine candidates while waiting for xenograft studies to mature, I have performed immunohistochemistry (IHC) studies in the VCaP preclinical model of prostate cancer progression for two important PCa growth factors, NPY and SDF-1α, that are also known to be degraded by DPP4. While IHC studies for SDF-1α were negative, NPY shows an inverse correlation with DPP4 protein expression as the VCaP xenograft progresses **(FIGURE 4)** as would be expected if DPP4 was degrading secreted NPY. This suggests that NPY might be the pro-survival growth factor/cytokine that drives early survival in prostate cancer undergoing ADT. These results are preliminary and will be extended into clinical specimens and validated in the inducible xenograft models.

Key Accomplishments/Main Conclusions:

1) Downregulation of DPP4 is tightly associated with PCa progression and the development of resistance to ADT both in preclinical models and in patient samples. (Russo et al, 2018). DPP4 is an AR stimulated gene, yet remains downregulated even when AR signaling is restored in castration resistance. As DPP4 is known to degrade a number of pro-survival growth factors and cytokines, these findings suggest that DPP4 downregulation might have a functional role in ADT resistance.
2) Inhibitors of DPP4 enzymatic activity decrease the effectiveness of ADT in xenograft and PDX models of PCa (Russo et al, 2018). This result has clear implications for men with Type II diabetes on a DPP4 inhibitor that are newly diagnosed with metastatic prostate cancer and about to start ADT. The combination of DPP4 inhibitor and ADT should be used with caution, as the DPP4 inhibitor might decrease the effectiveness of ADT.
3) NPY, a pro-survival growth factor and known target of DPP4 degradation, has an inversely correlated expression pattern compared to DPP4. DPP4 downregulation might cause increased local concentrations of NPY, allowing PCa tumors to resist ADT.

Future Plans

Experiments to define the growth factor/kinase cascade target of DPP4 are already underway for Aim 2 using the VCaP cell lines stably expression shRNA and cDNA to DPP4. Once a candidate growth factor/kinase cascade is identified, future studies will focus on using inhibitors of the signaling cascade in the VCaP xenograft and PDX animal model setting to counteract the effects of DPP4 downregulation/inhibition on tumor growth. As this signaling cascade might generally contribute to ADT resistance, any targeted therapy we identify could be used to treat patients whose tumors have become resistant to ADT.

As mentioned previously, the interaction of DPP4 inhibitors with ADT also has important implications in the treatment of metastatic PCa. I hope to start a collaboration with an epidemiologist to access medical Surveillance, Epidemiology, and End Results (SEER)-type databases and determine if men with Type II diabetes treated with DPP4 inhibitors who also have metastatic PCa treated with ADT have worse outcomes with decreased ADT efficacy.

As a prostate cancer early investigator my career goal is to become an independent academic research scientist with a focus on determining the causes of advanced metastatic prostate cancer resistance to ADT and developing therapies to combat this resistance. Downregulation of DPP4 protein expression in castration-resistant prostate cancer represents one of these mechanisms and my publications from this work will form the basis of future grant applications to establish myself as an independently funded prostate cancer researcher.

Challenges and Opportunities and Potential for Additional Funding

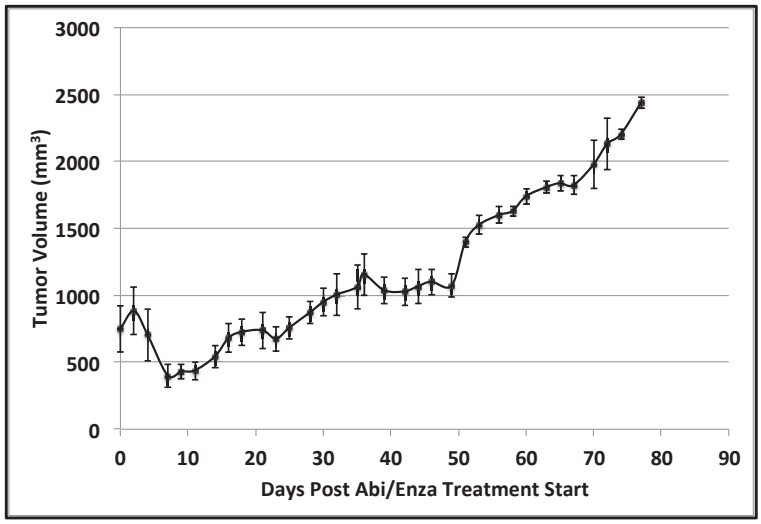
Challenges – Thus far it has been challenging to develop a collaboration with an epidemiologist to explore the interaction of DPP4 inhibitors with ADT in men with Type II diabetes and metastatic PCa. I am currently generating clinicopathological data relating DPP4 expression and clinical predictors of disease outcome in the primary prostate cancer setting. My hope is that if a positive correlation can be found between decreased DPP4 expression and pathological risk factors of aggressive primary prostate cancer, that it will generate more interest in the project from my epidemiologist colleagues.

Opportunities and Potential for Additional Funding – My published work on DPP4 and the additional work that will be completed over the next year will form the basis of an NIH/NCI RO1 application. The focus of the application will be determining the mechanism of DPP4 downregulation in the face of restored AR signaling and its role in PCa progression. As mentioned before, *DPP4* is an AR stimulated gene whose expression is not restored in castration-resistance. The mechanism by which this occurs could involve transcriptional cofactors, AR splice variants, alternative transcription factors, or epigenomic regulation. Understanding this mechanism will allow us to identify additional pro-survival genes important to ADT resistance regulated in the same fashion and more importantly will allow us to develop targeted therapy to stop the expression of these genes.

References

Russo JW, Gao C, Bhasin SS, Voznesensky OS, Calagua C, Arai S, Nelson PS, Montgomery B, Mostaghel EA, Corey E, Taplin ME, Ye H, Bhasin M, Balk SP. Downregulation of Dipeptidyl Peptidase 4 accelerates progression of castration-resistant prostate cancer. *Cancer Res*. 2018 Nov 15;78(22):6354-6362. PMID: 30242112

A. Figure 1



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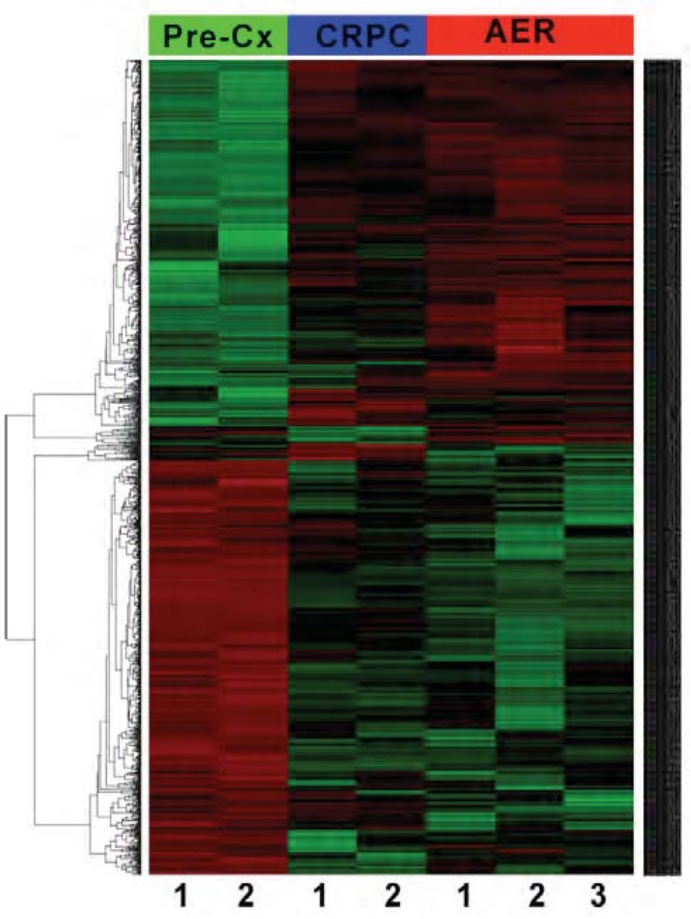


C. AER vs. Pre-Cx

Upregulated Genes		
Gene	Log2(FC)	P Value
TMEFF2	5.44	9.94E-08
OR51E1	5.39	1.39E-08
OR51E2	4.60	7.82E-08
AR	4.48	3.42E-15
BHLHE41	4.46	1.99E-06
NOV	4.42	1.74E-06
SCG2	4.16	1.62E-06
FOLH1	4.05	3.08E-06
FRAS1	3.98	3.30E-06
LOC100130417	3.84	4.98E-06
SDC2	3.80	1.29E-07
NR3C2	3.73	7.08E-10
EPHA4	3.53	5.79E-08
LRFN2	3.44	6.18E-06
DNAH7	3.28	5.44E-07
ANK2	3.27	1.35E-06
THBS1	3.20	4.41E-10
RTN1	3.10	9.74E-07
LINC02418	3.06	3.12E-06
COLEC12	2.97	2.17E-09
ADAM7	2.88	2.08E-07
SESN3	2.85	5.43E-09
VWA5B1	2.78	2.97E-09
KLF9	2.73	2.75E-09
NRXN2	2.69	1.51E-10
SAMD11	2.68	8.62E-07
PTGER2	2.67	4.50E-08
CACNA1C	2.63	2.54E-10
SATB1	2.61	1.37E-08

Downregulated Genes		
Gene	Log2(FC)	P Value
FAM189A2	-7.70	1.49E-08
DPP4	-6.83	5.40E-13
SH3RF2	-5.17	1.47E-06
CACNG4	-5.14	5.27E-08
VSTM2A	-5.01	5.87E-10
ST6GALNAC1	-5.00	7.85E-09
VSTM2A-OT1	-4.93	2.19E-06
TESC	-4.87	8.82E-11
HABP2	-4.84	9.82E-06
ALDH1A1	-4.74	3.71E-10
LUZP2	-4.62	1.80E-09
TRPM8	-4.23	1.32E-12
TMSB15A	-4.04	4.27E-06
LOC101929653	-3.98	1.85E-10
FRMPD3	-3.62	5.52E-07
SPOCK2	-3.57	3.09E-11
STEAP4	-3.56	1.78E-13
COL4A5	-3.49	5.26E-12
DIRAS2	-3.43	1.19E-10
CRISP2	-3.34	4.23E-06
ANKRD29	-3.33	8.85E-10
HLA-DMA	-3.19	1.60E-10
KRT14	-3.11	5.13E-06
NRCAM	-3.05	1.25E-12
LAMA3	-2.97	6.92E-15
UGT2B4	-2.96	9.56E-08
NNMT	-2.95	7.80E-08
SLC27A6	-2.93	1.54E-06
NTM	-2.88	4.30E-08
ESRRG	-2.86	1.00E-09
HGD	-2.85	1.30E-10
MCCC2	-2.84	4.74E-09
MCC	-2.80	4.26E-11
VWF	-2.77	7.72E-07
CUEDC1	-2.77	3.29E-07
ENSG00000213777	-2.77	3.00E-13
WISP1	-2.64	9.63E-06
TOX3	-2.62	8.55E-10
C4orf19	-2.62	2.52E-09
TG	-2.62	2.03E-09
ELOVL2	-2.61	6.56E-11
CNKSR2	-2.56	1.47E-09
MICALCL	-2.50	8.26E-07

D.

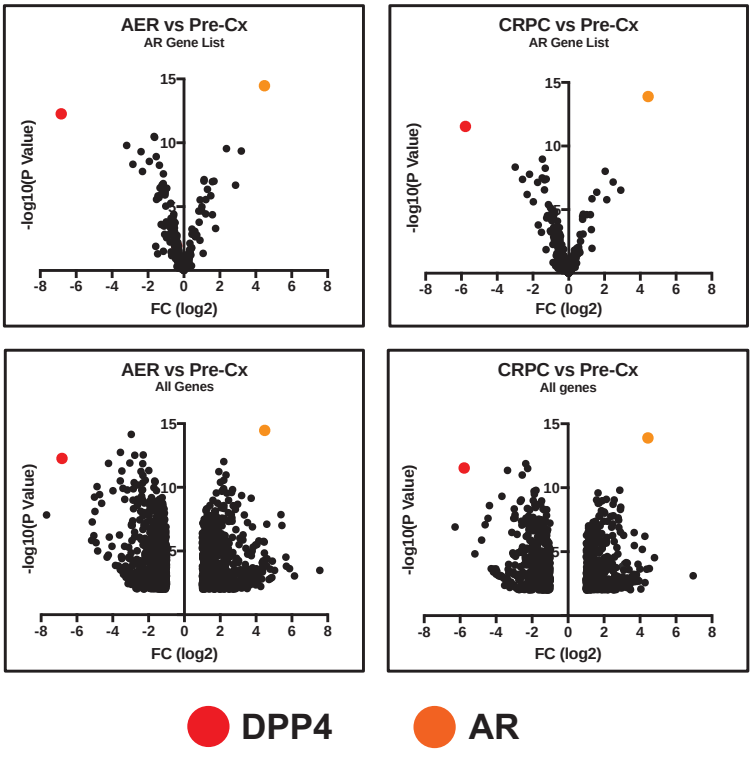
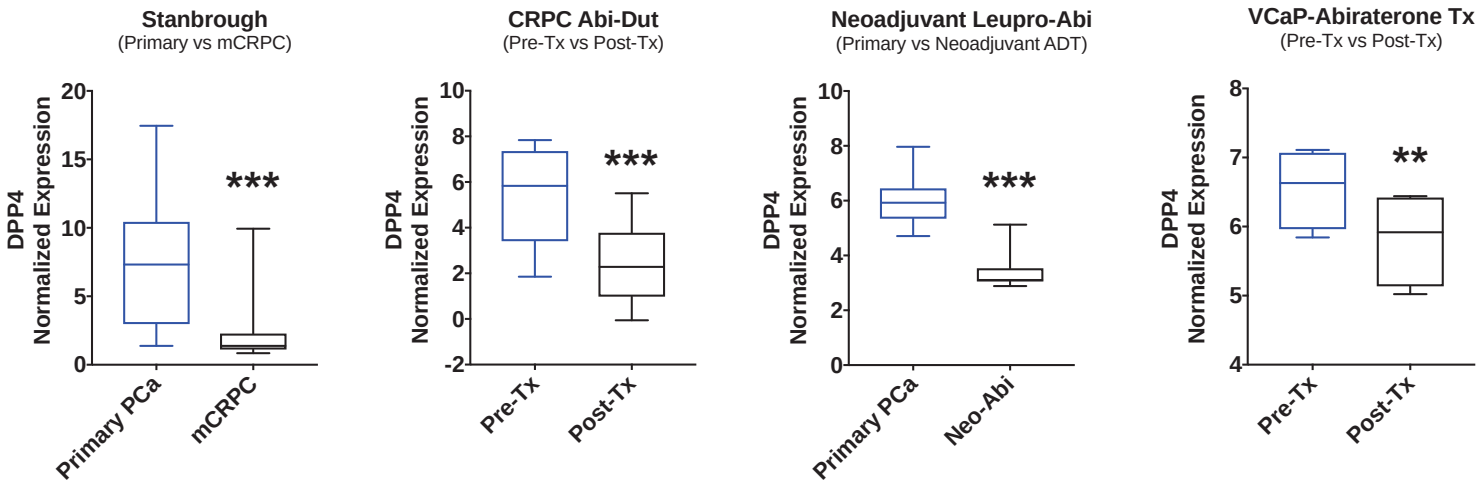


Figure 1 – Data analysis of AER VCaP xenograft serial biopsy RNA sequencing. AER VCaP tumors were serial biopsied prior to cx (Pre-Cx), at tumor relapse (CRPC), and when tumors exceeded 2000mm^3 (Abi/Enza resistant = AER). Initial cohort composed of 22 mice. **A)** VCaP CRPC xenografts are initially sensitive to Abi/Enza treatment for the first 10 days, but recover tumor volume by day 30. **B)** Unsupervised clustering of Pre-Cx, CRPC, and AER xenografts based on differential gene expression. **C)** Differentially expressed genes between AER and Pre-Cx xenografts that meet the stringent criteria of log2FC > 2.5 and P Value < 1.00x10^{-5}. **D)** Volcano plots depicting AR target genes (267 AR gene signature from Mendiratta et al. (12), supplemented with a selection of DHT-responsive genes from Xu et al. (4)) (upper) and all significantly differentially expressed genes (lower) in the AER vs. Pre-Cx and CRPC vs. Pre-Cx comparisons. The dots corresponding to *DPP4* and *AR* in each figure are highlighted.

Figure 2

A.



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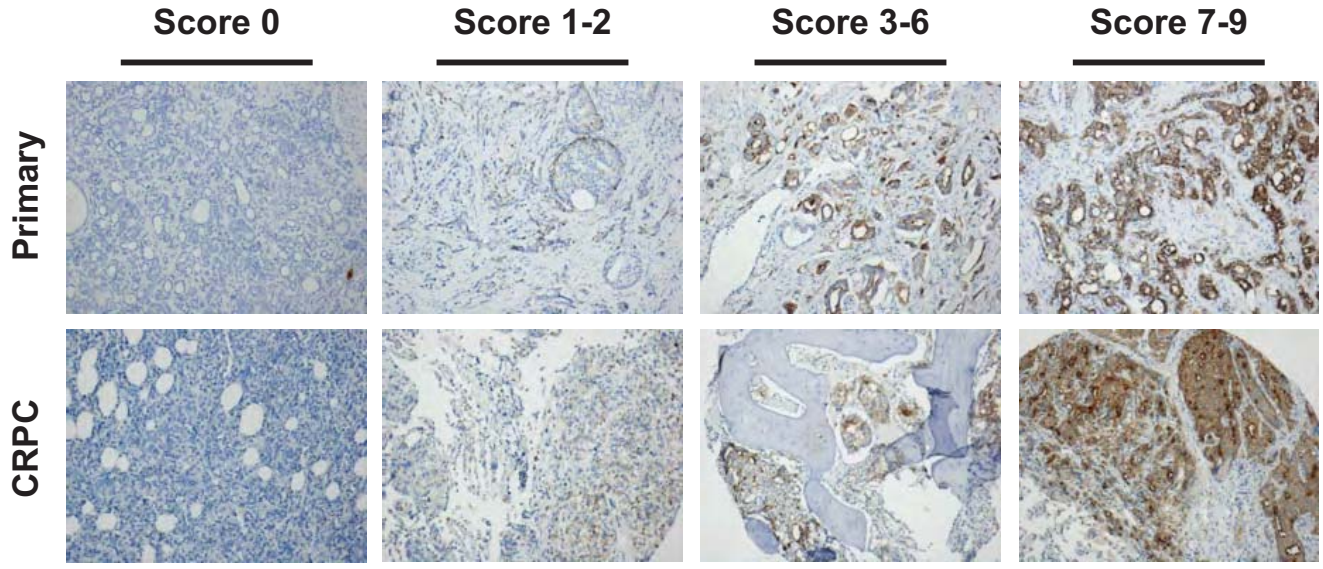
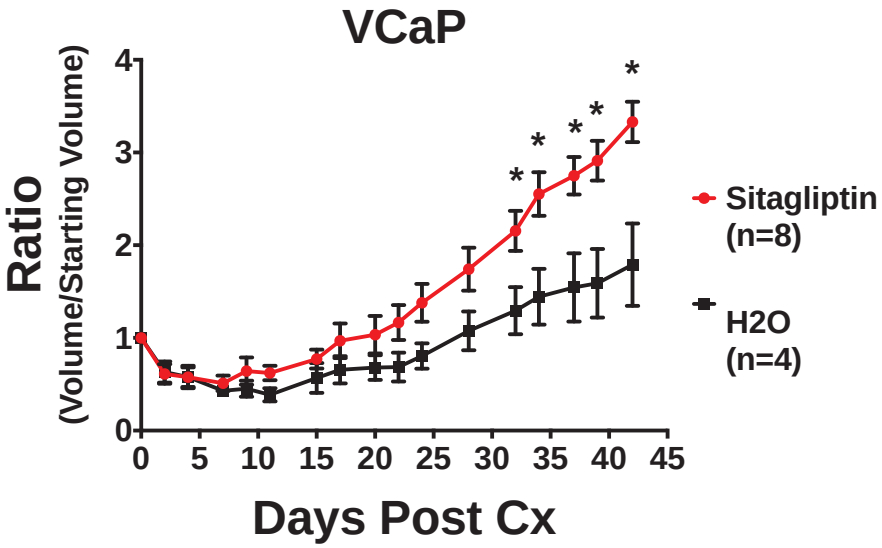


	Sample #	DPP4 Score 0	DPP4 Score 1-2	DPP4 Score 3-6	DPP4 Score 7-9	Avg. Score	Mann-Whitney U test
Primary	35	2	1	17	15	6.49	
CRPC	85	59	12	10	4	1.25	P = <0.0001

Figure 2 – DPP4 expression is decreased in ADT-resistant clininal specimens. A) RNA expression levels of *DPP4* obtained through Affymetrix microarray (Stanbrough) or RNA sequencing (CRPC Abi-Dut, Neoadjuvant Leupro-Abi, and VCaP Abiraterone-Tx) of material from several clinical and preclinical studies comparing Pre-ADT samples to resistant Post-ADT samples.*** = P<0.0001, ** = <0.001, limma for RNA sequencing studies and two-tailed student's t-test for microarray study. Abi – Abiraterone, Dut – Dutasteride, Leupro – Leuprolide. **B)** Representative images of DPP4 immunohistochemistry and DPP4 immunoscoring from a series of hormone-naïve primary prostate cancer (Primary) and CRPC clinical sections.

A.

Figure 3



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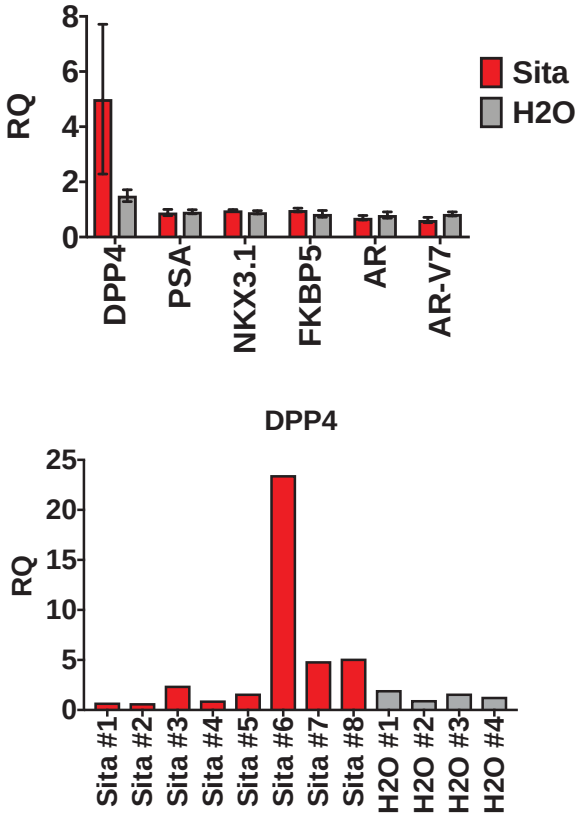

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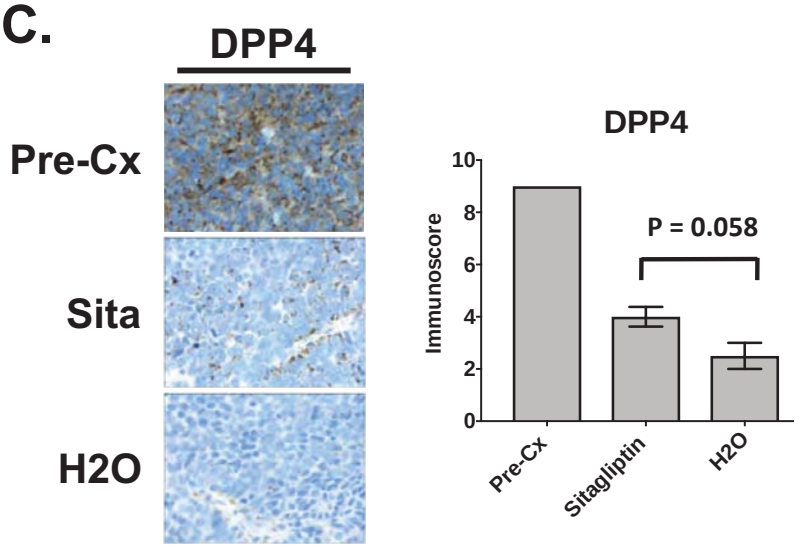

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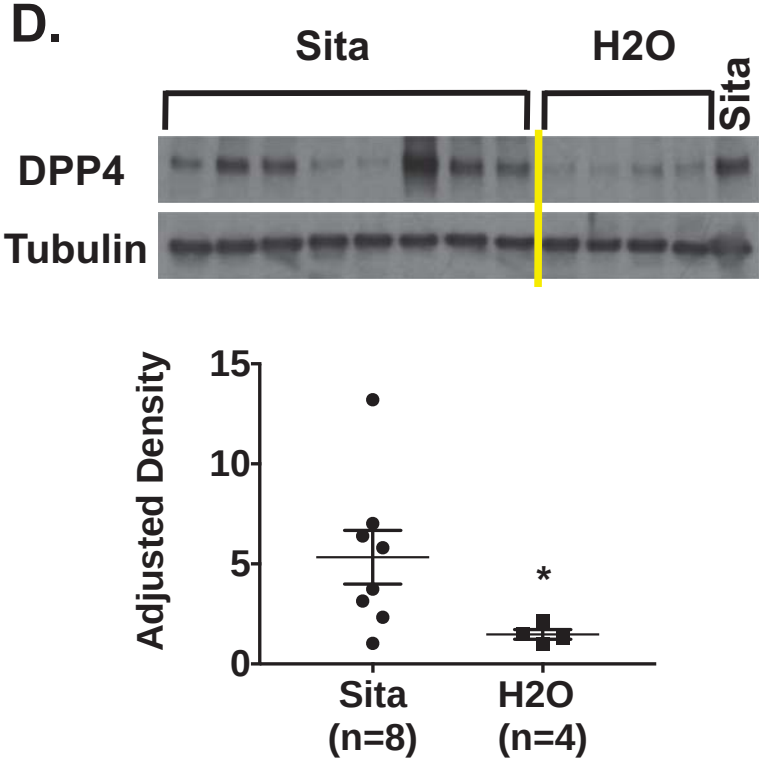

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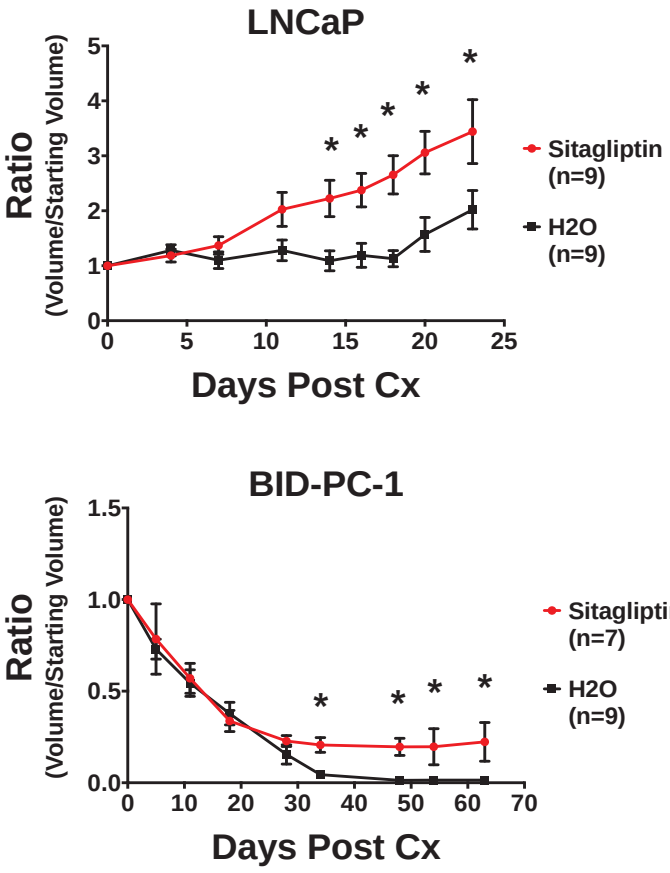


Figure 3 – DPP4 inhibitor increases VCaP tumor resistance to castration. A) VCaP subcutaneous xenografts were grown in intact male mice until tumors reached 500mm^3, then mice were castrated (Cx) and immediately began treatment with sitagliptin (120 mg/kg) or vehicle administered in drinking water. Y axis is the ratio of tumor volume at a given time point divided by the tumor starting volume. Bars = standard error of the mean (SEM). * = P<0.05, Mann-Whitney U. **B)** RT-PCR of *DPP4* and the AR regulated genes *PSA*, *NKX3*.1, and *FKBP5*, as well as the transcripts for AR and AR-V7 in VCaP xenografts harvested at Day 42 of the experiment represented in panel A. Each column represents the expression levels of xenograft tumors from eight mice (Sita) or four mice (H2O), with RT-PCR performed on each in technical triplicate. Bars represent standard error of the mean. H2O = water, Sita = Sitagliptin, RQ = Relative Quantification **C)** Representative high power images of DPP4 immunohistochemistry from Sita and H2O-treated tumors and DPP4 (left) and immunoscoring of DPP4 protein expression (right). P =0.058, Mann-Whitney U. **D)** Western blot of cell lysate from Sita and H2O-treated tumors probed with anti-DPP4 antibody (above) and densitometric quantification of bands (below). Bars = standard error of the mean (SEM). * = P<0.03, Mann Whitney U. **E)** LNCaP and BID-PC-1 subcutaneous xenografts were grown in intact male mice until tumors reached 500mm^3, then mice were castrated (Cx) and immediately began treatment with sitagliptin (120 mg/kg) or vehicle administered in drinking water. Y axis is the ratio of tumor volume at a given time point divided by the tumor starting volume. Bars = standard error of the mean (SEM). * = P<0.05, Mann-Whitney U.

Figure 4

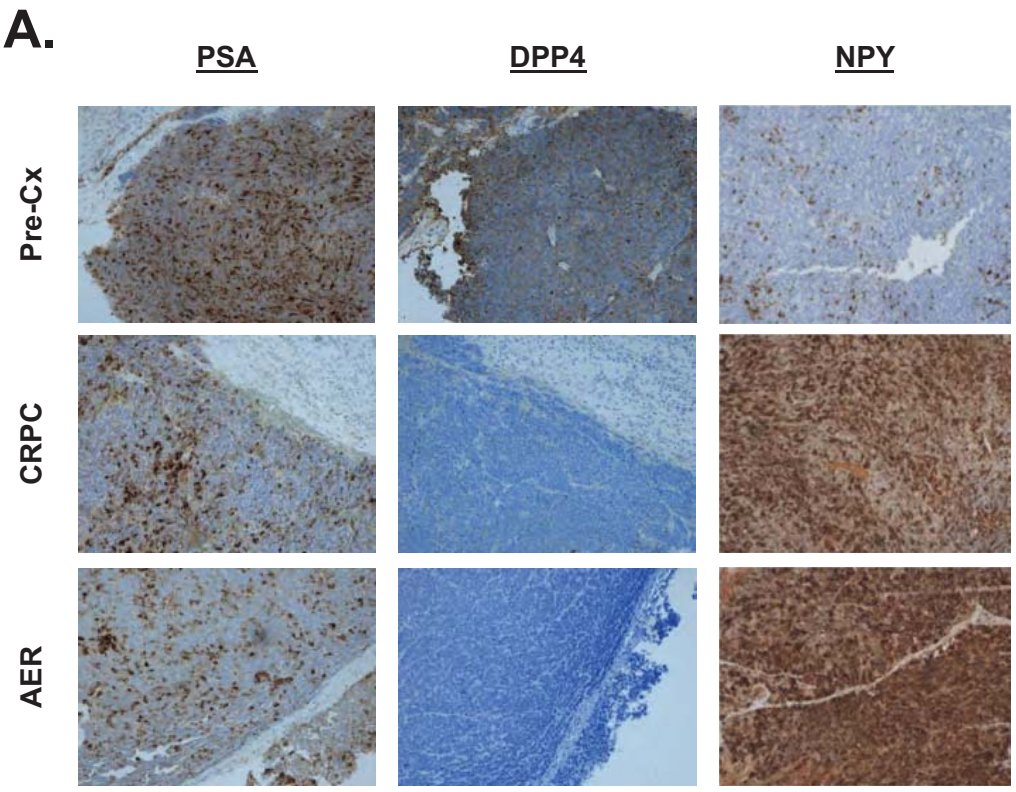


Figure 4 – NPY expression is increased in AER and CRPC VCaP xenograft serial biopsies compared to Pre-Cx. B) Representative images of PSA, DPP4, and NPY immunohistochemistry in serial biopsies of VCaP xenografts. Pre-Cx – precastration, CRPC – castration-resistant prostate cancer, AER – Abiraterone+Enzalutamide resistant.

Beth Israel Deaconess Medical Center

HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

FINAL INVOICE

Dana-Farber Cancer Institute
ATTN: Research Accounting
450 Brookline Avenue
Mail Stop BP437
Boston, MA 02215-5450

Your P.O. Number :
Sponsor Grant Number : 6192971
Sub Award No :

Invoice Number	:	RFO-01060706Final
Invoice Date	:	08/29/19
BIDMC Account Number	:	01060706
P.I. Name	:	Russo, Joshua
Prime P.I. Name	:	Myles Brown
Award-Direct Cost	:	53,000.00
Indirect Cost	:	-
Total Award	:	53,000.00
Grant Period	:	07/01/18-06/30/19

Balance Forward - Prior Invoice	52,457.72
Payments Since Prior Invoice	51,023.22
*Current Charges Due For Period From 07/01/18 to 06/30/19	542.28
Total Amount Outstanding	1,976.78

Direct Costs:	*Current Charges Due	Cumulative
SALARIES AND WAGES	-	51,349.46
SUPPLIES	542.28	1,640.54
MISCELLANEOUS	-	10.00
Total Direct Costs	$542.28	$53,000.00
Indirect Costs	-	-
Total Costs	$542.28	$53,000.00

Comments:

"I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812)."

