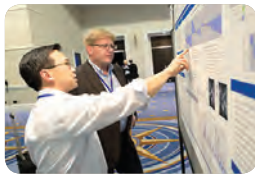


# STATE OF THE SCIENCE REPORT

Highlights from  
the 20<sup>th</sup> Annual PCF  
Scientific Retreat  
October 2013



Provided with the compliments of  
the Prostate Cancer Foundation



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## Introduction

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For the twentieth consecutive year, the Prostate Cancer Foundation (PCF) brought together the world's top prostate cancer physicians and scientists in a collaborative forum to share new data and concepts. The 20th Annual PCF Scientific Retreat was our best meeting ever, as measured by the novelty of findings, breadth of attendees, the quality and diversity of presentation topics, and attendee and Board member feedback.

The overarching goal of all research discussed at the Retreat was to accelerate the end of death and suffering for men with prostate cancer. With a prime focus on scientific presentations, knowledge exchange, and collaborative discussions, the 20th Annual Scientific Retreat featured the following:

- 66 presentations and panels in the Plenary Session
- 134 poster presentations
- 20 different scientific disciplines related to prostate cancer biology presented and discussed
- 32 speakers (48%) presented first-in-field, unpublished data at a PCF Scientific Retreat for the first time
- 41% of all attendees were first-time participants
- Attendance by 502 participants from 15 countries, including 201 PhDs, 170 MDs, 89 MD PhDs, 3 PharmDs, and 1 DVM PhD
- 119 academic institutions, 35 biopharmaceutical companies, and 10 medical research foundations were represented
- NIH, NCI, and Dept. of Defense research leaders present
- Attendance by 99 PCF Young Investigators
- Attendance by 18 PCF Board of Director members, major donors, and special guests

The PCF "Research Enterprise" is expanding globally with over 200 research projects in 18 countries. PCF currently funds a robust portfolio that totals \$101.9 million in innovative prostate cancer research projects. This includes \$50.5 million to PCF Challenge Award Teams, including \$11 million from Movember in support of 10 2012 and 2013 Movember-PCF Challenge Awards, \$20 million to fund two Dream Teams co-sponsored by Movember and Stand Up 2 Cancer, \$25.9 million to fund 124 PCF Young Investigator Awards, \$3.2 million to fund 9 centers in the PCF-DoD Therapy Consortium (<http://pcctc.org>), and \$2.3 million to fund Creativity Award projects. We are extremely grateful for \$7.7 million in ongoing research projects made possible by The Safeway Foundation.

We also thank the sponsors of the Retreat for their generous support: The Safeway Foundation, Sanofi-Aventis, Astellas/Medivation, Millenium Pharma, Genentech, BN Immunotherapeutics, Teva, Bayer, Johnson & Johnson, Exelixis, and Genomedx.

This PCF 2013 State of the Science Report summarizes each presentation individually. Highlights of each session provide a brief overview of the scientific discipline and a summary of the latest findings

Yours sincerely,

Jonathan W. Simons, MD  
President & CEO  
David H. Koch Chair

Howard R. Soule, PhD  
Chief Science Officer





## Session 1: Convergence of Engineering and Oncology

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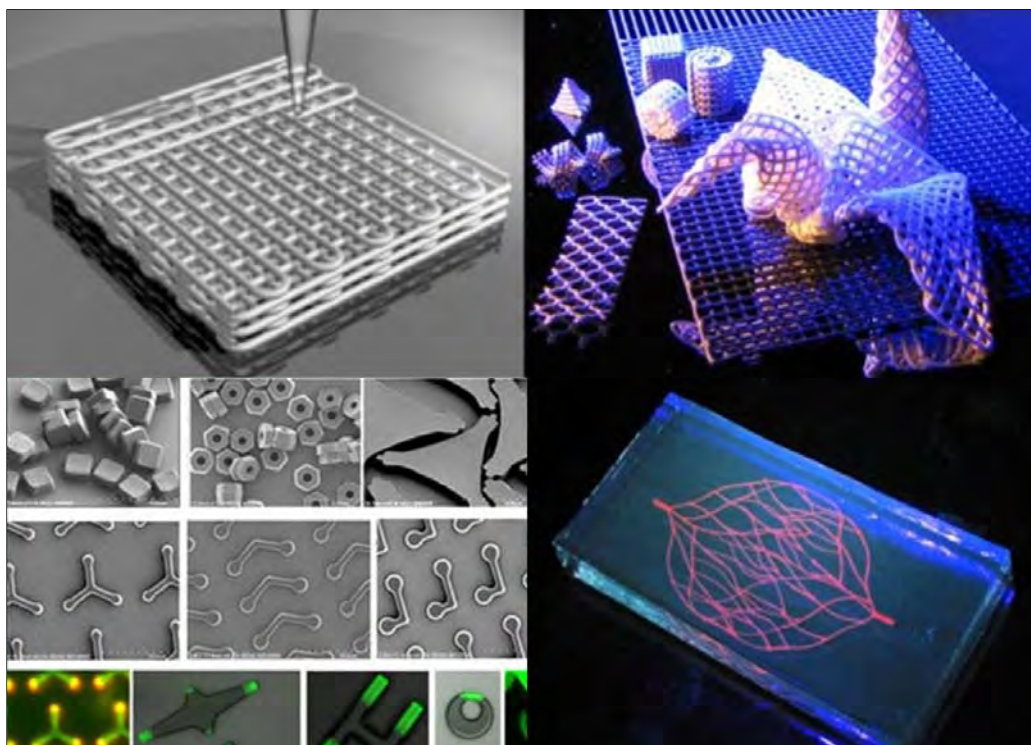
### Biomaterials Overview: Opening the Bioengineering Kimono

**Jeffrey Karp, PhD**  
**Brigham and Women's Hospital**

**Movember-PCF Challenge Award, 2012**

- Biomaterials are any substance that interacts with a biological system.
- Biomaterials have been used for thousands of years. Over 2000 years ago, the Romans, Chinese, and Aztecs were already using gold for dentistry. Wooden teeth and glass eyes are other historical examples of biomaterials.
- Modern medical applications of biomaterials include dental fillings, contacts, stitches, and joint replacements.
- The development of biomaterials accelerated in the 1990's with the formation of bioengineering departments, which brought together work of engineers, biologists, and clinicians.
- Dr. Jeffrey Karp discussed the development of various "designer" biomaterials that are revolutionizing science and medicine.
- Bioengineering methods now allow for the printing of various particles onto substrates at the micro and nano-scale.
- Research applications include the printing of substrates in arrays, patterns, or combinations for high-throughput screening. This has been used to identify the best surfaces or combination of matrix proteins for optimal cell culture conditions.
- Examples of medical applications include enzyme-responsive hydrogels in which an embedded pro-drug will only be released in its active form in the vicinity of tumor cells, following processing by tumor-enzymes.
- Micro-surgical tools can now be manufactured with no assembly required.
- Vaccine "scaffolds" have been produced, in which vaccine components (antigen and adjuvant) and immune cell recruitment proteins are embedded in a 3D porous polymer scaffold, creating a microenvironment to recruit and activate immune cells.
- Nanoparticles are particles with various applications that range in size from 1-100nm.

- New methods of synthesizing nanoparticles in a single step allows for scaling up of manufacturing processes.
- Nanoparticles can be used for drug delivery. Nanoparticles cloaked in membranes of red blood cells allow for “camouflage,” leading to an extended half-life in circulation.
- Novel cell-isolation methods are being developed, in which a “chip” is printed with molecules that are expressed on blood vessels to attract certain cell types. This allows for specific isolation of such cells from blood.
- Miniature urine-based diagnostic chips are also in development. Circulating levels of glucose, protein, leukocytes, nitrates, blood, bilirubin, urobilinogen, pH, and pregnancy markers can be assessed.
- There is currently an ongoing \$10 Million Qualcomm Tricoder competition between 41 teams to generate a “tricoder” that can detect 15 different medical conditions.
- “Physician screening gloves” are another idea in development, meant to enhance the sensitivity of a physician’s fingertips when doing a palpation-based examination, for instance in the detection of prostate cancer.
- Dr. Karp concluded his talk by encouraging collaborative efforts between basic scientists, clinicians, and bioengineers to facilitate and speed the development of the most useful and innovative applications of these new technologies.



**Figure 1:** Examples of nanoscale printing of biomaterials and manufactured micro-applications. Figure includes work by Drs. Jennifer Lewis, Chad Mirkin, and colleagues.

## Micro Scale Engineering for Modeling the Microenvironment and Rare Cell Analysis

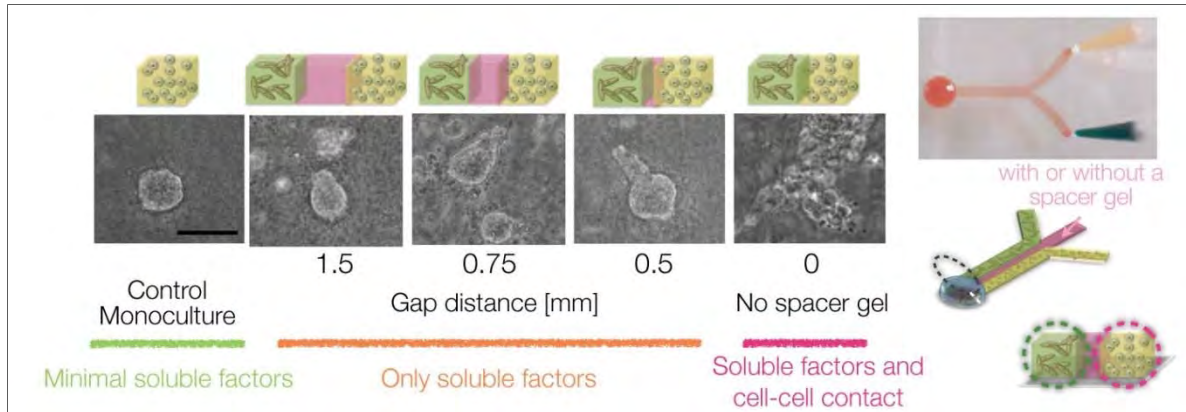
**David Beebe, PhD**  
**University of Wisconsin**

**Movember-PCF Challenge Award for circulating tumor cell (CTC) system, 2013-2015**

- The forces that dominate at the microscale can be different than at the macroscale. For instance, there is more pressure in a small droplet of water than a larger one, because at the microscale, surface tension dominates over gravity.
- Thus, microscale physics can be leveraged for these unique properties.
- Dr. David Beebe discussed creating a microscale platform for studying cancer cell properties with 2D or 3D compartmentalization, increased sensitivity, and needing only small numbers of cells (100s-1000s) per measurement.
- This is highly useful for patient-specific studies in which only a limited number of cells are available, such as using cells isolated from patient tumors to study interactions between tumor and stromal cells or evaluating tumor cell drug sensitivities.
- For example, in a study using a unique 3D micro-chamber, cells were cultured with or without a gel spacer of varying widths to separate different populations at defined distances. This allows the requirements for cell-cell contact vs. soluble factors, to be determined for various cell functions. Using this system, invasive properties of breast cancer cells were found to require direct interactions with stromal cells.
- Growth of cells in specially designed micro-chambers allowed the re-creation of the in vivo structure and function of tissues and tumor microenvironments. For instance, lumen structures can be grown and lined with endothelial cells, and bone metastatic sites can be modeled by co-culturing tumor cells with osteoblasts.
- Additionally, secretion of tumor-associated growth factors was significantly higher when cells were grown in microvessels compared with standard 2D, and 3D gel-embedded or flat gel cultures.
- Circulating tumor cells (CTCs) are tumor cells that have been shed from primary or metastatic tumors and circulate in the bloodstream. Analysis of CTCs can be used in diagnostic, prognostic, and experimental settings. A special micro-chip (VERSA) has been created for isolation of CTCs in order to enumerate them and measure protein and genomic endpoints.



- Dr. Beebe and colleagues have created many microscale 2D and 3D technologies for cell culture studies. A [foundry service](#) has been created at the University of Wisconsin to share these unique and specialized devices with investigators at production cost.



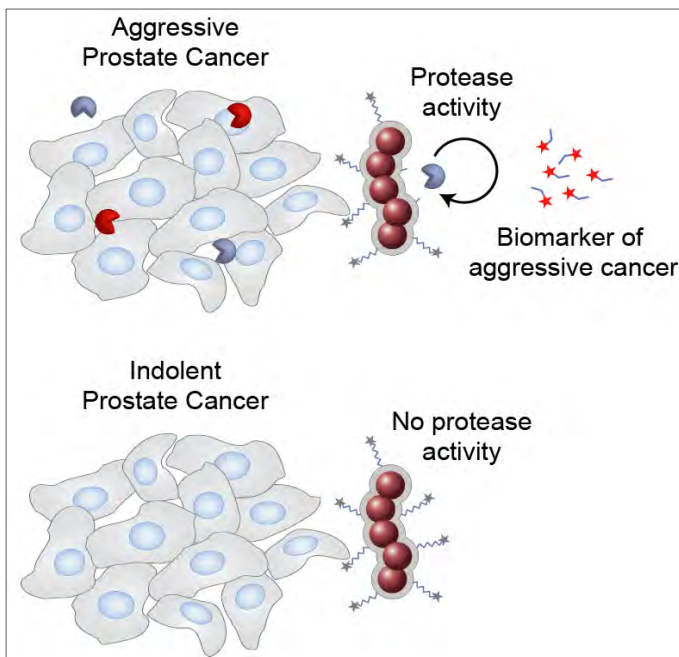
**Figure 2:** Left: In a unique 3D micro-chamber, cells were cultured with or without a gel spacer of varying gap widths to separate two different populations (tumor and stromal cells). This allows the requirements for cell-cell contact vs. soluble factors to be determined for various cell functions. Right: Schematic showing how the micro-chamber is structured to allow loading with two different cell populations (via green tip vs. beige tip).

## Engineering Cooperative Nanosystems for Cancer Diagnostics and Therapy

**Sangeeta Bhatia, MD, PhD**  
**Massachusetts Institute of Technology**

- Nanoparticles are a new class of biological tools that range in size from 1-100nm, and are being used for a multitude of research and medical applications.
- Dr. Sangeeta Bhatia discussed her work on engineering nanoparticles that can be used to detect and treat tumors. Such nanoparticles are designed to detect tumor cells, and within their structure carry a “payload,” such as a cytotoxic drug, that is then released.
- In many previous studies, 98-99% of nanoparticle payload never reached the tumor. Dr. Bhatia plans to solve this problem by designing nanoparticles that function collectively to accumulate in the tumor.
- In a “call and response” scheme, one nanoparticle contains specific targeting motifs to locate the tumor, and then sends a signal. A second nanoparticle is able to detect the signal, traffic to the site of the first nanoparticle, and release the payload.

- In an example, tumor-targeting gold nanorods were used as the first particle. At the nanoscale, the application of near-infrared light induces gold nanorods to undergo surface plasmon resonance and radiate heat. Thus, gold nanorods can be heated to 41°C, which activates the blood clotting cascade. Subsequently, a second nanoparticle that has been engineered to sense clotting factors, will be recruited to the clot (at the tumor site), and release the chemotherapeutic drug, doxorubicin.
- These nanoparticles were demonstrated to deliver higher levels of doxorubicin to the tumor site in mice, and were more effective at inhibiting tumor growth than non-communicating nanoparticles.
- Dr. Bhatia also demonstrated a diagnostic application for nanoparticles that can detect disease-associated enzyme activity. Nanoparticles are coated with peptides that can be specifically cleaved by disease-associated proteases. For blood clots, this is thrombin. Thus, under thrombotic conditions, the peptides are cleaved from the particle and emitted in the urine, where they can be detected by urinary analysis. For cancer, this can be matrix metalloproteases (MMPs) that are needed for cancer to invade and spread.
- Additional diagnostic specificity can be obtained by using multiple nanoparticles, each with unique peptides that are sensitive to cleavage by different disease-associated proteases, resulting in a specific urinary peptide signature.
- Dr. Bhatia has used these 'synthetic biomarkers' to detect primary and metastatic tumors, liver fibrosis, and pulmonary embolism in mice.
- Dr. Bhatia plans to apply this novel non-invasive, highly sensitive strategy toward understanding which proteases are involved in the progression of prostate cancer, and to develop more specific diagnostic tests for prostate cancer.

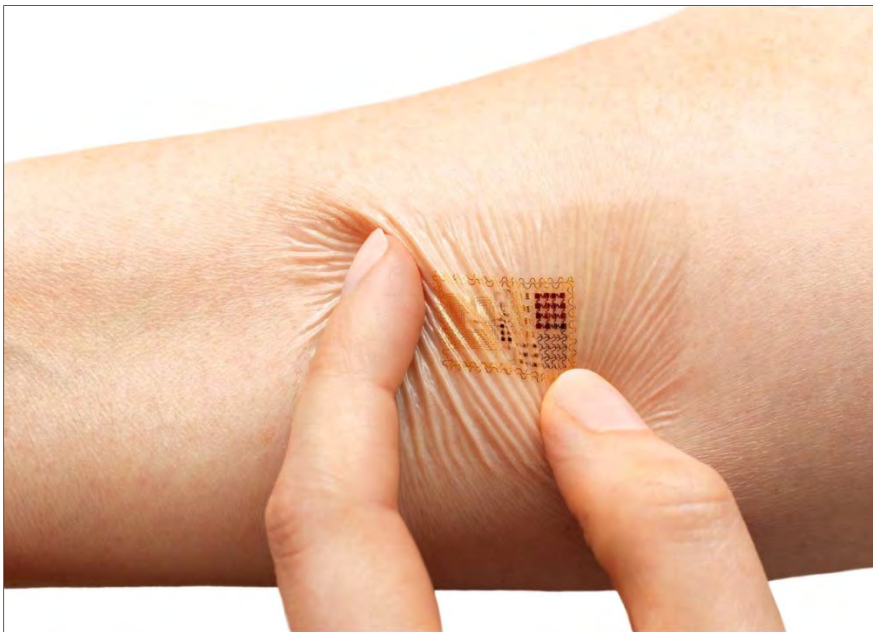


**Figure 3:** Nanoparticles can be coated with peptides designed to be specifically cleaved by proteases expressed by aggressive but not indolent tumors. Cleaved peptides would then serve as diagnostic biomarkers of aggressive cancer that can be detected by urinary analysis.