Approved:_____ Manager Research Finance Office

To assure prompt and accurate crediting of your payment, please refer to Invoice Number RFO-01060706Final and return the remittance copy of this invoice.

Please remit payment to:
Research Finance Office BR 109, Room 259
Beth Israel Deaconess Medical Center
330 Brookline Avenue
Boston, MA 02215
For questions about this invoice, please email: RFOBilling@bidmc.harvard.edu

A. David Mazzone Research Awards Program
Student Training Awards
Progress Report: Period of July 2018 -June 31, 2019

The overall goal of Dana-Farber/Harvard Cancer Center student's training program is to engage the scientific curiosity and promote the academic success and future research careers of promising young scientists from underrepresented communities. We are grateful to the A. David Mazzone Research Awards for helping to support our program and advance our mission.

Notable Accomplishments:

Student Recruitment and Selection

Our student training leadership team led on-site informational sessions at local high schools, colleges, and universities to promote the program. For 2018, 239 submitted applications were reviewed while in 2019, we received a total of 153. The applications represented a cross-section of local high-schools and area colleges and universities. The Advisory Committee interviewed and ranked these candidates based on aptitude, attitude, interest, motivation, and articulation of research-related career goals.

Research Experience

- We continued to be deliberate about student placement across the spectrum of basic, clinical, and population-based research. In addition, we have increased placement of our candidates in computational/bioinformatics and nursing research environments.
- Our orientation was comprehensive and built on the skills needed to be a successful researcher. This summer we required the completion of a learning plan, which facilitated early and ongoing discussion between the mentor and trainee, and we have continued our practice of visiting all of our students in their research environments twice during the summer program.
- We have maintained our partnership with Biogen, which provided a two-day career advising and lab skills experience in the Biogen Community Lab. This session continues to be highly regarded by our trainees in helping develop practice with professional and technical skills that they can apply within their CURE research environment.
- All of the 2018 and 2019 trainees participated in the hallmark end-of-summer scientific symposium that provided an opportunity for students to showcase their oral and poster presentation skills and to share their research with the DF/HCC community and beyond. We continued to offer students the opportunity to present their research on a digital poster platform. In addition to the lead poster, many students incorporated multimedia including animation videos and 3D elements which helped to illustrate the various dimensions of their research.
- The Annual Biomedical Research Conference for Minority Students (ABRCMS) was held in November 2018 in Indianapolis, Indiana. ABRCMS continues to be a great opportunity for our students to experience a conference for the first time, network with peers across the country, interact with representatives from most of the nation's graduate programs, and attend professional development seminars. Three students attended and presented their summer research projects.

Summer Programming

- Alongside their summer research experiences, our trainees participated in regular journal clubs, seminars, and career development workshops. This summer, trainees experienced a journal club that was focused on immunology. Participants read articles that provided insight through a lens of translation research. Many of the students participated in a book club for which they read When Breath Becomes Air by Paul Kalanithi, a memoir on the author's life and illness battling stage IV metastatic lung cancer.
- We hosted the fourth annual Beyond Academia: Conversations on Health and Life Science Careers event at Dana-Farber. Nearly 30 representatives from a number of local biotech and pharmaceutical companies as well as public health, government agencies and academic presses participated in small group informational interviews with attendees, providing our students with a valuable opportunity to learn about different STEM careers.

Advisory Committee

- Our advisory committee continues to meet bi-annually and is fully engaged in our student training efforts. Over the past year, the committee continues to help ensure the program offerings are on target and that we have the correct evaluation questions and tools.

Evaluation

- The response rate for the annual student survey administered in 2017 and 2018 was 63% and 65% respectively. Both surveys indicated at least 82% of our CURE participants have chosen to continue their education and career progression in the biomedical sciences.

Mentor Engagement

- Concurrent with student recruitment processes, a letter of recruitment was sent to all Dana-Farber/Harvard Cancer Center (DF/HCC) members from Dr. Laurie Glimcher, President of DF/HCC, requesting volunteer mentors. Upon volunteering, mentors were notified of program expectations and provided a description of their proposed research project. Mentors and students were matched based on common research interests.

CURE Alumni Network

- Over the past two years, the CURE leadership planned at total of three networking events for current and past CURE students to explore the opportunity to create a catalyst which could lead to sustainable relationships and partnerships among CURE participants. Each of the sessions were well attended, and further validated that the common thread of being a CURE provided many attributes. CURE alums continue to assist with college coaching activities and a variety of summer presentations.

Funded Students in 2018

Four students received the support and funding from the A. David Mazzone Research Awards Program to participate in the CURE Program:

Destiny Porte – Destiny completed her senior year at Kipp Academy and was accepted in the class of 2022 at Tufts University. Destiny returned to the lab of Dr. Keisha McCall to focus on the reproducibility of molecular imaging of glucose metabolism.

Graciella Ortega – A rising Freshman at Simmons College, Graciella completed her second-year ins the CURE program. Her focus this summer was on survivin, a highly expressed protein in many cancer malignancies.

Robert Pepen – Robert returned for a second summer in the research environment of Larissa Nekhlyudov. He continued his research on establishing worldwide cancer survivorship guidelines.

Edmilson (Ianic) Pires – Ianic is a rising sophomore at Boston College. For the past two summers he investigated the association between probiotic intake and microbiome composition under the guidance of Kerry Ivey, PhD.

Funded Students in 2019

A total of two students were recommended and selected to participate in our 2019 summer research training program with funding support from the A. David Mazzone Research Awards Program:

They included:

David Bamgbowu – David is a rising sophomore at UMass Amherst majoring in biology.
This past summer, he worked in the lab of Dr. Alejandro Gutierrez, at Boston Children's Hospital. His research focuses analyzing the localization of CHKA in relation to treatment with nitrogen mustard, the active metabolite of cyclophosphamide.

Arlin Arias – Arlin is a rising sophomore at Boston College and majors in chemistry. Under the direction of Othon Iliopoulos, MD his research focus included testing therapeutic agents to treat patients with hemangioblastoma.

MAZZONE Student Training
2018-2019

		Spent 2018-2019
Arlin Arias	$	2,769.00
David Bamgbowu	$	3,016.00
Graciella Ortega	$	3,660.00
Robert Pepen	$	2,890.00
Emilson (Ianic) Pires	$	3,720.00
Destiny Porte	$	2,929.00
Professional Development activities	$	1,016.00
Total	**$**	**20,000.00**
Remaining funds	$	-

Appendix 5. Presentations from Program Retreat on September 13, 2019

Cancer Center

Beth Israel Deaconess Medical Center

DF/HCC Dana-Farber/Harvard Cancer Center
NCI Comprehensive Cancer Center
A Cancer Center Designated by the National Cancer Institute

HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

Prostate Cancer Molecular Heterogeneity and Response to Intensive Androgen Deprivation Therapy

Steven P. Balk MD, PhD
Beth Israel Deaconess Medical Center

A. David Mazzone Awards Program
A Program of DF/HCC and the Prostate Cancer Foundation
Retreat September 13, 2019

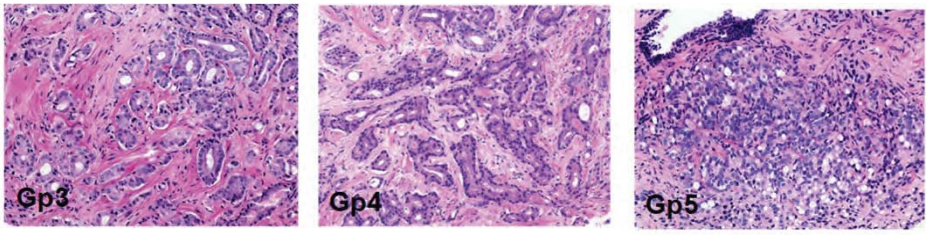
1

Impact Award: Molecular Characterization of Gleason 3 Tumors that Progress to Gleason 4

Prostate cancer (PCa) is identified based on loss of normal basal cell layer and nuclear atypia, and graded by Gleason pattern (Gleason score is sum of two major patterns)

Gp3 - well formed and separated glands
Gp4 - glands fusing, cribiform or intraductal growth
Gp5 – single cells and sheets of cells invading stroma

Gleason pattern matters: men who are confirmed to have only Gp3 (Gleason score 3+3=6) after radical prostatectomy almost never relapse

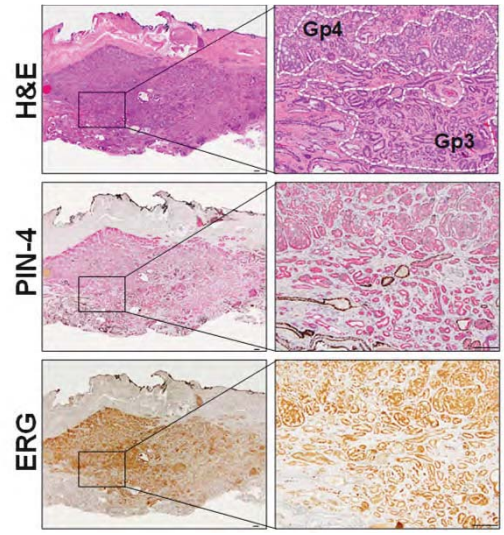


Can we identify Gp3 tumors that can just be monitored (Active Surveillance) versus those that are likely to progress?

2

Analyze the Gp3 foci in tumors in which Gp3 appears to have progressed to Gp4 (Gleason score 4+3=7)

- Identify cases with clear adjacent foci of Gp3 and Gp4 (cribiform)
- PIN-4 multiplex IHC to definitively identify PCa cells
- Laser-capture microdissect adjacent Gp3 and Gp4
- Initially selected ERG positive cases and confirmed identical *TMPRSS2:ERG* breakpoints - indicating Gp3 and Gp4 emerged from same clone
- Whole exome sequencing



Adam Sowalsky

Huihui Ye

Prostate Cancer Foundation
Curing Together.

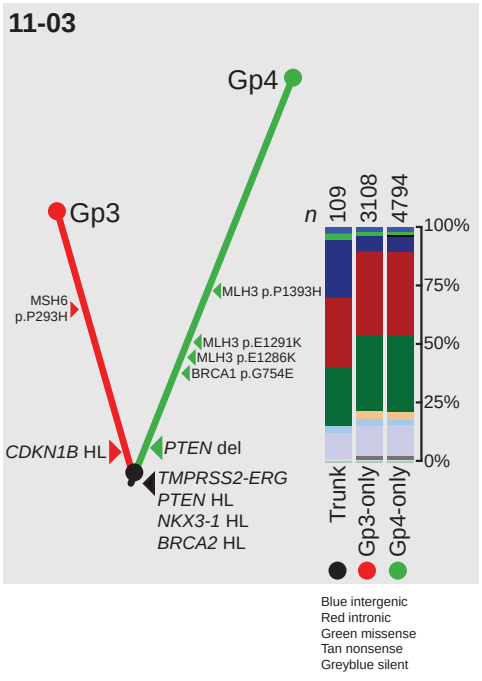
PCF Young Investigators

Truncal: *TMPRSS2:ERG* fusion, heterozygous losses (HL) of *PTEN*, *NKX3.1*, *BRCA2*

Gp4 specific: 2nd copy *PTEN* loss in Gp4 (high allele frequency), subclonal mutations in *BRCA1* and *MLH3*

Gp3 specific: Heterozygous loss of *CDKN1B* (p27), subclonal MSH6 mutation

Comparable number of distinct mutations in Gp3 and Gp4 (line length based on unique mutations and reflecting evolutionary distance from common precursor)

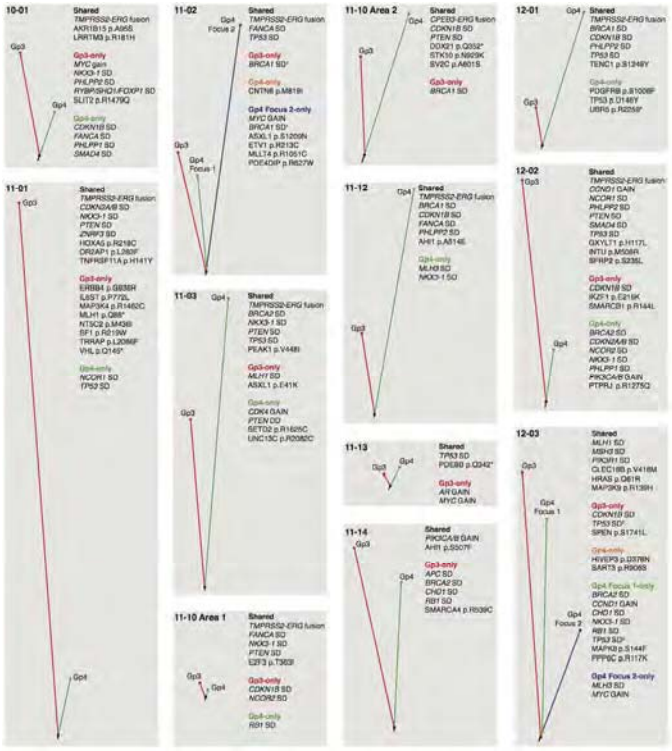


Similar pattern in all cases - Gp3 and Gp4 have shared (truncal) and unique driver alterations

Indicates that Gp3 tumors do not simply progress to Gp4 through acquisition of one more oncogenic alteration

Suggests that Gp3 and Gp4 emerge early from common precursor and extensively diverge

No clear consistent genomic features of these Gp3 tumors that distinguish them from Gp3 that are not associated with Gp4 (Gleason score 3+3=6)

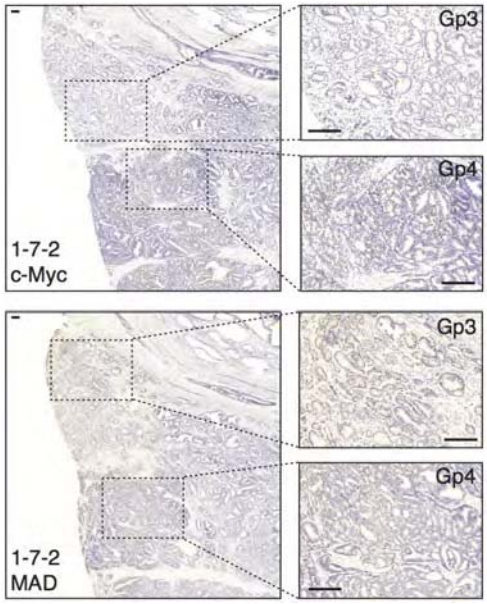


5

What is the molecular basis for the distinct morphology (and invasive potential) of the Gp4 tumor foci versus the adjacent Gp3?

Direct comparison of mRNA in adjacent Gp3 versus Gp4 foci (whole transcriptome analysis) indicated increased MYC activity in Gp4 foci

IHC showed increased expression of MYC or decreased expression of MAD/MXD1 (negative regulator of MYC) in most cases



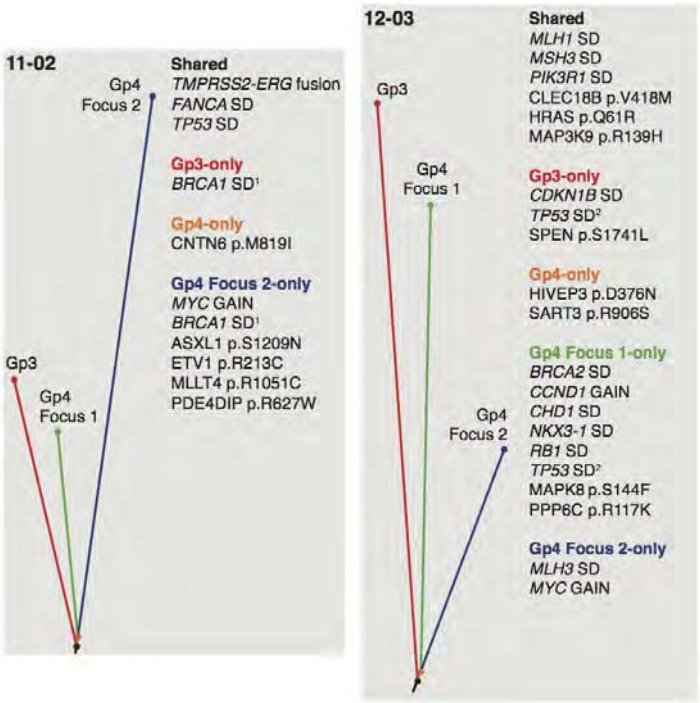
6

In addition to heterogeneity between Gp3 and Gp4, there was substantial heterogeneity between Gp4 foci in the same tumor - *MYC gain in only one Gp4 focus*

Is this heterogeneity clinically important?

Does it play a role in resistance to therapy?

Addressed this question in a neoadjuvant trial of intensive androgen deprivation therapy

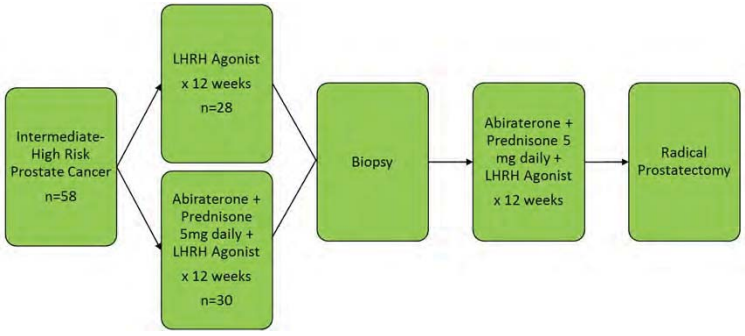


7

Androgen deprivation therapy (LHRH agonist to suppress testicular androgen synthesis) has been standard treatment for metastatic PCa

Early addition of abiraterone (further suppresses androgen synthesis) or a direct androgen receptor antagonist can markedly enhance responses to LHRH agonist

Neoadjuvant trial of LHRH agonist combined with abiraterone to determine whether early use of intense androgen deprivation would enhance responses



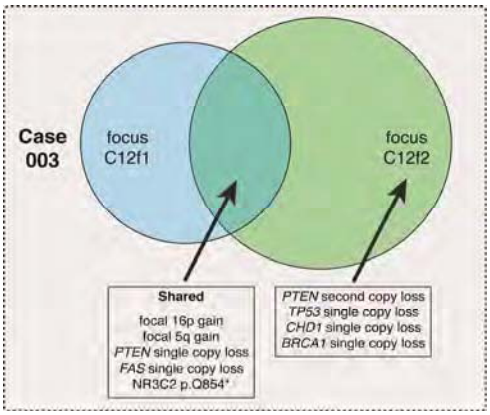
Analysis of residual tumor in radical prostatectomy specimens to determine mechanisms of resistance

Mary-Ellen Taplin

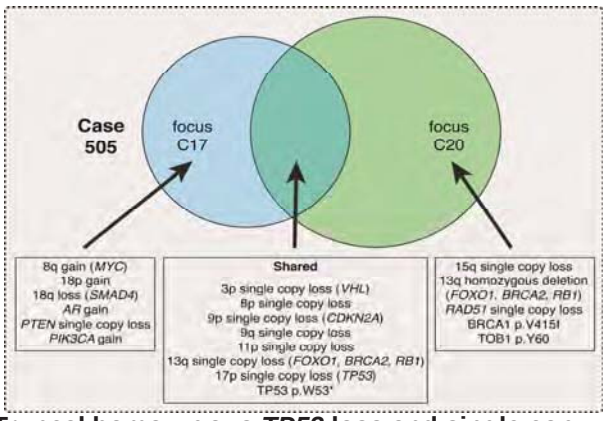
Prostate Cancer Foundation
Curing Together.

8

Laser capture microdissection of residual tumor foci followed by whole exome sequencing



Truncal single copy *PTEN* loss with homozygous loss in one focus



Truncal homozygous *TP53* loss and single copy *BRCA2, RB1 (13q) losses*; homozygous *BRCA2, RB1* loss in one focus, *MYC* gain in another focus

Tumor heterogeneity is important: Intensive androgen deprivation is selecting for resistant subclones present in the primary tumor

Can we use this information to improve therapy?

Metastatic castration-resistant PCa that emerged in this patient was derived from a subclone that was identified in his radical prostatectomy after neoadjuvant therapy

May have benefited from adjuvant PARP or ATR inhibitor, cisplatin

Oncopanel report of lymph node biopsy

- Low resolution copy number data of metastasis
- AR amplified
- RB homozygously deleted
  - Hemizygous deletion in 2 foci in RP
  - Additional copy deleted 3rd focus in RP
- ATM frameshift mutation *ATM c.4692delT (p.P1566Lfs*35), exon 31* - in 5% of 296 reads *
  - Same mutation detected in 2/3 foci (not truncal)
- AR amplification was not observed in residual tumor: this event drove resistance to later therapy, not neo-abi

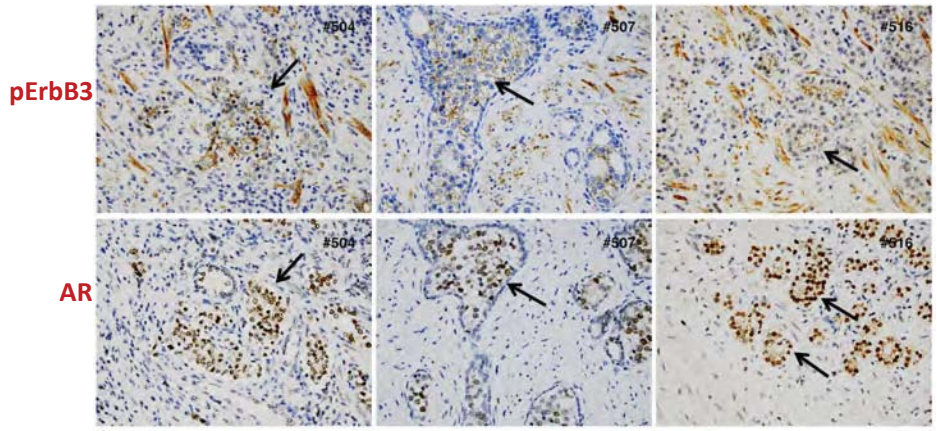
May be able to use analysis of residual tumor after neoadjuvant therapy to predict the most effective adjuvant therapy to prevent relapse with metastatic disease

DoD Impact Award to address this and related questions

Additional actionable resistance mechanisms: ErbB2 activation (phospho-ErbB3) in ~20% of cases after neoadjuvant leuprolide + abiraterone

Associated with increased androgen receptor expression

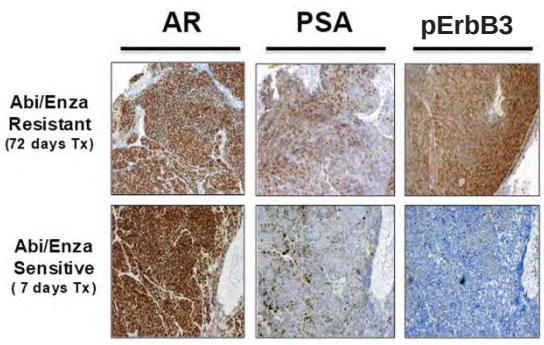
ErbB2 is also activated in ~20% of metastatic castration-resistant prostate cancers (not shown)



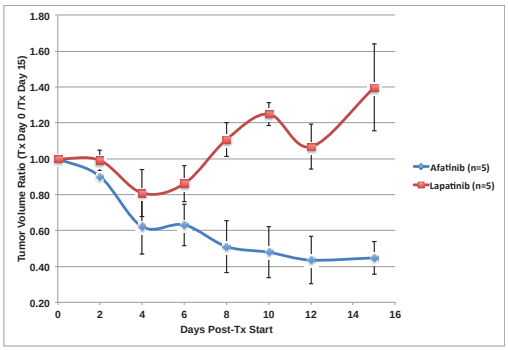
	Total sample#	pErbB3 score=1	pErbB3 score=2	pErbB3 score=3	Total %	Pearson's χ^2 test
Naïve	43	1	1	0	4.7%	
Abi-treated	48	2	6	1	18.8%	p=0.039

p-ErbB3 score:
1=weak
2=moderate
3=strong

11



Increased ErbB2 activity in VCaP xenografts that progress after castration and treatment with abiraterone + enzalutamide (Abi/Enza)



Castration-resistant VCaP xenografts respond transiently to reversible ErbB2 inhibitor (Lapatinib); more robust response to covalent inhibitors (Afatinib) or Neratinib (not shown)

Pursuing biomarker driven clinical trial of ErbB2 inhibitor (neratinib) in metastatic castration-resistant PCa, and possibly as adjuvant therapy in selected patients

Prostate Cancer Foundation
Curing Together.

PCF Young Investigator

12

Balk lab and affiliates (present and past)
Seiji Arai
Changmeng Cai
Carla Calagua
Sen Chen
Shaoyong Chen
Shuai Gao
Yanfei Gao
Sean Gerrin
Xiaming Liu
Fen Ma
Josh Russo
Rachel Schaefer
Adam Sowalsky
Olga Voznesensky
Ziyang Yu
Xin Yuan
Jacob Zhang

BIDMC GU Medical Oncology
Glenn Bubley
Rupal Bhatt
Marc Garnick
Kathleen Mahoney

BIDMC Urologic Oncology
Peter Chang
William DeWolf
Andrew Wagner

BIDMC Pathology
Huihui Ye

BIDMC Radiation Oncology
Joseph Aronovitz
Irving Kaplin

BIDMC Radiology
Mary-Ellen Sun

DFCI GU Oncology
Myles Brown
Philip Kantoff
Massimo Loda
Mary-Ellen Taplin

Funding
NCI
DOD
Prostate Cancer Foundation
V Foundation

A. David Mazzone Awards Program

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Curing Together.

CDMRP

THE BRIDGE PROJECT

DANA-FARBER CANCER INSTITUTE | BROAD INSTITUTE | CFCE CENTER for FUNCTIONAL CANCER EPIGENETICS | HARVARD MEDICAL SCHOOL

Charting the Prostate Cancer Epigenome

Matthew Freedman, MD

September 13, 2019

Mazzone Retreat

1

Genome-wide association studies

As of 2019-09-05, the GWAS Catalog contains 4131 publications and 128550 associations.

2

Unanticipated result:

The vast majority of GWAS loci are *outside* of protein coding regions

GWAS loci are *enriched in regulatory elements*

Trait-Associated SNPs Are More Likely to Be eQTLs: Annotation to Enhance Discovery from GWAS

Dan L. Nicolae[1,2,3], Eric Gamazon[1], Wei Zhang[1], Shiwei Duan[1,4], M. Eileen Dolan[1,3], Nancy J. Cox[1,3,*]

Systematic Localization of Common Disease-Associated Variation in Regulatory DNA *Science* **337**, 1190 (2012)

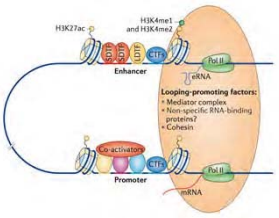
Matthew T. Maurano,[1*] Richard Humbert,[1*] Eric Rynes,[1*] Robert E. Thurman,[1] Eric Haugen,[1] Hao Wang,[1] Alex P. Reynolds,[1] Richard Sandstrom,[1] Hongzhu Qu,[1,2] Jennifer Brody,[3] Anthony Shafer,[1] Fidencio Neri,[1] Kristen Lee,[1] Tanya Kutyavin,[1] Sandra Stehling-Sun,[1] Audra K. Johnson,[1] Theresa K. Canfield,[1] Erika Giste,[1] Morgan Diegel,[1] Daniel Bates,[1] R. Scott Hansen,[4] Shane Neph,[1] Peter J. Sabo,[1] Shelly Heimfeld,[5] Antony Raubitschek,[6] Steven Ziegler,[7] Chris Cotsapas,[7,8] Nona Sotoodehnia,[9,10] Ian Glass,[11] Shamil R. Sunyaev,[12] Rajinder Kaul,[1] John A. Stamatoyannopoulos[1,13]†

Linking disease associations with regulatory information in the human genome

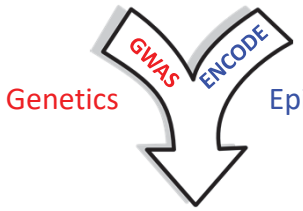
Marc A. Schaub,[1] Alan P. Boyle,[2] Anshul Kundaje,[1] Serafim Batzoglou,[1,3] and Michael Snyder[2,3,4] *Genome Res.* 2012 22: 1748-1759

Combinatorial effects of multiple enhancer variants in linkage disequilibrium dictate levels of gene expression to confer susceptibility to common traits

Olivia Corradin,[1] Alina Saiakhova,[1] Batool Akhtar-Zaidi,[1] Lois Myeroff,[2] Joseph Willis,[2,3] Richard Cowper-Sal-lari,[4] Matthieu Lupien,[4] Sanford Markowitz,[1,2,3] and Peter C. Scacheri[1,2,5] *Genome Res.* 2014 24: 1-13

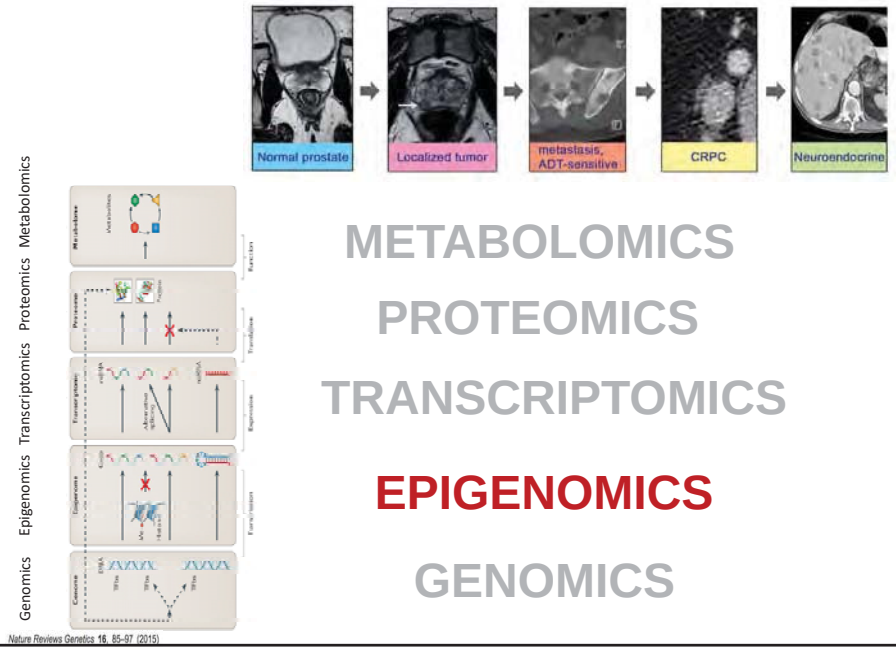


Nat Rev Mol Cell Biol. 2015 Mar;16(3):144-54.