## Stretchy Electronics and Sensors That Can Dissolve in Your Body

**John Rogers, PhD**  
**University of Illinois**

- Dr. John Rogers is a chemist and materials science engineer, interested in designing electronics that have the same flexibility as skin, which is softer than standard silicon electronics by six orders of magnitude.
- To create electronic surfaces with this level of flexibility, Dr. Rogers has generated sheets of silicon so thin, that they become bendable. Applying these silicon sheets in a buckled configuration onto a rubber substrate adds the properties of flexibility and stretchability.
- Such highly flexible electronics are being developed for a variety of biological applications, including as sensors of skin conditions, and application onto organs such as the brain or the heart to function as treatments for epilepsy or for mapping.
- Dr. Rogers is further developing these flexible electronic sensors to be dissolvable by body substances, for use in temporary internal functions. These sensors will disappear over time and not need to be surgically removed. Applications include non-antibiotic thermal therapy devices for treatment of surgical site infections.
- These new bio-integrated electronics are likely to enable a multitude of clinical applications for prostate cancer patients including diagnosis, treatment, and monitoring.



**Figure 4:** Electronic sensors have been developed that have the same flexibility as skin.

## Session 1 – What This Means for Patients:

- Nanomedicine is an emerging technology for the diagnosis and treatment of diseases.
- This session presented several new technologies that will benefit patients in the near term.
- A new and efficient method and device for isolation of circulating tumor cells will help diagnose cancer and provides a new technology for precision medicine, better matching patients to effective therapy.
- New biomaterials were described that can actually be applied to a patient's skin to diagnose disease and deliver drugs.
- Finally, gold nanorods can be used to seek and deliver chemotherapy to tumors.

## Special Lecture: Experimental Scientific Integrity in the New Millennium

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### Promoting a Culture of Reproducible Research

#### Elizabeth Iorns, PhD Science Exchange

- Dr. Elizabeth Iorns of the Science Exchange discussed a huge problem in science: the irreproducibility of published studies.
- One of the main principles of the scientific method is reproducibility: the ability of an experiment or study to be reproduced by the original researcher or by someone else using the same methods.
- In the process of peer-reviewed publication of scientific studies, other experts on the topic review the study for the integrity of the evaluation, interpretation, and impact of the data presented. However, it is difficult if not impossible, for reviewers to assess the integrity of the methods that produced the data.
- Recent reports have demonstrated that a significant number of published scientific studies are irreproducible.
  - In a survey of scientists at MD Anderson Cancer Center, 50% of respondents had at some point, been unable to reproduce the results of published studies.
  - The ALS Therapy Development Institute tested >70 drugs from 221 independent studies and could not reproduce results from a single study.

- In a National Institute of Neurological Disorders and Stroke sponsored study, only 2 of 12 spinal cord injury studies could be replicated.
- Bayer reported on in-house target validation studies in which 14 of 67 could be reproduced.
- In an Amgen study, only 6 of 53 “landmark” oncology publications were reproducible.
- The expectation of scientists is that the scientific literature will eventually “correct itself,” via publication of studies refuting previous results or by retraction of papers when investigators discover results are flawed.
- However, despite the estimated 70% irreproducibility rate, only 0.2% of literature is retracted, less than 30% of scientists publish their failure to reproduce their own results, and only 14% of reports include negative results.
- Furthermore, evaluations of irreproducible reports found that citation rates (the amount of times a scientific report is referenced by another report) were no different than reproducible reports.
- Dr. Iorns’ proposed solution to this problem is to change the scientific culture to one that incentivizes the publication of reproducible results.
- This would likely require the creation of independent agencies specifically for this purpose. The expense of science makes it financially difficult for academic scientists to repeat experiments, while politics make it uncomfortable for scientists to publish refuting data.
- The Science Exchange is leading a collaborative “reproducibility initiative” to provide fee-for-service replication studies performed outside of the academic incentive system. Over 1000 expert facilities and over 1000 experimental services are available by this provider network.
- Studies that have been validated by this initiative will then be highlighted by journals, which would likely have a strongly positive influence on the study’s impact.
- The Science Exchange is currently funded to replicate 50 cancer biology studies published in high impact journals.
- Hopefully these and similar efforts will change the scientific culture to one that incentivizes the publication of reproducible studies and discourages publication of studies lacking integrity.





## Best Practices in Cell Line Authentication

Howard Soule, PhD

Prostate Cancer Foundation

- Dr. Howard Soule, Chief Science Officer of the Prostate Cancer Foundation, discussed the problems, solutions, and the new stance PCF is taking on the authentication of cell lines in prostate cancer research.
- Cell lines used in science research are cells that originated from human or animal tissues and have adapted to living in *in vitro* tissue culture systems. Tumor cell lines can also be propagated and maintained *in vivo* using certain types of mice.
- A huge problem in cell line studies is that lines can easily be either mixed up or “contaminated” with cells from other lines. If not periodically authenticated, these mistakes could lead to the production of significant false or misleading information as the wrong cell type is being studied. In fact, reports have estimated that 15-20% of cell lines are not from the supposed source.
- An even more frequent problem with cell line studies is contamination with *mycoplasma* bacterium. *Mycoplasma* does not cause the easily observable changes in cell cultures that other contaminating microorganisms do, and thus are easily overlooked.
- However, *mycoplasma* contamination of cell cultures can cause:
  - alterations in cell growth rate
  - inhibited or stimulated cellular transformations
  - potentially harmful morphological changes
  - altered DNA, RNA, and protein synthesis
  - altered enzyme actions
  - chromosomal abnormalities
  - reduced or increased virus yields
  - depleted nutrients from growth media
  - altered cell surface antigenic characteristics
  - decreased malignancy of tumor cells
- The scientific community needs to raise awareness of these problems and their effects on the integrity of scientific research.
- Cell line authentication and tests for *mycoplasma* contamination need to be encouraged as Best Practices and expected by scientific journals and funding agencies.

- To establish these as Best Practices by our researchers and to ensure high integrity research and publications in our field, beginning in 2014, PCF will expect all PCF-funded researchers to include the following in annual progress reports:
  - Baseline and one year cell line authentication, using the ATCC genotype as the reference.
  - *Mycoplasma* testing results.
- The DNA Diagnostic Center has offered 1000 free cell line authentications and *mycoplasma* testing panels to PCF-funded researchers, at up to 5 per investigator. [www.ddcmedical.com/pcf](http://www.ddcmedical.com/pcf)
- PCF thanks the DNA Diagnostic Center for this generous support and our investigators for their cooperation.

### What This Means for Patients:

- A responsibility of the Prostate Cancer Foundation is to ensure donors of the veracity and reproducibility of funded research.
- This session addressed laboratory issues related to cell line contamination and the reproducibility of cancer research.
- Donors and patients deserve studies to be performed to the highest degree of scientific integrity that may lead to new cancer treatments.
- From 2014, PCF will mandate Best Practices for all foundation-funded research.

## Special Lecture: The Project Data Sphere Initiative— Advancing Data Sharing in Cancer Research

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**Charles Hugh-Jones, MD, MCRP**  
**Member Life Science Consortium, CEO Roundtable on Cancer**

- Dr. Charles Hugh-Jones discussed the Project Data Sphere Initiative, a broad-access, not-for-profit, voluntary initiative by the CEO Roundtable on Cancer, to share, integrate, and analyze historical phase III cancer trial data for the purposes of expediting research.
- The easy to use platform designed by the business analytics specialist SAS, provides data, protocols, blank case report forms which describe all types of data collected by the trial, and descriptors. It is based on many examples of data and

project sharing in other fields that have led to significantly faster problem solutions.

- Goals of the project include:
  - Identification of patient sub-groups
  - Establishing real-world data standards
  - Improving design, operation, and FDA submission processes of clinical trials
  - Reducing study duplications and being able to run smaller trials
  - Gaining new insights, methodologies, and hypotheses
- The initial datasets being provided are comparator arm only.
- Patient privacy is critical, and thus all data will need to be responsibly de-identified.
- Future questions to be addressed for the initiative include:
  - How much data is sufficient?
  - How should the data be provided?
  - How to optimize the data?
  - How to build an ecosystem through social tools and competition-based challenges?
- This is an ongoing project with a launch in early 2014.
- Visit [www.projectdatasphere.org](http://www.projectdatasphere.org) for more information.



## What This Means for Patients:

- Transparency for clinical trial results for oncology medicines, especially those that fail in advanced clinical development, is frequently withheld from the public.
- The datasphere project, which is a program of the CEO Roundtable on Cancer, intends to reverse this unacceptable trend.
- Clinical trial data from pharmaceutical company and academic trials will be de-identified and deposited in the datasphere.
- Advanced analytical tools will enable any qualified investigator to test new ideas across clinical trials.
- The overall goal is to better understand why clinical trials are successful or have failed, which will enable more efficient future clinical trial design.

## **Session 2: Stem Cells & "Stemness" in Prostate Cancer**

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### Signaling Pathways for Prostate Stem Cells

**Owen Witte, MD**  
**University of California, Los Angeles**

**PCF Challenge Award, 2008**

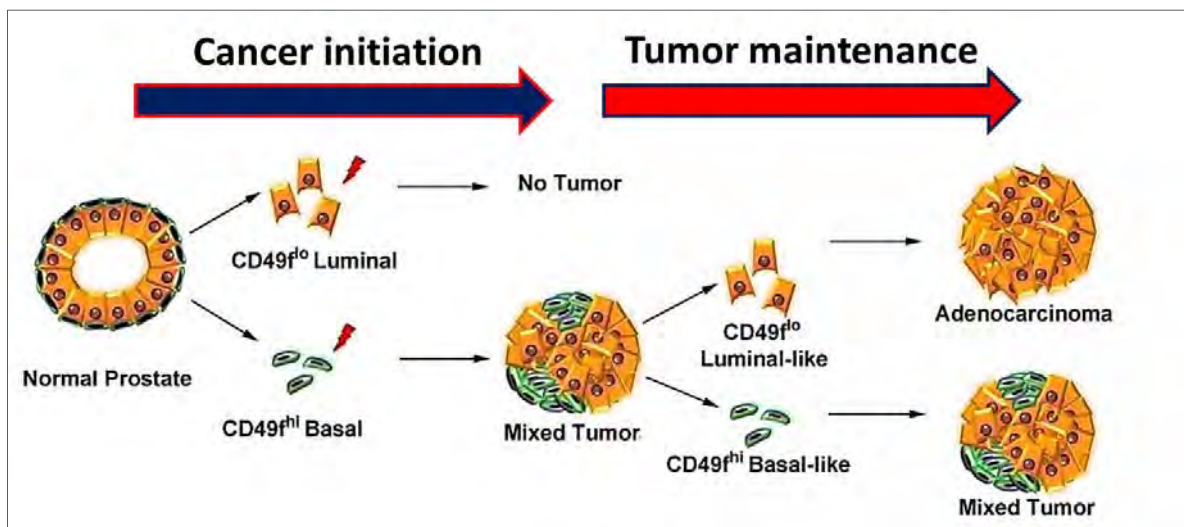
**The Ben Franklin-PCF Creativity Award, 2011**

**Movember-PCF Challenge Award, 2012**

**PCF "Targeting Adaptive Pathways in Metastatic Treatment-Resistant Prostate" Dream Team, 2012**

- The ductal glands of the prostate are composed of two epithelial cell layers: secretory luminal cells and underlying basal cells. Prostate tumors exhibit luminal morphology and express luminal cell markers. However, whether cancer cells originate from the basal or luminal cells of the prostate is a critical question for understanding disease initiation and progression.
- Dr. Owen Witte discussed his studies on determining the prostate cancer cell of origin.
- Luminal and basal cells can be differentiated by expression levels of the CD49f gene, and isolated from human prostates.

- To create possible prostate cancer initiating cells, human prostate luminal (CD49<sup>fl0</sup>) and basal (CD49<sup>flhi</sup>) cell types were made to express prostate cancer oncogenes: androgen receptor (AR) and activated versions of AKT and ERG.
- Luminal and basal cells were implanted into the kidney capsule of immune-deficient mice, an environment highly supportive of transplanted cells. Under these conditions, only basal cells developed into prostate adenocarcinomas and squamous tumors, while luminal cells could not develop into tumors.
- Thus, following oncogenic transformation, basal epithelial cells develop either the luminal characteristics of adenocarcinomas, or become a mixed tumor type with both basal and luminal cells (squamous).
- From these tumors, basal and luminal cancer cells can be isolated and made to form new tumors when implanted into different mice. However, isolated luminal cancer cells can only form adenocarcinomas, while the basal cells will again form both adenocarcinomas and squamous tumors.
- Thus, basal cells are the prostate tumor cell of origin, but once transformed, these distinct cell types can support maintenance of discrete tumor cell populations.

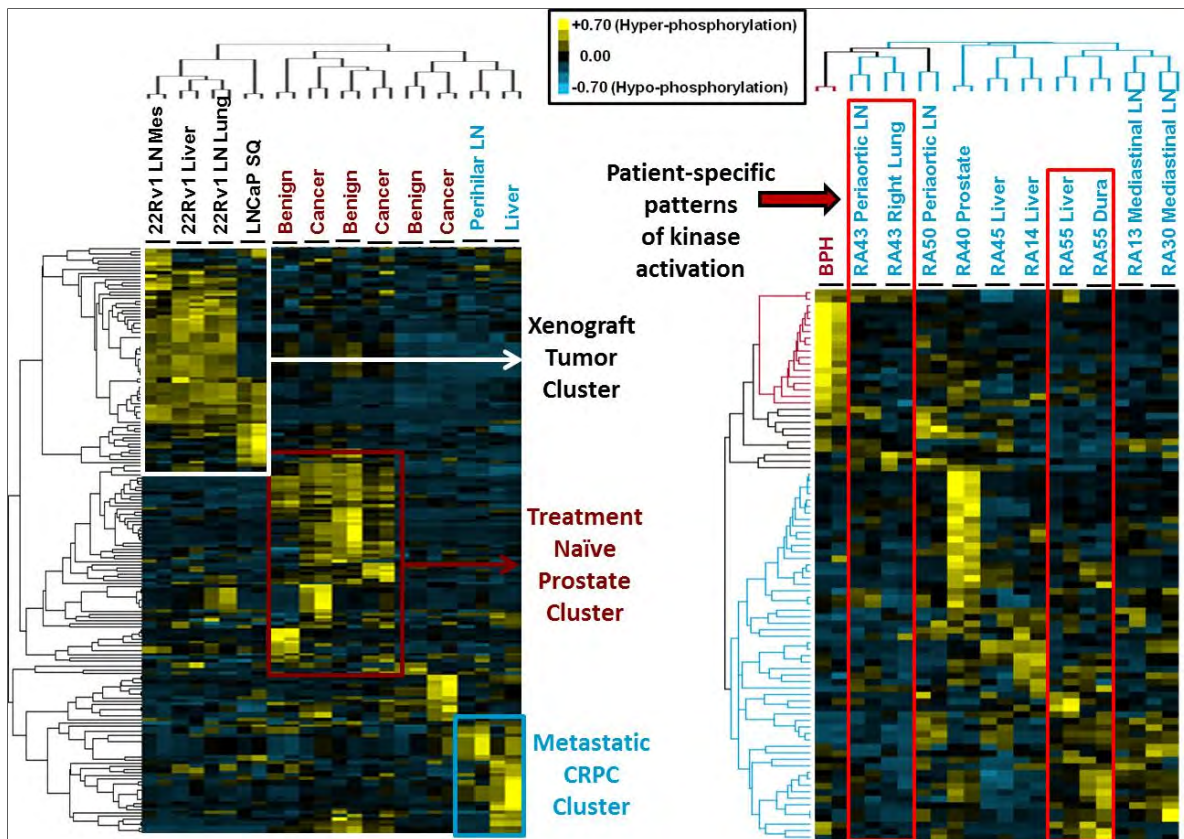


**Figure 5:** Prostate gland ducts are composed of a layer of basal and luminal epithelial cells. Dr. Witte's studies have found that basal but not luminal cells are the prostate cancer cell of origin. Following oncogenic transformation, basal cells develop either the luminal characteristics of adenocarcinomas, or become a mixed tumor type with both basal and luminal cells.

- In many types of cancer, including CML and ALL, activated tyrosine kinases act as essential drivers of disease progression. Tyrosine kinases are signaling molecules which regulate the function of other proteins by phosphorylating tyrosine residues.



- It is unknown if kinase-dependent pathways play any role in prostate cancer propagation.
- Mutation rates of tyrosine kinases are low in prostate tumors. However, the activity of the androgen receptor (AR) is regulated by tyrosine phosphorylation, and a global elevation of tyrosine phosphorylation events was observed in prostate tumors.
- Dr. Witte's group has begun studying the role of tyrosine kinases in prostate cancer.
- Tyrosine-phosphorylated proteins were isolated from metastatic tumors of 12 prostate cancer patients and compared with treatment-naïve prostate tumors and cell-line derived xenograft tumors (tumors grown in immunodeficient mice from human prostate cancer cell lines). Mass spectrometry was performed to identify the proteins which are tyrosine phosphorylated in these tumors.
- Analysis of tyrosine phosphorylated proteins found different patterns in metastatic vs. treatment-naïve vs. cell-line derived xenograft prostate tumors, as well as patient-specific patterns.
- The functions of activated kinase pathways in prostate cancer and treatment strategies using combinations of targeted kinase inhibitors are being explored. In particular, data from 10/13 metastatic castrate-resistant prostate cancer (CRPC) patients has indicated that combinatorial targeting of AKT, SRC, and/or MAPK1/3 kinases may be of benefit.
- Similar studies are also being performed to identify serine/threonine phosphorylated proteins from prostate tumors. Thus far, 20 associated kinases have been identified that are associated with metastatic but not treatment-naïve prostate tumors.
- Dr. Witte's lab is also working to develop new tools to study protein kinase signaling, including a strategy to develop antibodies that specifically recognize phosphorylated versions of proteins.



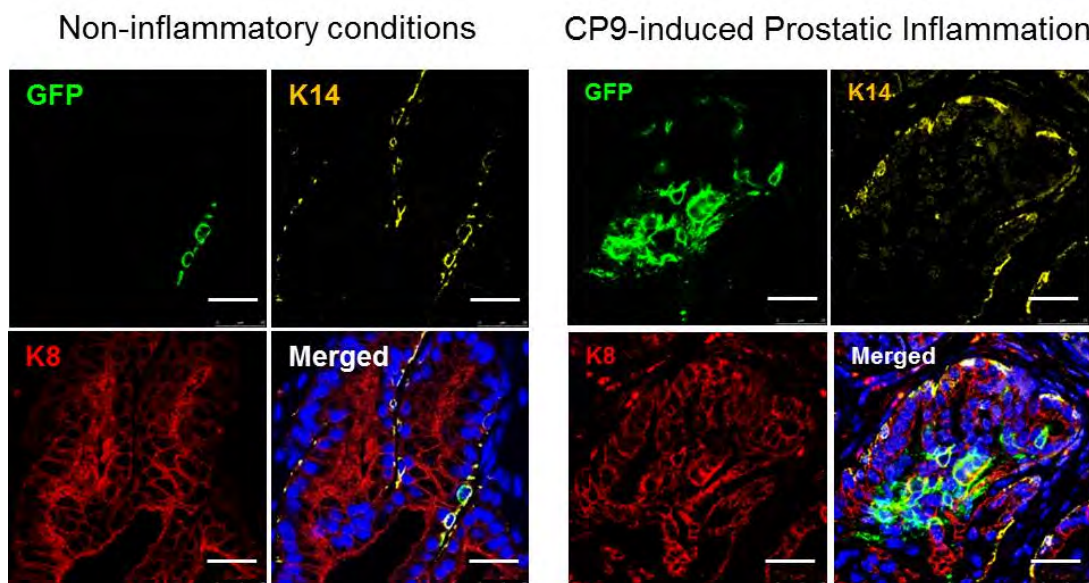
**Figure 6:** Analysis of tyrosine phosphorylated proteins found different patterns in metastatic vs. treatment-naïve vs. cell-line derived xenograft prostate tumors (Left), as well as patient-specific patterns (Right).

## Prostate Epithelial Lineage Hierarchy and Cells of Origin for Prostate Cancer

**Li Xin, PhD**  
**Baylor College of Medicine**

- The prostate gland is composed of self-sustaining lineages of basal and luminal epithelial layers. Basal cells are considered as one origin for prostate cancers, which are often luminal in phenotype. Thus, delineating conditions under which cancerous basal cells differentiate into luminal cells is critical for understanding prostate cancer progression.
- Dr. Li Xin's research is focused on understanding the "lineage hierarchy" of the prostate epithelium. Specifically, whether prostate basal cells can differentiate into luminal cells under various conditions, and how this relates to prostate cancer development.

- These studies use an *in vivo* lineage tracing approach, in which prostate basal cells were labeled with green fluorescence protein (GFP), and their fate was tracked *in vivo*.
- Prostatitis is an infection-mediated inflammatory condition of the prostate gland. To determine if inflammation promotes basal cell differentiation into luminal cells, mice were given prostatitis by injecting the uropathogenic bacterium, *E. coli* CP9 strain into the bladder of mouse prostates via the urethra.
- Under non-inflammatory conditions, GFP-labeled (green) basal cells remained basal (express the basal K14 gene). However, following *E. coli* CP9 injection, some basal cells began to express signature luminal genes (K8). Thus, during prostatitis, basal cells can differentiate into luminal cells.
- Dr. Xin next wanted to know if the basal into luminal cell differentiation caused by prostatitis/inflammation promotes the development of prostate cancer. In a mouse model, the tumor suppressor gene PTEN was deleted specifically in basal cells. PTEN-deletion alone was not enough to significantly enhance cancer development, thus under non-inflammatory conditions, prostate cancer development was slow. However, prostatitis substantially accelerated the development of prostate cancer in these mice.
- These studies demonstrate that under normal conditions, basal and luminal cells maintain their native state. However, prostatitis/inflammation promotes basal to luminal cell differentiation and accelerates the development of prostate cancer.



**Figure 7:** Left: under non-inflammatory conditions, transplanted (GFP/green) basal cells in the regenerated prostate remained basal (express the basal K14 gene - yellow). Right: following *E. coli* CP9-induced prostatic inflammation, approximately half of the transplanted (GFP/green) basal cells express luminal genes (K8 - red).

## **Session 2 – What This Means for Patients:**

- Identification of the cell of origin for prostate cancer (cancer-stem cells), as well as cells that resist therapy, is important for the development of new therapies.
- In this session, a strain of bacteria known to cause prostatitis was shown to promote cellular changes consistent with transformation of normal prostate cells to a malignant state.
- In addition, a novel technology was developed to identify activated cancer promoting cell signaling molecules (kinases) in metastatic prostate cancer.

## **Session 3: The Patho-Epidemiology of Prostate Cancer— Translating Population Science to Prevention and Treatment of Advanced Prostate Cancer**

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### **Patho-Epidemiology and its Promise to Reduce Suffering from Prostate Cancer**

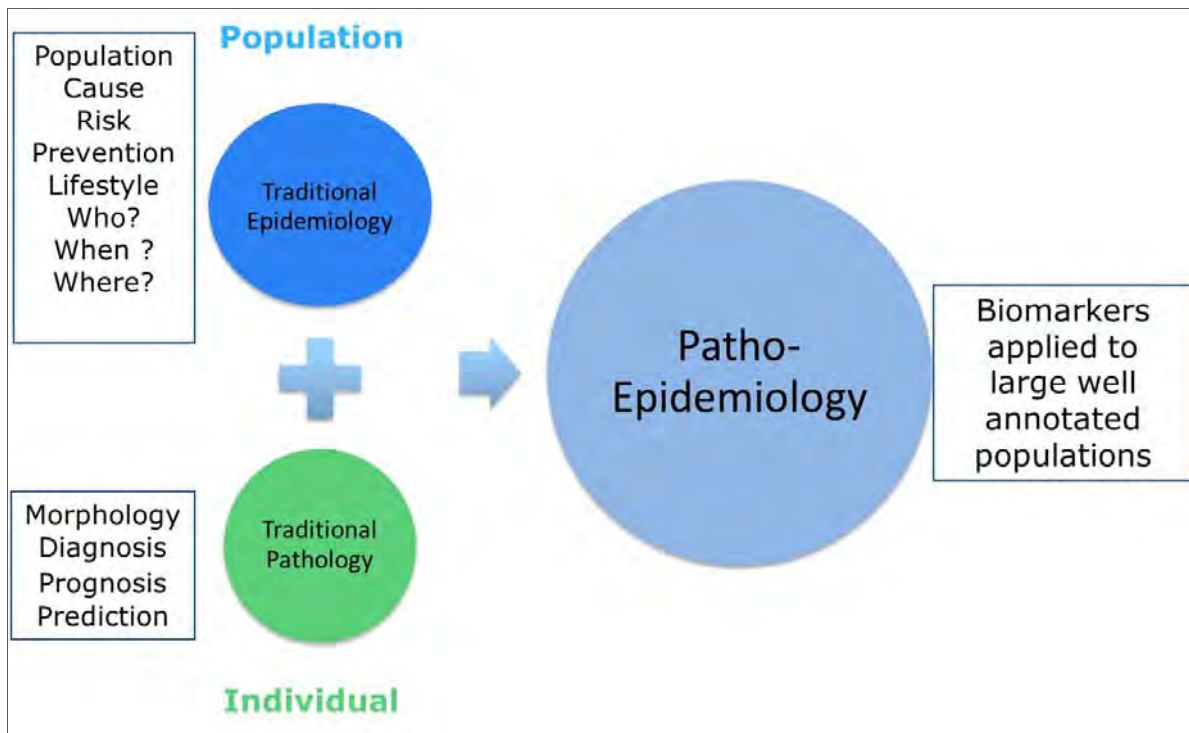
**Stephen Finn, MBBS, PhD**  
**University of Dublin, Trinity College**

**PCF Young Investigator Award, 2012**  
**Movember-PCF Challenge Award, 2012**

- Dr. Stephen Finn introduced the concept of patho-epidemiology, an integration of scientific disciplines.
- In traditional epidemiology, defined populations are studied to understand the patterns of disease cause, risk, prevention, and lifestyle.
- In traditional pathology, tissues from patients are studied for their morphology and to support diagnosis, prognosis, and prediction of disease course and response to treatments.
- Patho-epidemiologic studies also integrate the expertise of statisticians, bioinformaticians, clinicians, and researchers in fields including molecular biology and genetics to support data and sample acquisition, study design, analysis, and interpretation.
- Patho-epidemiology studies apply biomarkers to large, annotated populations. The influence of lifestyle, genetic, and/or environmental factors on the molecular

characteristics of cancer can be determined. Biomarkers that differentiate or identify new cancer subtypes, predict outcome or response to therapy, or lend insight into factors associated with etiology or disease progression can be identified.

- Patho-epidemiology is a growing discipline. These types of studies are routinely integrated into clinical trials, contribute to the generation of novel databases, and support the advancement of precision medicine.



**Figure 8:** Patho-epidemiology is an integrated discipline combining traditional epidemiology and traditional pathology.

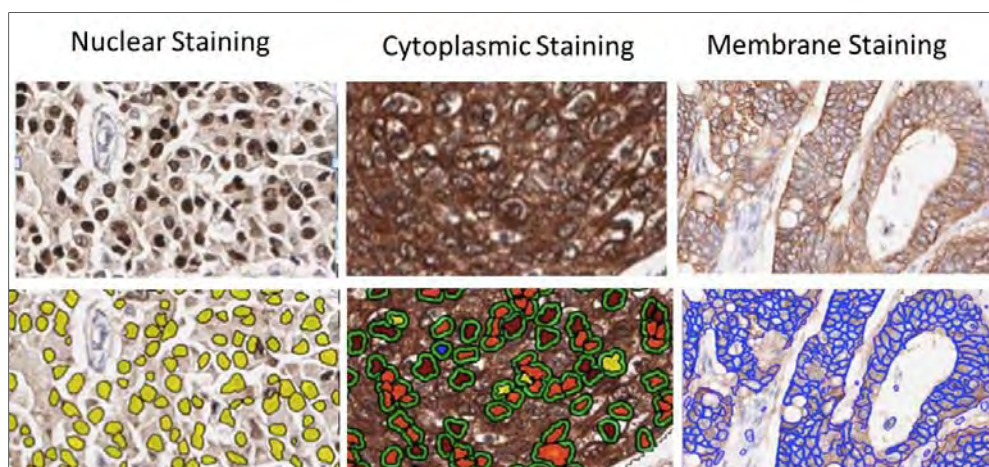
## Digital Pathology and Tissue Imaging for Conducting Patho-Epidemiology Studies

**Peter Hamilton, PhD**  
**Queens University Belfast**

- Dr. Peter Hamilton discussed digital pathology, tissue imaging technologies, and methods for high-throughput biomarker analysis of cancer patient tissues.



- There are millions of preserved patient tumor tissues that can be studied using histology methods to gain insight into disease mechanisms in large-scale epidemiology studies.
- In traditional histology methods, resected patient tissues are assessed for morphology or stained with dye-labeled antibodies against biomarkers. These tissues are then visualized and assessed by the researcher using microscopes.
- Tissue microarrays are histological slides consisting of an array of many hundreds of cores. Each core can be from a different patient's tumor, thus providing a way to more quickly analyze many samples.
- The assessment of biomarker staining by eye can be highly subjective. These assessments are also time consuming and not amenable to high-throughput studies.
- This can be improved using "digital pathology," which utilizes computer algorithms to recognize and score tissue morphology and the regions and intensities of biomarker expression. The derived results are quantitative and can pick up changes in cancer not visible to the naked eye.
- PathXL has developed a range of digital pathology software to support research and discovery of new cancer biomarkers in tissue. Additionally, TissueMark from PathXL uses computers to automatically and rapidly identify the cancer in tissue samples – again helping to identify cancer patients that will benefit from new and emerging drugs.
- Digital pathology technologies are being applied to many cancer types to examine the heterogeneity and architectural structure of tissues, and quantitative expression of biomarkers to facilitate the development of personalized medicine.
- This technology has the additional advantage of allowing expert pathologists to view and score slides on-line anywhere in the world.
- The digital aspects of this technology thus allow for many institutions to perform identical methodological analyses, to easily share data, and to perform high-throughput analyses for furthering the understanding of prostate cancer.



**Figure 9:** Nuclear, cytoplasmic, and membrane staining of histological tissues (top) can be identified by digital pathology algorithms (bottom).

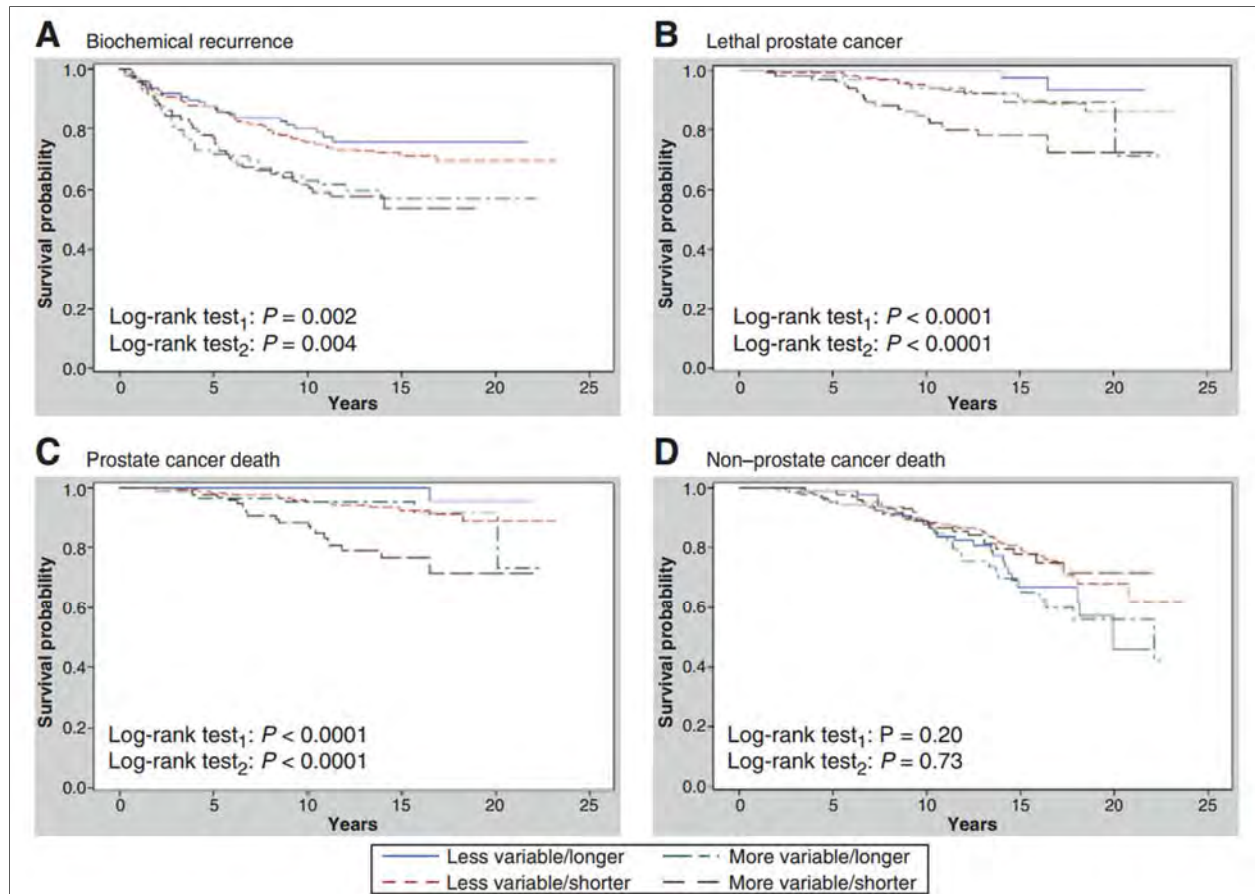
## Future Directions in Patho-Epidemiology and Discussion

**Angelo De Marzo, MD, PhD**  
**Johns Hopkins University**

**Movember-PCF Challenge Award, 2013**

- Dr. Angelo De Marzo discussed several recent findings from collaborative patho-epidemiology studies.
- Telomeres are protective repetitive nucleotide sequences located at the ends of chromosomes. Chromosomal ends are lost with each cell division and thus telomeres act as a sequence buffer to protect from loss of gene sequences at the chromosome ends, consequentially becoming shortened themselves.
- Shortened telomeres are associated with cells that have undergone many divisions and contribute to chromosomal instability, a common feature of metastatic cancers.
- In a recently published study, Dr. De Marzo, along with Drs. Elizabeth Platz, Alan Meeker, Christopher Heaphy, and colleagues, explored the connection between the risk of prostate cancer progression and telomere length and variability in surgically removed primary prostate tumors.
- Combinations of shorter telomere length in prostate cancer-associated stromal cells and greater telomere variability in prostate cancer cells were found to contribute to a significantly higher risk of metastatic progression and death from prostate cancer.

- In another study, a significant association was found between inflammation in benign tissue and the development of high-grade prostate cancer.
- These studies demonstrate the significance of collaborative patho-epidemiology studies in elucidating factors that contribute to prostate cancer progression.



**Figure 10:** Combinations of shorter telomere length in prostate cancer–associated stromal cells and greater telomere variability in prostate cancer cells (dashed brown line) were found to contribute to a significantly higher probability of progression to metastasis and death from prostate cancer.