presents testable hypothesis

Characterization of primary prostate samples



METABOLOMICS

PROTEOMICS

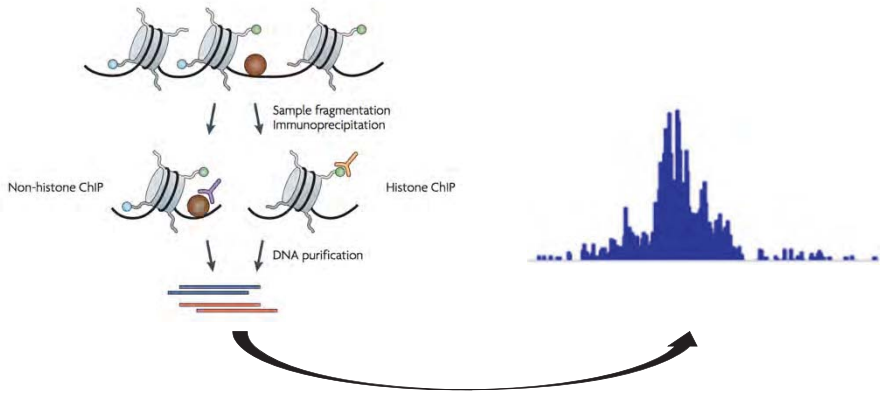
TRANSCRIPTOMICS

EPIGENOMICS

GENOMICS

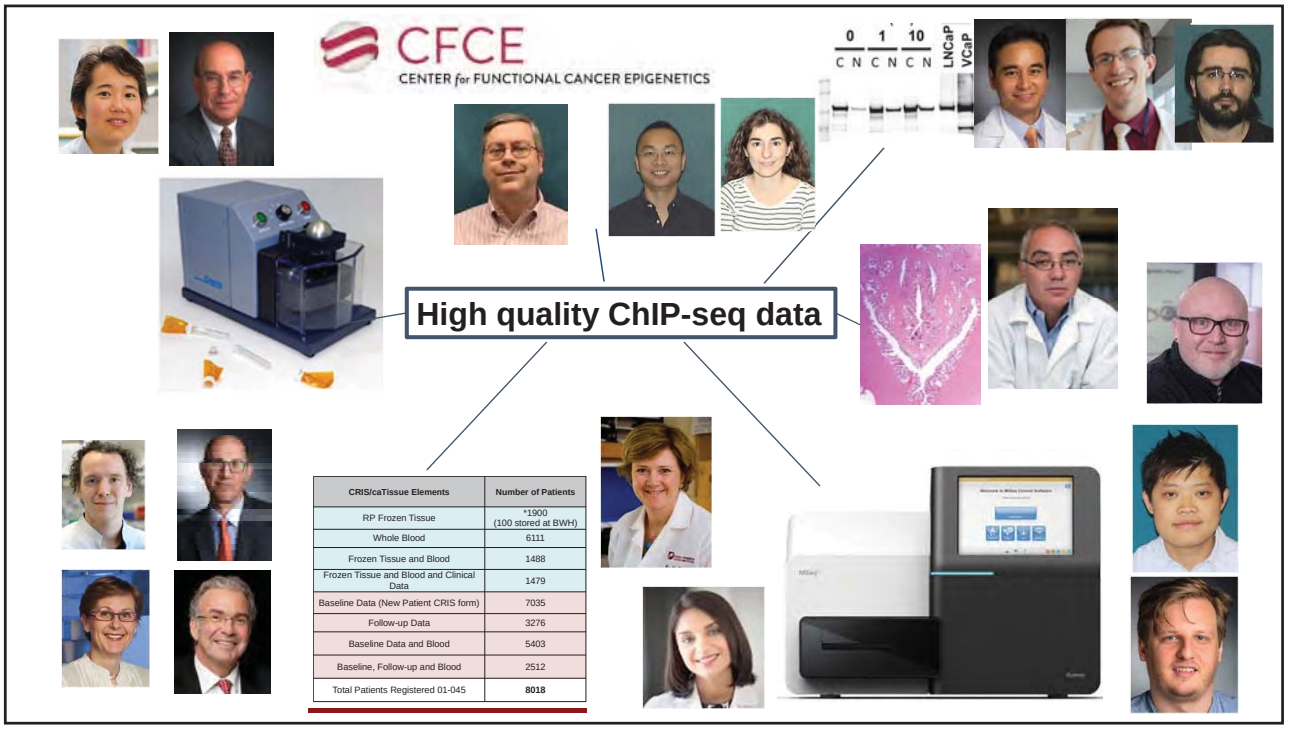
5

ChIP-seq

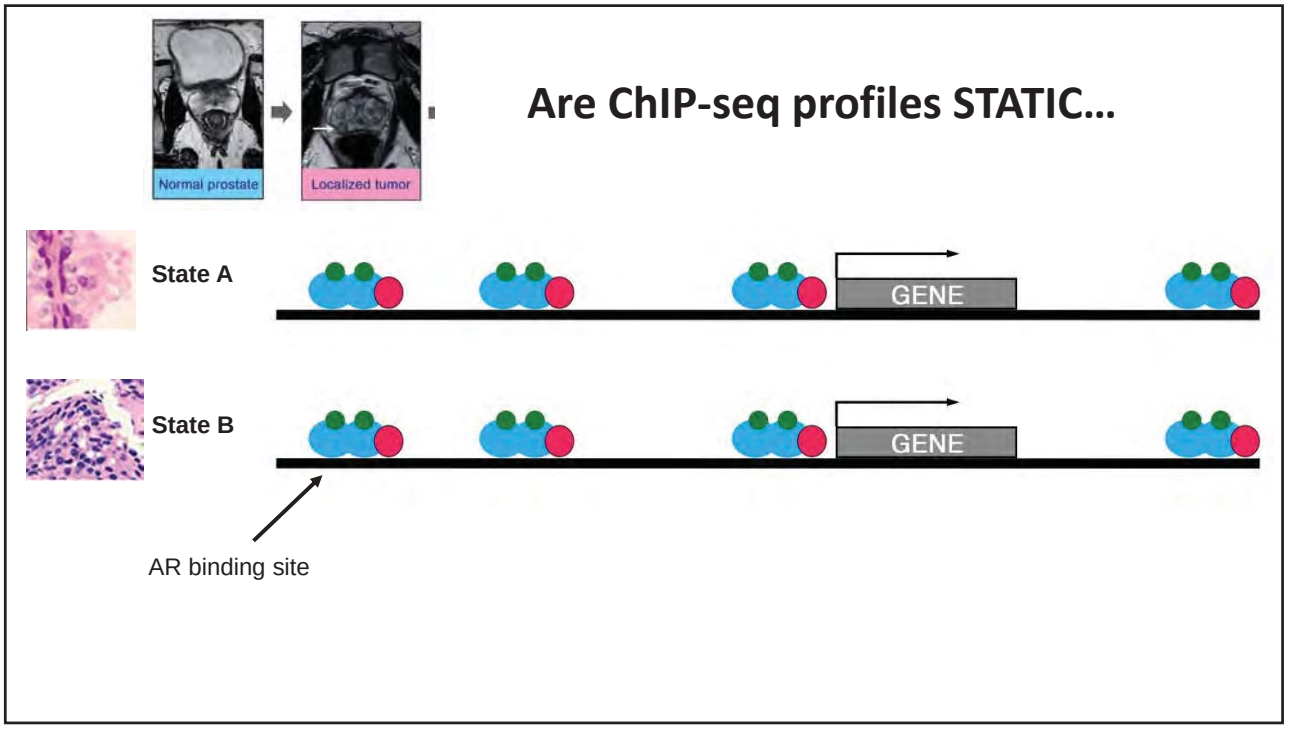


Sequence, align to genome, identify peaks

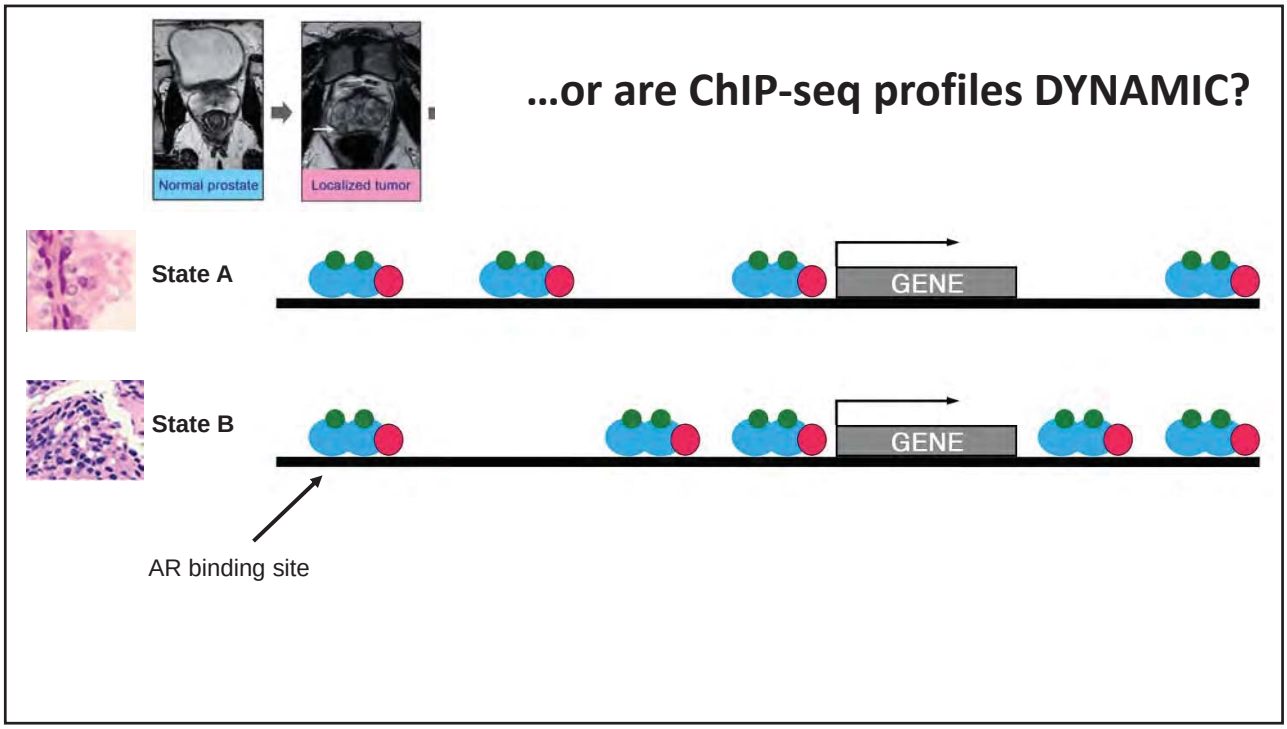
6


CFCE
CENTER for FUNCTIONAL CANCER EPIGENETICS
0 1 10
C N C N C N
LNCaP
VCaP
High quality ChIP-seq data
CRIS/caTissue Elements | Number of Patients
RP Frozen Tissue | *1900 (100 stored at BWH)
Whole Blood | 6111
Frozen Tissue and Blood | 1488
Frozen Tissue and Blood and Clinical Data | 1479
Baseline Data (New Patient CRIS form) | 7035
Follow-up Data | 3276
Baseline Data and Blood | 5403
Baseline, Follow-up and Blood | 2512
Total Patients Registered 01-045 | 8018

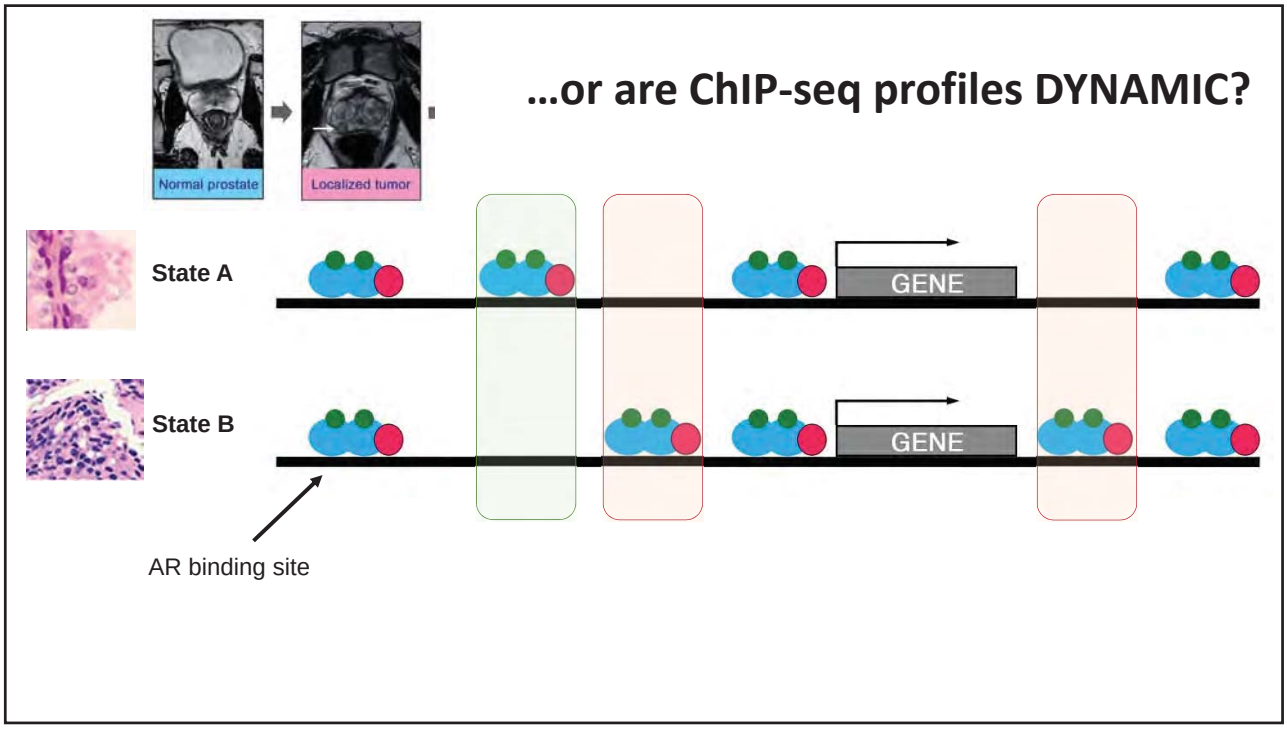
7


Normal prostate
Localized tumor
Are ChIP-seq profiles STATIC…
State A
GENE
State B
GENE
AR binding site

8

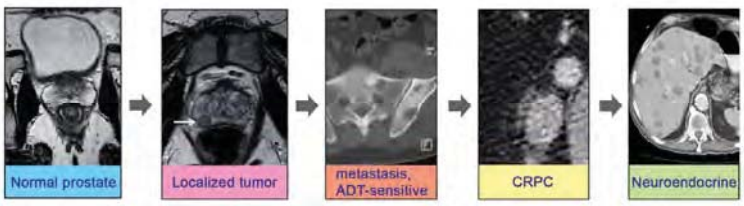

…or are ChIP-seq profiles DYNAMIC?
Normal prostate
Localized tumor
State A
GENE
State B
GENE
AR binding site

9


…or are ChIP-seq profiles DYNAMIC?
Normal prostate
Localized tumor
State A
GENE
State B
GENE
AR binding site

10

Datasets

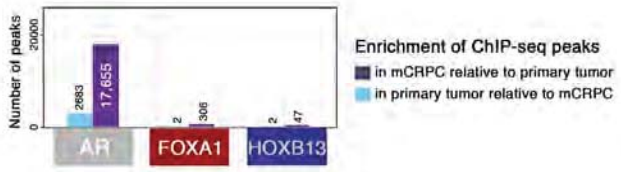


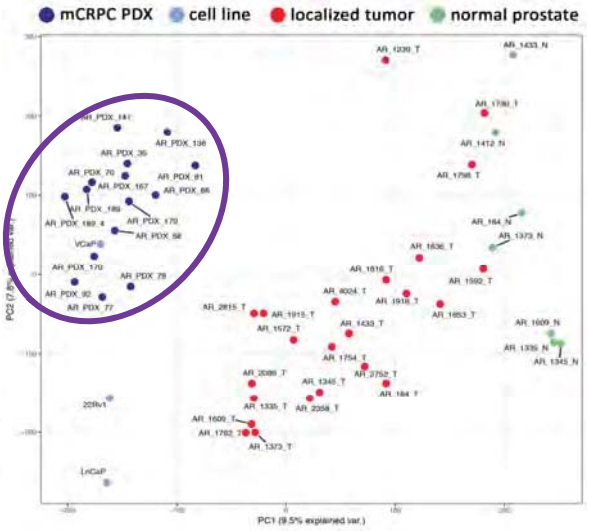
Over 250 sequenced libraries

H3K27ac	**AR**	ATAC-seq
H3K4me2	HOXB13	
H3K4me3	FOXA1	
H3K27me3		

Point 1: AR reprogramming likely reactivates developmental programs in CRPC

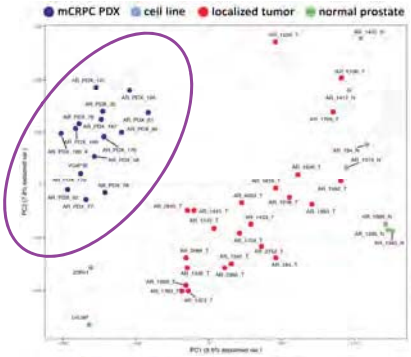
AR is extensively reprogrammed across state transitions

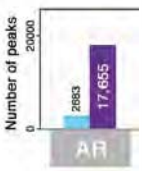


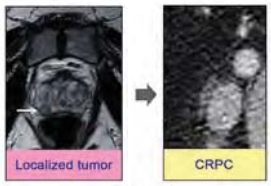


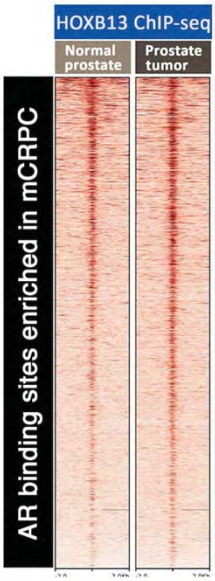
13

AR reprogrammed metastatic sites are not created *de novo*



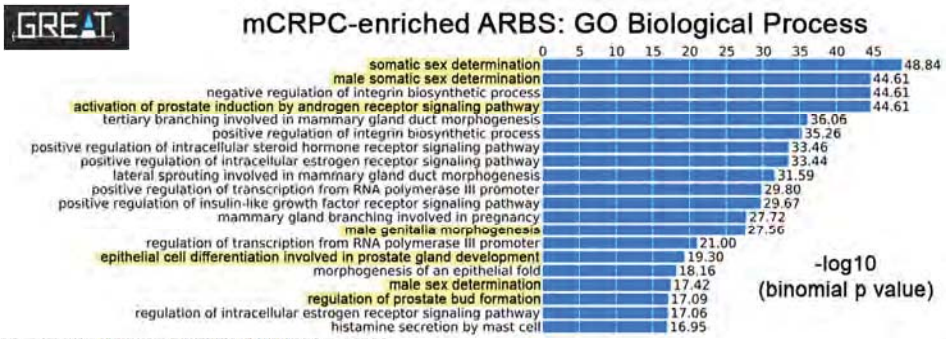






14

AR reprogrammed sites appear to (re)activate developmental programs



Rank	Motif	Name	P-value	
1		ZEB1(Zf)/PDAC-ZEB1-ChIP-Seq(GSE64557)/Homer	1e-155	

3		Lhx2(Homeobox)/HFSC-Lhx2-ChIP-Seq(GSE48068)/Homer	1e-136	-3.144e+02	0.0000	3144.0	28.76%	11251.9	18.90%
4		Lhx1(Homeobox)/EmbryoCarcinoma-Lhx1-ChIP-Seq(GSE70957)/Homer	1e-128	-2.964e+02	0.0000	3268.0	29.89%	11988.2	20.14%
5		E2A(bHLH),near_PU.1/Bcell-PU.1-ChIP-Seq(GSE21512)/Homer	1e-124	-2.865e+02	0.0000	2987.0	27.32%	10760.6	18.07%
6		ATHB33(ZFHD)/col-ATHB33-DAP-Seq(GSE60143)/Homer	1e-116	-2.680e+02	0.0000	3503.0	32.04%	13377.4	22.47%
7		THRb(NR)/Liver-NR1A2-ChIP-Seq(GSE52613)/Homer	1e-116	-2.677e+02	0.0000	6584.0	60.23%	29335.7	49.28%
8		Erra(NR)/HepG2-Erra-ChIP-Seq(GSE31477)/Homer	1e-113	-2.605e+02	0.0000	4189.0	38.32%	16826.9	28.26%
9		AP-2gamma(AP2)/MCF7-TFAP2C-ChIP-Seq(GSE21234)/Homer	1e-111	-2.558e+02	0.0000	2439.0	22.31%	8509.6	14.29%
10		Nanog(Homeobox)/mES-Nanog-ChIP-Seq(GSE11724)/Homer	1e-110	-2.539e+02	0.0000	7180.0	65.68%	32839.5	55.16%

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Point 2: Somatically acquired enhancers are functionally relevant

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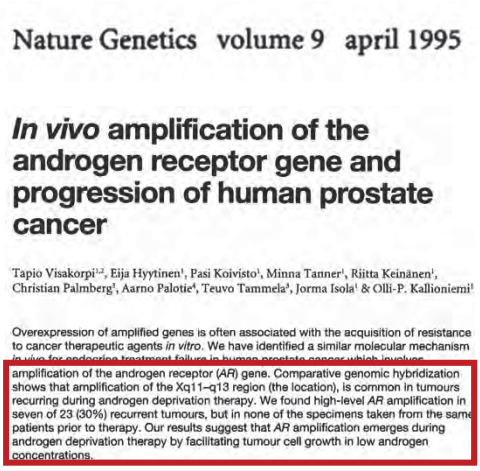
Genetics of prostate cancer

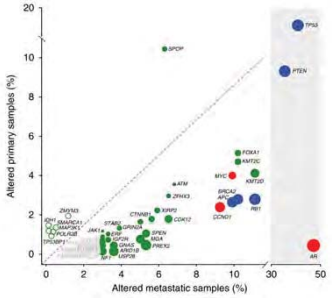


Nature Genetics volume 9 april 1995

***In vivo* amplification of the androgen receptor gene and progression of human prostate cancer**

Tapio Visakorpi[1,2], Eija Hyytinen[1], Pasi Koivisto[1], Minna Tanner[1], Riitta Keinänen[1], Christian Palmberg[3], Aarno Palotie[4], Teuvo Tammela[3], Jorma Isola[1] & Olli-P. Kallioniemi[1]

Overexpression of amplified genes is often associated with the acquisition of resistance to cancer therapeutic agents *in vitro*. We have identified a similar molecular mechanism *in vivo* for endocrine treatment failure in human prostate cancer which involves amplification of the androgen receptor (*AR*) gene. Comparative genomic hybridization shows that amplification of the Xq11–q13 region (the location), is common in tumours recurring during androgen deprivation therapy. We found high-level *AR* amplification in seven of 23 (30%) recurrent tumours, but in none of the specimens taken from the same patients prior to therapy. Our results suggest that *AR* amplification emerges during androgen deprivation therapy by facilitating tumour cell growth in low androgen concentrations.



- Amplification/Mutation
- Deletion/Mutation
- Mutation
- Significance

Armenia et al. Nature Genetics 2018

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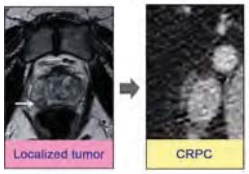
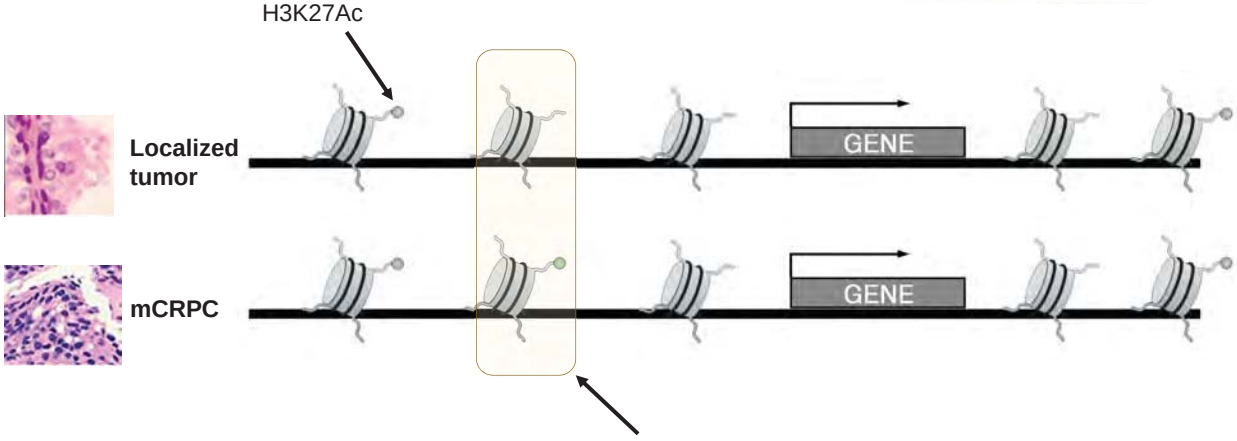
H3K27Ac across disease states





genome-wide: 16,047 H3K27Ac sites enriched in mCRPC

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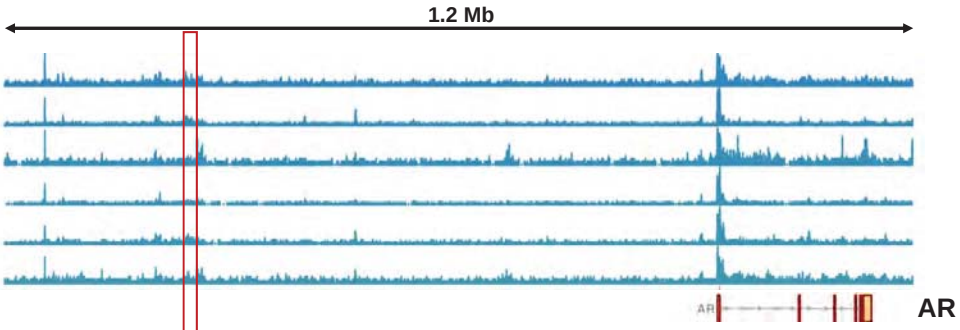
H3K27Ac and the discovery of an AR enhancer

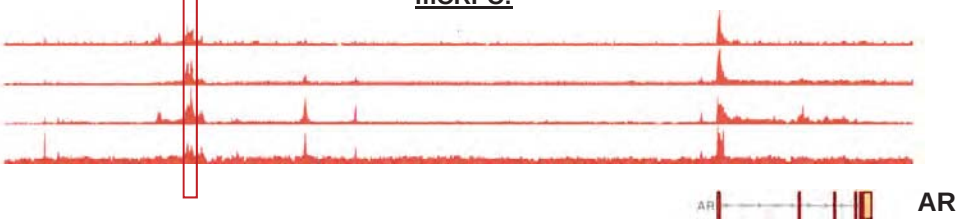
Cell

Takeda et al., 2018, Cell *174*, 422–432

Localized Prostate Cancer:

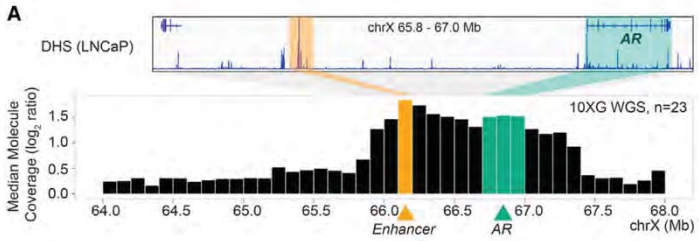


mCRPC:



19

Putative regulatory element is amplified in castration-resistant prostate cancer



Cell *174*, 433–447, July 12, 2018

Srini Viswanathan – Meyerson lab

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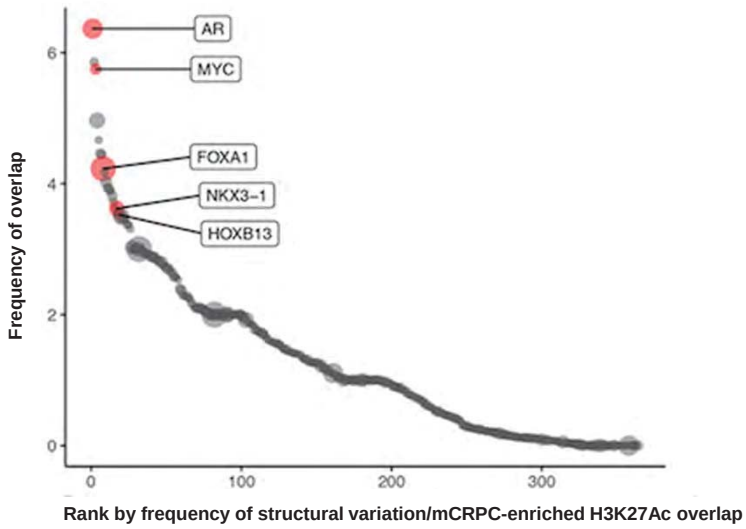
Somatically amplified regions in metastatic disease coincide with gain of acetylation



Srini Viswanathan
Gavin Ha

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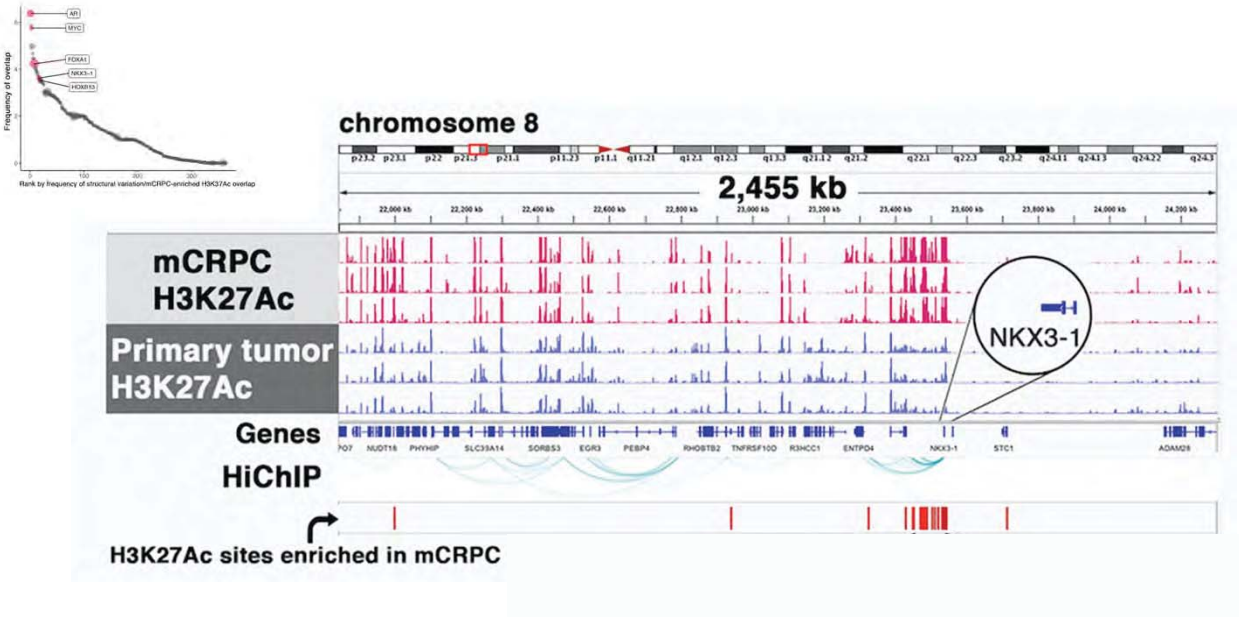
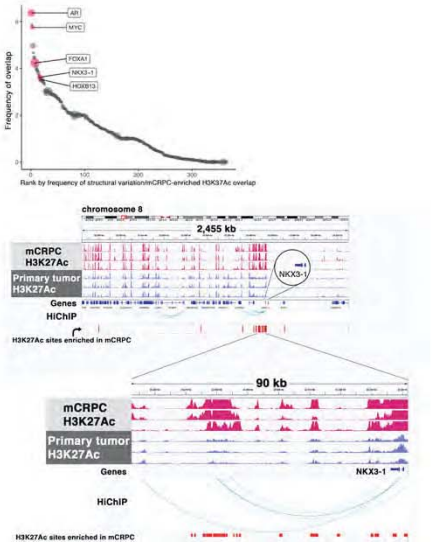
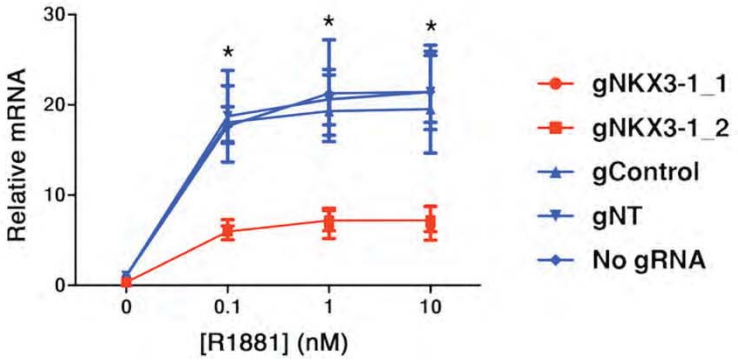
Somatically amplified regions in metastatic disease coincide with gain of acetylation



22

Somatically amplified regions in metastatic disease coincide with gain of acetylation impact expression





David Takeda

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Point 3: Epigenomic analyses can identify important genes missed by other methods

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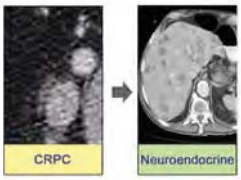
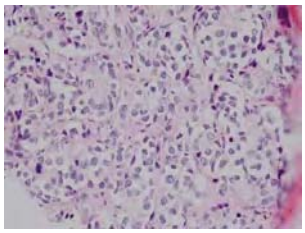
Neuroendocrine prostate cancer

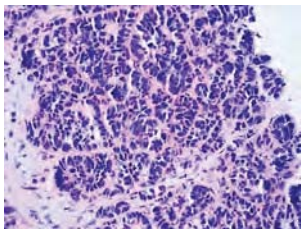


Prostate adenocarcinoma (**PRAD**)



Lineage plasticity

Neuroendocrine prostate cancer (**NEPC**)



www.cancer.gov

- Clinically heterogeneous
- High response rate to androgen deprivation
- Hormone receptor (AR)-dependent

- Uniformly aggressive
- AR-independent
- Aberrant expression of neural lineage and EMT programs
- Increasing incidence (treatment-related)

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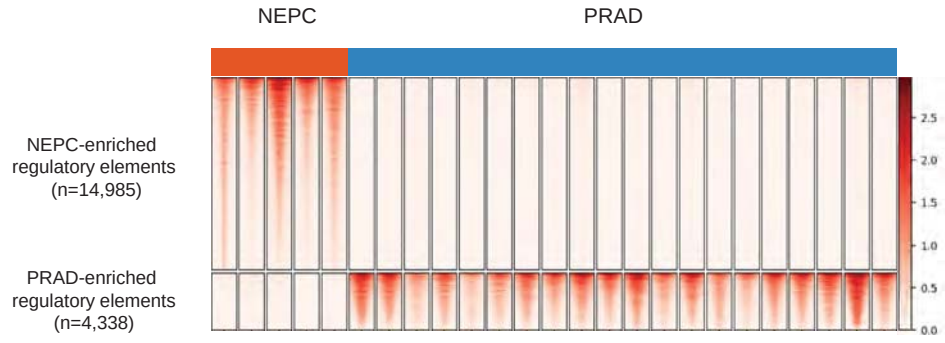
H3K27ac tag densities at candidate enhancers with differential H3K27 acetylation between NEPC and PRAD



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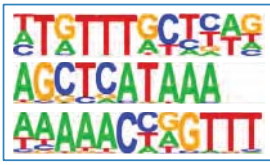
Top significantly enriched motifs at candidate regulatory elements

	Motif	Enrichment *p*-value	TF Candidate
PRAD-enriched regulatory elements		1 x 10^{-30}	**FOXA1**
		1 x 10^{-20}	HOXB13
		1 x 10^{-18}	GRHL2
NEPC-enriched regulatory elements		1 x 10^{-159}	ASCL1
		1 x 10^{-136}	**FOXA1**
		1 x 10^{-67}	NKX2-1

<u>FOXA1</u>

- Transcription factor – pioneer
- Involved in embryonic development of endodermal tissues
- Established importance in adenocarcinoma – interaction with androgen receptor

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Cold Spring Harb Perspect Med 2019;9:a030593