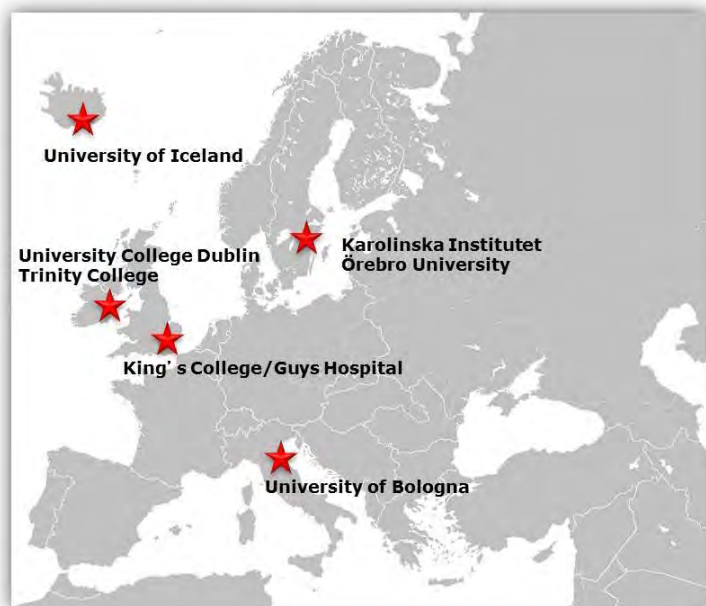
## Future Directions in Patho-Epidemiology and Discussion

**Massimo Loda, MD**  
**Dana-Farber Cancer Institute**

**PCF Challenge Award, 2012**

- Dr. Massimo Loda summarized the components, gains, and future directions of patho-epidemiology studies in prostate cancer research.

- Patho-epidemiology combines the rigorous experimental design, analyses, and annotation methods of epidemiology with the morphologic and molecular specimen analysis methods of pathology.
- Brought together, these disciplines promote the discovery and validation of powerful prognostic, predictive, and longitudinal biomarkers of disease mechanisms, outcome, and therapeutic responses.
- These types of studies can gain even more power by integration with other disciplines. For instance, “omics” data including genomics, epigenomics, proteomics, and metabolomic profiles, as well as the incorporation of other experimental *in vitro* and *in vivo* biological methods to validate biomarkers and understand mechanisms.
- Future directions of patho-epidemiology studies include longitudinal studies, integrating additional multidisciplinary approaches, understanding and applying findings on the effects of genetics and lifestyle to therapy, and developing and improving technology, databases, and infrastructure for these studies.
- PCF supports multiple investigators as well as a collaborative international research team in this field through PCF Young Investigator and PCF Challenge Award mechanisms.
- Dr. Loda is a co-leader with Dr. Lorelei Mucci (Harvard School of Public Health), of an international team of patho-epidemiology investigators (USA, Ireland, UK, Sweden, and Italy), supported by a 2012 PCF Challenge Award aiming to shed light on stromal-epithelial interaction in prostate cancer carcinogenesis and mortality.



### **Session 3 – What This Means for Patients:**

- Epidemiology is a powerful science that generates many important hypotheses concerning public health.
- Patho-epidemiology is a new science that intends to attach the power of observational research with molecular endpoints.
- Although this discipline is early in its scientific development, this team intends to increase the power of epidemiologic findings related to cause of initiation and progression of prostate cancer by applying tissue, blood, and urine testing from patients under observation.

### **Session 4: The Role of Long Non-Coding RNAs in Prostate Cancer Progression**

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#### **Integrative Annotation and Functional Characterization of Long Non-Coding RNAs**

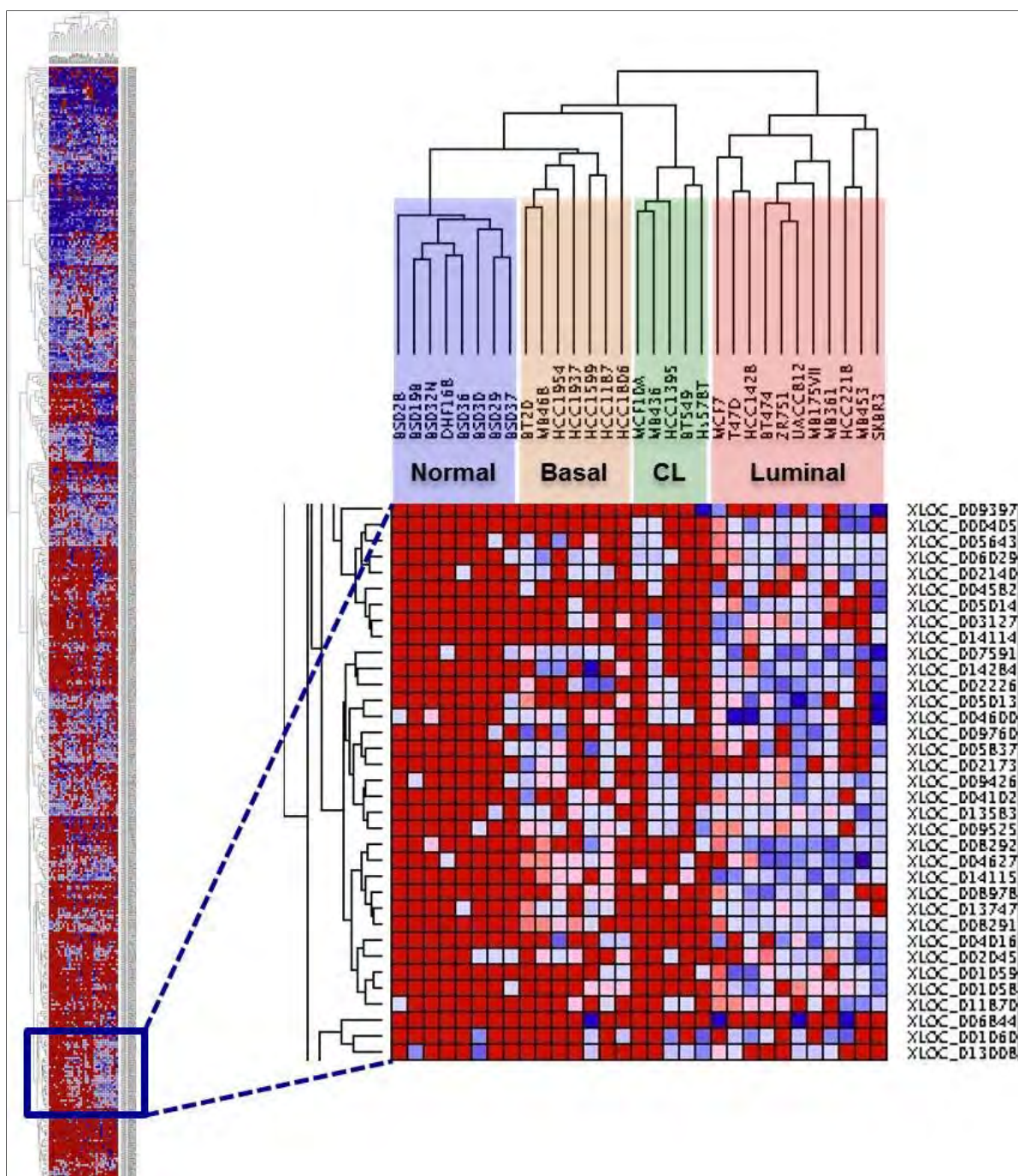
**W. Lee Kraus, PhD**

**University of Texas Southwestern Medical Center at Dallas**

- Long non-coding RNAs (lncRNA) are a recently appreciated class of genes that are transcribed into RNA but are not translated into proteins. LncRNAs are expressed in a variety of cell-types, and they perform specialized regulatory functions as non-coding RNA molecules.
- Known regulatory functions of lncRNAs include regulation of genome structure, RNA transcription (gene expression), and protein translation.
- The biological functions of only a few lncRNAs are known.
- Dr. Lee Kraus is interested in determining the biologic role of lncRNAs in tumor cells.
- LncRNAs expressed by a human breast cancer cell line were identified by subcellular RNA fractionation, sequencing, and bioinformatics methods. 1888 lncRNAs were identified, about half of which were not previously known.
- Like prostate cancer cells, breast cancer cells are hormone-regulated. To determine the effects of estrogen on lncRNA expression, breast cancer cells were grown with or without estrogen, which altered the expression of about 25% of the lncRNAs.



- To further classify breast cancer cell lncRNAs, the expression of lncRNAs were determined in 22 breast cancer cell lines, one normal breast cell line, and 8 primary breast cell cultures.
- Breast cancers can be classified into several distinct molecular subtypes by features including protein expression, morphology, and clinical behavior. In unsupervised hierarchical clustering, a bioinformatics method in which gene expression patterns are used to group samples, the 500 most variably expressed lncRNAs defined known molecular subtypes of breast cancer. This suggests that lncRNAs have roles in the regulation of breast cancer subtype behavior.
- To discover potential functions of breast cancer lncRNAs, their expression patterns were correlated with the expression patterns of protein-coding messenger RNA (mRNA). The functions of the associated proteins included DNA replication, cellular division, RNA processing, and gene regulation. LncRNAs may also play a role in all of these functions.
- One lncRNA, LncRNA-152, was highly expressed in breast cancer cells, particularly the luminal subtype. LncRNA-152 was required for optimal breast cancer cell growth, proliferation, and expression of cell cycle regulatory proteins.
- Overall, these studies identified and characterized lncRNAs in cancer cells. This is an emerging field in science and detailed understanding of lncRNA functions in cancer will lead to insights in cancer biology and treatment targets and strategies.



**Figure 11:** The expression of lncRNAs were determined in 22 breast cancer cell lines, one normal breast cell line, and 8 primary normal breast cell cultures. In unsupervised hierarchical clustering, the 500 most variably expressed lncRNAs (in rows) were able to differentiate the normal breast cells and breast cancer cell lines (in columns) by their known molecular subtype (basal, luminal, claudin-low (CL)).

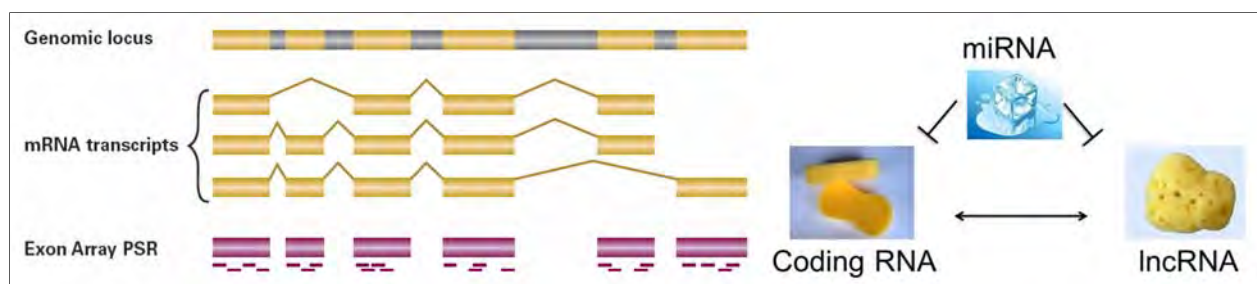
## Oncogenic and Tumor Suppressive Long Non-Coding RNAs in Prostate Cancer

**X. Shirley Liu, PhD**

**Dana-Farber Cancer Institute, Harvard School of Public Health**

- Long non-coding RNAs (lncRNA) are a recently described class of RNA that are transcribed from DNA into RNA but are not translated into proteins.
- Over 15,000 lncRNAs in the human genome have been annotated and are available from the genome-mapping ENSEMBL database, using data primarily from high-throughput RNA sequencing (RNA-Seq), a method in which all transcribed cellular RNA is sequenced. This method allows for identification of new genes, i.e., sequences of the human genome that are transcribed.
- Data on lncRNA expression in tumor samples is limited, as RNA-Seq methodology is optimized for specific types of RNA, which does not encompass all lncRNAs, including nuclear-localized lncRNAs. RNA-Seq also has insufficient “sequencing depth” as lncRNAs can be expressed at very low levels and thus are not detectable by this method. Finally, the computer algorithms that identify lncRNA sequences from the RNA-Seq data are still under-developed.
- DNA microarray technology works by attaching or synthesizing DNA sequence fragments (probes) from genes onto a special surface, each probe sequence applied to a specific and identifiable spot on the array. Dye-labeled-DNA or RNA derived from the cells being analyzed is then placed over the array and will find and hybridize to complementary probe sequences. The intensity of dye at each probe-spot is quantitated to determine expression levels of the corresponding gene.
- Dr. Shirley Liu has developed methods to identify prostate cancer-associated lncRNAs from publicly available RNA expression datasets generated by the Affymetrix human exon 1.0 ST Microarray platform. This Microarray was originally developed to assess the expression of different mRNA isoforms (different gene versions created by the transcription of alternate/different exons from any given gene).
- This Microarray has ~6 million probes, with an average of 4 probes per known or predicted gene exon, to be sure that every exon is sufficiently assessed. Of these exons, only ~25% correspond to known protein-expressing genes, while most other exons are predicted from the human genome based on their sequence features.
- Exons from over 10,000 lncRNAs were found to have at least 4 probes on this Microarray.

- Dr. Liu's team used bioinformatics methods to reassess a publicly available dataset from a study that had analyzed gene expression in normal prostate, prostate cancer, and metastatic tissues using this Microarray. This analysis identified ~200 prostate cancer-associated lncRNAs. Many were frequently altered in copy number in prostate cancer cells.
- Several lncRNAs with potential oncogenic functions were identified. PCAN1 & PCANR2 were most highly expressed in metastatic prostate cancer and lowest in normal prostate tissues. PCANR2 was negatively associated with expression of genes involved in adhesion. Inhibiting expression of PCAN1 or PCANR2 reduced tumor cell growth.
- Several lncRNAs with potential tumor-suppressor functions were also identified.
- Micro RNAs (miRNA) are a type of regulatory RNA that influences the stability and protein-translation rates of messenger RNA (mRNA) by binding mRNA via hybridization to complementary sequences.
- By sharing complementary miRNA target sequences with mRNAs, some lncRNAs may function as "competing endogenous" targets for miRNAs (ce-lncRNAs): acting as "sponges" that soak up miRNAs to allow expression of the mRNA.
- The tumor-suppressor gene PTEN has many miRNA target sequences. Four ce-lncRNAs that may enhance PTEN expression by competing for PTEN-regulating miRNAs were computationally predicted, based on sharing >10 miRNA sites with PTEN and correlating with PTEN expression.
- Inhibiting expression of these predicted ce-lncRNAs caused a reduction in PTEN expression and enhanced tumor cell growth. These effects required miRNA processing enzymes and miRNA regulatory sequences in PTEN, confirming these as ce-lncRNAs.
- Thus, these studies demonstrated the successful repurposing of the Affymetrix human exon 1.0 ST Microarray to identify prostate-cancer associated oncogenic and tumor-suppressive lncRNAs.



**Figure 12:** (L): Alternate mRNA exons (yellow) can be transcribed together to generate alternate proteins with different functions. Multiple probe selection regions (PSR) from each known and predicted exon in the human genome were used to create the Affymetrix human exon 1.0 ST Microarray platform, and many of these exons actually are from lncRNAs. (R): Competing endogenous lncRNAs (ce-lncRNAs) act as "sponges": soaking up miRNAs to allow expression of protein coding mRNAs with shared miRNA target sequences.

# Identification of SChLAP1, an Unannotated Long Non-Coding RNA That Promotes Metastases in Prostate Cancer

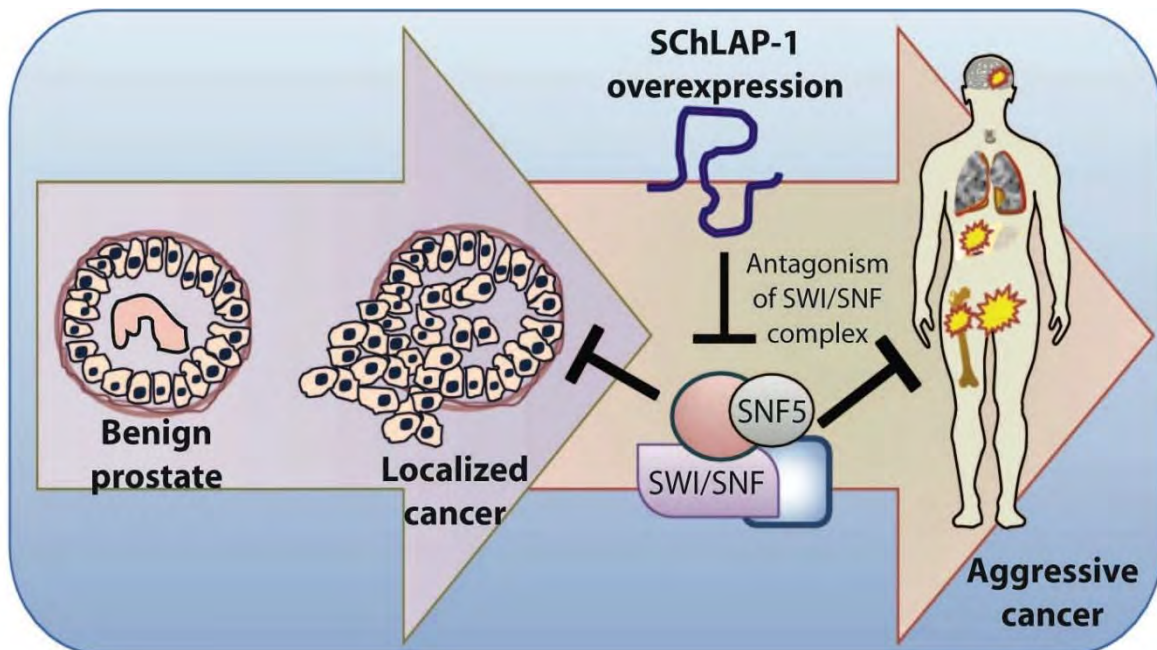
Felix Feng, MD  
University of Michigan

Young Investigator Award, 2010  
Movember-PCF Challenge Award, 2012  
Movember-PCF Challenge Award, 2013-2015  
Movember-PCF Challenge Award, 2013-2015

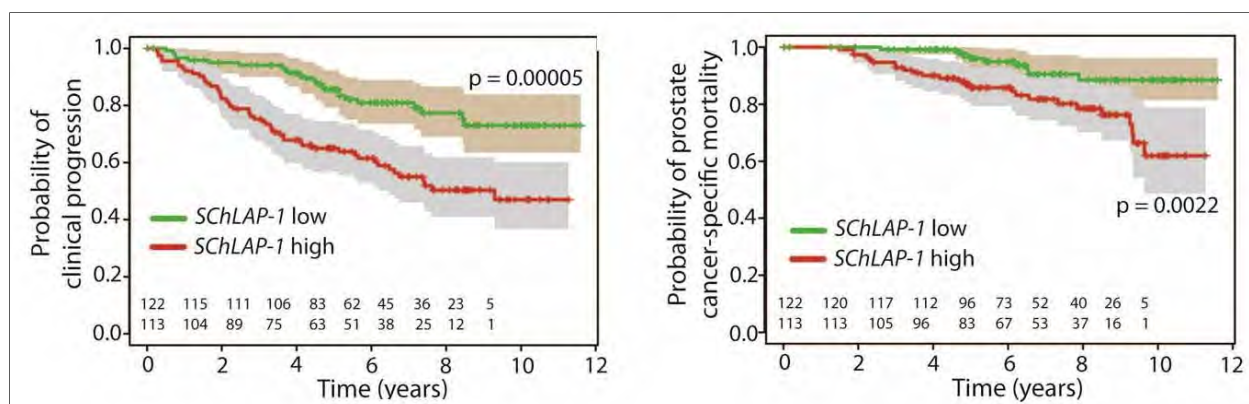
- Analysis of the human genome by the ENCODE consortium has found that although ~60% of our genome is transcribed into RNA, only 1.5% encodes for proteins. Thus, the purpose of the remainder of transcribed RNA is an important question in human biology.
- Long non-coding RNAs (lncRNA) are a recently described class of non-translated RNAs that share many structural features of mRNA.
- Drs. Felix Feng and Arul Chinnaiyan are interested in identifying lncRNAs that are expressed in prostate cancer and determining their functions. High-throughput RNA sequencing (RNA-Seq), a method of sequencing and quantitating the expressed RNA from cells, was used to find lncRNAs preferentially expressed in localized or metastatic prostate cancer compared to benign prostate tissues from a cohort of patients, as well as various prostate cancer cell lines.
- Of the 121 lncRNAs that were differentially expressed in prostate cancer, the novel lncRNA SChLAP1 was identified as the top outlier lncRNA.
- SChLAP1 was highly expressed in ~25% of localized and metastatic prostate tumors but not in benign prostate disease tissues.
- The function of SChLAP1 was analyzed by inhibiting its expression in prostate cancer cells. SChLAP1 promoted invasive properties *in vitro*, and was required for metastasis *in vivo*.
- To gain further insight into the SChLAP1 role in prostate cancer, microarray gene expression analysis was performed on prostate cancer cell lines in which SChLAP1 expression was inhibited vs. uninhibited.
- An inverse correlation was found between genes whose expression changed with SChLAP1 expression, and genes regulated by the SWI/SNF nucleosome remodeling complex, a tumor-repressor that acts by altering the structure of genomic DNA.
- SChLAP1 was found to antagonize the SWI/SNF tumor-repressor complex by interacting with the SNF5 component and inhibiting its binding to genomic target sites.



- Importantly, SChLAP1 was demonstrated to have significant clinical relevance, as gene expression profiling of 235 high-risk prostate cancer patients who had undergone radical prostatectomy, found that out of all protein-coding and non-coding exons, SChLAP1 expression was the highest predictor of metastatic progression.
- Patients with high SChLAP1 expression had significantly higher incidence of biochemical recurrence, clinical progression, and prostate cancer-specific mortality.
- SChLAP1 maintained high prognostic significance even on multivariable analysis accounting for standard clinical prognostic variables.
- Methods have been developed to identify SChLAP1 lncRNA expression in prostate cancer tissues by histological methods.
- Thus, SChLAP1 lncRNA expression is being developed into a clinical prognostic test.



**Figure 13:** The SWI/SNF nucleosome remodeling complex is a tumor-repressor, which the oncogenic SChLAP1 lncRNA molecule acts to inhibit. Patients with SChLAP1 overexpression are demonstrated to have more aggressive prostate cancers and worse clinical outcome.



**Figure 14:** In a gene expression profiling study of 235 high-risk prostate cancer patients who had undergone radical prostatectomy, patients with high SChLAP1 expression had significantly higher incidence of clinical progression and prostate cancer-specific mortality.

## Session 4 – What This Means for Patients:

The study of non-coding RNA molecules is a relatively new scientific discipline. These molecules account for over 80% of the coding potential of the human genome and yet their biological significance is still in very early stages of discovery. Long non-coding RNAs (lncRNA) are proving to be a very valuable tool for diagnosis and prognosis of prostate cancer as well as drivers of disease progression. In this session, several candidate lncRNAs were described that promote invasive behavior of prostate cancer and predict the most clinically significant forms of the disease.

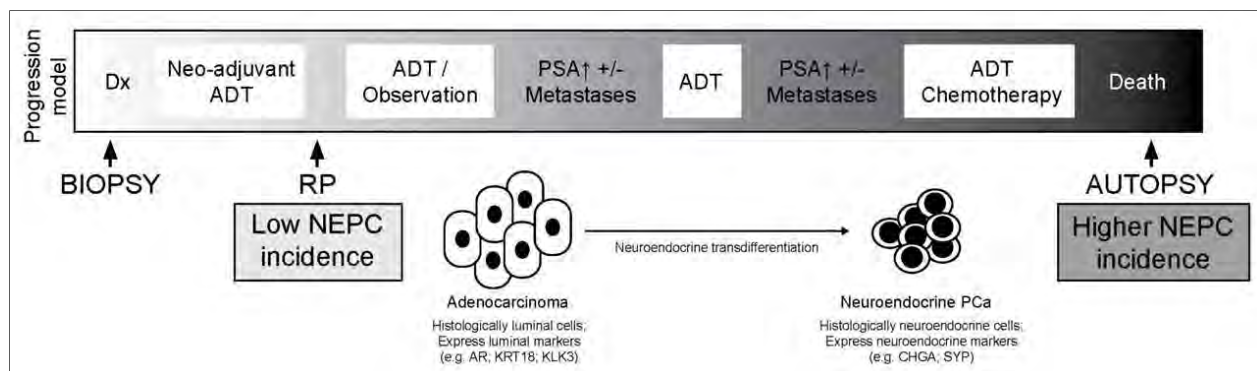
## Session 5: Game-Changing Research—PCF High-Achieving Young Investigators I

### Translational Modeling of Neuroendocrine Trans-differentiation

Alexander Wyatt, DPhil  
Vancouver Prostate Centre

PCF Young Investigator Award, 2012

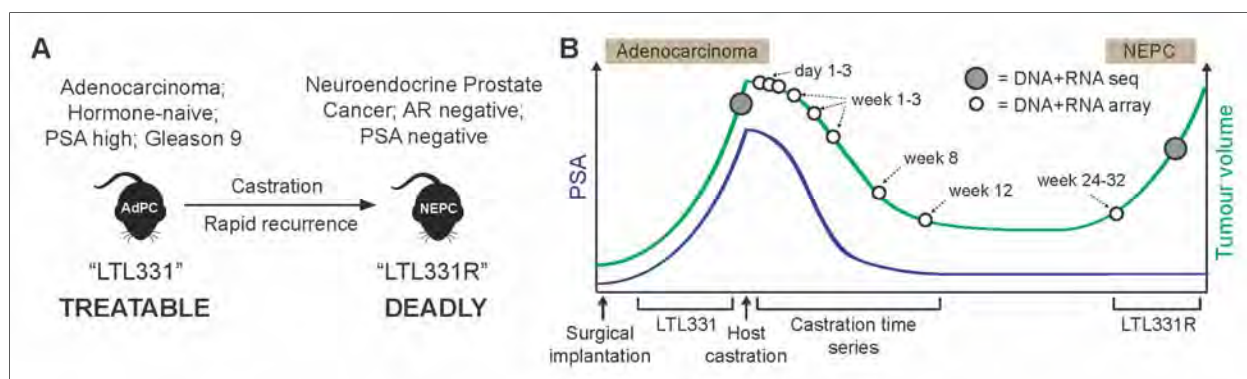
- Prostate cancers require growth and survival signals that rely on androgens (i.e. testosterone). Thus, the primary treatment for prostate cancer is androgen-deprivation therapy (ADT).
- However, while ADT induces initial tumor regression, over time, prostate cancers become resistant to androgen-deprivation and relapse as aggressive castration-resistant prostate cancers (CRPC), which are androgen-independent derivatives.
- In some patients, this involves a change from prostate adenocarcinomas into a neuroendocrine prostate cancer (NEPC) phenotype where the prostate cancer cells now take on phenotypic and gene expression properties of neuronal tissue.
- The frequency of prostate cancer cells with this phenotype increases with disease progression and NEPC-type tumors are observed in 25% of patient autopsies.



**Figure 15:** Course of prostate cancer patient diagnosis, treatment with androgen-deprivation therapy (ADT), and progression to NEPC.

- Dr. Alexander Wyatt's studies seek to identify and characterize the genes involved in the "trans-differentiation" (differentiation of one cell type into another) of prostate adenocarcinoma into NEPC.

- Dr. Wyatt has developed a clinically relevant experimental system to study the progression and transitional phases of adenocarcinomas into CRPC and NEPC.
- In this system, hormone-naïve prostate cancer cells from patients are extracted from biopsy specimens or surgically removed tumors, and transplanted into immune-deficient mice. Human cells are able to grow in certain types of immune-deficient mice without host immune system-mediated rejection of the “foreign” human tissue (xenografts). Thus, this system enables the study of growing human tumors in an experimental setting.
- Following growth of the implanted human cancer cells, the mice are castrated. Similar to the human setting, androgen-deprived tumors initially regress and then recur as CRPC that can progress to NEPC.
- NEPC tumors that developed in these mice had an irreversible phenotype, as supplementation of mice with testosterone did not change their phenotype, which includes loss of expression of the androgen receptor (AR), the transcription factor that responds to androgens and which the original adenocarcinomas were dependent on for expression of growth and survival genes.
- NEPC derivatives had the same set of gene-fusion rearrangements as the adenocarcinomas from that patient, indicating that NEPC trans-differentiation did not involve the development of additional gene-fusions.
- To understand the genes involved in this process of trans-differentiation, gene expression was compared over time in xenografts from the same patient’s tumor as initial adenocarcinomas prior to mouse castration and as tumors regressed and then progressed to NEPCs following castration.
- Two classes of genes were expressed during NEPC development.
- One class of genes was only transiently expressed between castration and NEPC emergence. These genes included the Wnt/ $\beta$ -catenin pathway (a pathway involved in embryonic development and oncogenesis), and anti-apoptosis (resistance to cell death).
- A second class of genes persistently rose with the development of NEPC. These genes were involved in processes including cell fate, growth, and neuroendocrine functions. Furthermore, these genes overlapped with those identified in gene expression analyses of clinical NEPC tumors.
- These genes are promising candidates as prognostic biomarkers and therapeutic targets for the prevention of development and growth of NEPC.



**Figure 16:** Modeling neuroendocrine trans-differentiation in a patient-derived xenograft. A) Overview of Dr. Wyatt's system. B) Schematic depicting the tumor growth curve in mice with prostate cancer patient-derived xenograft tumors that first grow as adenocarcinomas, but later develop into NEPC following mouse castration. Indicated are the times at which RNA gene expression and genomic DNA mutations were assessed.

## What This Means for Patients:

A subset of very advanced lethal prostate cancers assume the properties of nerve tissue (neuroendocrine). Dr. Alexander Wyatt described a model for the development of neuroendocrine prostate cancer and described properties of these highly invasive tumor cells that could lead to new therapies.

## Proteome-based Biomarker Discovery in Prostate Cancer; Building the Heme-oxygenase (HO-1) Interactome

**Geraldine Gueron, PhD**

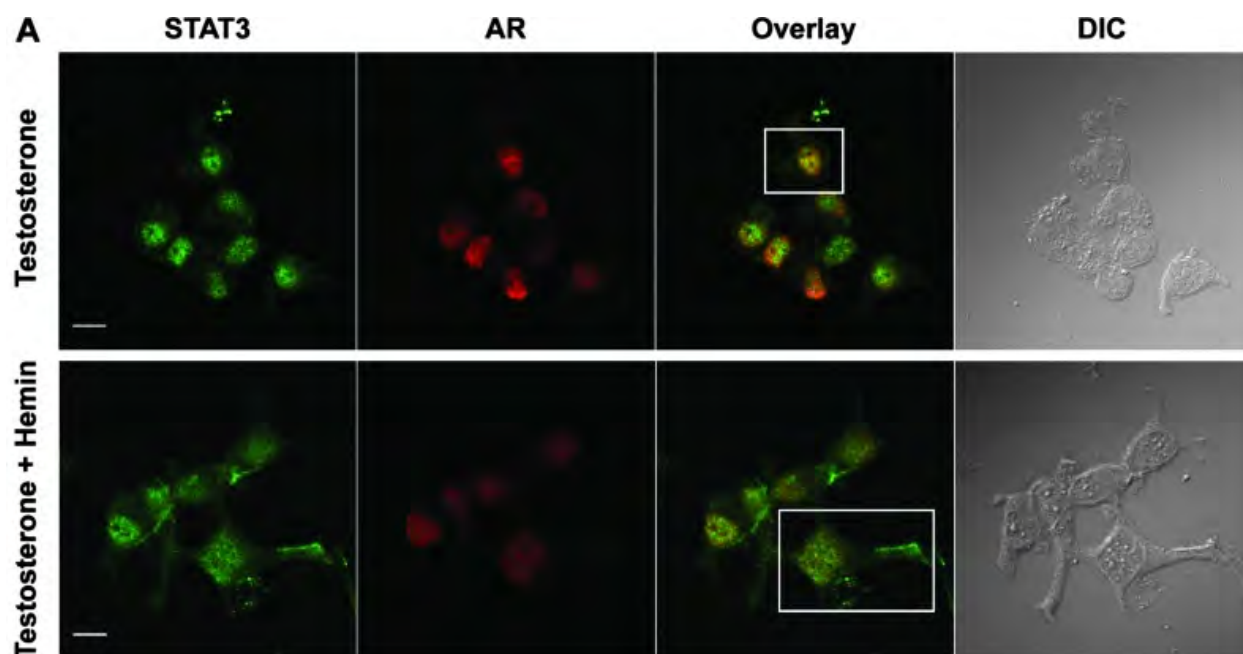
**IQUIBICEN-CONICET, University of Buenos Aires**

**PCF Young Investigator Award, 2013**

- Proteomics methods enable researchers to determine the proteins expressed by a cell, as well as all protein: protein interactions.
- Heme Oxygenase-1 (HO-1) is an anti-oxidant and anti-inflammatory enzyme that degrades heme, a component of hemoglobin that transports gases such as oxygen into tissues.
- Dr. Geraldine Gueron discussed her studies examining the tumor-suppressor functions of HO-1 in prostate cancer.



- Experimentally enhancing expression of HO-1 in prostate cancer cells greatly reduced the ability of these cells to develop into tumors in mice. Examination of these tumors found that HO-1 reduced blood vessel development (angiogenesis) in tumors.
- Dr. Gueron questioned what proteins interact with HO-1 to generate these anti-tumor functions. Using proteomics methods, many potential HO-1 interacting proteins were identified in prostate cancer cells.
- The oncogenic transcription factor STAT3 was identified as an HO-1 interacting protein. This interaction was enhanced when HO-1 was activated by hemin, a potent and specific HO-1 inducer.
- The androgen receptor (AR), the master oncogenic transcription factor in prostate-cancer cells, also interacted with STAT3 in the cell's nucleus, when AR was activated by androgens.
- However, when HO-1 was also activated, the interaction between STAT3 and AR was reduced. Thus, inhibiting interactions between these two oncogenic transcription factors may be an important anti-cancer function of HO-1.
- Metacore is an integrated bioinformatics analysis method that combines many kinds of gene expression and protein interaction data, and is a powerful tool for discerning the functions of genes. Metacore analysis of data from prostatic intraepithelial neoplasia tissues, indicated significant associations between HO-1 regulated genes and STAT3 and AR, implicated in the regulation of cellular processes including angiogenesis, cellular adhesion and the migratory and invasive capacity of cells.
- Another protein that was identified as a candidate HO-1 interacting protein is Muskelin, a gene involved in regulating cellular morphology. HO-1 interaction with Muskelin was enhanced when HO-1 was activated.
- Because of the role of Muskelin, Dr. Gueron examined the effect of HO-1 on cell morphology. When HO-1 was activated, cells changed shape and their branch-like cytoplasmic extensions became condensed.
- The expression of genes that changed with high levels of HO-1 were also examined. HO-1 reduced the expression of genes that function in regulating the extracellular space, as well as regulating immune responses and responses to cell communication and migration signals.
- These studies are ongoing, and in future work, Dr. Gueron will continue to unravel the proteins that HO-1 interacts with and the mechanisms underlying the anti-tumor properties of HO-1 in prostate cancer.



**Figure 17:** Top row: AR (red) co-localized in the nucleus of cells with STAT3 (green) when activated by testosterone. Bottom row: when HO-1 was activated by Hemin, the co-localization between STAT3 and AR was impaired. Co-localization is indicated by the yellow color upon overlay of green & red.

## What This Means for Patients:

Metabolism, inflammation, and angiogenesis are important in cancer development and progression. Heme Oxygenase-1 (HO-1) is an enzyme that functions in these processes as a tumor-suppressor. Dr. Geraldine Gueron has discovered many HO-1 interacting proteins. HO-1 appears to antagonize interactions between the oncogenic transcription factors STAT3 and AR. This work will lead to new discoveries of prostate cancer mechanisms and therapies.