Genes that have been nominated through differential expression analysis

FOXA1 is <u>not</u> on this list

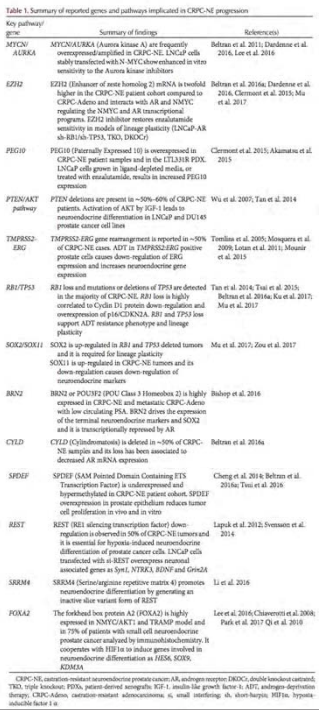

Table 1. Summary of reported genes and pathways implicated in CRPC-NE progression

Key pathway/ gene	Summary of findings	Reference(s)
MYCN/ AURKA	*MYCN/AURKA* (Aurora kinase A) are frequently overexpressed/amplified in CRPC-NE. LNCaP cells stably transfected with N-MYC show enhanced in vitro sensitivity to the Aurora kinase inhibitors	Beltran et al. 2011; Dardenne et al. 2016, Lee et al. 2016
EZH2	EZH2 (Enhancer of zeste homolog 2) mRNA is twofold higher in the CRPC-NE patient cohort compared to CRPC-Adeno and interacts with AR and NMYC regulating the NMYC and AR transcriptional programs. EZH2 inhibitor restores enzalutamide sensitivity in models of lineage plasticity (LNCaP-AR sh-RB1/sh-TP53, TKO, DKOCs)	Beltran et al. 2016a; Dardenne et al. 2016, Clermont et al. 2015; Mu et al. 2017
PEG10	PEG10 (Paternally Expressed 10) is overexpressed in CRPC-NE patient samples and in the LTL331R PDX. LNCaP cells grown in ligand-depleted media, or treated with enzalutamide, results in increased PEG10 expression	Clermont et al. 2015; Akamatsu et al. 2015
PTEN/AKT pathway	*PTEN* deletions are present in ~50%–60% of CRPC-NE patients. Activation of AKT by IGF-1 leads to neuroendocrine differentiation in LNCaP and DU145 prostate cancer cell lines	Wu et al. 2007; Tan et al. 2014
TMPRSS2-ERG	*TMPRSS2-ERG* gene rearrangement is reported in ~50% of CRPC-NE cases. ADT in TMPRSS2:ERG positive prostate cells causes down-regulation of ERG expression and increases neuroendocrine gene expression	Tomlins et al. 2005; Mosquera et al. 2009; Lotan et al. 2011; Mounir et al. 2015
RB1/TP53	*RB1* loss and mutations or deletions of *TP53* are detected in the majority of CRPC-NE. *RB1* loss is highly correlated to Cyclin D1 protein down regulation and overexpression of p16/CDKN2A. *RB1* and *TP53* loss support ADT resistance phenotype and lineage plasticity	Tan et al. 2014; Tsai et al. 2015; Beltran et al. 2016a; Ku et al. 2017; Mu et al. 2017
SOX2/SOX11	SOX2 is up-regulated in *RB1* and TP53 deleted tumors and it is required for lineage plasticity SOX11 is up-regulated in CRPC-NE tumors and its down-regulation causes down-regulation of neuroendocrine markers	Mu et al. 2017; Zou et al. 2017
BRN2	BRN2 or POU3F2 (POU Class 3 Homeobox 2) is highly expressed in CRPC-NE and metastatic CRPC-Adeno with low circulating PSA. BRN2 drives the expression of the terminal neuroendocrine markers and SOX2 and it is transcriptionally repressed by AR	Bishop et al. 2016
CYLD	*CYLD* (Cylindromatosis) is deleted in ~50% of CRPC-NE samples and its loss has been associated to decreased AR mRNA expression	Beltran et al. 2016a
SPDEF	SPDEF (SAM Pointed Domain Containing ETS Transcription Factor) is underexpressed and hypermethylated in CRPC-NE patient cohort. SPDEF overexpression in prostate epithelium reduces tumor cell proliferation in vivo and in vitro	Cheng et al. 2014; Beltran et al. 2016a; Tsai et al. 2016
REST	REST (RE1 silencing transcription factor) down-regulation is observed in 50% of CRPC-NE tumors and it is essential for hypoxia-induced neuroendocrine differentiation of prostate cancer cells. LNCaP cells transfected with si-REST overexpress neuronal associated genes as *Syn1, NTRK3, RUNF* and *Grin2A*	Lapuk et al. 2012; Svensson et al. 2014
SRRM4	SRRM4 (Serine/arginine repetitive matrix 4) promotes neuroendocrine differentiation by generating an inactive slice variant form of REST	Li et al. 2016
FOXA2	The forkhead box protein A2 (FOXA2) is highly expressed in NMYC/AKT1 and TRAMP model and in 75% of patients with small cell neuroendocrine prostate cancer analyzed by immunohistochemistry. It cooperates with HIF1α to induce genes involved in neuroendocrine differentiation as *HES6, SOX9, KDM5A*	Lee et al. 2016; Chiaverotti et al. 2008; Park et al. 2017 Qi et al. 2010

CRPC-NE, castration-resistant neuroendocrine prostate cancer; AR, androgen receptor; DKOCs, double knockout castrated; TKO, triple knockout; PDXs, patient-derived xenografts; IGF-1, insulin-like growth factor-1; ADT, androgen-deprivation therapy; CRPC-Adeno, castration-resistant adenocarcinoma; si, small interfering; sh, short-hairpin; HIF1α, hypoxia-inducible factor 1 α

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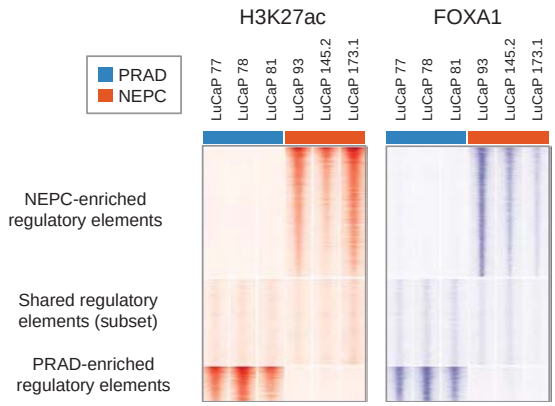
FOXA1 is reprogrammed to neuroendocrine regulatory elements in NEPC



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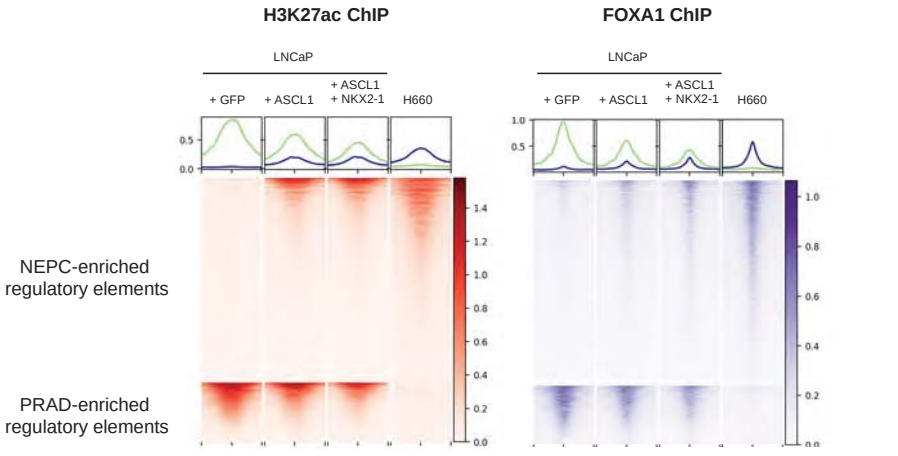
Reprogramming of FOXA1 binding in the presence of NEPC transcription factors



David Takeda, Sarah Abou Alaiwi, Ji-Heui Seo

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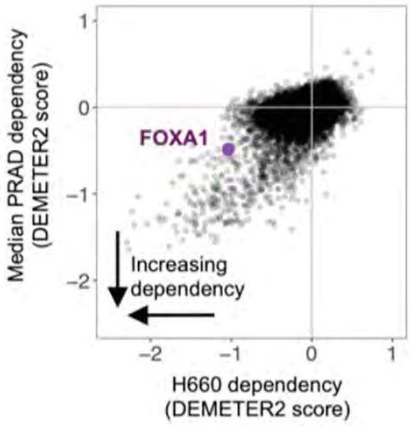
Both adeno- and NEPC cell lines are FOXA1 dependent



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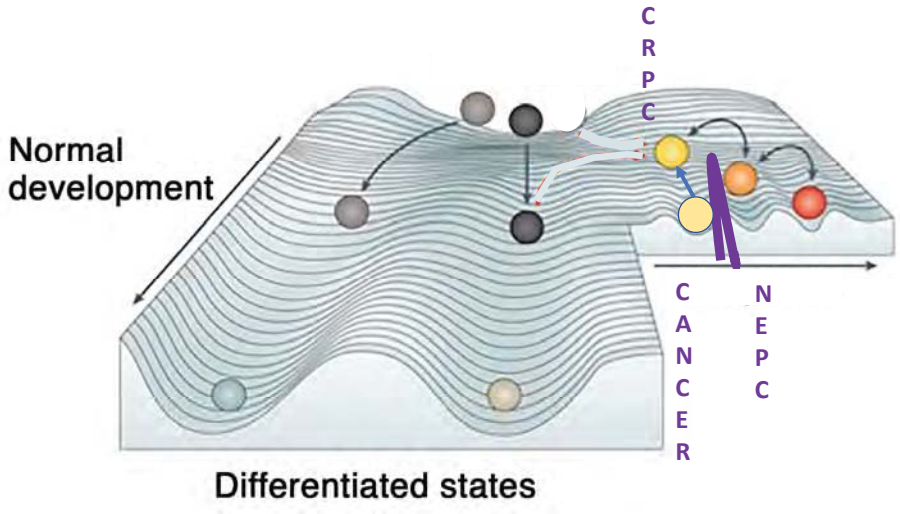
The Waddington landscape of development as it relates to cancer



adapted from Feinberg et al., Nat Rev Genet 2016

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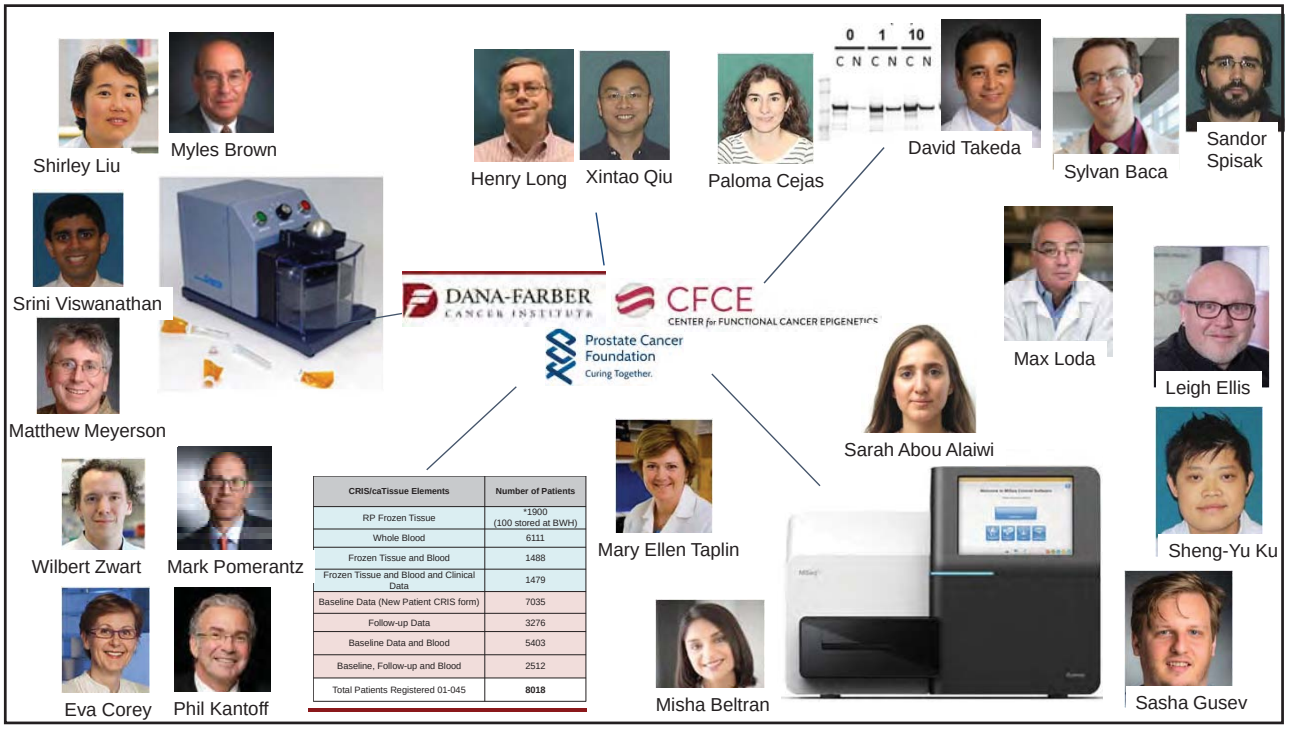
Summary

- AR undergoes extensive reprogramming in the during progression of prostate adenocarcinoma
  - Metastatic prostate adenocarcinoma programs do not arise *de novo*
  - Reprogramming appears to (re)activate fetal programs
- Integration of genetic and epigenetic datasets can identify state-specific, functionally relevant, non-coding regulatory loci
- Epigenomic profiling identifies FOXA1 as a critical mediator of prostate cancer lineage plasticity



A Teaching Affiliate of Harvard Medical School

Molecular Characterization of Circulating Tumor Cells in Prostate Cancer

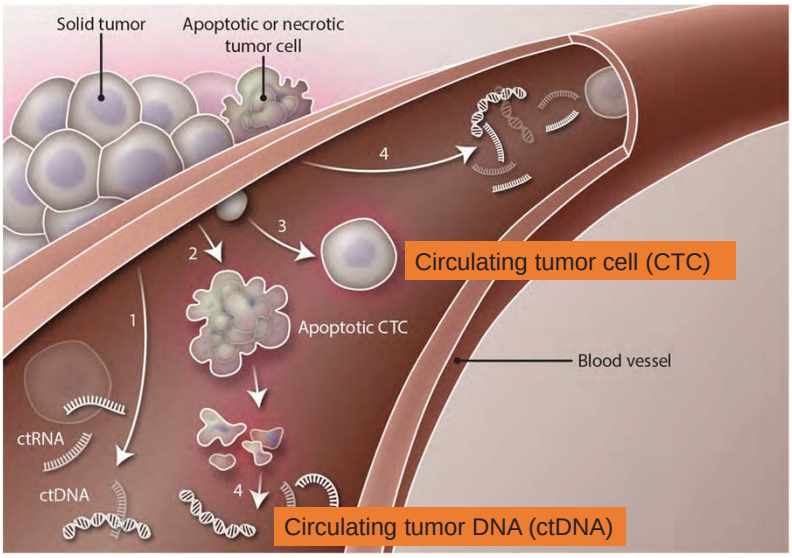
David T. Miyamoto, MD, PhD
Department of Radiation Oncology
Center for Cancer Research
Massachusetts General Hospital
Harvard Medical School

DF/HCC

Mazzone Awards Program
September 13, 2019

MASSACHUSETTS GENERAL HOSPITAL CANCER CENTER

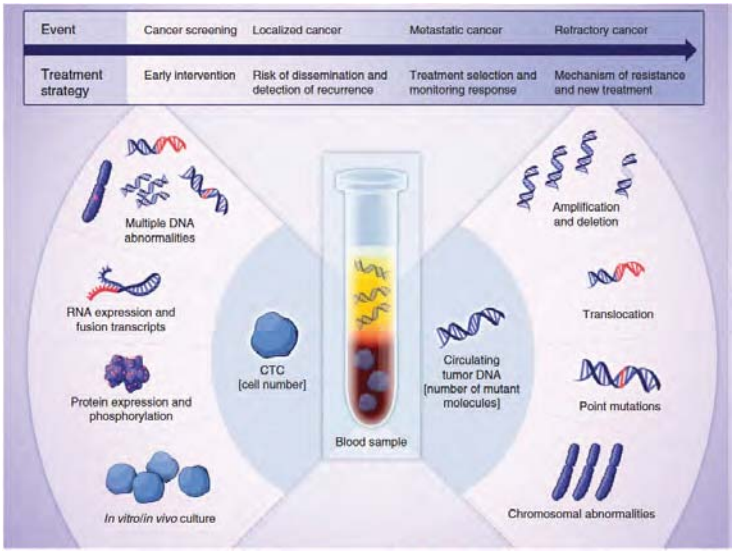
Liquid Biopsies



Schweizer and Antonarakis *Sci Transl Med* 2015

- Non-invasive blood draw, compared to traditional tissue biopsies
- May be performed repeatedly during and after therapy
- May be more representative of multiple heterogeneous tumors throughout the body

CTCs and ctDNA: complementary liquid biopsies



Haber and Velculescu, *Cancer Discovery* 2014

The Challenge:

10 mL Whole Blood
50 billion RBCs
50 million WBCs
0-10 CTCs

CTC Isolation Strategy: “Negative Selection”

Microfluidic depletion of hematopoietic cells ⟶ **untagged CTCs**

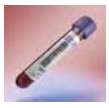
- No assumptions about cell surface epitopes
- Untagged, viable cells can be isolated
- Complex molecular analyses are feasible


Mehmet Toner Daniel Haber

5

CTC-iChip: “Negative Selection”

Microfluidic depletion of hematopoietic cells ⟶ **untagged CTCs**


Whole blood

Anti-CD45 magnetic beads

RBCs, platelets

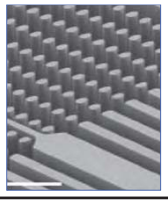
WBCs

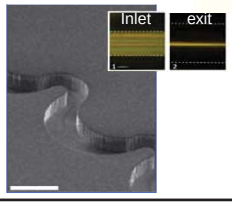
CTCs

Deterministic Lateral Displacement

Inertial Focusing

Magneto-phoresis






Mehmet Toner Daniel Haber

Ozkumur et al., *Sci Transl Med*, 2013
Karabacak et al. *Nature Protocols*, 2014
Fachin et al., *Scientific Reports*, 2017

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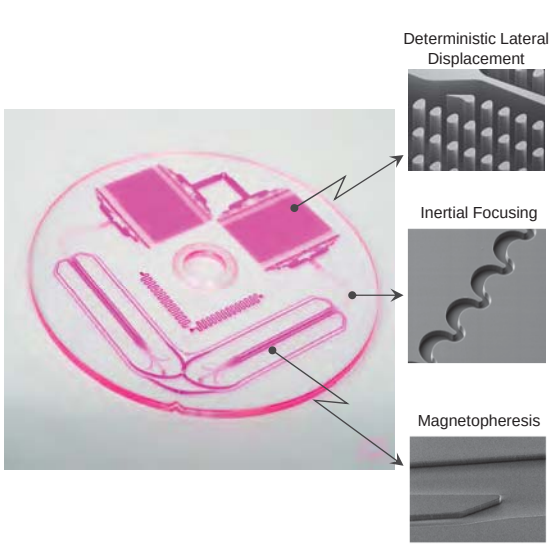
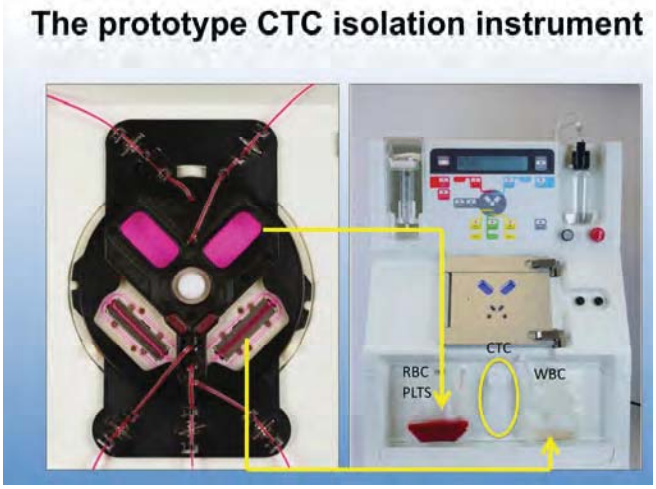
CTC-iChip: Micro-engineering

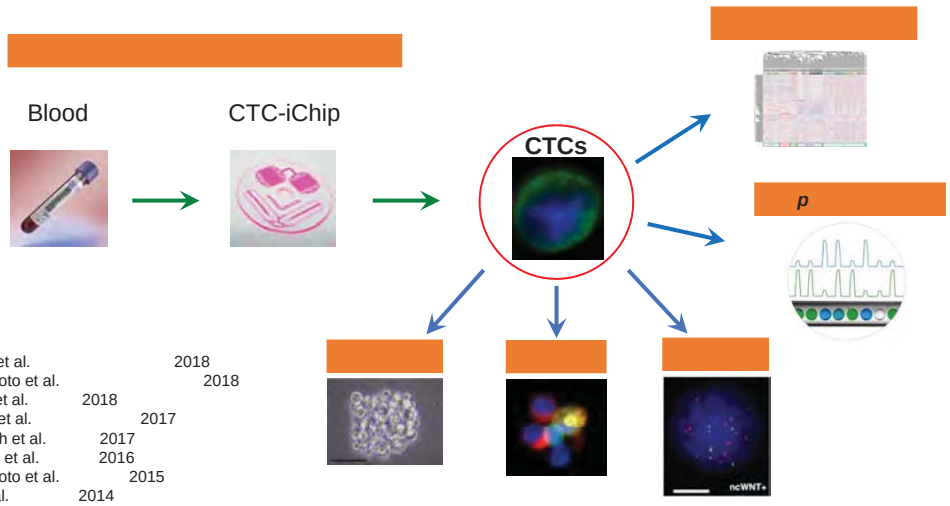




Automated sample processing
Rate: 10 ml blood/hour
Volume: up to 30 ml

7

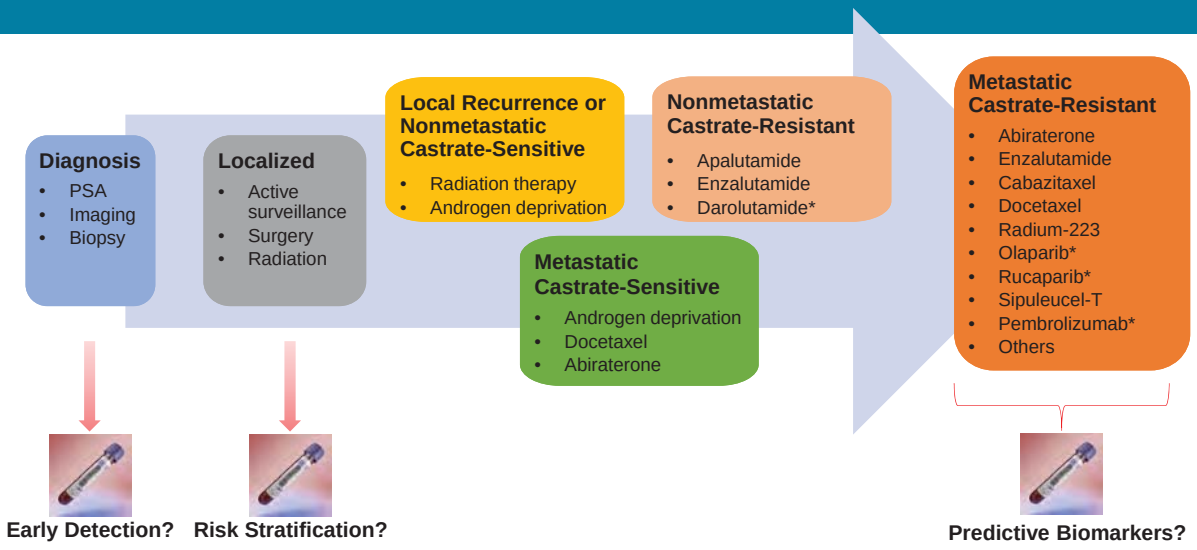
Beyond Enumeration: Molecular Analyses of CTCs



Kwan et al. 2018
Miyamoto et al. 2018
Hong et al. 2018
Wong et al. 2017
Kalinich et al. 2017
Jordan et al. 2016
Miyamoto et al. 2015
Yu et al. 2014
Aceto et al. 2014
Ting et al. ***p*** 2014
Ozkumar et al. 2013

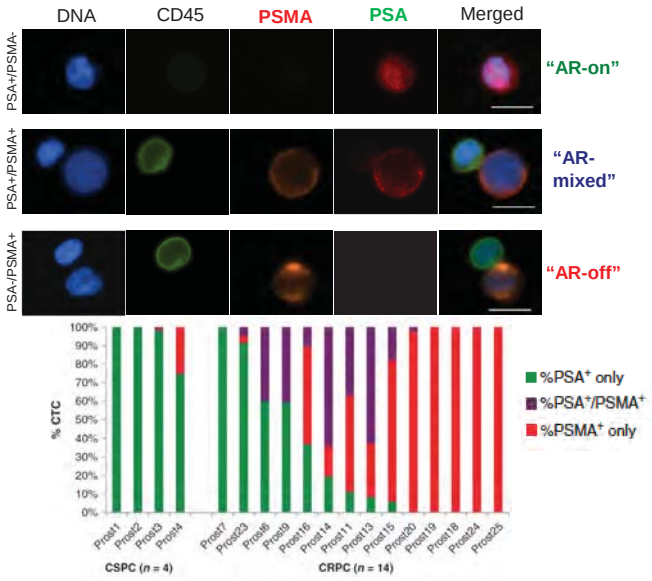
8

Liquid Biopsy Guided Treatment of Prostate Cancer?

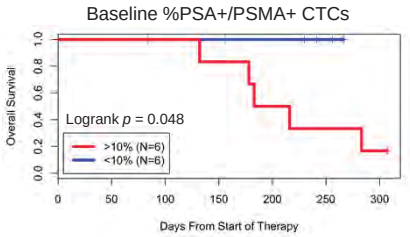


9

PSMA expression in CTCs is heterogeneous



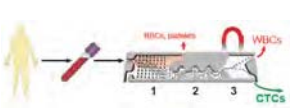
mCRPC: treatment with abiraterone

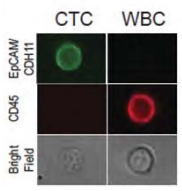


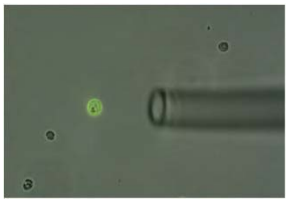
Miyamoto, Lee, Stott, et al. *Cancer Discovery* 2012

10

Prostate CTC Single Cell RNA-Seq

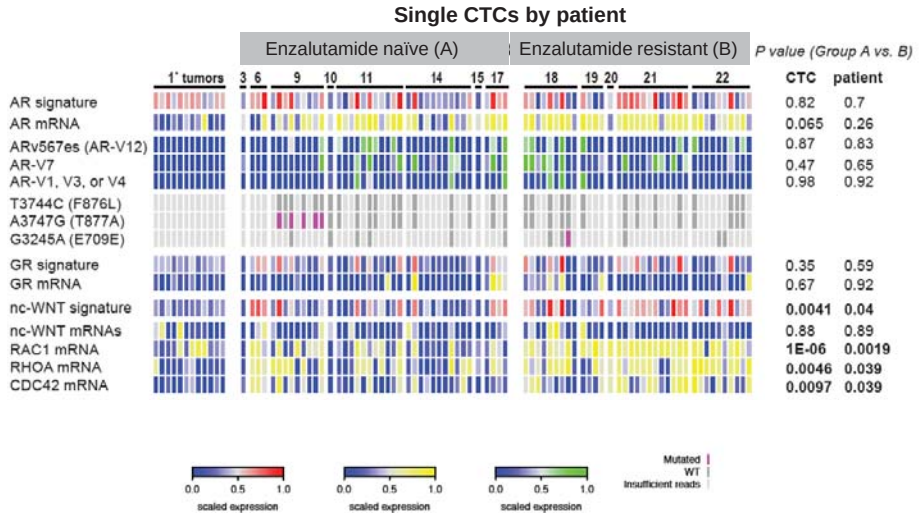






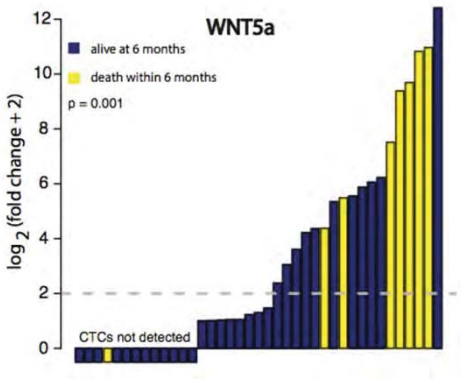
Miyamoto, Zheng, et al. *Science* 2015

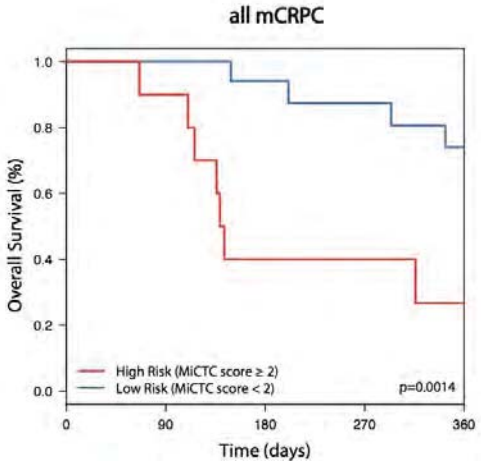
Prostate CTC Single Cell RNA-Seq: Studying Mechanisms of Enzalutamide Resistance



Miyamoto, Zheng et al. *Science* 2015

Independent Validation of WNT5A in CTCs as Prognostic Marker

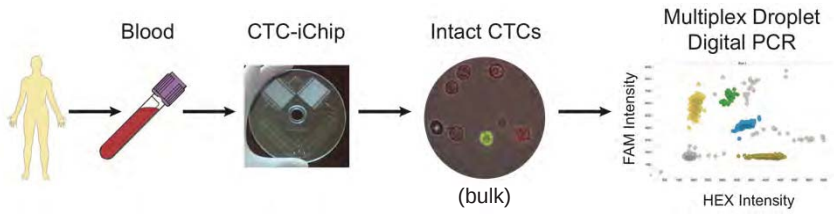




Courtesy of Todd Morgan (Univ of Michigan)

Singhal, Wang, et al. *Molecular Cancer Research* 2018

Towards Clinical Implementation: Digital RNA Readouts for CTC Detection



Miyamoto, Lee, et al., *Cancer Discovery* 2018

Lineage-specific transcripts in prostate CTCs

Bioinformatic ID of candidates: **(+) in prostate cancer, (-) in WBCs** (**40 genes**, 107 probes)

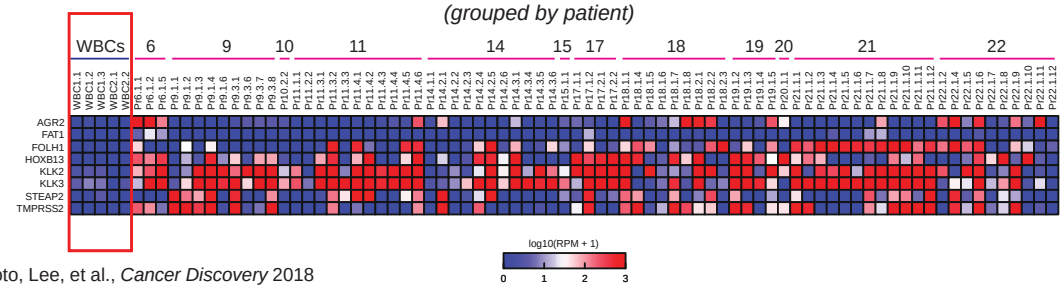
qRT-PCR: **(+) in cell lines** and **(-) in WBCs** (**22 genes**, 34 probes)

CTC-iChip/ddPCR: **(-) signal in healthy donor blood** (**11 genes**, 11 probes)

Final testing: **(+) signal** in spiked blood samples and **mCRPC patients** (**8 genes**, 8 probes)

Single prostate CTCs

(grouped by patient)



Miyamoto, Lee, et al., *Cancer Discovery* 2018

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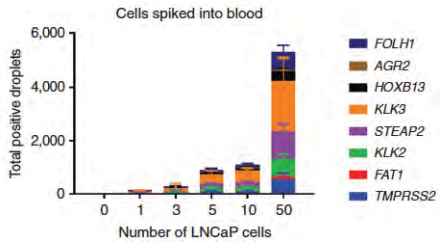
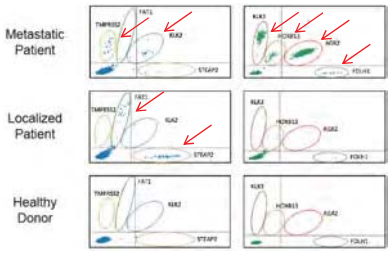
Measuring multiple RNA transcripts from CTC-enriched product



Spiking LNCAP cells into blood

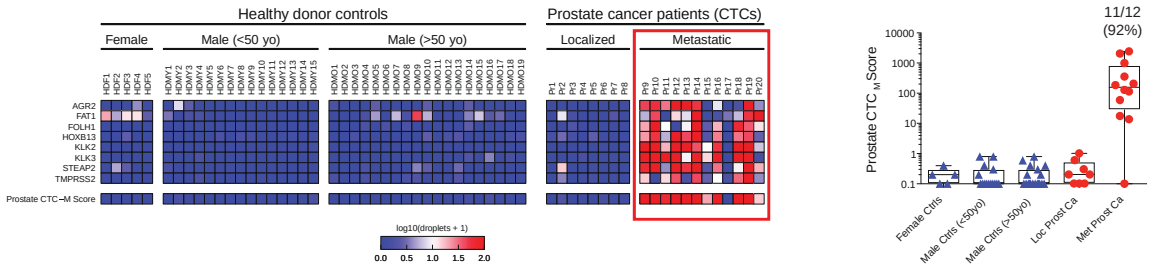


Patients with prostate cancer

Miyamoto, Lee, et al., *Cancer Discovery* 2018

16

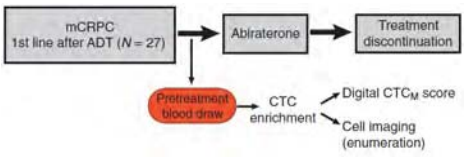
Digital detection of prostate CTCs



Miyamoto, Lee, et al., *Cancer Discovery* 2018

17

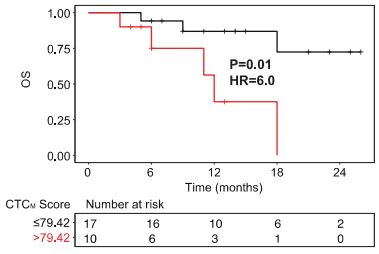
Predicting outcome of AR-targeted therapy: metastatic prostate cancer



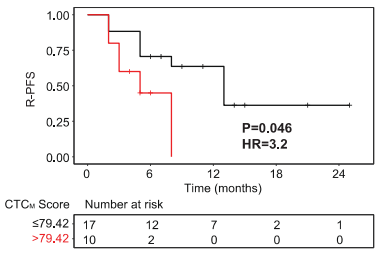
Baseline CTC Score predicts response to AR-targeted therapy

- *Prospective study of first-line abiraterone in mCRPC*

CTC_M Score pretreatment (OS)



CTC_M Score pretreatment (R-PFS)



Miyamoto, Lee, et al., *Cancer Discovery* 2018

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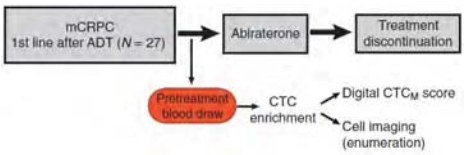
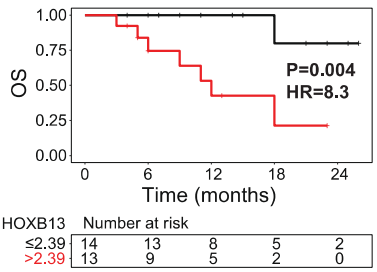
Predicting outcome of AR-targeted therapy: metastatic prostate cancer



Baseline HOXB13 and AR-V7 within CTCs

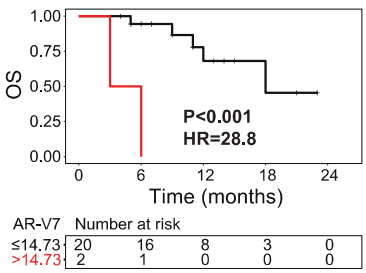
- *Prospective study of first-line abiraterone in mCRPC*

HOXB13



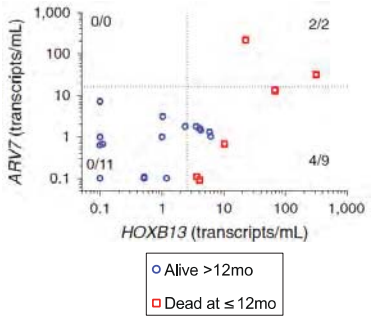
HOXB13	Number at risk				
≤2.39	14	13	8	5	2
>2.39	13	9	5	2	0

AR-V7



AR-V7	Number at risk				
≤14.73	20	16	8	3	0
>14.73	2	1	0	0	0

HOXB13 vs. AR-V7



Miyamoto, Lee, et al., *Cancer Discovery* 2018

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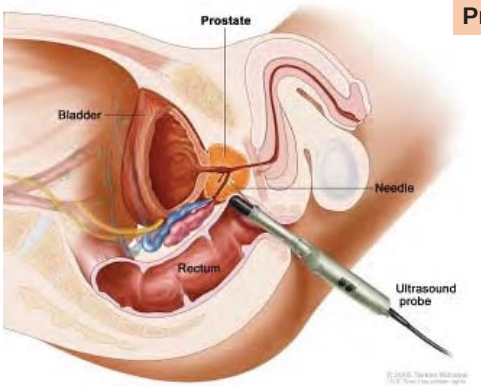
Prostate Biopsy – Problem of Upgrading and Upstaging



Prostate Needle Biopsy

12 biopsy cores
1x10 mm

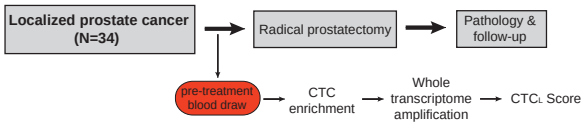
Radical prostatectomy

~20-40% upgraded
~10-20% upstaged

Dinh et al *J Urol* 2015
Caster et al *IJROBP* 2015

20

Predicting pathologic stage in localized prostate cancer



Pre-op CTC analysis in men scheduled for radical prostatectomy

- *Prediction of pathologic seminal vesicle invasion or microscopic lymph node involvement*

Miyamoto, Lee, et al., *Cancer Discovery* 2018

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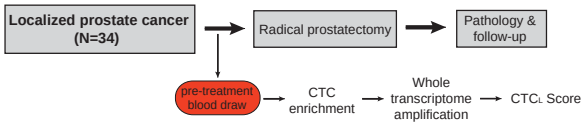
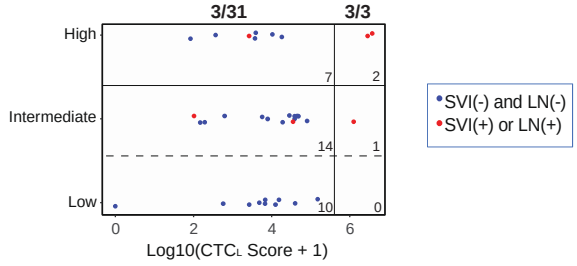
Predicting pathologic stage in localized prostate cancer



Pre-op CTC analysis in men scheduled for radical prostatectomy

- *Prediction of pathologic seminal vesicle invasion or microscopic lymph node involvement*

CTC_L Score vs. D'Amico Risk Group



CTC_L Score

Invasion	High (N=3)	Low (N=31)
+	3	3
-	0	28

P < 0.001

PPV 100% **NPV 90%**

Results after Leave-One-Out Cross Validation (LOOCV)

Miyamoto, Lee, et al., *Cancer Discovery* 2018

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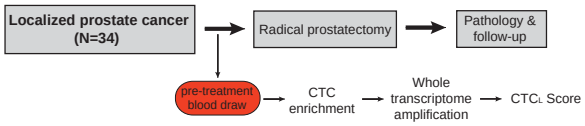
Predicting pathologic stage in localized prostate cancer



Pre-op CTC analysis in men scheduled for radical prostatectomy

- *Prediction of pathologic seminal vesicle invasion or microscopic lymph node involvement*

	D'Amico (high)	CAPRA (high)	CTC_L score (high)
PPV	33% (3/9)	60% (3/5)	100% (3/3)
NPV	88% (22/25)	90% (26/29)	90% (28/31)

Miyamoto, Lee, et al., *Cancer Discovery* 2018

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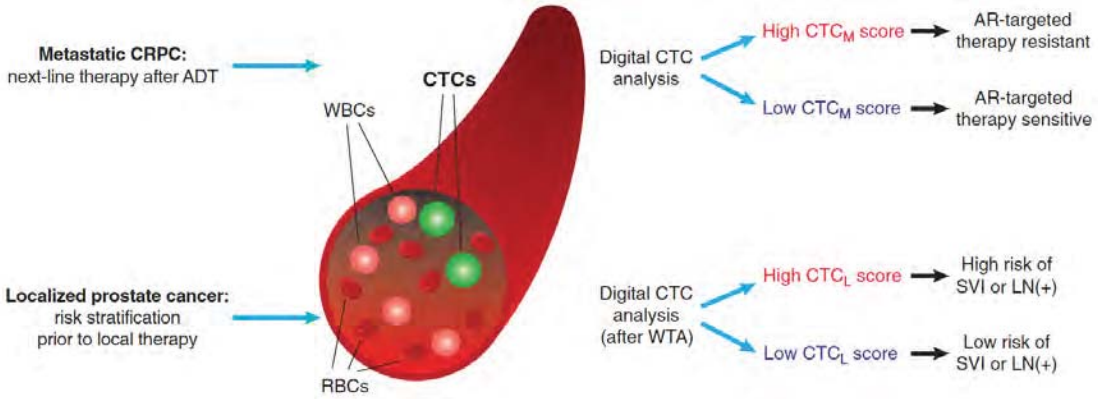
RNA-based analysis of prostate CTCs



Miyamoto, Lee, et al., *Cancer Discovery* 2018

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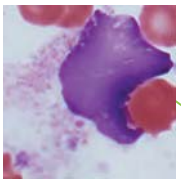
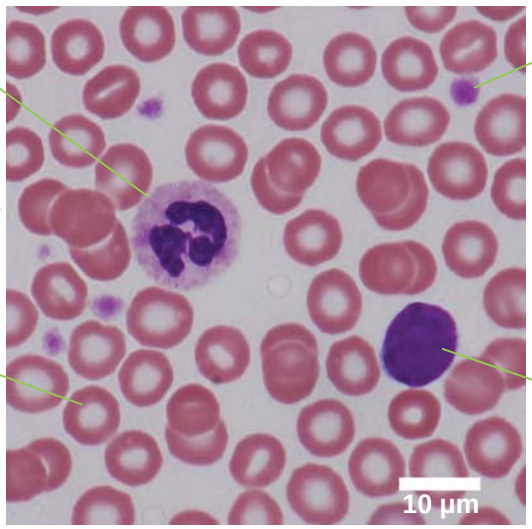
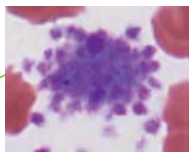
Blood degradation damages target cells & cripples precise microfluidic technologies

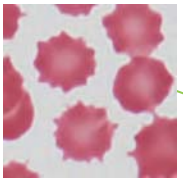


Oxidative bursts
Extracellular traps





Aggregation
Clotting



Hemolysis

Activation
Cytokines

Courtesy of Shannon Stott

Preservation of Blood for CTC RNA Analyses

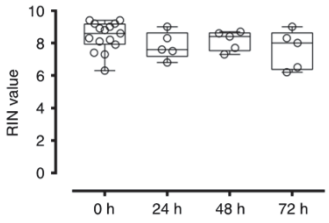
ACD (acid citrate dextrose) tube

+

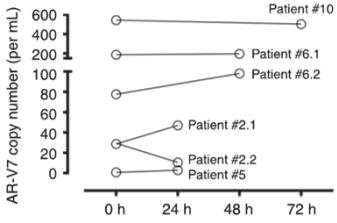
Platelet inhibition (GPIIb/IIIa inhibitor - tirofiban)
Calcium chelation (EDTA)
Hypothermic storage (4˚C)

CTC-iChip

CTC RNA integrity number (RIN)

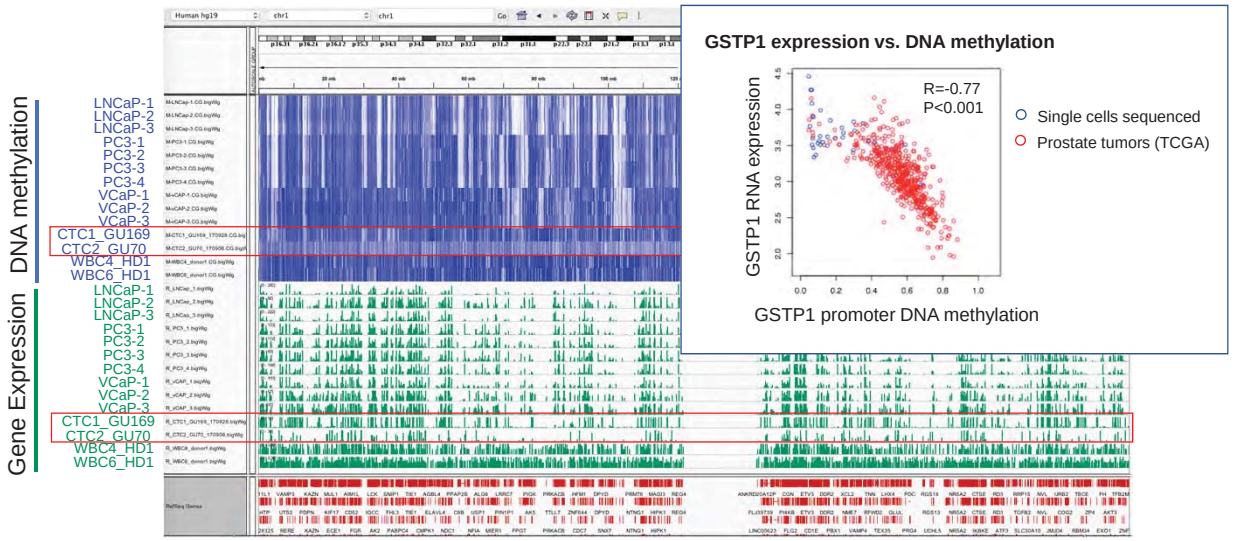


CTC AR-V7



Wong, Tessier, Miyamoto, et al., *Nature Communications* 2017

Epigenetics of Prostate CTCs: Single cell DNA Methylation



Unpublished data, Hongshan Guo

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Conclusions

- High quality CTC RNA can be isolated via CTC-iChip microfluidics
- Inter and intra-patient heterogeneity in CTCs
- Pre-treatment CTC digital RNA score predicts response to therapy in metastatic prostate cancer
- Pre-treatment CTC digital RNA score in localized prostate cancer predicts microscopic dissemination and pathologic stage
- Advantages of RNA-based analyses:
  - Snapshot of the cell: real-time biological states (e.g. EMT, signaling, etc.)
  - RNA assays do not require prior knowledge or sequencing of mutations
  - CTC RNA assays can provide universal non-invasive cancer biomarkers that are mutation agnostic

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MGH Circulating Tumor Cell Team

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Tanya T. Kwan
Tilak Sundarasen
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Erin Emmons
Uyen Ho
Joe LiCausi
John Milner

Mehmet Toner
Shannon Stott
Jon Edd
Ravi Kapur
Rebeca Sandlin
Shannon Tessier
John Walsh
Keith Wong
Linda Nieman
Lauren Bookstaver
Thomas Carey
Kathleen Miller
Cleo Stannard

Douglas Dahl
Jason Efstathiou
Richard Lee
Lecia Sequist
Matthew Smith

Anita Giobbie-Hurder
Tianqi Chen
Steven Skates

p
Ben Wittner
Vishal Thapar

Chin-Lee Wu

Prostate Cancer Foundation
Curing Together.

A. David Mazzone Research Awards Program

DF/HCC
DANA-FARBER / HARVARD CANCER CENTER

Charles Evans Foundation

STAND UP TO CANCER

NATIONAL CANCER INSTITUTE

janssen

Epidemiology studies of racial and ethnic disparities in prostate cancer

Lorelei Mucci, ScD

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Harvard T.H. Chan School of Public Health

Leader of Cancer Epidemiology
Dana-Farber/Harvard Cancer Center

Twitter: @loreleimucci

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HARVARD T.H. CHAN SCHOOL OF PUBLIC HEALTH Department of Epidemiology

ToPCaP Transdisciplinary Prostate Cancer Partnership

1

Three vignettes

1. **Are there racial and ethnic disparities in prostate cancer risk in a socioeconomically homogenous population?**
   - Health Professionals Follow-up Study (HPFS)

2. **Can differences in lifestyle and dietary factors account for racial disparities in prostate cancer?**
   - Health Professionals Follow-up Study (HPFS) and National Health and Nutrition Examination Survey (NHANES)

3. **Is there diversity in prostate cancer clinical trials**
   - IRONMAN: International Registry of Men with Advanced Prostate Cancer

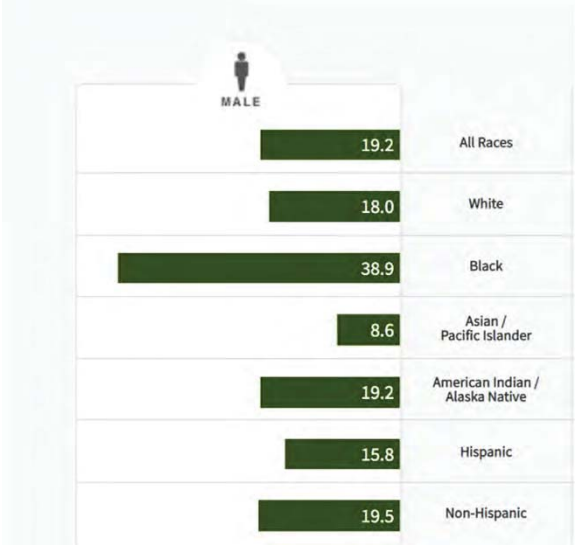
2

VIGNETTE 1. HPFS Cohort

ARE THERE DISPARITIES IN PROSTATE CANCER BY RACE/ANCESTRY IN ECONOMICALLY HOMOGENEOUS POPULATION?

3

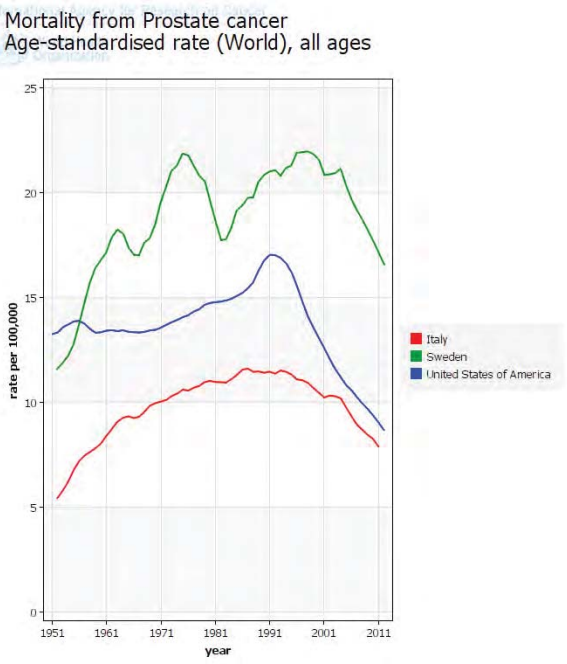
Number of Deaths per 100,000 Persons by Race/Ethnicity: Prostate Cancer



Hispanics in Puerto Rico 26.4/100,000

SEER, 2012-2016
Miller et al, 2018 CA: A Cancer Journal for Clinicians

4



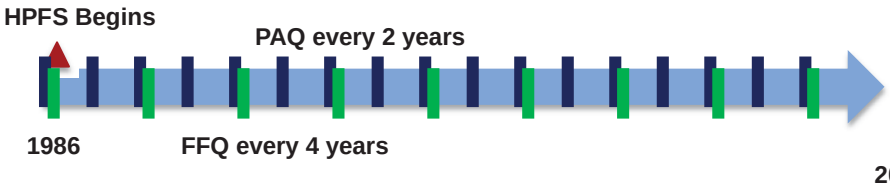
GLOBOCAN, 2018

5

Methods: Health Professionals Follow-up Study


Nadine amieh Megan ansen

- 51,529 US male health professionals age 40-75 years in 1986
- Ongoing follow-up for lifestyle, health-related factors, disease outcomes every two years
- Cancer incidence and mortality for 32 years follow-up



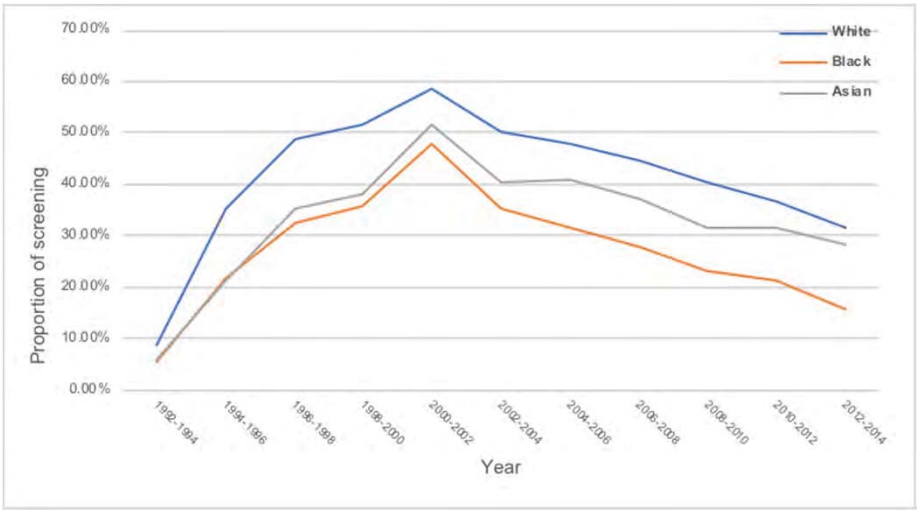
6

Age-adjusted characteristics of 46,108 men in the HPFS study population at baseline in 1986 baseline by race

	Race				
	White (n=44,785)			Black	Asian
	Southern European (n=11,740)	Scandinavian (n=5,046)	Other white (n=28,009)	(n=479)	(n=834)
Mean (SD) age, years	53.9 (9.7)	53.7 (9.6)	56.0 (9.8)	54.8 (8.6)	52.7 (9.0)
Mean (SD) height, inches	69.7 (2.9)	70.7 (2.8)	70.3 (2.8)	70.1 (3.4)	66.9 (3.0)
Overweight, %	55%	53%	52%	58%	34%
Current or quit smoking ≤10 years, %	22%	21%	23%	29%	21%
Mean (SD) vigorous activity, MET-h/week	13.2 (28.4)	12.3 (26.3)	12.4 (25.2)	14.5 (33.9)	11.5 (21.1)
Family history of prostate cancer, 1990, %	12%	13%	12%	12%	7%
Ever had a PSA test by 2012, %	84%	84%	85%	73%	75%

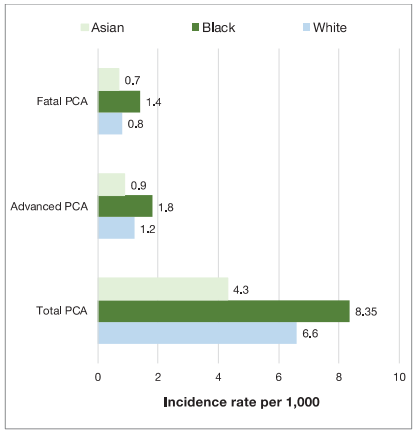
7

Trends in frequency of PSA screening in past two years among white, black, and Asian-American men in the Health Professionals Follow-up Study



8

Age-standardized incidence rates and Hazard Ratio (95% CI) for prostate cancer risk in HPFS

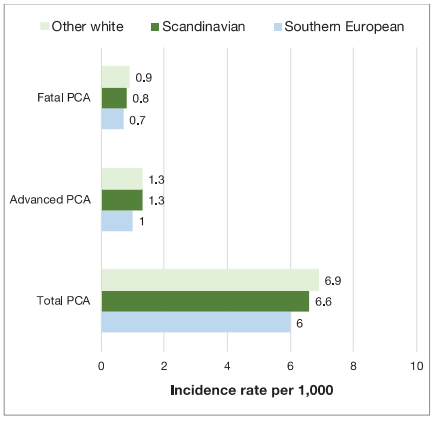


	white	Black	Asian
Fatal prostate cancer†, N	**773**	**14**	**13**
Multivariable-HR (95% CI)*	1	1.69 (0.98 to 2.91)	1.28 (0.73 to 2.26)
Advanced prostate cancer†, N	**1176**	**18**	**18**
Multivariable-HR (95% CI)*	1	1.41 (0.88 to 2.28)	1.10 (0.68 to 1.78)
Total prostate cancer, N	**6461**	**82**	**84**
Multivariable-HR (95% CI)*	1	1.36 (1.09 to 1.71)	0.77 (0.62 to 0.96)

igh-grade PCA Gleason 4+3 or higher
Multivariable-ad usted *R*: age, calendar time, height, body mass index, smoking, vigorous activity, family history of prostate cancer, prostate-specific antigen testing (yes/no), and history of physical examinations

9

Age-standardized incidence rates and Hazard Ratios (95% CI) for prostate cancer risk in Health Professionals Follow-up Study



	Southern European	Scandinavian	Other white
Fatal prostate cancer†, N	**170**	**86**	**517**
Multivariable-HR (95% CI)*	1	1.14 (0.92 to 1.39)	1.15 (1.00 to 1.32)
Advanced prostate cancer†, N	**260**	**140**	**776**
Multivariable-HR (95% CI)*	1	1.21 (0.98 to 1.49)	1.13 (0.98 to 1.31)
Total prostate cancer, N	**1568**	**733**	**4160**
Multivariable-HR (95% CI)*	1	1.08 (0.98 to 1.18)	1.07 (1.01 to 1.14)

Multivariable-ad usted *R*: age, calendar time, height, body mass index, smoking, vigorous activity, family history of prostate cancer, prostate-specific antigen testing (yes/no), diet, and history of physical examinations

10

VIGNETTE 2. NHANES and HPFS

CAN DIFFERENCES IN LIFESTYLE AND DIETARY FACTORS ACCOUNT FOR DISPARITY IN PROSTATE CANCER INCIDENCE

11

Study Methods


Sam Peisch Travis er e

National Health And Nutrition Examination Study	Health Professionals Follow-up Study
Prevalence of lifestyle and dietary factors in black and white men	Multivariable relative risks for lifestyle factors and lethal prostate cancer

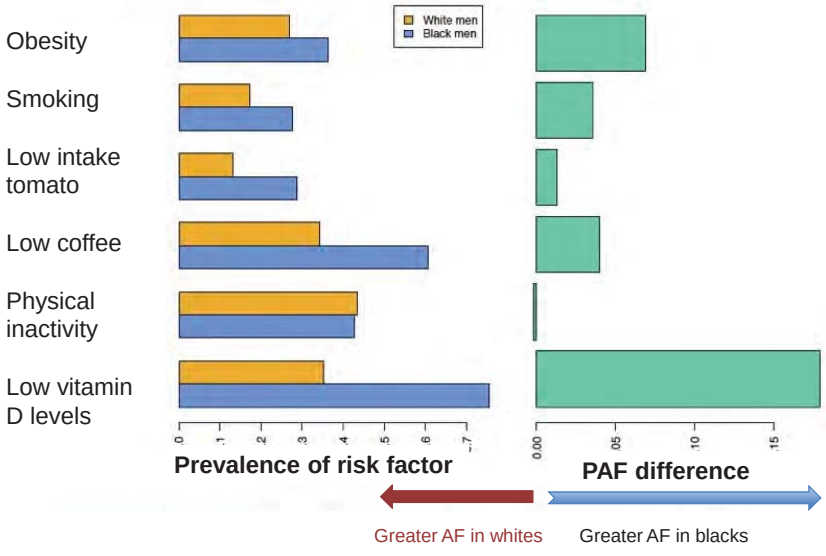
Population attributable fraction

$$AR = P(E^+)(RR_c - RR_c\text{-}RR_a)/[1 + P(E^+)(RR_c - 1)],$$

$P(E^+)$ denotes exposure prevalence, RR_c is the crude risk ratio, and RR_a is the confounder-adjusted risk ratio

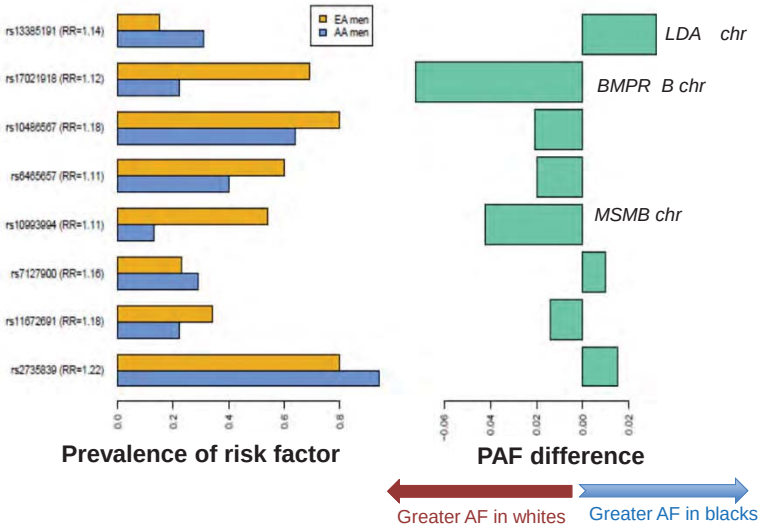
12

Population attributable fraction differences of fatal prostate cancer in black and white men
Lifestyle and diet



13

Population attributable fraction differences in prostate cancer in black and white men
Genetic factors



14

VIGNETTE 3. IRONMAN Registry

LACK OF DIVERSITY IN PROSTATE CANCER TRIALS

15

Is there diversity in prostate cancer clinical trials?

Trial Information	Treatment Trials N=61	Prevention Trials N=4	Screening Trials N=5	All Trials N=70
Earliest Recruitment Start Date	1994	1993	1987	
Latest Recruitment End Date	2016	2012	2016	
Availability of Race/Ethnicity				
Trials with Available Race	39 (63.9%)	4 (100%)	1 (20%)	
Trials with Separate Ethnicity	6 (9.8%)	1 (25%)	0 (0%)	
Participant Information	N=35,913	N=62,424	N=792,757	**N=891,094**
Availability of Race/Ethnicity Data,				
Available Race Data Participants	25,619 (71.3%)	62,424 (100)	76,702 (9.7)	**164,745 (18.5)**
with Separate Ethnicity Data	4,039 (11.2%)	423 (0.7)	0 (0)	**4,462 (0.5)**

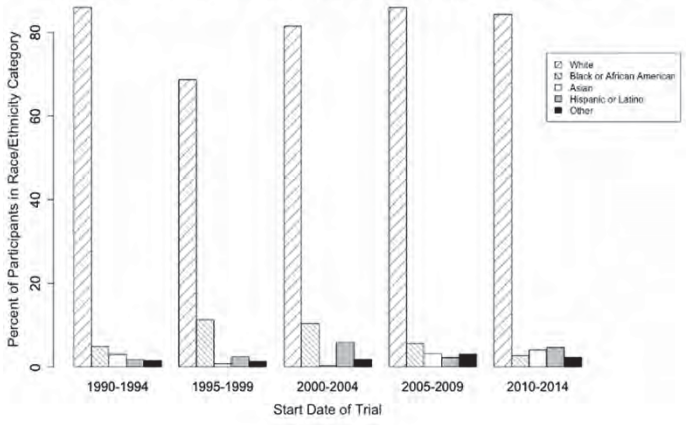
2 screening trials w/o reported data were 100% white men (personal communication)

Rencsok et al, under review (Cancer)

16

Given racial disparities, is there diversity in prostate cancer clinical trials?

Figure 1. Representation of Races/Ethnicities in Prostate Cancer Clinical Trials over Time



Rencsok et al, under review (JAMA)

17

IRONMAN

An International Registry for Men with Advanced Prostate Cancer

18

Our **vision** is to create the global IRONMAN study to understand differences in patient care, experiences, and outcomes among men with advanced prostate cancer

Our **goal** is to create a global study of men with advanced disease to represent the diversity of patients globally

Ixqglqj2Sduwqhuvkls

19

IURQP DQ#Vwxg|#Srsxodwlrq

Uhfuxlw#A8/333#p hq#z lwk#dgydqfhg#survwdwh#fdqfhu

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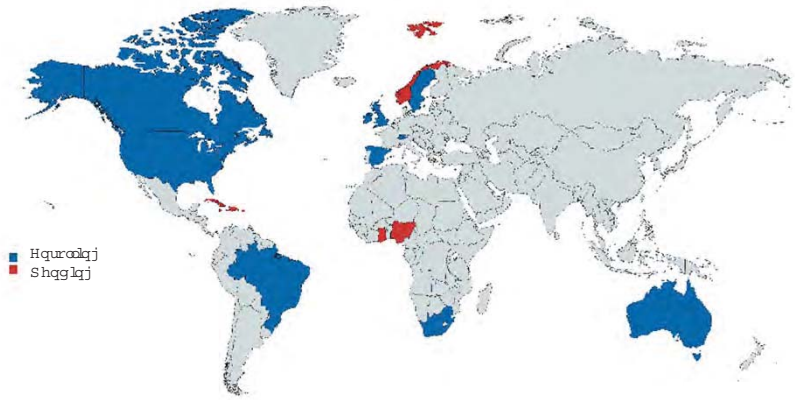

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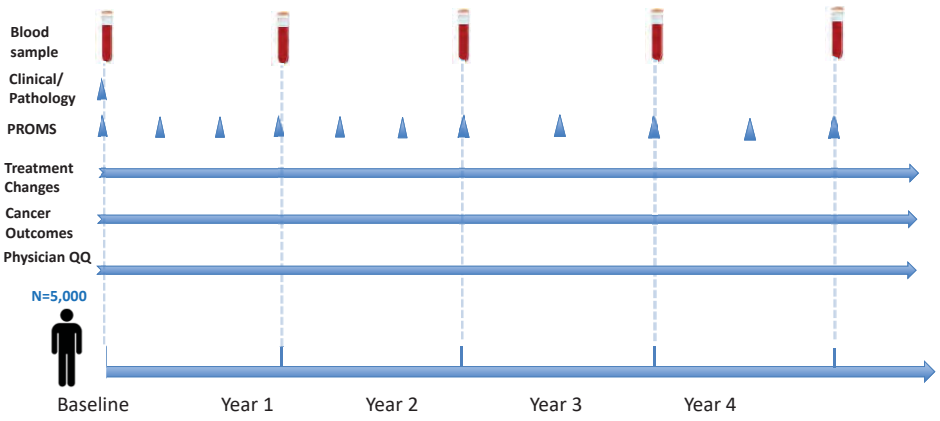

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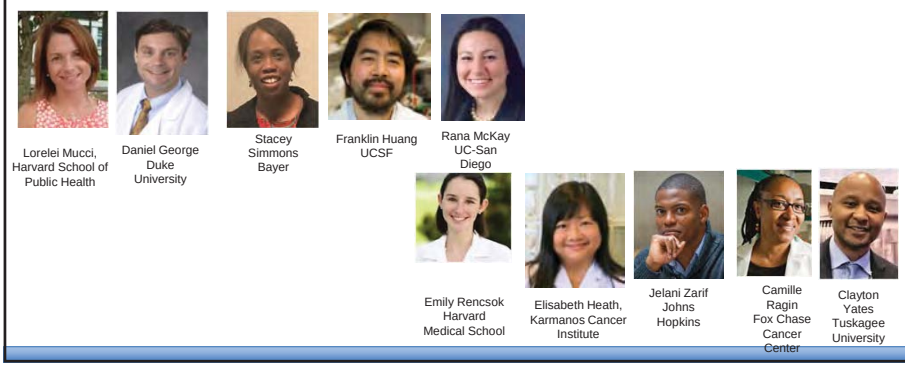

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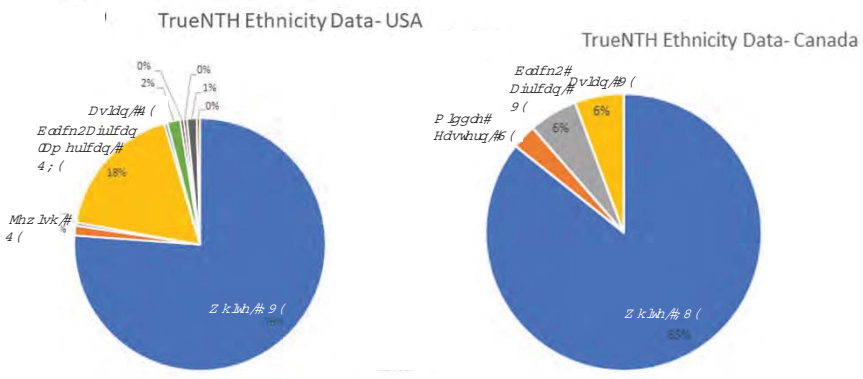

23

Summary of Findings

- Even after adjusting for risk factors, screening patterns, and socioeconomic status
  - Excess risk of prostate cancer overall (and fatal) in black men and men of Scandinavian descent
- Differences in prevalence of obesity, smoking, and vitamin D status account for part of disparity in risk of prostate cancer
- Prostate cancer clinical trials to date have lacked racial/ethnic diversity
- Collaborate with us on IRONMAN!