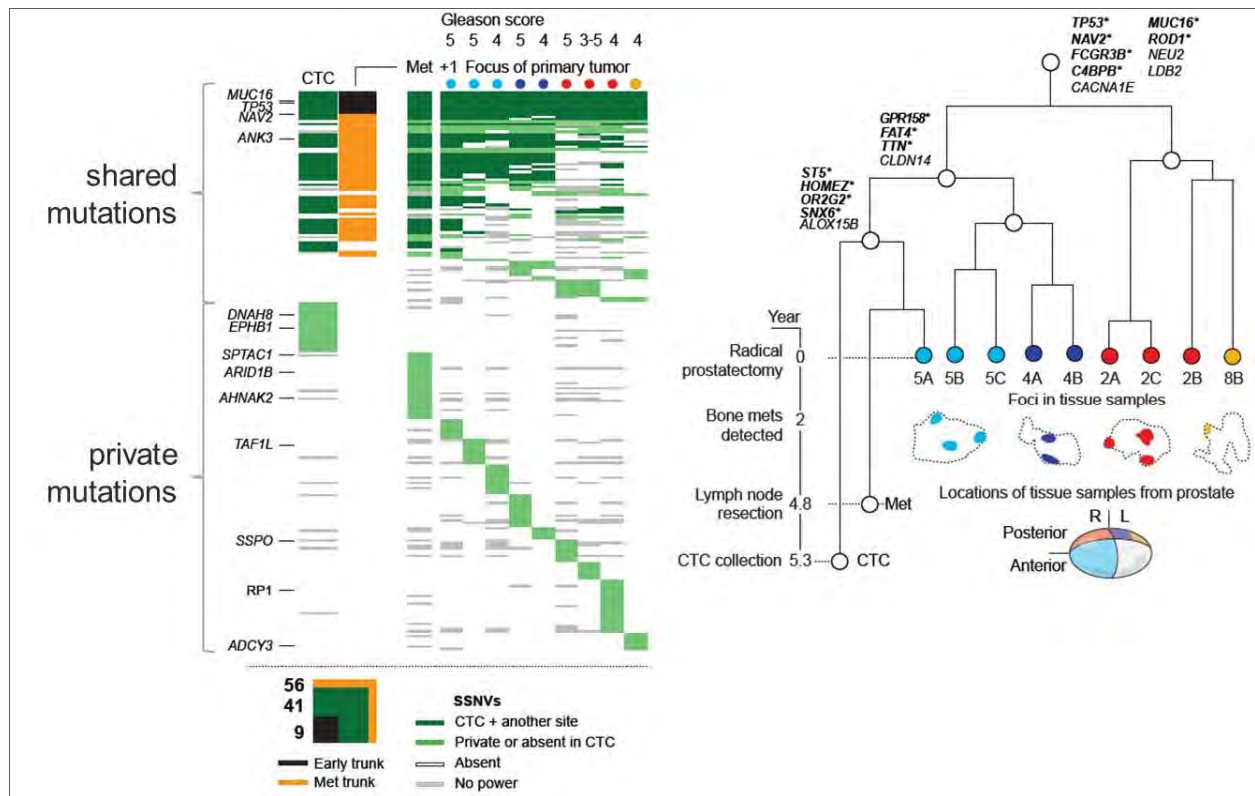
## Functional and Genomic Characterization of Viable CTCs Enabled by Nanowells

**Atish Choudhury, MD, PhD**  
**Dana-Farber Cancer Institute**

**PCF Young Investigator Award, 2008**

- Circulating tumor cells (CTCs) are tumor cells that have been shed from primary or metastatic tumor sites and can be found in peripheral blood.
- Because drawing blood is a significantly less invasive procedure than prostate biopsy, many studies have aimed to characterize CTCs via “liquid biopsy,” as an alternative method to gain insight into the molecular properties of tumors. However, issues including purification methods have made this a difficult task.
- Dr. Atish Choudhury collaborated with Dr. Christopher Love at the Koch Institute of MIT, who has established a nanowell-based approach to assess CTCs on a single cell level. Cells expressing the epithelial cell marker EpCam but not the hematopoietic cell marker CD45 (EpCam<sup>+</sup>CD45<sup>-</sup> cells) were isolated into a specialized nanowell apparatus.
- Assessment of cell viability demonstrated that many of the captured cells remain alive after the isolation procedure, particularly from patients with a high burden of disease.
- CTCs were assessed for multiple phenotypic characteristics, such as invasiveness and secretion of prostate cancer proteins including prostate-specific antigen (PSA) and protein-degrading enzymes. This demonstrated that CTCs isolated in this manner can be used to examine functional properties of the patient’s cancer cells.
- Genetic mutations in CTCs were characterized in single cells. Detection of a mutation in a minimum of 3 individual CTCs ensured that these were indeed tumor-cell mutations and not technical errors in genome sequencing.
- Mutations in CTCs were compared with metastatic tumors and different regions of the primary tumor from the same patient, to determine their differences and evolutionary relationships.
- CTCs and metastatic tumors had both shared (“trunk”) and unshared (“private”) mutations, indicating that CTCs were not directly derived from the metastatic site sampled, but shared an ancestor population that generated both pools of cells.
- Different primary tumor foci also had private mutations, indicating expansion of derived mutants in different regions of the primary tumor. In the example shown, one of these primary tumor foci was related to the metastatic site and the CTCs.

- This project was enabled by a collaboration between the Gelb Center for Translational Research at the Dana-Farber Cancer Institute, the Broad Institute of Harvard and MIT, and the Koch Institute of MIT.



**Figure 18:** Left: Shared mutations and private mutations can be detected in CTCs, metastatic sites, and different foci of the primary tumor from the same patient. Right: By analyzing mutations, the evolutionary relationships between primary tumor foci, metastatic tumors, and CTCs can be determined.

## What This Means for Patients:

Precision medicine, the matching of therapy to the mutations in a patient's tumor, requires an invasive biopsy procedure. Circulating tumor cells (CTCs) on the other hand, are representative of the patient's tumor and are easily obtained with a simple blood draw. Dr. Atish Choudhury presented a novel technology for isolating single CTCs and methodology for genomic analysis which may lead to improved precision medicine decision making.

## Epithelial Progenitor Cells and Inflammation

Andrew Goldstein, PhD

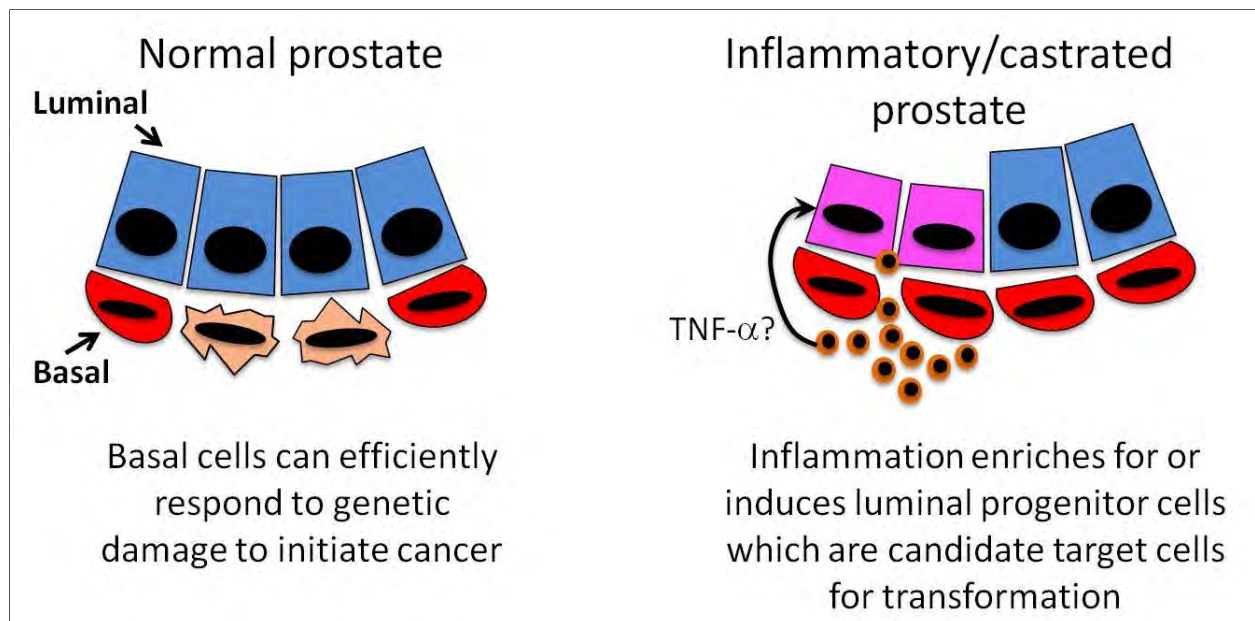
University of California, Los Angeles

PCF Young Investigator Award, 2011

- The prostate gland is composed of layers of luminal epithelial and basal epithelial cells.
- Prostate cancer can arise from either luminal or basal cells in mice, while in humans, basal cells are the demonstrated tumor-initiating cell type.
- However, studies have identified long-lived luminal progenitor cells in models of human prostate cancer and these cells are readily capable of generating new tumors.
- The proliferation of prostate luminal cells has been associated with inflammation in patients with Proliferative Inflammatory Atrophy, an inflammatory prostate condition, and in murine prostatitis models.
- Dr. Andrew Goldstein is interested in the connection between inflammation and prostate cancer initiation, and hypothesized that under inflammatory conditions, luminal progenitor cells gain proliferative and tumor-initiating capacities.
- Luminal progenitor cells have been described to have no or low expression of the androgen receptor (AR) transcription factor and of prostate specific antigen (PSA), a prostate cancer-associated gene that is regulated by AR.
- Dr. Goldstein was able to identify and viably isolate AR<sup>low</sup>PSA<sup>low</sup> luminal progenitor cells from benign regions of prostate tissues obtained from patients who underwent radical prostatectomy. These cells were confirmed to have a luminal phenotype, expressing luminal but not basal cell genes.
- To determine what genes are involved in the functions of AR<sup>low</sup>PSA<sup>low</sup> luminal progenitor cells vs. “mature” AR-expressing luminal cells, microarray gene expression analysis was performed. Genes involved in immune responses and inflammation were highly expressed in the luminal progenitors. Visualization of prostate tissues revealed that luminal progenitors are in close proximity to immune cells, indicating that interactions with immune cells may induce luminal progenitors to express immune-regulated genes.
- Castration of mice also leads to an increase in the prevalence of cells with a luminal progenitor phenotype. Dr. Goldstein found that castration also initiated prostate inflammation, with increased numbers of T cells and myeloid cells observed in prostates from castrated mice.



- $\text{TNF}\alpha$  is a cytokine that is released from cells during inflammatory conditions and regulates the process of inflammation. In cell culture experiments,  $\text{TNF}\alpha$  enhanced the progenitor activity of luminal but not basal cells.
- Thus, under inflammatory conditions, factors including  $\text{TNF}\alpha$  induce luminal progenitor cells to proliferate and gain tumor-initiating capacities.
- Finally the clinical relevance of luminal progenitor cells was examined. In a watchful waiting cohort of 281 Swedish men with prostate cancer who were followed for 30 years, patients with  $\text{AR}^{\text{low}}$  luminal progenitor-like tumors had significantly worse overall survival than patients with  $\text{AR}^{\text{high}}$  luminal-like tumors.
- Thus, while basal cells may be the typical prostate cancer initiating cell type, in the context of inflammation, luminal progenitor cells may initiate and promote the progression of prostate cancer.



**Figure 19:** Left: Basal cells may be the typical prostate cancer initiating cell type. Right: During inflammation, which can be induced by castration, luminal progenitor cells may initiate prostate cancer.

## What This Means for Patients:

Understanding prostate tumor cells with tumor initiating properties is important in understanding cancer development and treatment resistance. Dr. Andrew Goldstein described a type of prostate tumor initiating cell with very low expression of the androgen receptor (AR, the engine of prostate cancer progression and survival), with a worse overall survival rate than patients with high AR activity. During inflammatory processes, this alternate cell type can initiate and promote prostate cancer.

## Session 7: The Prostate Cancer Epigenome and Epigenomic Therapy

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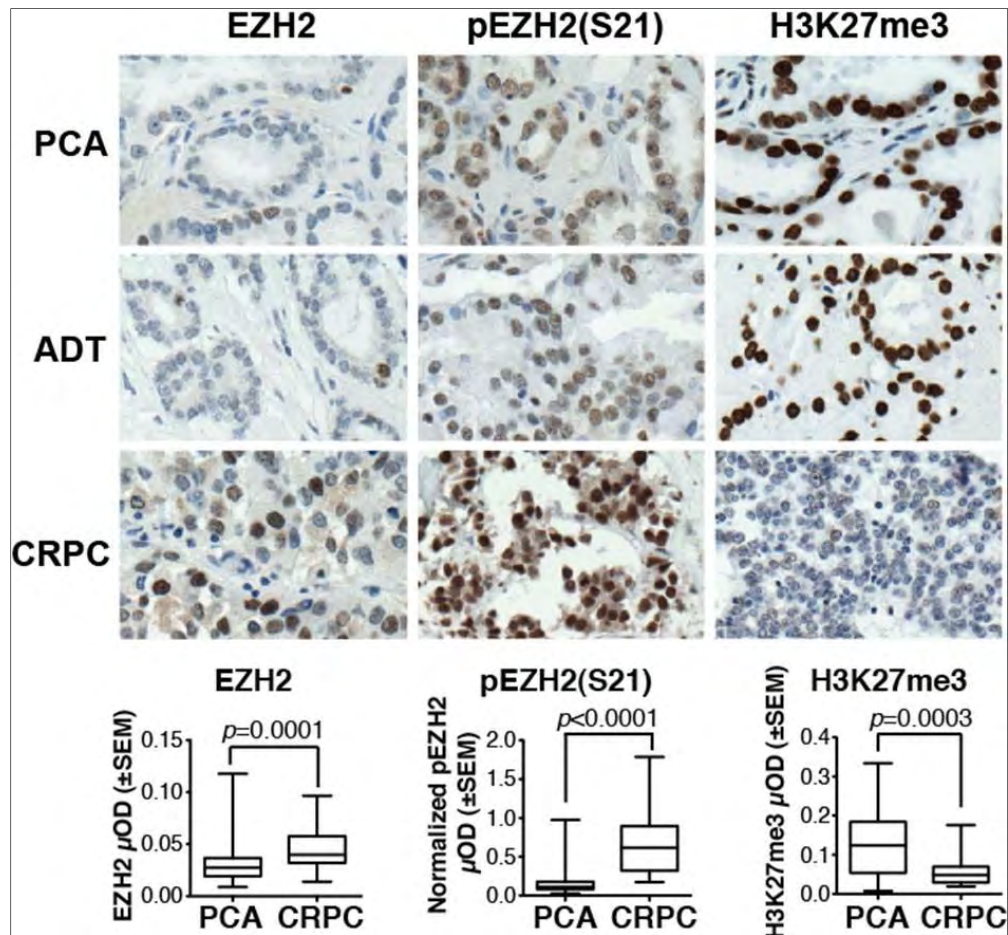
### Targeting Epigenetic Regulators in Prostate Cancer

**Myles Brown, MD**  
**Dana-Farber Cancer Institute**

**PCF Challenge Award, 2008**

- Prostate tissue and prostate cancers are dependent on androgen-receptor signaling pathways for growth and survival.
- Castration-resistant prostate cancer (CRPC) is an aggressive recurrent prostate cancer phenotype that develops after patients are treated with androgen-deprivation therapy, allowing for androgen-independent growth.
- The role of epigenetic modulation vs. genomic mutations in the development of CRPC is relatively unknown.
- Epigenetic regulation is mediated in part by the addition of activating vs. repressive chemical modifications, such as methylation of histones which form the protein complexes around which chromosomal DNA is wound.
- The heritable states of repressive histone methylation are maintained in part by polycomb repressor complexes. EZH2 is a histone methyltransferase enzyme that is a component of the polycomb repressor complex 2 (PRC2). EZH2 adds repressive methylation “marks” to lysine 27 of histone H3 (H3K27).
- EZH2 is considered oncogenic and its expression increases during prostate cancer progression, particularly in metastatic CRPC, and correlates with poor clinical prognosis.
- Dr. Myles Brown discussed his group’s studies examining the role of EZH2 in prostate cancer progression.
- Interestingly, while EZH2 expression levels were increased in CRPC cells compared with androgen-dependent prostate cancer cells, H3K27 methylation was decreased.
- Phosphorylation of EZH2 on serine-21 (pS21) inhibits its histone H3 methyltransferase activity, and pS21 levels were correspondingly increased in CRPC cells along with EZH2 expression levels in opposition to decreased H3K27 marks. This indicates that the role for EZH2 in promoting CRPC may be independent of its ability to methylate histone H3.

- GSK-126 is a specific small-molecule inhibitor of EZH2 methyltransferase activity.
- When CRPC cells vs. androgen-dependent prostate cancer cells were treated with GSK-126, both cell types exhibited growth inhibition. However, even though CRPC cells had very low amounts of H3K27 marks, these cells were much more sensitive to GSK-126. Thus, EZH2 methyltransferase activity was required in CRPC cells, but H3K27 methylation did not appear to be a factor.
- Analysis of genes altered by GSK-126 treatment revealed many cell cycle regulators.
- The growth and survival of the CRPC cell line LNCaP-abl, is “androgen-independent.” Growth of these cells is only inhibited by high levels of the androgen receptor inhibitor enzalutamide.
- At low doses of enzalutamide that inhibit the androgen-dependent growth of LNCaP cells yet hardly affect LNCaP-abl cells, the addition of GSK-126 inhibited CRPC cell growth to a greater degree than treatment with either enzalutamide or GSK-126 alone.
- Thus, inhibiting both EZH2 and the androgen receptor (AR) simultaneously may provide a therapeutic strategy for the treatment of CRPC, and EZH2 phosphorylation at S21 may be a predictive biomarker for the utility of EZH2 inhibitors in this disease.



**Figure 20:** EZH2 expression (left) and EZH2 phosphorylation on serine-21 (pEZH2(S21), middle) was increased while H3K27 methylation (right) was decreased in CRPC tumors vs. primary prostate cancer (PCA) or post ADT-treated tumors from patients. The brown color indicates intensities of the indicated markers, which are quantitated in the graphs below. Figures from: Xu et al., *Science*. 2012, Dec 14;338(6113):1465-9.