24

Harvard
Ericka Ebot Claire Pernar
Kathy Wilson Sam Peisch
Emily Rencsok Tim Rebbeck
Meir Stampfer
Ed Giovannucci

Case Western
Sarah Markt

University of Michigan
Latifa Bazza

Johns Hopkins
Elizabeth Platz

Moffitt Cancer Center
Travis Gerke

IRONMAN
Dan George Philip Kantoff
Jake Vinson Paul Villanti
Rana McKay Elisabeth Heath
Theresa Gold Camille Ragin
Clayton Yates Franklin Huang
Jelani Zarif Konrad Stopsack

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DFHCC SPORE in Prostate Cancer

Movember Foundation
Bayer
Astellas
Sanofi
Astra Zeneca

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LY6D links castration-resistant prostate luminal cells to prostate progenitors and cancer

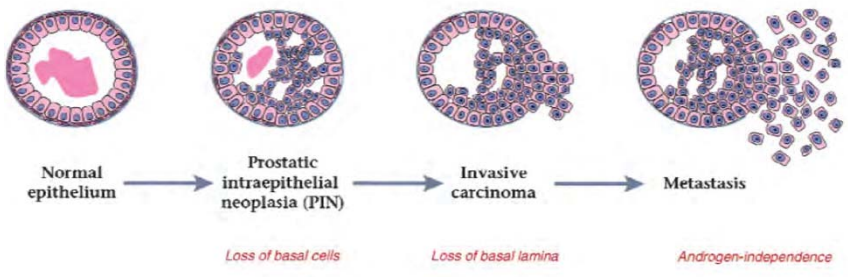
Zhe Li, PhD

Division of Genetics, Brigham & Women's Hospital
Harvard Medical school

September 13, 2019

1

Major challenges in Prostate Cancer (PCa)

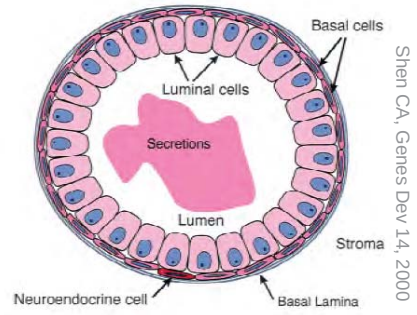


Shen MM, Genes Dev 14, 2000

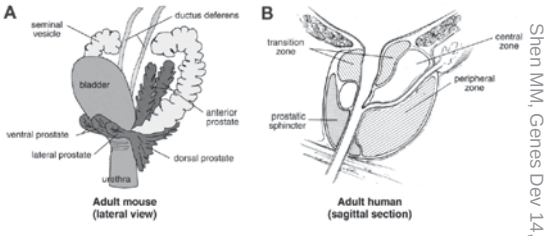
- **Prostate cancer is 2nd leading cause of cancer-related death among Western males**
- **Critical need to distinguish indolent versus aggressive disease**
- **Critical need to treat castration-resistant PCa**
- **Advanced PCa: androgen deprivation therapy**
- **Castration-resistance: adaptation or selection?**

2

Prostate architecture



Shen CA, Genes Dev 14, 2000



murine **human**

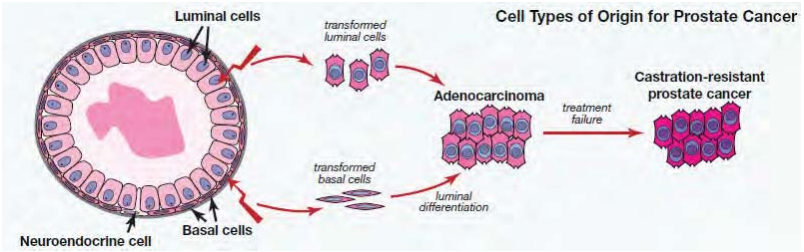
Shen MM, Genes Dev 14, 2000

- **Anatomical differences between murine and human prostates**
- **Ducts consist of luminal (K8/K18) and basal cells (K5/K14)**
- **Gland can undergo series of regression/regeneration through androgen withdrawal/restoration**
  - **Most luminal cells are sensitive to castrate conditions**
  - **Existence of castration-resistant luminal cells**

3

Defining cellular origins of prostate cancer

- Both lineages can serve as cells of origin in PCa
  - Luminal cells may be preferred origin for PCa
  - Basal cells are more resistant to oncogenic transformation
- PCa has a strikingly luminal phenotype
- **<u>Better characterization of prostate hierarchy is needed, especially castration-resistant luminal cells</u>**

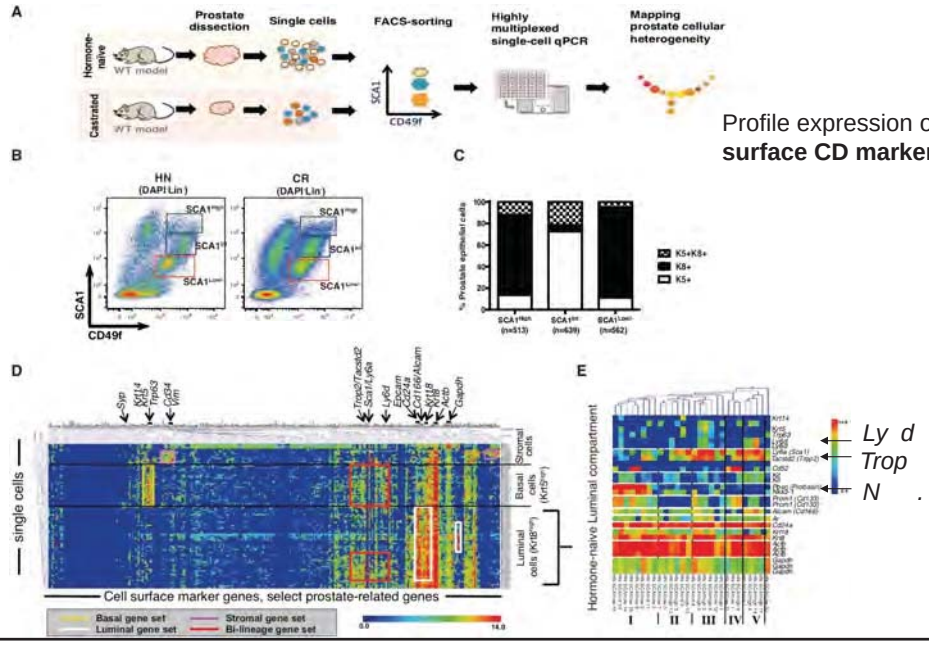


Barlow LJ, Cancer Cell 2013

Single cell profiling is a valuable tool to address prostate epithelial heterogeneity

4

Single cell gene expression profiling



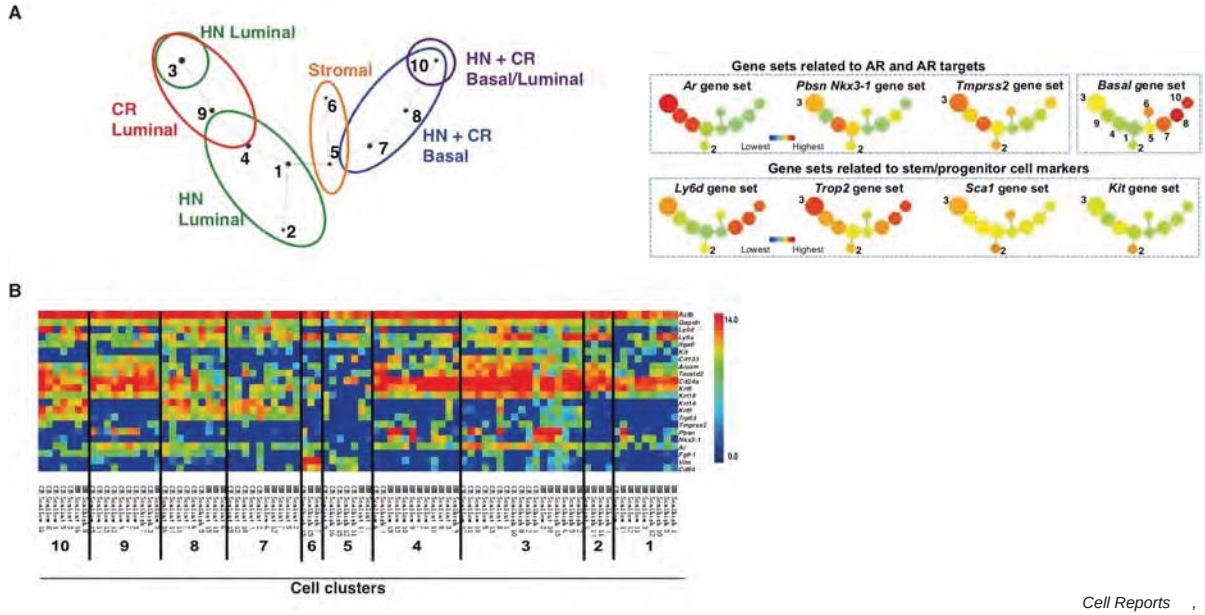
Profile expression of most **cell surface CD marker genes**

Cell Reports ,

5

Mapping lineage relation of HN and CR prostate cells

SPADE analysis (dimension reduction)

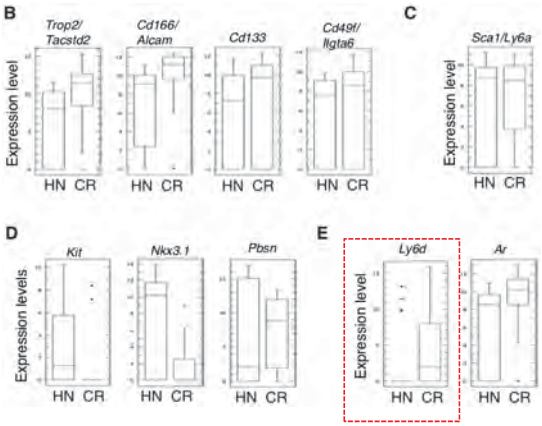


Cell Reports ,

6

Candidate marker of castration resistance

Enriched in castrated vs. intact luminal (K8+) cells:

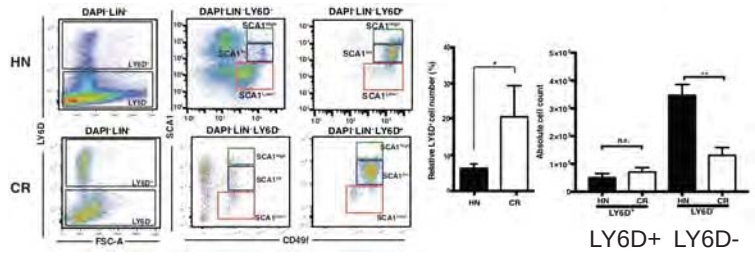


LY6D (E48/Thb):

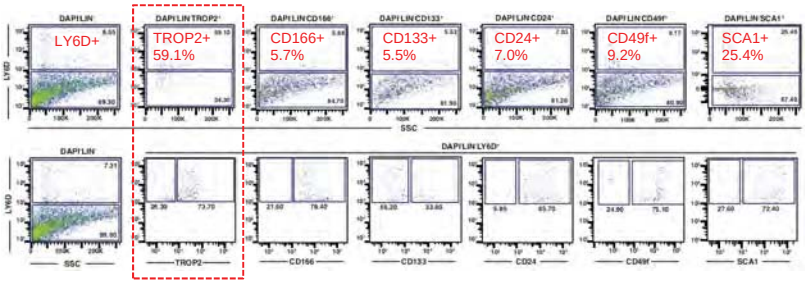
- **GPI-anchored receptor;**
- **marker of B-cell lineage specification;**
- **has a human ortholog.**

7

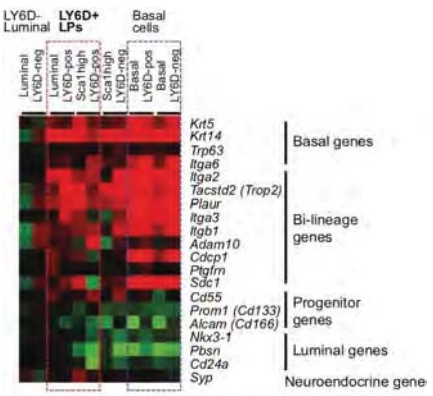
Characterization of LY6D+ prostate cells



Relation with other prostate stem/progenitor markers:

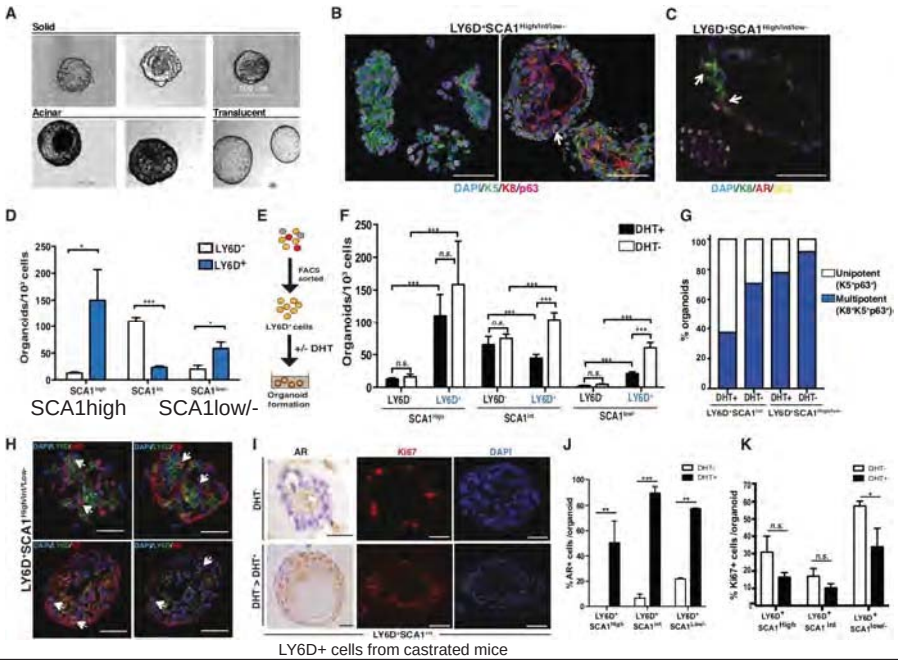


Expression profiling of sorted LY6D+ subpopulations:



8

LY6D+ prostate cells are enriched for organoid-forming potential

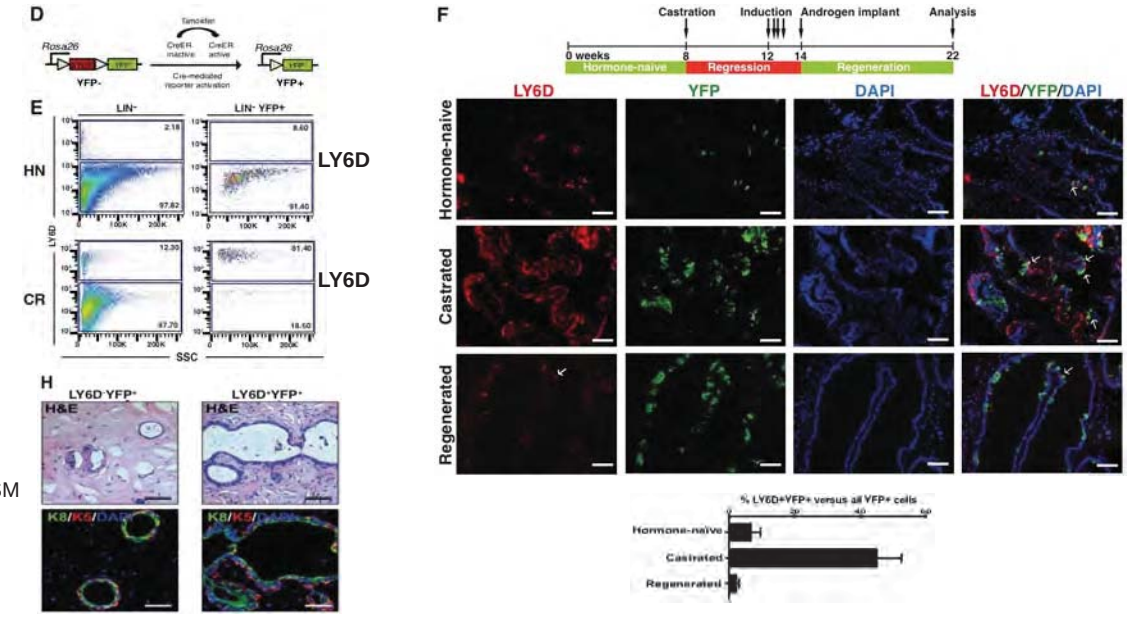


LY6D+ cells from castrated mice

sther Baena et al., Cancer Research U Cell Reports ,

9

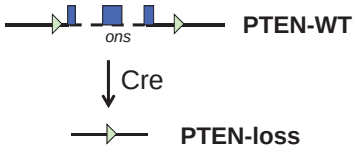
LY6D marks castration-resistant luminal cells (K8) that may possess regenerative capacity

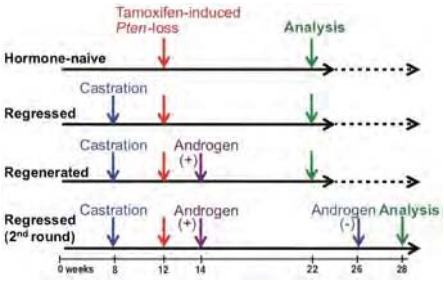


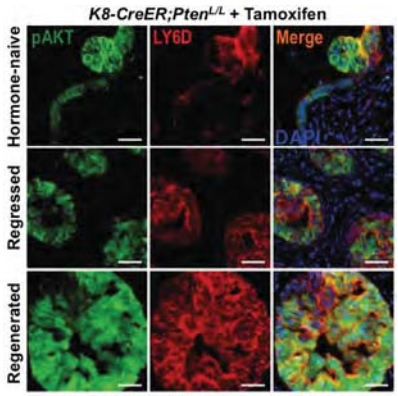
With UGSM cells

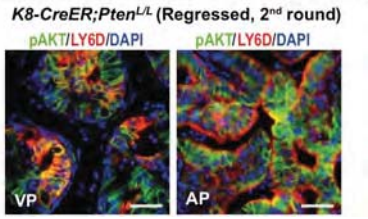
10

LY6D+ prostate cells in the luminal lineage are involved in prostate cancer initiation and progression



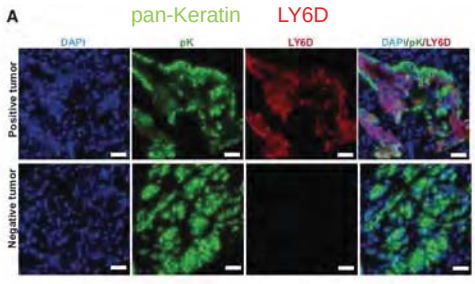


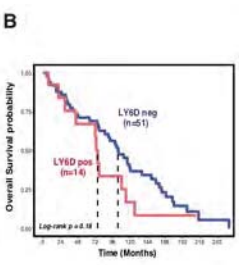




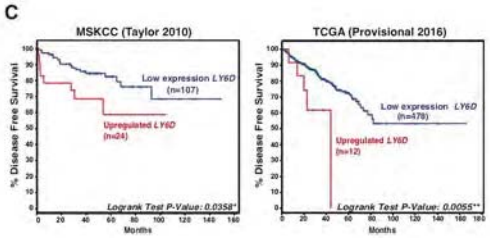
11

LY6D is associated with advanced human prostate cancer

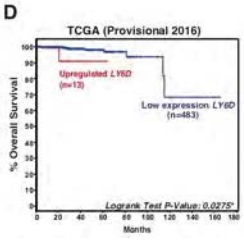




Tissue microarrays
Christie Hospital



Time to biochemical recurrence from diagnosis

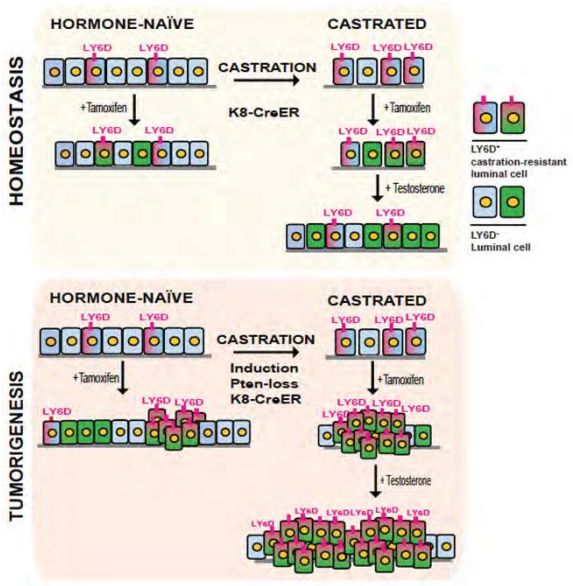


Overall survival

sther Baena et al., Cancer Research U Cell Reports ,

12

Model and Summary



1. **Single-cell analysis identifies a highly heterogeneous luminal compartment.**
2. **LY6D marks luminal cells that are resistant to castration with bi-lineage capacity.**
3. **LY6D correlates with prostate cancer development from the luminal lineage.**
4. **LY6D expression in human prostate cancer correlates with early disease progression.**

Cell Reports ,

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Adnan Ali

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Michael Brown

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Stuart Orkin Lab, BCH/DFCI

Esther Baena

Guoji Guo

GC Yuan Lab, DFCI

Guo-cheng Yuan

BWH flow cytometry core

Yiling Qiu

DF/HCC Rodent pathology core

Rod Bronson

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A. David Mazzone Awards Program
Project Development Award

Localized Chemo Radiation Therapy for Prostate Cancer

RA Cormack
Division of Medical Physics and Biophysics
Department of Radiation Oncology
Brigham and Women's Hospital / Dana-Farber Cancer Institute

Disclosures

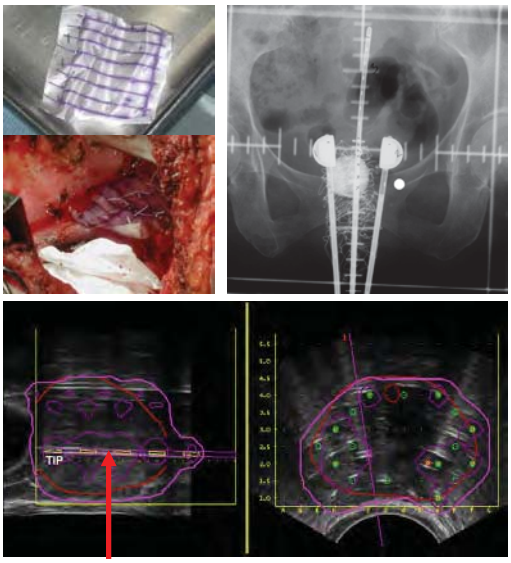
- I accept honoraria from professional societies
- IP assigned to my employer may be discussed in this presentation. I may benefit from royalties flowing from such IP

Outline

- Prostate brachytherapy
- Mazzone Goals
- Mazzone Accomplishments
- Current efforts
  - Brachytherapy spacers
  - Other sites

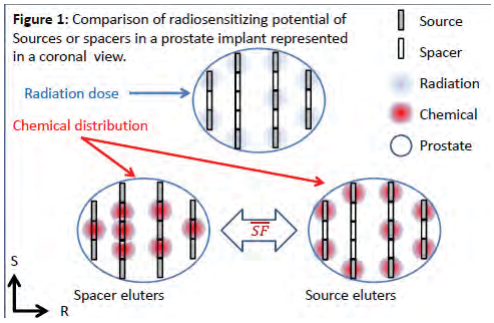
Types of Brachytherapy

- Brachythearpy: Radiation sources in or proximal to target
  - Permanent
  - Temporary
- Sites: prostate, gynecologic, breast, lung, sarcoma, skin
- TRUS permanent prostate implants archetypical image guided radiation therapy
  - Real time visualization of prostate and needles
  - Permanent placement of sources and SPACERS



Brachytherapy: An opportunity for implants to inform/improve radiation

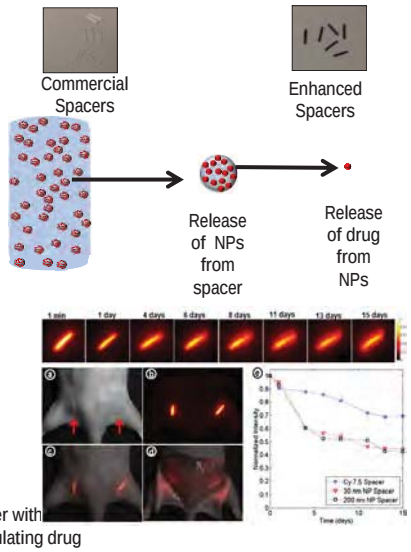
Sustained Radiosensitization:
DFCI/BWH & Northeastern University



- Team
  - DFCI/BWH
    - Robert Cormack PhD (PI)
    - Mike Makrigiorgos PhD
    - Anthony D'Amico MD
    - Department of Radiation Oncology
  - NU
    - Sri Sridhar PhD
    - Nanotechnology Science and Technology Center

Future: Therapeutic enhancement

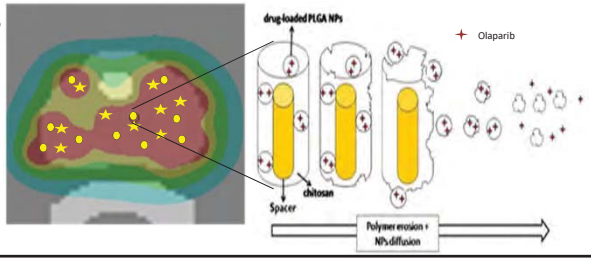
- Biologic enhancement of radiation effect
  - radiosensitizers
  - repair inhibitors
  - optimization of spatial and temporal release
- Physical enhancement
  - gold NP
  - Auger electons
- Localized sustained distributions



Degragable Spacer with Encapsulating drug

Goals of Project

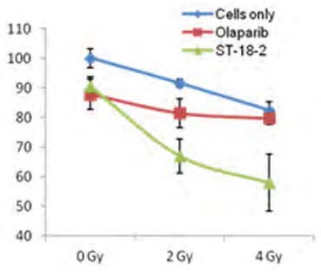
- Develop a drug eluting spacer for sustained release of olaparib in the setting of permanent prostate brachytherapy.
  - **SA1: Optimization of the coated brachytherapy spacers with fluorescent, Olaparib loaded PLGA nanoparticles, and in vitro radio sensitization of Olaparib released from spacers**
  - **SA2: *In vivo* evaluation of synergistic image guided chemo- and radiation therapy with coated spacers implanted into the subcutaneously xenografted prostate tumor in mice.**



Accomplishments: In-vitro

- Drug releasing nanoOlaparib
- Radiosensitization

NanoOlaparib	Physico-chemical Parameter
Particle Size	~ 120 nm
Zeta Potential	~ +15mV
Polydispersity Index	~ 0.05
Drug loading	~ 400mM to 2.3mM. Lower concentrations can be obtained by dilution



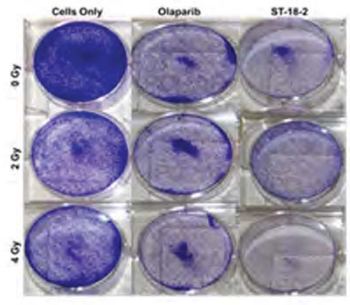
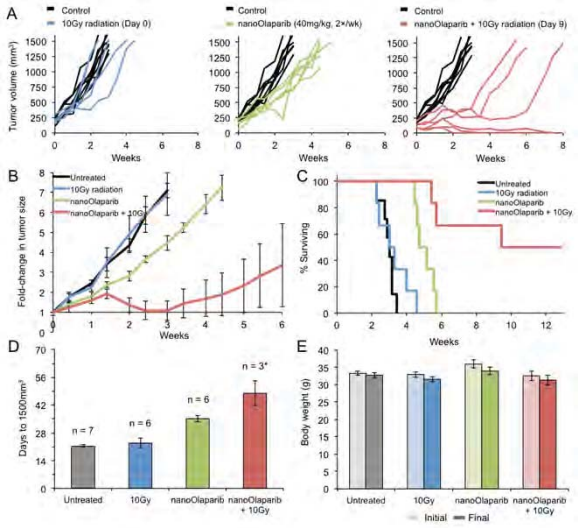


Figure 3 : (Left) Short-term effect of radio-sensitization on PC3 cell line, determined by MTS assay at 20 µM Olaparib and NanoOlaparib concentrations (Right) Long-term delayed effect of radio-sensitization at 2 and 4 Gy on PC3 cell line (Qualitative Clonogenic assay). The blue color represents the crystal violet stain taken up by the glutaraldehyde fixed cells after 6 doubling cycles of PC3 (1.5 weeks). The bar graph represents quantitative estimate of stained cells in the graphics on right. ST-18-2 represents Nano-olaparib

Accomplishments: In-vivo

- Drug releasing nanoOlaparib
- Radiation enhancement

- ~~Effective when released from spacer~~

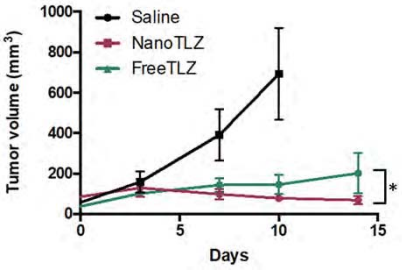


Redirection of Efforts

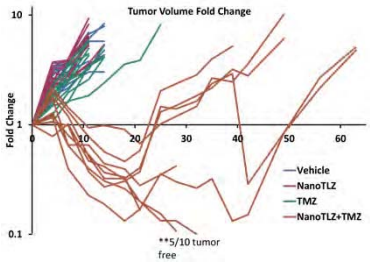
- NU: other PARP inhibitors for other sites
- DFCI/BWH:

SUMMARY OF RESULTS

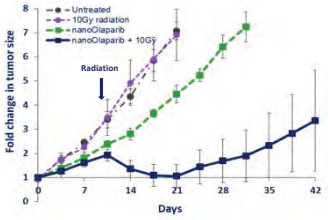
Breast Cancer



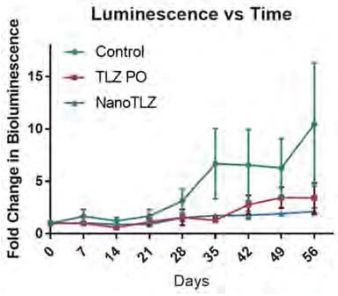
Ewing's Sarcoma



Prostate Cancer



Ovarian Cancer



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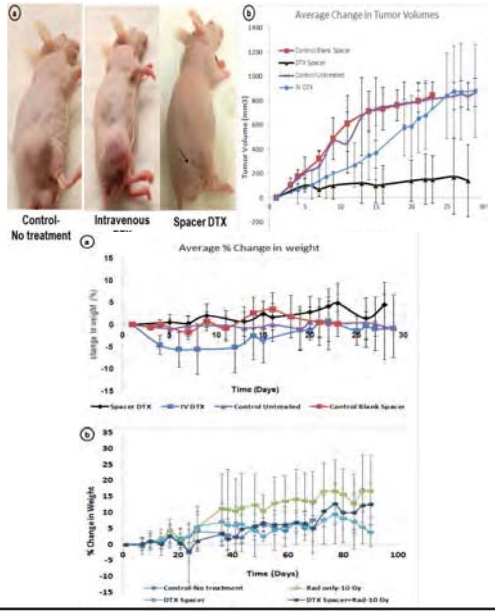
In-situ Docetaxel Release

- Prolonged release
- Less toxic than equivalent IV delivery
- Comparable behavior when combined with radiation



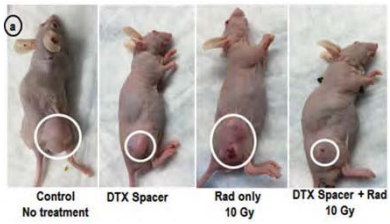
12

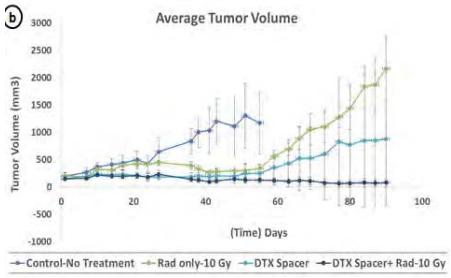
12

In-situ Docetaxel Release+Radiation

- Prolonged release & focal radiation
- Less toxic than equivalent IV delivery
- Combined response greater than individual
- DTX+Rad mice aging well

- Starting soon: in-vivo evaluation of docetaxel spacers in intact prostates





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Summary

- Olaparib nanoformulation increases effect of radiation in cells
- Olaparib nanoformulation and radiation delay tumor growth compared to individually
- Dose enhancing effect not seen when released from polymer spacer
- Efforts have evolved to
  - different sites and PARP inhibitors
  - different sensitizer in spacers for prostate

Nanoformulation of Olaparib Amplifies PARP Inhibition and Sensitizes *PTEN/TP53*-Deficient Prostate Cancer to Radiation, van de Ven et al, Mol Cancer Ther. 2017 Jul;16(7):1279-1289. doi: 10.1158/1535-7163

First-in-class Approach to Sensitize Prostate Cancer to Immunotherapy

Jennifer Wu, PhD
Mary and Patrick Scanlan Professor of Urology
Professor of Immunology
Northwestern University
Robert Lurie Comprehensive Cancer Center

Northwestern Medicine
Feinberg School of Medicine

Robert H. Lurie Comprehensive Cancer Center of Northwestern University

Highly Immune Suppressive Tumor Microenvironment in Prostate Cancer

- **Lack of immune infiltrates –"Cold Tumor"**
- **No NK cell activity**
- **Impaired T cell function – impaired CD3z expression**
- **Accumulation of MDSC**
- **Accumulation of TAM**
- **Low PD-L1 expression**

(Reviewed by Boattcher et al., , rontiers Oncology)

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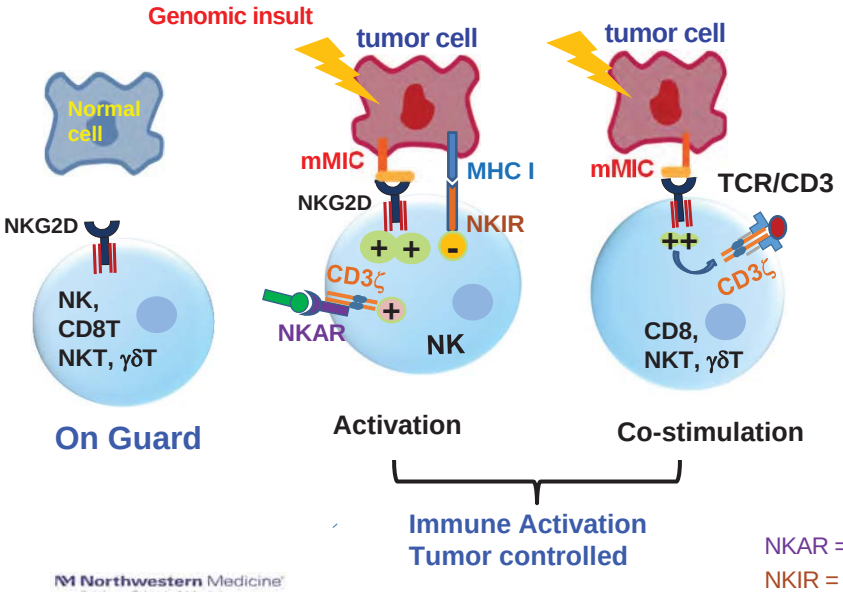
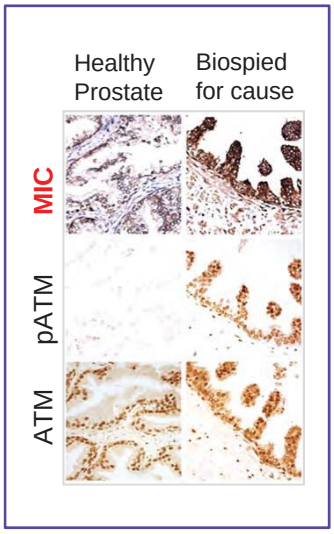
Induction of MIC at early tumorigenesis provides immune protection





NKAR = NK Activating Receptor
NKIR = NK Inhibitory Receptor

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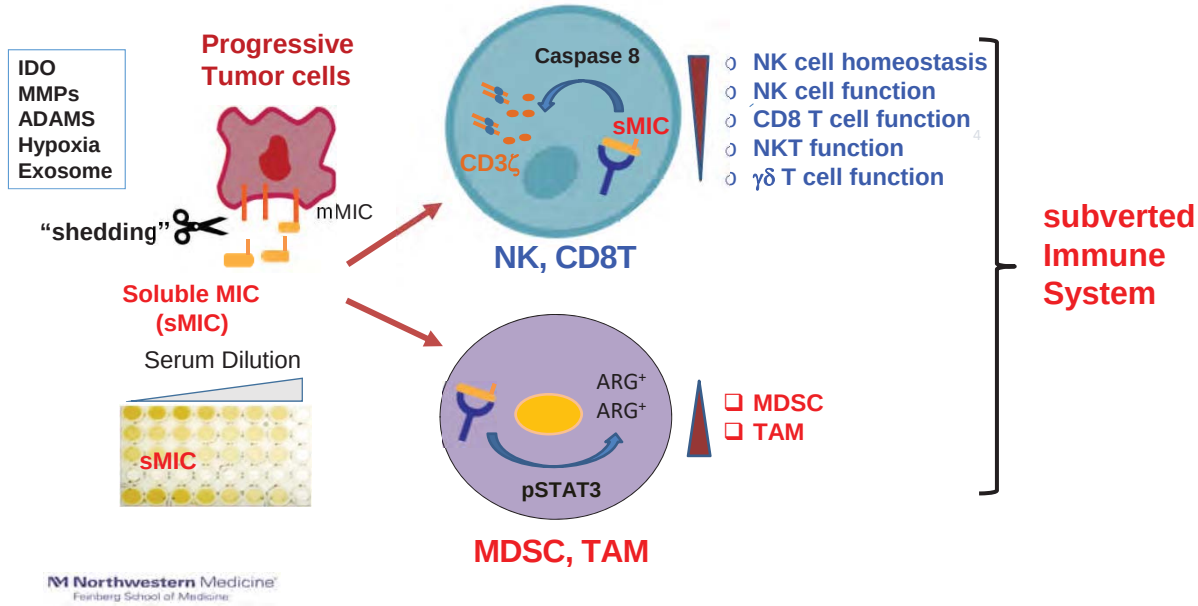
Tumor-shed sMIC induces multiple immune suppression



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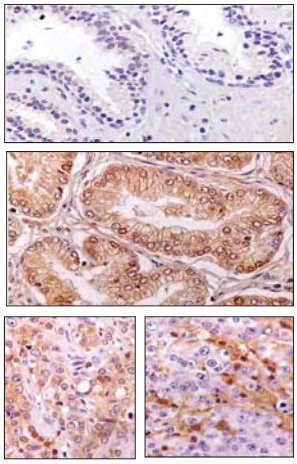
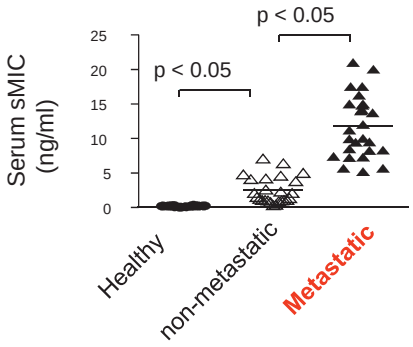
Increased serum sMIC associates with metastatic PCa



(JCI, ,)



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Is sMIC a therapeutic target for metastatic PCa?

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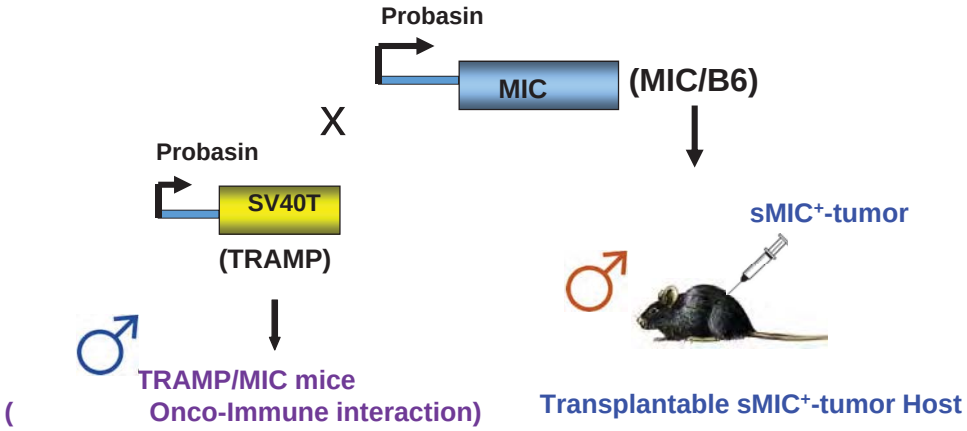
Animal models recapitulate onco-immune dynamics in men

The Hurdle: MIC is a human-specific molecule

The Way out: Human MIC can serve as mouse NKG2D Ligand



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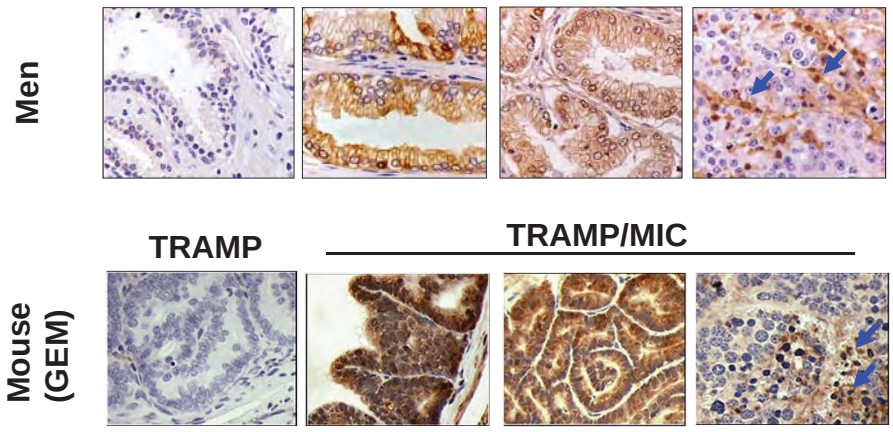
MIC expression in the prostate: Men and GEM



Liu et al, JCI 2013

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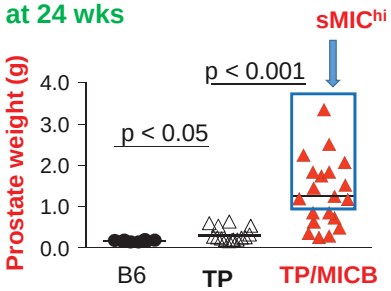
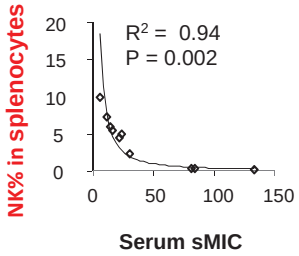
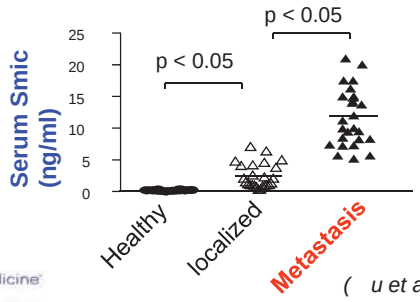
Onco-Immunological Resemblance: Men and GEM

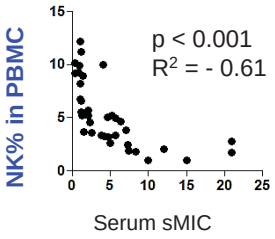
GEM (TRAMP/MIC)





MEN





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(u et al, JCI , Liu et al, JCI , and unpublished)

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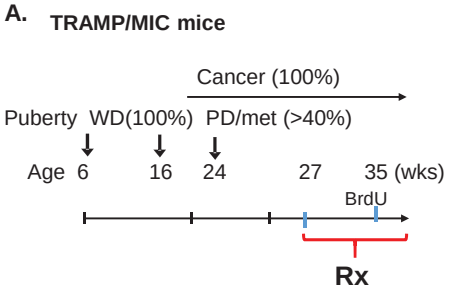
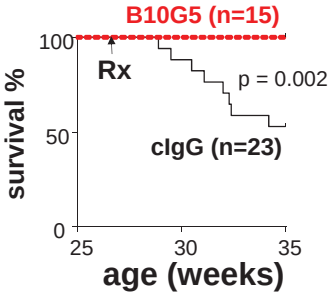
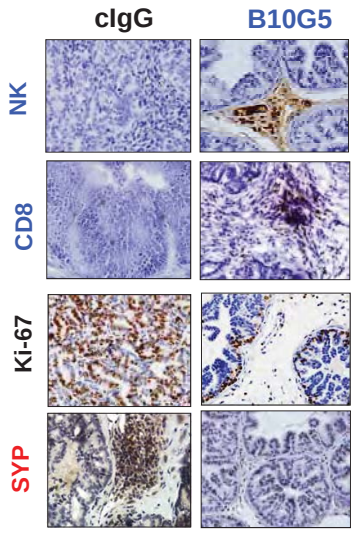
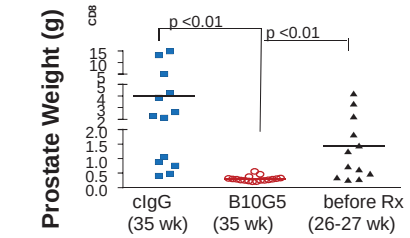
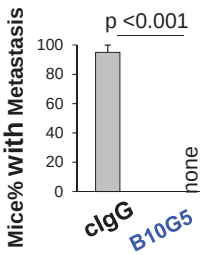
Targeting sMIC debulks primary tumor and eliminates metastasis





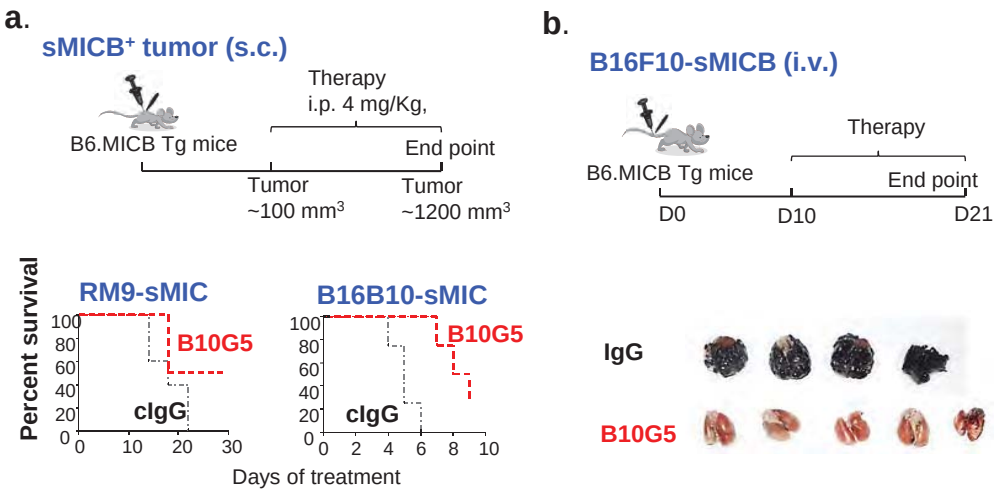






10

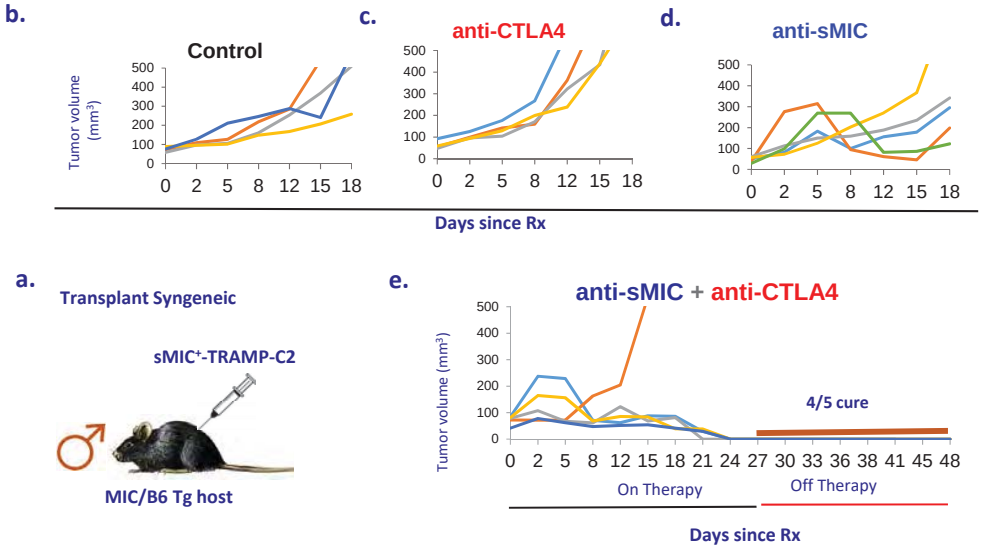
B10G5 targeting-sMIC therapy is effective in multiple animal models



(Basher et al., , Oncotarget Basher et al., submitted)

11

Anti-sMIC therapy synergizes with ICI



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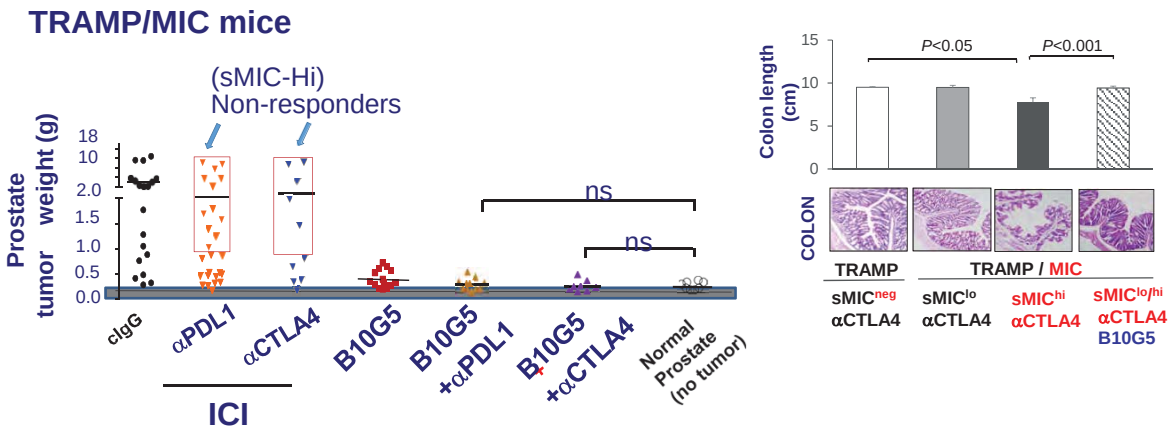
B10G5 enables response to ICI and eliminates colitis

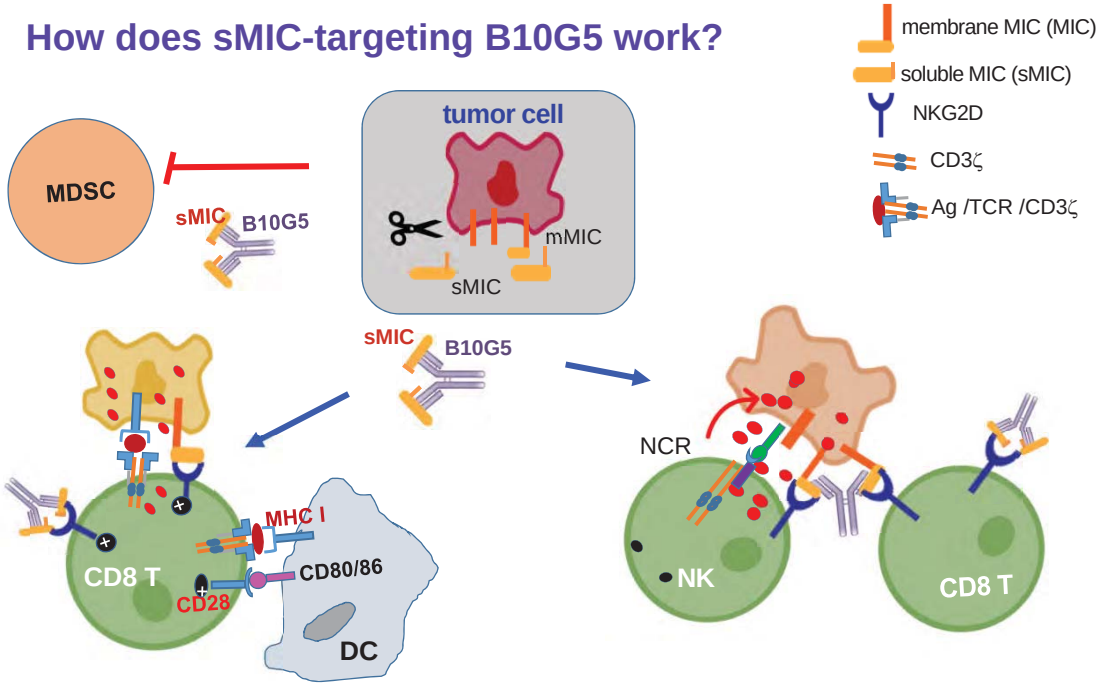


(hang et al., Science Advances JITC,)

13

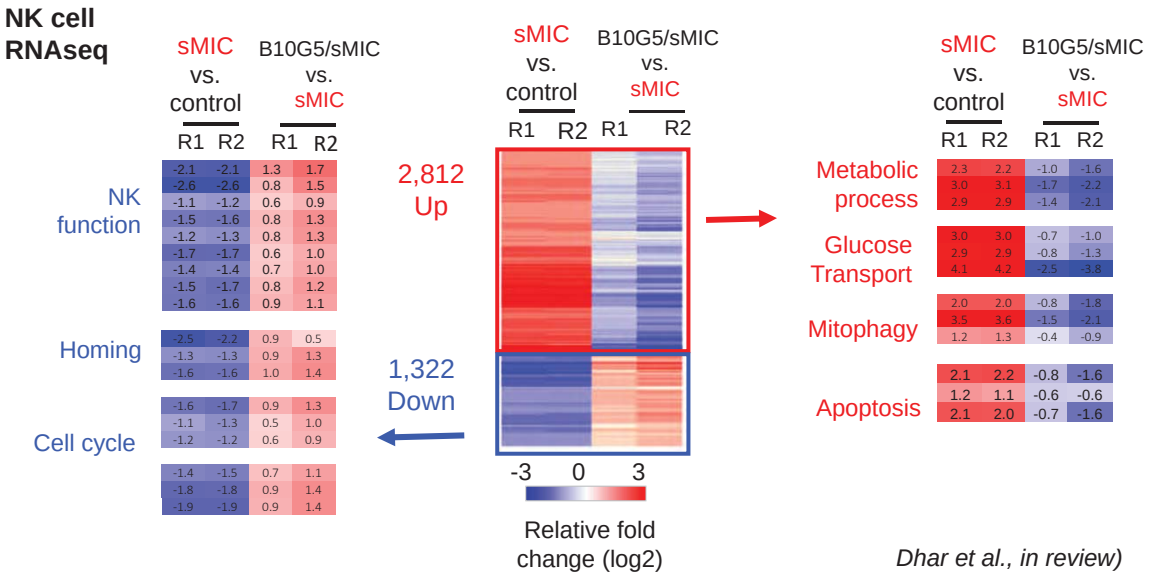
13

How does sMIC-targeting B10G5 work?



14

sMIC and anti-sMIC differentially regulate NK cell function and fate



Dhar et al., in review)

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Milestones for B10G5 to Enter Clinic (Collaboration with CanCure LLC and Partners)

MILESTONES			2018	2019-2022
Humanization (CuraB10), stable cell line, RCB, pre-CMC, Pilot tox non-GLP in NHP, PK study		Completed		
GLP/cGMP Manufacturing				
IND-Enabling Toxicology and IND application				
Phase I Clinical Trial				

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Addressing key questions for moving the therapy to clinic (within next 3 years)

- **Who** are the best patients for target ?
  - define selection criteria for serum sMIC level.
- **When** is the best window for therapy?
  - define disease stages?
- **How** to integrate with Standard Care of Pca?

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Acknowledgements

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Matthieu Besneau, PhD

Past members
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Fahmin Basher
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Gang Xiao
Gavin Liu
Xuangjun Wang

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Washington University
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Knights Cancer Center / OHSU
Julie Graff

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NIH National Cancer Institute

Congressionally Directed Medical Research Programs CDMRP Department of Defense

Prostate Cancer Foundation Curing Together.

Scanlan Family Endowment

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Outcome of the David A. Mezzone PCF Challenge Award

- Renewal of R01
- Two New R01s
- Two DOD Awards
- Small Business Awards (Phase I and II)
- Northwestern Prostate SPORE Renewal submission - Project 2
- Professional Growth: Promotion and Recruitment by NU
- Publications and Invited Presentations at National Meetings

THANK YOU!

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Prostate Cancer Mazzone Awards Program Retreat
September 2019

Precision Imaging in Prostate Cancer:
re **in** patient care

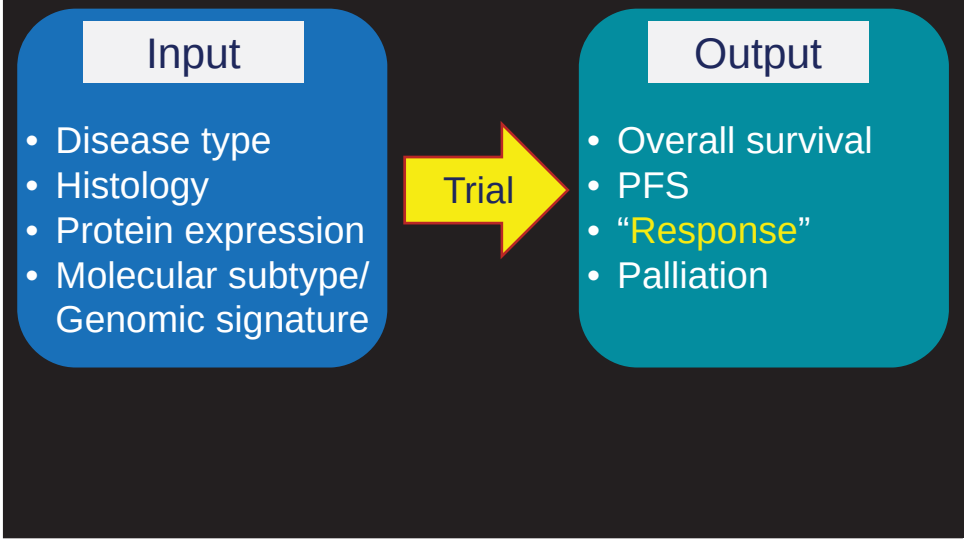
Glenn Liu, MD
Professor of Medicine and Medical Physics
Head, Genitourinary Oncology
Director, Genitourinary Oncology Research
Leader, Developmental Therapeutics Program
Chair, Prostate Cancer Subcommittee, ECOG-ACRIN

Disclosures

- Co-inventor patents US9161720 and US9603567: System and method for evaluation of disease burden
- Co-Founder and consultant, AIQ Solutions

- Funding for presented work:
  - PCF Creativity Award 2011
  - David Mazzone-PCF Challenge Award 2011
  - PCF Challenge Award 2014
  - Medivation/Pfizer

Drug development

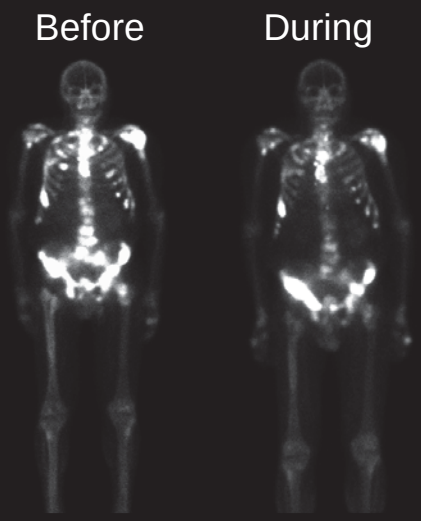


3

BONE IMAGING

4

Response or progression?