## Targeting the Prostate Cancer Epigenome for Development of Biomarkers and Therapeutics

Vasan Yegnasubramanian, MD, PhD  
Johns Hopkins University

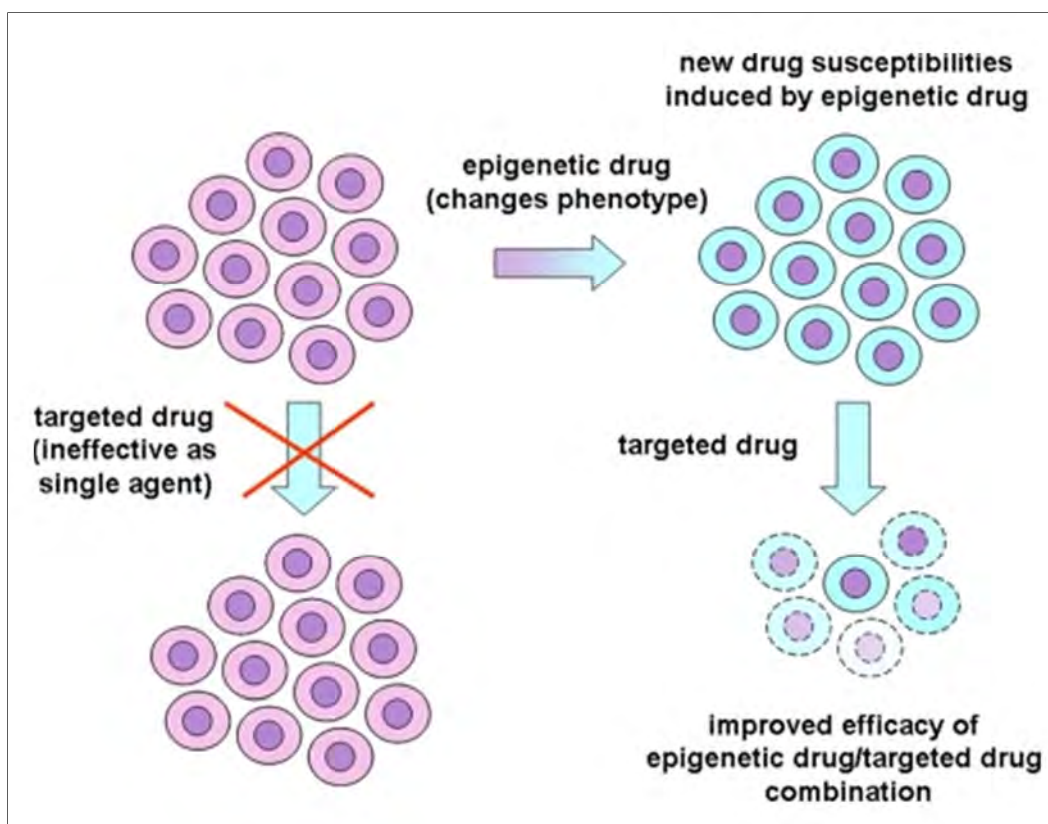
2011 A. David Mazzone-PCF Challenge Award  
2013-2015 Movember-PCF Challenge Award

- Epigenetics is a major mechanism of gene regulation that involves the chemical modification of DNA and the proteins that organize DNA.
- Chromosomal DNA is wound around protein bodies called histones, to create genomic structure. Epigenetic regulation includes the addition and subtraction of activating vs. repressing types of chemical modifications, such as methylation, to the DNA and to histones. Such modifications, or epigenetic marks, can regulate how tightly wound the DNA is, and therefore how accessible genes in that genomic region are for transcription.
- Epigenetic configurations are heritable as cells divide. This is a critical mechanism governing the maintenance of cell type identity.
- Epigenetics of prostate cancer is an important area of study for understanding disease biology and identifying therapeutic targets.
- For example, a urine test evaluating methylation patterns of three genes, significantly outperformed serum PSA tests in predicting positive results of diagnostic biopsies for prostate cancer. In this test, the positive predictive value was 100% (positive results were correct 100% of the time), while the negative predictive value was 79% (negative results were correct 79% of the time, but missed cancers in the other 21%). Thus, while epigenetic tests have already started to outperform PSA screening in some clinical trials, significant improvements need to be made in developing epigenetics-based diagnostic tests to realize their full potential.
- Dr. Vasan Yegnasubramanian is studying the “epigenetic cityscape” of prostate cancer to develop clinically applicable biomarkers and identify drug targets. He asks whether epigenetic factors are therapeutically targetable, the degree to which epigenetics can be inherited or altered, and the role that epigenetic maintenance plays in clonal selection during tumor progression and therapeutic resistance.
- By using a series of genomics and informatics methods, Dr. Yegnasubramanian has created a map of hyper-methylated and hypo-methylated regions of the prostate cancer genome. These patterns were compared across patients and in multiple metastatic sites from the same patients. Methylation patterns were



highly maintained across metastatic sites from the same individual, while between individuals there was significant heterogeneity.

- Genes with consistent methylation patterns in prostate cancer metastases included EDNRB and PTGS2. The degree of hyper-methylation of these genes was highly associated with late stage and high Gleason score cancers, and PTGS2 methylation strongly predicted the risk of cancer recurrence.
- Some anti-cancer drugs work by altering epigenetic states. However, reactivation of oncogenes or suppression of anti-tumor genes will reduce the effectiveness of these drugs. A goal of Dr. Yegnasubramanian's studies is the "induction of synthetic lethality with epigenetic therapy" (ISLET). In other words, targeting genes whose expression is activated by epigenetic therapy, to create therapeutic synergy.
- For example, expression of the oncogenic gene COX2 was reactivated by several epigenetic drugs. Combining a COX2 inhibitor (Celecoxib) with these drugs significantly enhanced blockade of tumor cell growth.
- In another example, epigenetic drugs reactivated expression of anti-tumor retinoic acid response genes, and when combined with activators of this pathway, tumor cell inhibition was enhanced.
- To discover more drug targets, prostate cancer cells were screened for genes whose inhibition would synergize with epigenetic drugs. In this assay system, a population of prostate cancer cells was fed a "library" of gene-silencing shRNA molecules, each inhibiting the expression of a different gene in different cells. The population was then treated with the DNA methyltransferase inhibitor, decitabine. Cells that survived were screened for the shRNA molecules that "dropped out," indicating that inhibition of those genes synergized with decitabine to promote cell death. ~100 genes were identified as potential synergistic killing targets in combination with decitabine.
- The Aurora A kinase (AURKA), a regulator of cell division, was a candidate target identified, with available clinical inhibitors. AURKA inhibitors synergized with decitabine in blocking prostate cancer cell growth *in vitro* and tumor growth in mice.
- Thus, these studies have mapped the epigenome of prostate cancer cells and have demonstrated that synergy can be achieved between epigenetic drugs and other targeted drugs.
- Ongoing studies are exploring additional synergistic drug targets. Clinical trials in lung cancer have observed enhanced responses in patients who received epigenetic therapy, and later were treated with the immunotherapeutic drug anti-PD1. Thus, combining epigenetic drugs with immunotherapy may have therapeutic promise and is being further evaluated.



**Figure 21:** Induction of synthetic lethality with epigenetic therapy (ISLET): Drugs that alter epigenetic states may be ineffective alone. Combining this treatment with drugs that target genes whose expression is reactivated by epigenetic therapy will create therapeutic synergy.

## Session 7 – What This Means for Patients:

Epigenetics is the study of chemical modification of DNA that regulates expression of genes. For example, different genes are expressed in the liver than the brain, making these two organs unique. Dr. Myles Brown presented data concerning the role of EZH2, a master regulator of the cell epigenome, and the mechanisms by which EZH2 causes androgen-independent growth of castrate-resistant prostate cancer cells. Dr. Vasanth Yegnasubramanian is studying the reactivation of genes previously silenced by cellular epigenetic changes. In this research, he has discovered approximately 100 genes may be targetable for treatment. Clinical trials with targeted medicines as well as immunotherapeutic approaches will be initiated based on this research.

## Session 8: Predictive Biomarkers for Prostate Cancer Progression and Survival

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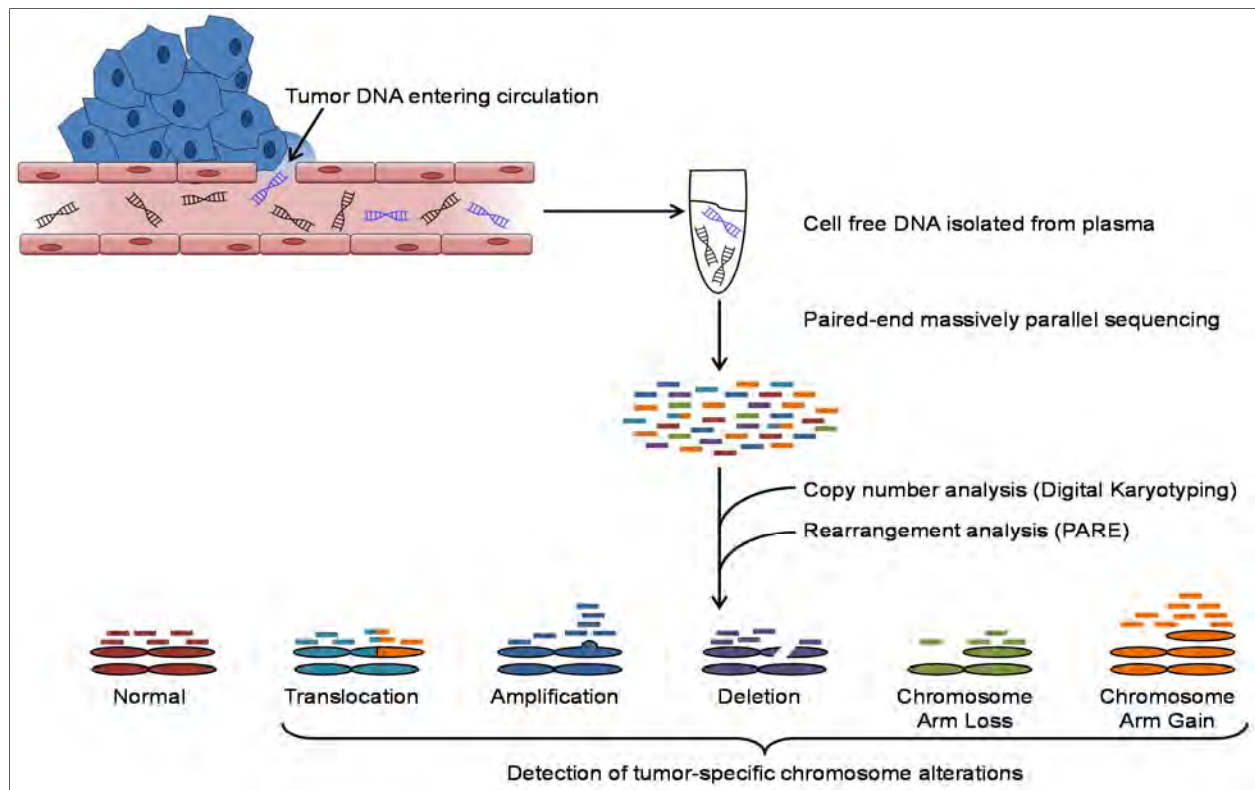
### Personalized Genomic Biomarkers for Cancer Progression and Survival

**Victor Velculescu, MD, PhD**  
**Johns Hopkins Kimmel Cancer Center**

- Precision or personalized medicine in cancer treatment is the application of patient-specific therapeutic strategies based on phenotypic, molecular, and genomic characteristics of the patient and patient's tumors.
- An additional arm of precision medicine is the development of personalized biomarkers to assess therapeutic responses and disease progression.
- Dr. Victor Velculescu discussed the development of blood-based genomic analyses as "liquid biopsies." Each cancer patient's tumor not only has a unique mutational landscape, but significant genetic heterogeneity within the cancer cell population.
- Both circulating tumor cells (CTCs) and shed tumor DNA can be detected and isolated from a patient's blood, allowing genetic analysis of the tumor.
- Plasma from patient blood, which contains shed tumor DNA, was assessed for unique genetic alterations.
- DNA copy number alterations were observed in colorectal and breast cancer patient plasma but not in plasma from healthy donors, and a scoring method was developed based on the number of alterations. In a colorectal cancer patient with no disease detectable by standard clinical examination, DNA copy number alterations could be detected. This patient later had a recurrence, demonstrating the sensitivity of this assay.
- Chromosomal rearrangements were detected in the plasma from patients with various cancer types, while no rearrangements were found in plasma or peripheral blood immune cells from healthy individuals. The utility of this analysis was examined as a monitor of tumor progression. In one example, the fraction of rearranged DNA in plasma followed the clinical course of a colorectal cancer patient undergoing therapy, dropping following surgical removal of the tumor, rising thereafter, and again falling with chemotherapy and surgical removal of a liver metastasis.
- These analyses can be used to identify patient-specific alterations that may present unique actionable targets or identify disease-resistance mechanisms

following therapy. For instance, amplification of HER2 in a colorectal cancer patient, a rare event, may indicate the use of anti-HER2 therapy in this patient.

- As a comparison to circulating tumor cells, more tumor DNA could be obtained from total plasma than from CTCs alone, indicating that similar assays can be done on CTCs, but plasma offers enhanced detection ability.
- Thus, these methods allow sensitive and patient-specific noninvasive assessment of cancer progression and tumor genomic characteristics, lending to the implementation of early cancer detection and precision medicine for both treatment and monitoring.



**Figure 22:** DNA is shed from tumor cells and enters the circulation from which it can be isolated and analyzed for genomic properties including copy number alterations and chromosomal rearrangements. Figure is from: Leary RJ, et al., *Science Translational Medicine*. 2012, Nov 28;4(162).

## Circulating DNA and MicroRNAs as Biomarkers in Cancer Patients

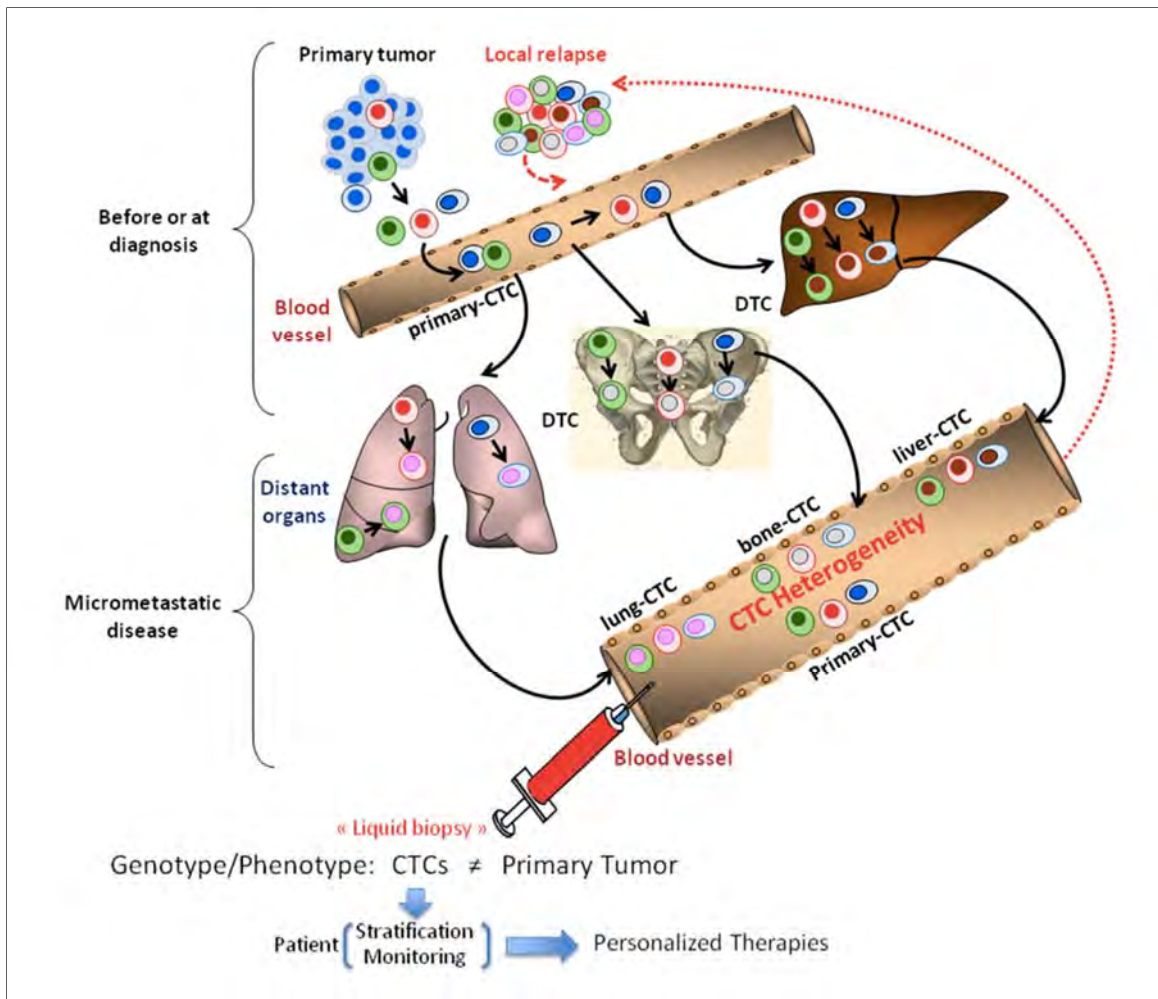
Klaus Pantel, MD, PhD

University Medical Center Hamburg

- Prostate cancer most often metastasizes to the bone and lymph nodes, followed by the lungs, liver, and brain. Compared with the primary tumor, metastatic tumors acquire new phenotypes and molecular and genetic aberrations, which are critical to characterize for successful treatment. However, biopsies of metastatic sites can be painful and difficult, if not impossible.
- Dr. Klaus Pantel discussed the application of “liquid biopsies,” to gain insight into tumor characteristics and as diagnostic or prognostic tools. From patient plasma, it is possible to obtain cells and molecules that have originated from tumors and have entered the circulation. These include circulating tumor cells (CTCs) that have left primary or metastatic sites, and tumor DNA and RNA shed from dying tumor cells.
- Both CTCs and circulating tumor DNA can be assessed for genomic anomalies including mutations, copy number aberrations, and genomic translocations.
- CTCs can also be analyzed for in-depth molecular and phenotypic characteristics including gene expression and growth *in vitro* or *in vivo*.
- Circulating levels of a number of microRNAs, a type of regulatory RNA molecule, have been demonstrated to have diagnostic as well as prognostic potential. For instance, discrimination between benign and malignant tumors was demonstrated for breast and lung cancer.
- One challenge with these assays is that CTC numbers and the concentrations of circulating DNA can often be low and thus difficult to detect or obtain enough of for high quality assays, particularly in patients with early stage cancer or a low tumor burden.
- ERA-NET TRANSCAN is a collaborative effort between researchers in Germany, France, Greece, Poland, and Austria, to detect and assess CTCs from prostate cancer patients. In a cohort of early stage prostate cancer patients, those with no detectable CTCs had a significantly better PSA recurrence-free survival than patients with any CTCs detectable by the FDA-approved CellSearch assay (Janssen/Veridex, USA).
- In a new approach to collect a higher numbers of CTCs from patients, an *in vivo* capture method was engineered, in which a nanodetector capture apparatus (Gilupi, Germany) is inserted into a patient's vein. The inserted tip is coated with antibodies targeting an epithelial cell marker, EpCAM, expressed by cancer cells. Over the course of 30 minutes, CTCs in the bloodstream that flow past the nanodetector are captured by the antibodies, thus enabling the collection of significantly more CTCs than would be obtained from a single blood draw.