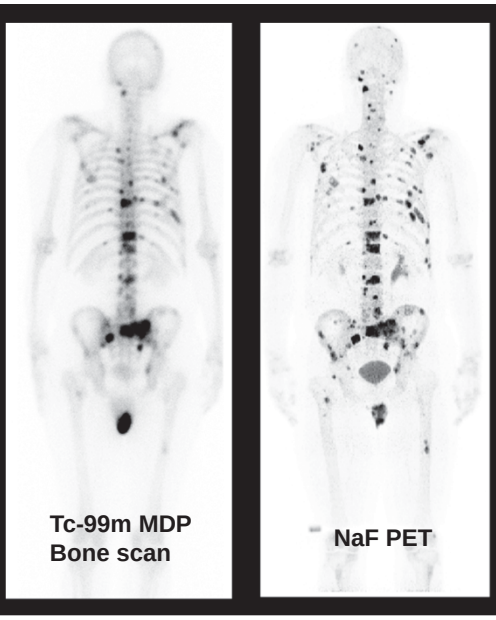
5

Technical advances

- **99mTc-MDP**
  - 2D, planar-based imaging
- **SPECT**
  - 3D, single photon emission CT
  - Can see smaller lesions (e.g. in spine) due to anatomic localization
- **NaF PET/CT**
  - Captures physiologic activity with attenuation correction and anatomic localization

6

Bone scintigraphy vs NaF PET

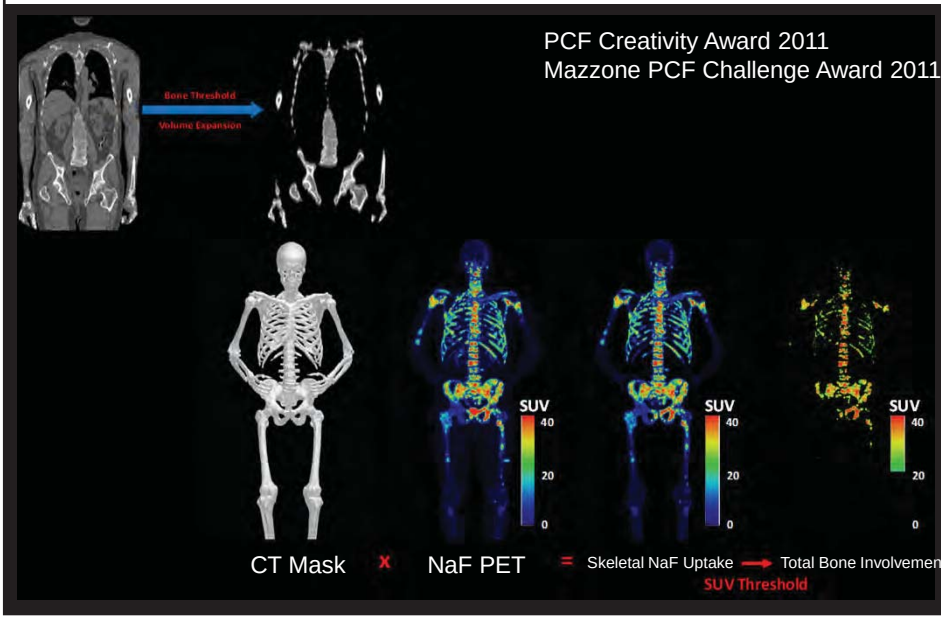


7

QUANTITATIVE TOTAL BONE IMAGING (QTBI)

8

Quantitative Total Bone Imaging (optimized for precision)



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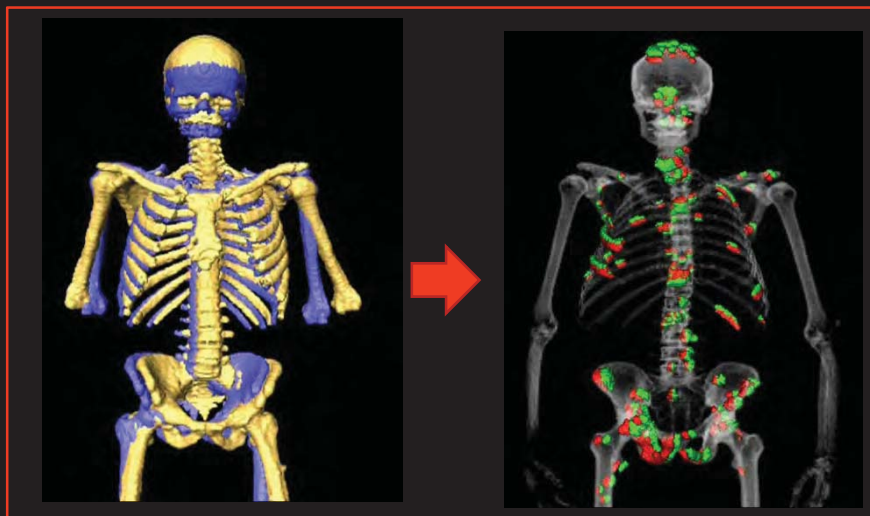
QTBI – Bone Registration

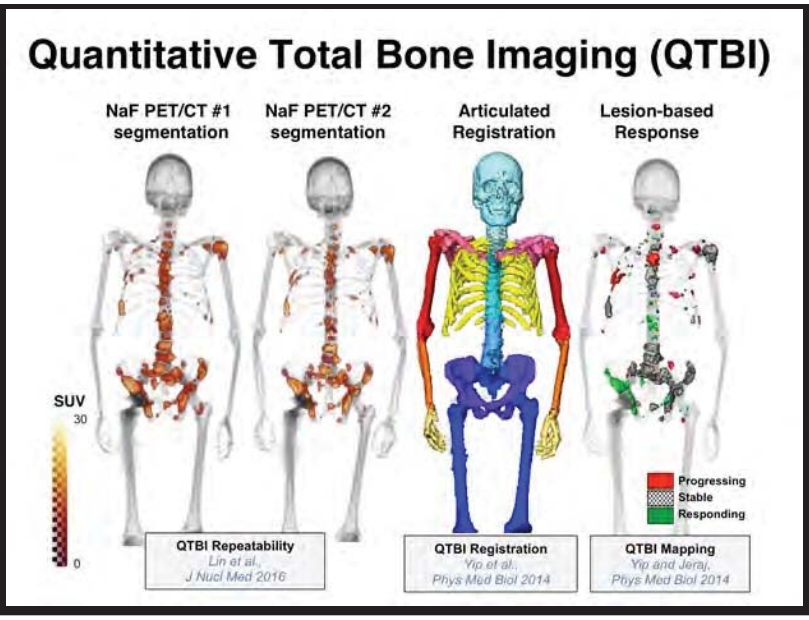


Articulated Registration

Bone Lesion Matching

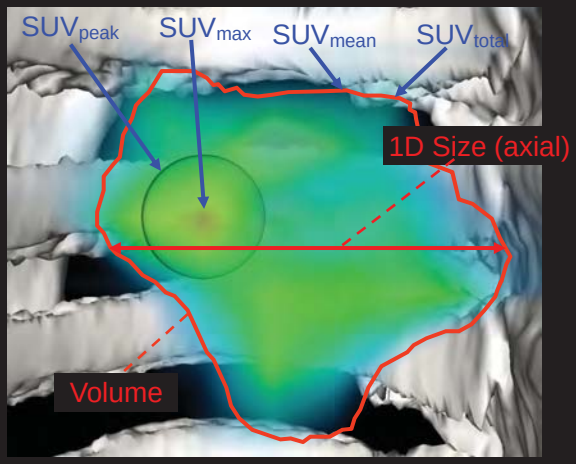
10

Treatment response



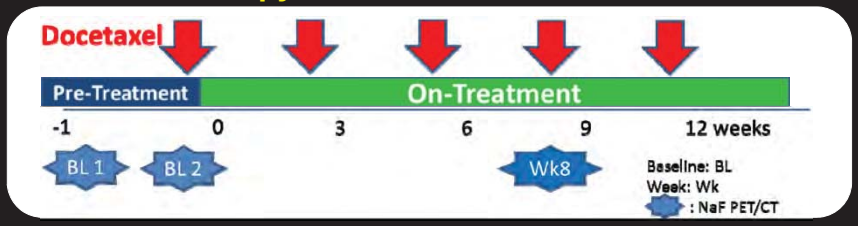
PET response assessment metrics

- Size measures
  - Volume
  - 1D size (axial)
- Standardized Uptake Value (SUV) measures:
  - SUV_{mean}
  - SUV_{total}
  - SUV_{max}
- Uptake non-uniformity measures:
  - SUV_{hetero}

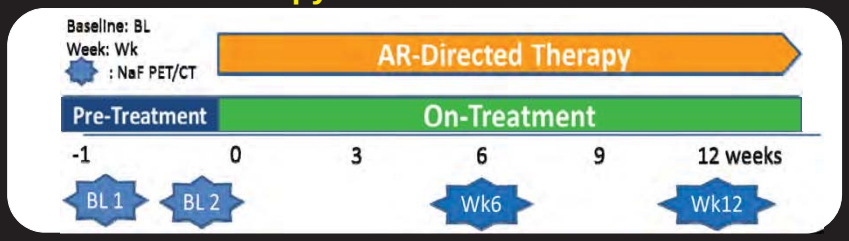


Schema

Docetaxel Therapy Cohort



AR-Directed Therapy Cohort



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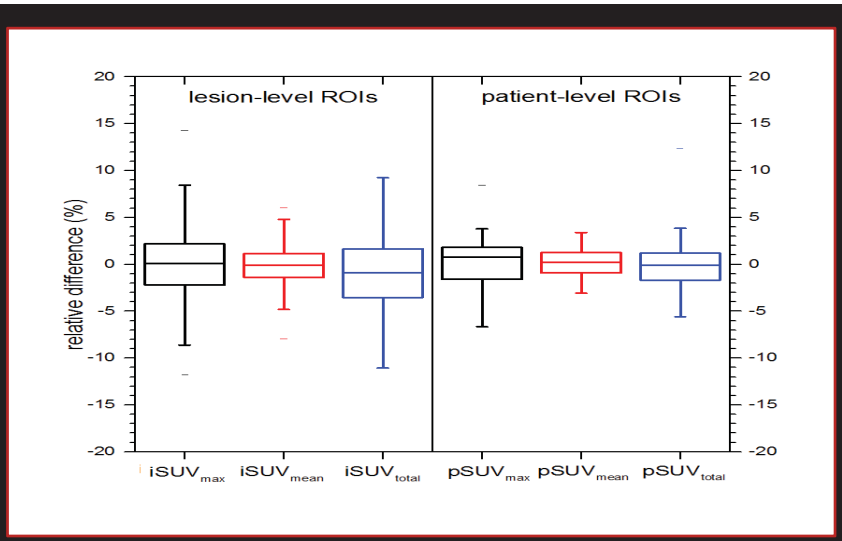
NaF PET/CT repeatibility



Lin C,…Liu G, and Jeraj R. J Nucl Med 116, 2016

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Case 1

- 61 yo male Dx 2003 with PSA 7.9 ng/mL; Gleason 4+3
- Prior therapies: RP, sacral XRT, ADT, ZD4054

- Enrolled on NaF Trial 8/16/2012 with right shoulder pain
  - PSA 26.6 ng/mL
  - Bone scan: right scapular uptake and sacrum
  - CT: increased sclerosis in right scapula, T12

- Therapy: Docetaxel with prednisone started 8/27/2012 (stopped after 5 cycles due to toxicity)
  - PSA 6.44 (10/2012)
  - PSA 1.36 (12/2012)

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Case 1 response

Responding lesion
Non-responding lesion

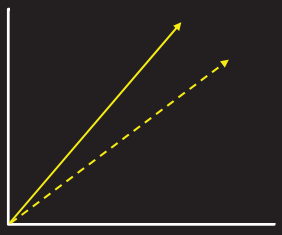
SUV_{max} SUV_{total}

16

Case 2

- 67 yo male Dx 1998 with PSA 9.2 ng/mL; Gleason 3+3
- Prior therapies: ADT, steroid sulfatase inhibitor, XRT left shoulder, docetaxel + AZD2171
- Enrolled on NaF Trial 8/2/2012 with rising PSA
  - PSA 137.3 ng/mL
  - Bone scan: increased axial and appendicular mets
- Therapy: Abiraterone with prednisone started 8/6/2012
  - PSA 38.6 (9/2012)
  - PSA 55 (10/2012)
  - PSA 132 (11/2012)
  - Treatment discontinued 12/26/2012 for increasing pain

Case 2 response



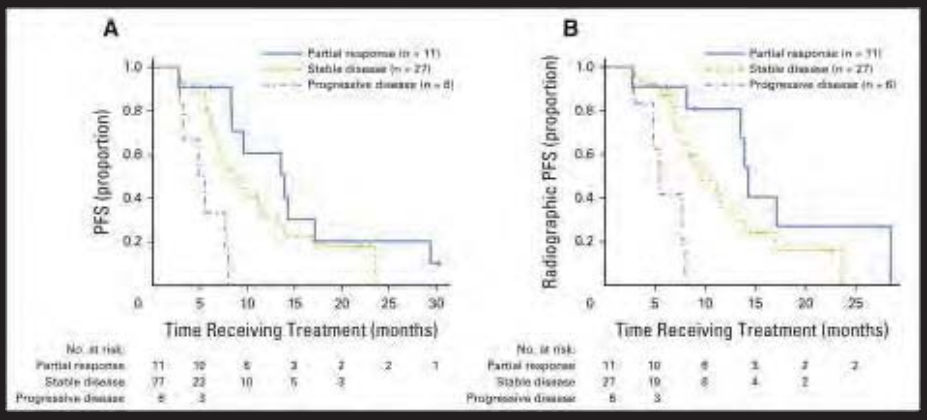
Responding lesion

Non-responding lesion

SUV_{max}

SUV_{total}

Change in skeletal burden on PFS



Harmon S,,…Liu G, Jeraj R. J Clin Oncol 35(24), 2017

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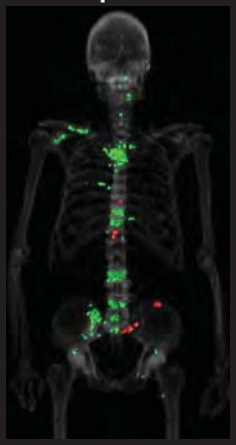
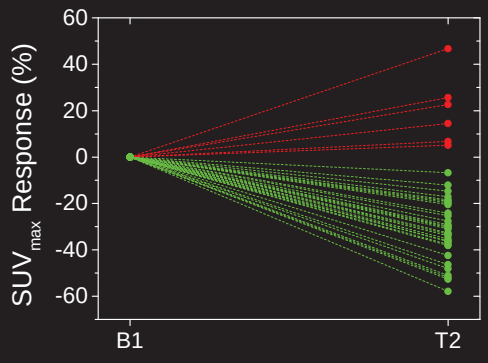
Inter-lesion response heterogeneity

Treatment Response



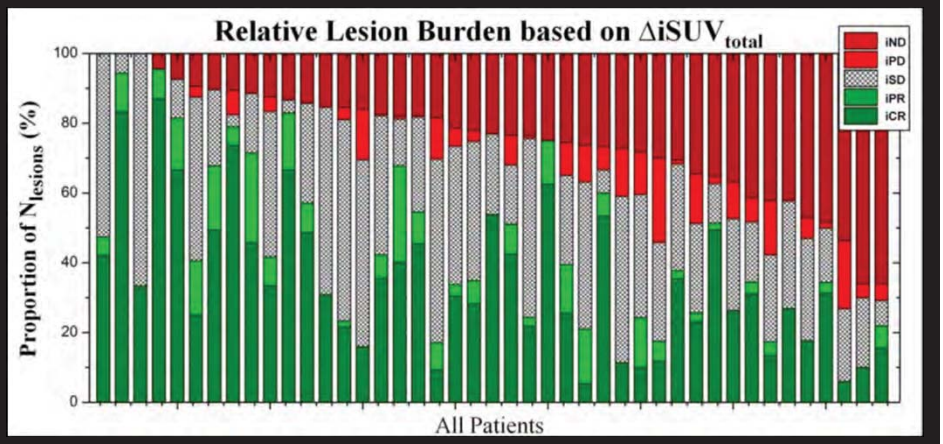


Responding lesion

Non-responding lesion

20

Response heterogeneity



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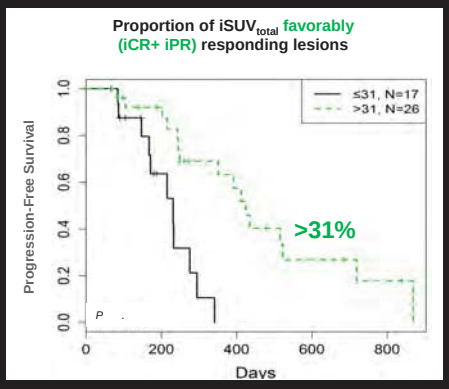
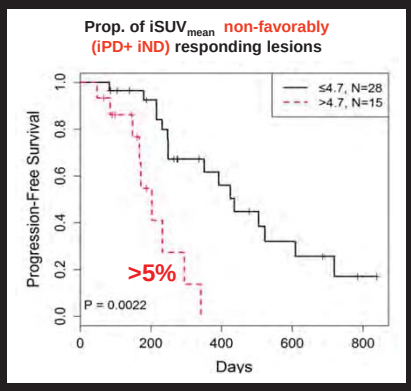
Interlesional response

Although increasing response improves benefit…

Overall outcome predominantly driven by resistance!





armon et al., AAPM ( )

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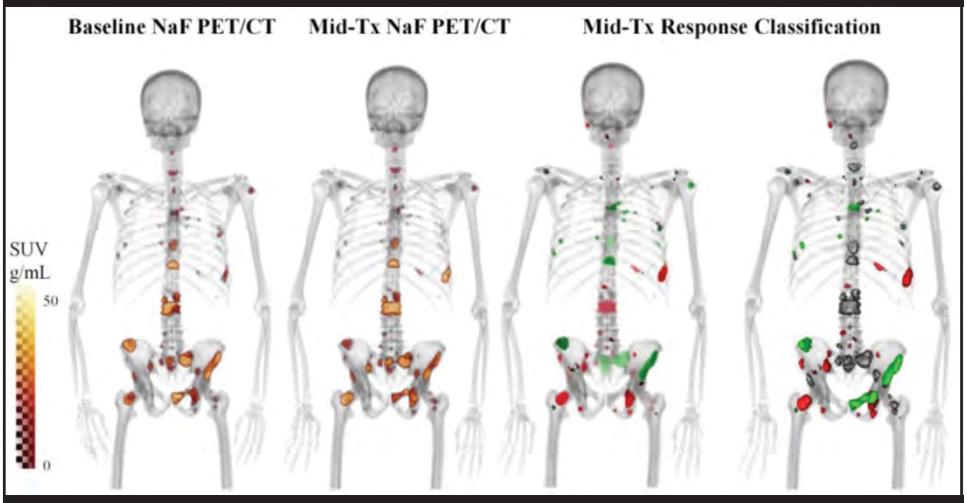
Response heterogeneity (spatial)



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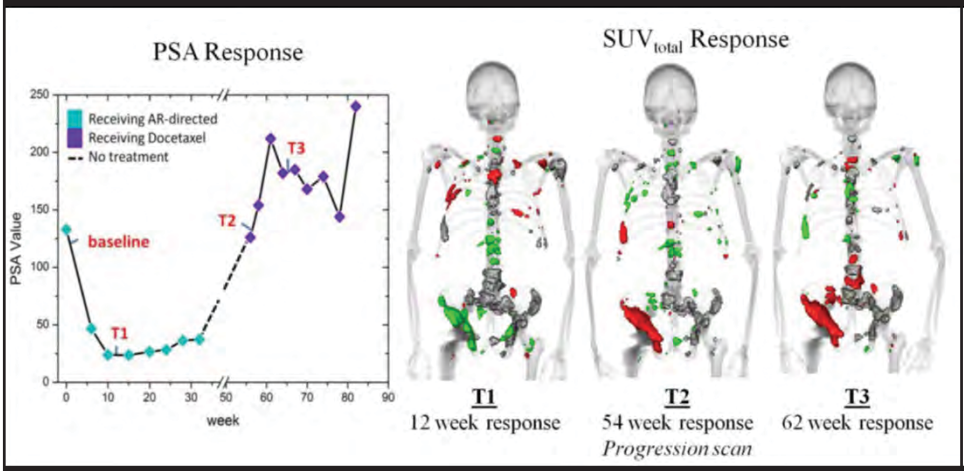
Temporal response dynamics



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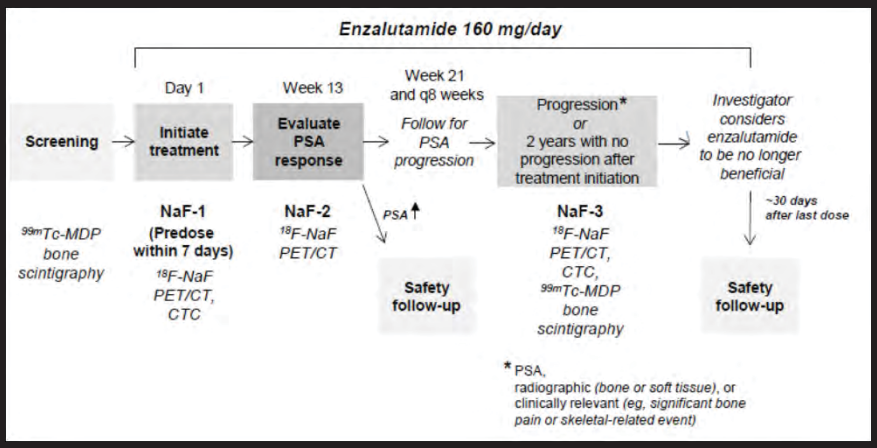
A Phase 2, Open-label, Single-arm Study Of ^{18}F-sodium Fluoride Pet/ct Bone Imaging In Enzalutamide-treated Chemotherapy-naïve Patients With Bone-metastatic Castration-resistant Prostate Cancer

- DOD PCCTC: UWisc (PI), Karmanos, Rutgers



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Results

- The study met its primary objective; 22 of 22 (100%) evaluable men had ≥ 1 responding bone lesion on QTBI at PSA progression
- The proportion of progressive lesions increased from a mean 7.8% (range, 0-29) at PET2 to 9.4% (range, 0-32) at PSA progression

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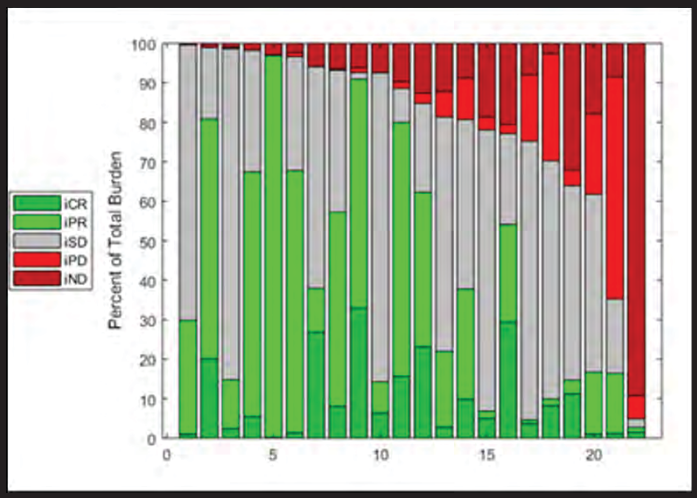
Change in disease burden at PSA Progression

Treatment response classification by SUVtotal normalized to baseline burden



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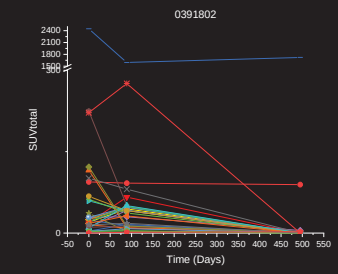
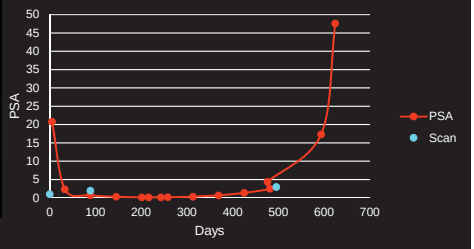
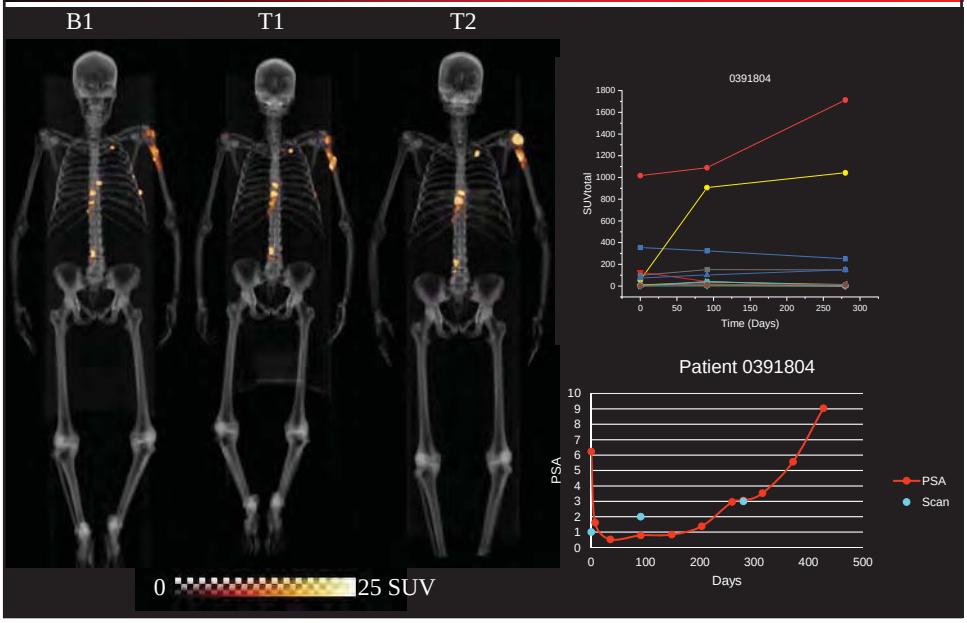
Most lesions improving



Patient 0391802



Most lesions non-responding



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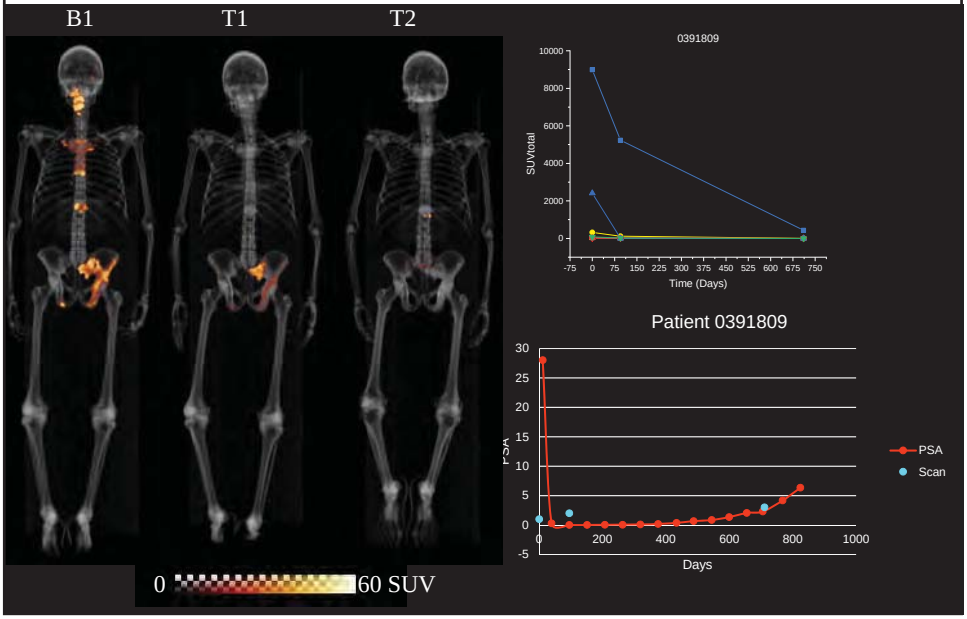
Treatment beyond progression



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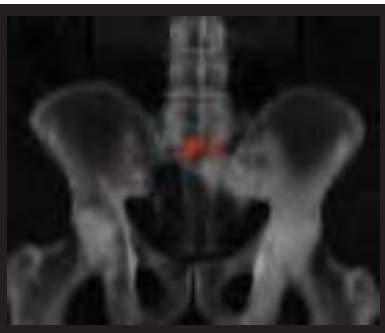
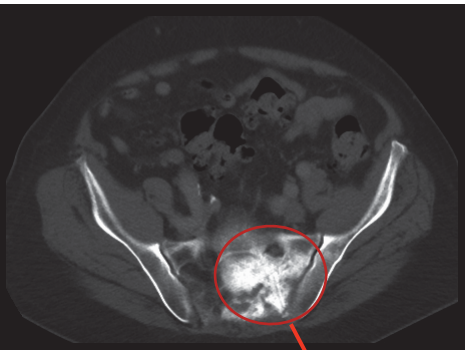
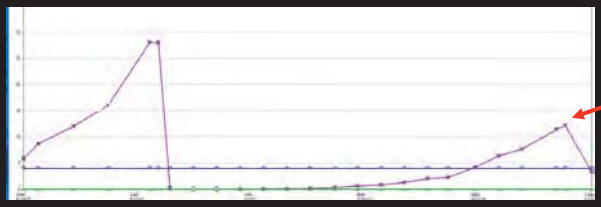
Oligo-progression







Radiotherapy

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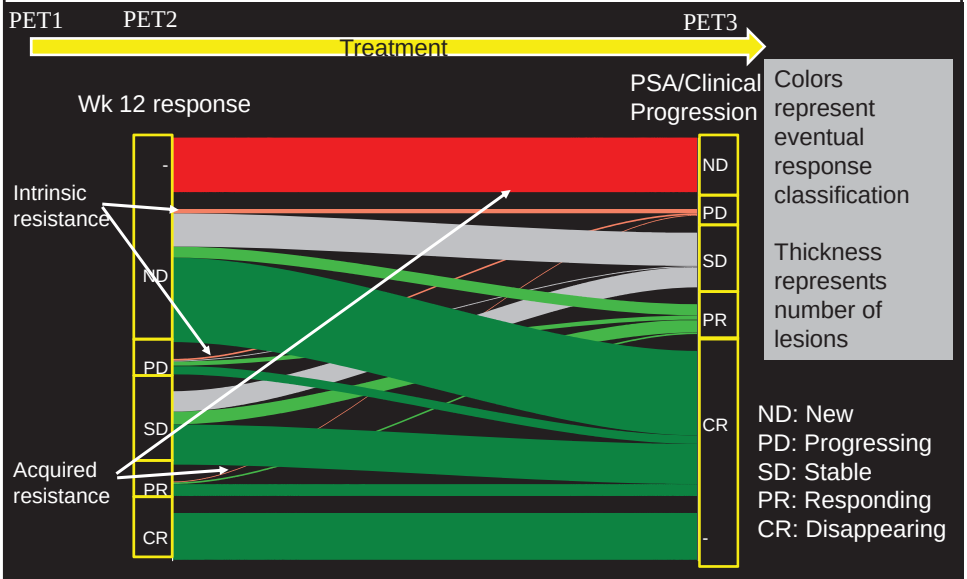
Dynamics of lesion response



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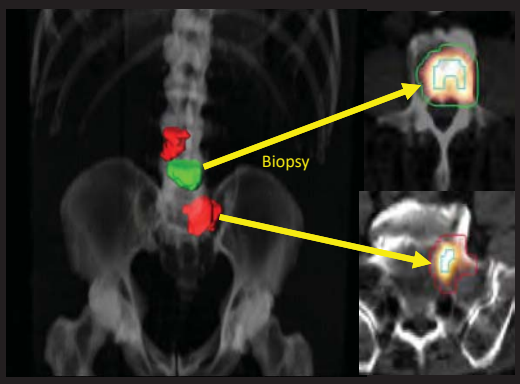
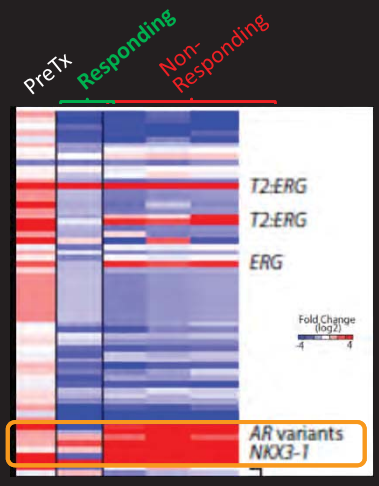
Understanding Resistance

Enzalutamide Treatment





ASCO 2019

PCF Challenge Award 2014

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Quantitative RNA – AR splice variants

Patient	Final Lesion Response Classification	AR-V7	AR-V1,V3,V4	AR-V3	AR-V9
3	PR	Negative	Negative	Positive	Negative
3	PD	Negative	Negative	Positive	Negative
4	SD	Negative	Negative	Negative	Negative
4	PD	Positive	Positive	Positive	Positive
5	PR	Negative	Negative	Positive	Positive
5	PD	Positive	Positive	Positive	Positive
6	PD	Positive	Positive	Positive	Positive

AR-V7 was detected in only PD lesions.

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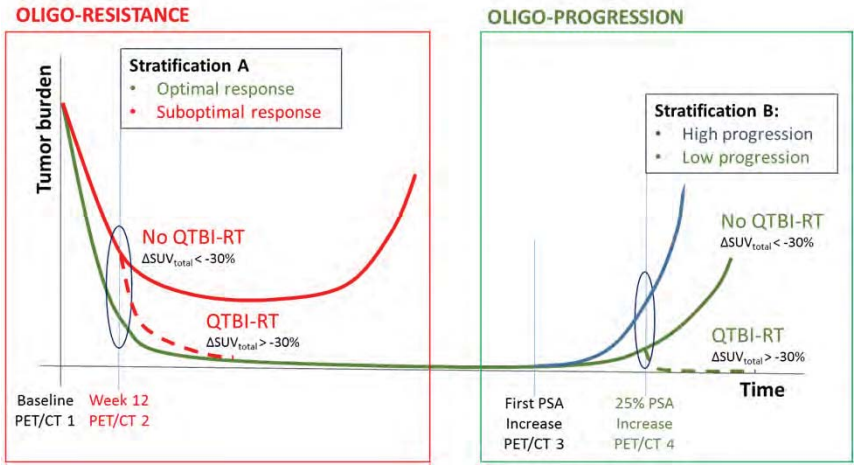
Applications



QTxI as a Platform

- QTxI NaF
  - FDA 510 (k) approval July 2018
  - Licensed to AIQ Solutions
- QTxI PSMA
  - Being developed for bone and non-bone
- QTImmuno-Imaging
  - For immunotherapeutic agents

Manuscripts

- Simoncic U, Perlman S, Liu G, Staab MJ, Straus JE, and Jeraj R. Comparison of NaF and FDG PET/CT for Assessment of Treatment Response in Castration-Resistant Prostate Cancer with Osseous Metastases. Clin Genitourin Cancer (Epub ahead of print), Jul 14, 2014.
- Muzahir S, Jeraj R, Liu G, Hall LT, Rio AM, Perk T, Jaskowiak C, and Perlman SB. Differentiation of metastatic vs degenerative joint disease using semi-quantitative analysis with (18)F-NaF PET/CT in castrate resistant prostate cancer patients. Am J Nucl Med Mol Imaging 5(2):162-8, 2015.
- Simoncic U, Perlman S, Liu G, and Jeraj R. Optimizing and 18F-NaF and 18F-FDG Cocktail for PET Assessment of Metastatic Castration-Resistant Prostate Cancer. Nucl Med Commun (EPub ahead of print), Sept 16, 2015.
- Lin C, Bradshaw TJ, Perk TG, Harmon S, Eickhoff J, Jallow N, Choyke P, Dahut W, Larson SM, Humm JL, Perlman S, Apolo AB, Morris MJ, Liu G, and Jeraj R. Repeatability of quantitative 18F-NaF PET: a multicenter study. J Nucl Med (Epub ahead of print), July 21, 2016.
- Harmon SA, Perk T, Lin C, Eickhoff J, Choyke PL, Dahut WL, Apolo AB, Humm JL, Larson SM, Morris MJ, Liu G, and Jeraj R. Quantitative Assessment of Early [18F]Sodium Fluoride Positron Emission Tomography/Computed Tomography Response to Treatment in Men with Metastatic Prostate Cancer to Bone. J Clin Oncol (Epub ahead of print), August 20, 2017.
- Weisman AJ, Harmon SA, Perk TG, Eickhoff J, Choyke PL, Kurdziel KA, Dahut WL, Humm JL, Apolo AB, Larson SM, Morris MJ, Perlman SB, Liu G, and Jeraj R. Quantification of bone flare on 18F-NaF PET/CT in metastatic castration-resistant prostate cancer. Prostate Cancer Prostatic Dis (Epub ahead of print), Nov 9, 2018.
- Perk T, Chen S, Harmon S, Lin C, Bradshaw T, Perlman S, Liu G, and Jeraj R. A statistically optimized regional thresholding method (SORT) for bone lesion detection in 18F-NaF PET/CT imaging. Phys Med Biol (Epub ahead of print), Nov 20, 2018.
- Perk T, Bradshaw T, Chen S, Im HJ, Cho S, Perlman S, Liu G, and Jeraj R. Automated classification of benign and malignant lesions in 18F-NaF PET/CT images using machine learning. (Epub ahead of print), Nov 20, 2018.
- Lin C, Harmon SA, Bradshaw TJ, Eickhoff J, Perlman S, Liu G, and Jeraj R. Response-to-repeatability of quantitative imaging features for longitudinal response assessment. Phys Med Biol. (Epub ahead of print), Dec 19, 2018.

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Collaborators

PCF Creativity Award Team 2011:

- Glenn Liu, MD
- Robert Jeraj, PhD
- Scott Perlman, MD

Mazzone-PCF Challenge Team 2011:

- Glenn Liu, MD
- Robert Jeraj, PhD
- Scott Perlman, MD
- Jens Eickhoff, PhD
- Stephanie Harmon, PhD (YI)
- Tim Perk, PhD (YI)
- Michael Morris, MD (MSKCC)
- Steve Larson, MD (MSKCC)
- William Dahut, MD (NCI)
- Peter Choyke, MD (NCI)

PCF Challenge Team 2014:

- Robert Jeraj, PhD
- Glenn Liu, MD
- Scott Perlman, MD
- Michael Tuitte, MD
- Jens Eickhoff, PhD
- Urban Simoncic, PhD (YI)
- Alison Roth, (YI)
- Scott Tomlins, MD (UMich)

pctc The Prostate Cancer Clinical Trials Consortium

Prostate Cancer Foundation Curing Together.

AIQ Solutions

A. David Mazzone Research Awards Program

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