- These and other methods of liquid biopsy will allow for the development of precision medicine decision making platforms, particularly useful in metastatic cancer patients.

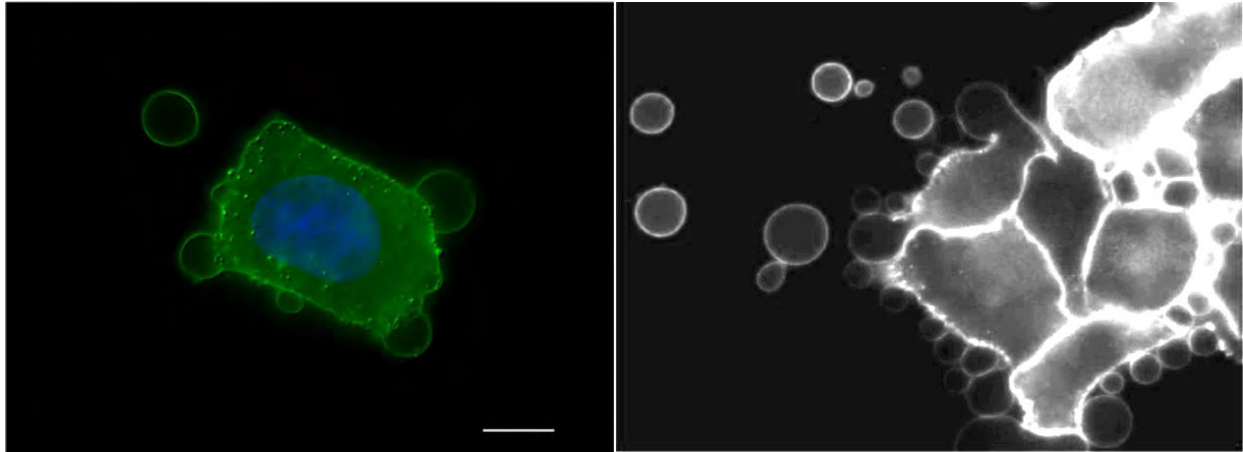


**Figure 23:** The principles of liquid biopsies: Circulating tumor cells (CTCs) that have left primary or metastatic sites can be collected from patient plasma with a blood draw. These can be assessed for genomic anomalies and molecular and phenotypic properties to discover tumor characteristics that support precision medicine decisions. Figure from Alix-Panabieres and Pantel, *Clin. Chem.* 2013.

## Circulating Large Oncosome Profiling and Prostate Cancer Progression

**Dolores Di Vizio, MD, PhD**  
**Cedars-Sinai Medical Center**

- Cancer cells can release various types of extracellular vesicles (EV) into the surrounding environment. These include exosomes, which are 50-100nm in diameter, and large oncosomes, which are 1-10uM in diameter.
- EVs contain various types of intracellular molecules and nucleic acids and are a means of cell-cell communication, as contents are transferred from one cell to another.
- Dr. Dolores Di Vizio discussed her studies identifying and characterizing oncosomes released from prostate cancer cells. When added to other cell types, these oncosomes could activate signaling cascades, gene expression, and promote activity including cell migration.
- Oncosomes can be visualized as non-apoptotic membrane blebs emerging from prostate cancer cells, and were detectable in tissue sections from patients with prostate cancer.
- The frequency and number of oncosomes increased with disease burden and tumor progression. The prevalence of oncosomes increased in patients with high grade cancer (Gleason score >7) or with castrate resistant prostate cancer, and in mice with progressive prostate cancer.
- Microscopic analyses showed that when added to stromal cells, oncosomes were able to enter cells and appeared to reach the cell's nucleus.
- Proteomic analysis of the contents of oncosomes compared with the cells they were derived from, found an enrichment of proteins involved in cytokinesis, motility, and secretion.
- The contents of oncosomes were significantly different than smaller microvesicles, with certain proteins found only in one vesicle type but not the other. This indicates specificity in formation and function of these unique microvesicles.
- Oncosomes also carry nucleic acids including microRNA and DNA. Genomic profiling of oncosome DNA found the same mutations as in the tumor cells.
- These studies indicate that oncosomes play a significant and specific role in prostate cancer progression, and have prognostic value. Further studies will reveal additional biology and the clinical significance of these recently discovered structures.



**Figure 24:** Oncosomes emerging from tumor cells.

## **Session 8 – What This Means for Patients:**

Evaluation of circulating tumor cells (CTC) and circulating tumor DNA are emerging methodologies that allow the analysis of solid tumor characteristics without the necessity of an invasive biopsy procedure. These liquid biopsy technologies will greatly enable precision medicine, the determination of best treatment for individual patients. Furthermore, it is believed that measuring CTCs and circulating tumor DNA will provide new endpoints to facilitate clinical trials. The utility of CTC enumeration as a surrogate biomarker of survival would greatly speed clinical trials and eliminate the need for years of follow-up to measure the effect on overall survival.

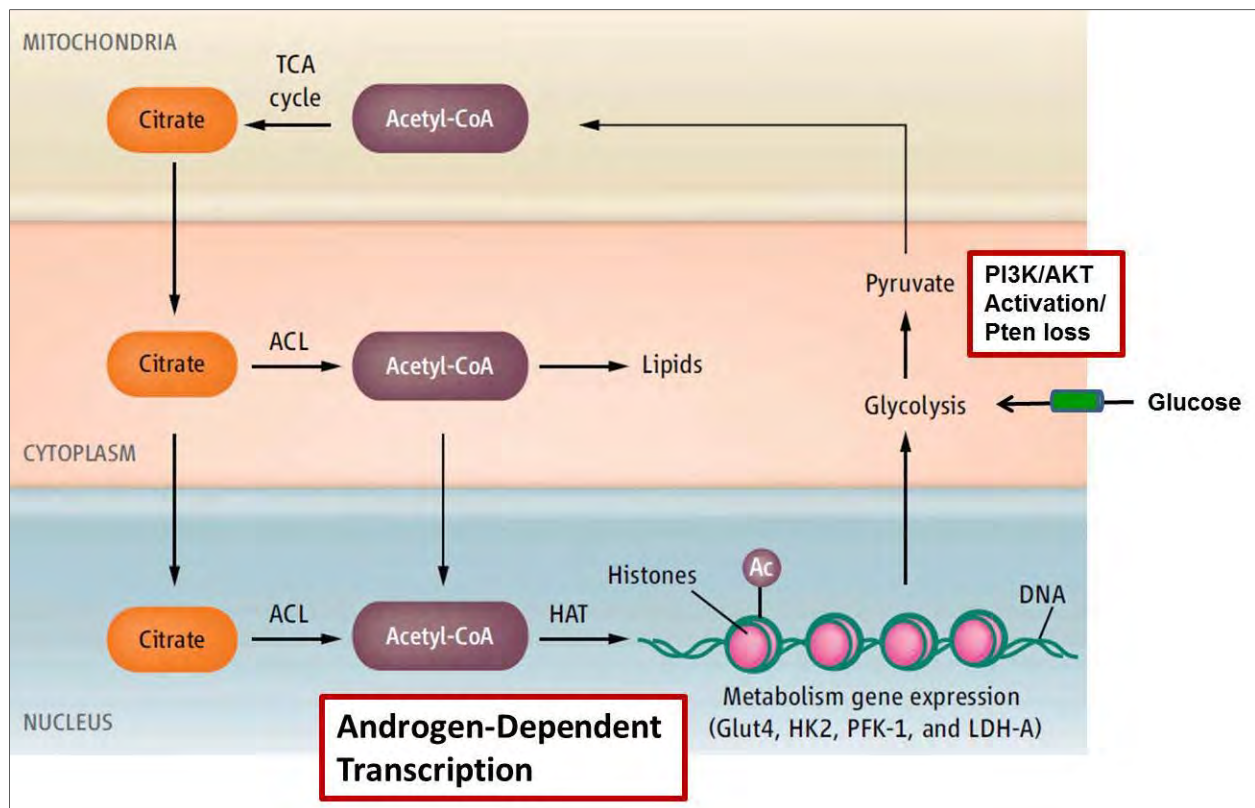
# Special Lecture: Prostate Epithelium—Why is it Androgen-Dependent?

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**Craig Thompson, MD**  
**Memorial Sloan-Kettering Cancer Center**

- Multicellular organisms are composed of a multitude of cell types organized in a very specific way. This “social system” includes a built-in blockade against cells making autonomous decisions that would compromise the organism’s biology.
- To ensure maintenance of this social/environmental dependency, cells require specific extrinsic signals for continued functions. Loss of these signals leads to “death by neglect.”
- For example, if cells lose the ability to take up glucose, they begin to access stored intracellular metabolites. This begins a “clock,” in which the activation of cellular stress pathways will either induce programmed cell death (apoptosis) within days, or turn on an alternate autophagy-dependent survival pathway, in which cellular proteins are degraded and recycled, maintaining survival for another 3-4 weeks, before the cell ultimately dies.
- For cellular proliferation, two signals are required: a growth signal and fuel signal, thus enacting a dual barrier against unchecked proliferation. However, cancer cells acquire mechanisms to bypass these “rules of conduct.”
- Cancer cells metabolize glucose at a higher rate than any other cell type in the body except for the brain. This phenomenon is the basis for PET imaging for the detection of cancer, in which a radioactively-labeled glucose analog (<sup>18</sup>Fluoro-2-deoxyglucose) is administered and cells with the greatest glucose metabolism rates are imaged.
- Dr. Craig Thompson discussed his studies on why prostate cancers are dependent on the androgen receptor (AR) pathway: both the growth and fuel signal for prostate cells require expression of genes regulated by the AR transcription factor.
- The metabolism of glucose for cell growth requires the PI3K/AKT/TOR pathway. However, in prostate cells, this pathway additionally requires AR.
- AR was found to regulate many genes required for glucose uptake and metabolism.
- Thus, for prostate cancer to develop, metabolism pathways are altered by evolving either mechanisms for hyper-activation of PI3K/AKT, or by inactivating PTEN, a suppressor of AR, thus enabling an enhancement in the function of AR.

- Acetyl-CoA is a product of glucose metabolism and acts as a signal of glucose-metabolism rates.
- The ability of transcription factors to access DNA and transcribe genes, is regulated by histone acetylation, a process where acetyl groups are attached to DNA-associated proteins (histones). Acetylation of histones loosens their interactions with DNA, allowing increased transcription factor activity.
- Acetyl-CoA is a source of these acetyl groups, and therefore, higher glucose metabolism increases acetyl-CoA levels and increases gene transcription. This process was found to specifically regulate the expression of genes involved in glucose metabolism, including those by AR.
- Overall, these studies demonstrate that prostate cells rely on AR for transcription of glucose metabolism genes, and in turn, require glucose metabolism for AR transcriptional activity. These processes are enhanced in cancer cells by mutations that accelerate this pathway to enable unrestrained growth and proliferation of prostate cancer.



**Figure 25:** Prostate cells rely on the androgen receptor (AR) for transcription of glucose metabolism genes. In turn, AR transcriptional activity relies on glucose metabolism to produce Acetyl-CoA, used for histone acetylation which allows gene transcription. These processes are enhanced in prostate cancer cells by mutations (PI3K/AKT activation or PTEN loss) that accelerate this pathway, thereby allowing unrestrained growth and proliferation of prostate cancer cells. Figure adapted from Rathmell and Newgard, *Science*, 2009.



## What This Means for Patients:

Tumor cells acquire the ability to thrive and survive by metabolizing glucose at a higher rate than any other tissue in the body except for the human brain. Dr. Thompson has invested his scientific career in understanding the nature of metabolic dysfunction in cancer. He has described numerous biochemical pathways and survival mechanisms related to tumor cell metabolism. In prostate cancer, Dr. Thompson has determined that the androgen receptor (the engine that drives prostate cancer) is a master metabolic regulator. This knowledge will result in new therapeutic targets for the treatment of advanced prostate cancer.

## Session 9: Understanding Mechanisms of Prostate Cancer Initiation and Progression

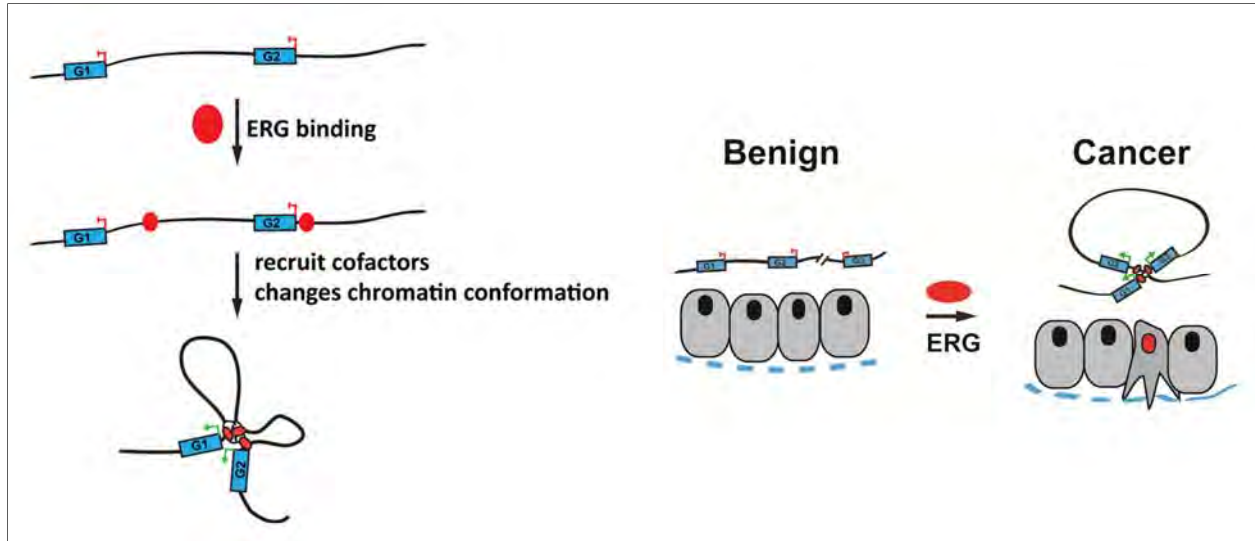
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### Implications of ERG-Mediated Alterations in Chromatin Conformation

**David Rickman, PhD**  
**Memorial Sloan-Kettering Cancer Center**

- Each type of cancer is often associated with mutations in specific genes or pathways. In prostate cancer, chromosomal fusion between two genes, TMPRSS2 and ERG (TMPRSS2:ERG), is an early and common event.
- The expression of TMPRSS2 is induced by the androgen receptor (AR), the major transcription factor required for normal and cancerous prostate cells.
- This fusion results in an aberrantly high expression of the oncogenic ERG transcription factor in prostate cells, now driven by AR.
- Dr. David Rickman discussed the relevance of high ERG levels in prostate cancer. To understand how altered gene expression regulated by ERG promotes associated phenotypes (e.g. cell invasion), the genomic sites that ERG binds were mapped in prostate cells that overexpressed ERG.
- ERG was found to bind to gene promoters and introns, but primarily to distal enhancers: regulatory regions located further than 50kb from the transcriptional start sites of genes.
- Dr. Rickman hypothesized that ERG might be functioning at these distant sites to affect gene expression by altering the conformation of DNA.

- A genome-wide “chromosome configuration capture” method (Hi-C) was performed, which identifies close physical associations between regions of the genome. ERG was found to promote specific long-range interactions between sites on the same chromosome and on different chromosomes.
- ERG also affected physical associations between local genomic regions.
- Additional studies assessed the genomic locations that ERG binds to, and the genes expressed with high levels of ERG.
- ERG was found to coordinate expression of genes that it bound both locally and distantly.
- These genes are involved in processes including cell adhesion, migration, skeletal and urogenital development, structure, localization, and embryonic morphogenesis.
- The involvement of co-factors in ERG activities was assessed. Interactions between ERG and the transcription factor AP-1 were found at a significant number of transcriptionally active genomic sites in promoters and distal enhancers.
- Overall, these studies find that overexpression of ERG by the TMPRSS2:ERG fusion in prostate cancer cells affects distant and local chromosome topology to coordinate expression of oncogenic genes.



**Figure 26:** ERG binds to gene-regulatory sites of DNA to alter distant and local chromosome configurations. This occurs in prostate cancer cells in which ERG is overexpressed to promote expression of oncogenic genes.

## Context-Specific Oncogenesis of Aberrantly Expressed Transcription Factors in Prostate Cancer

**Yu Chen, MD, PhD**

**Memorial Sloan-Kettering Cancer Center**

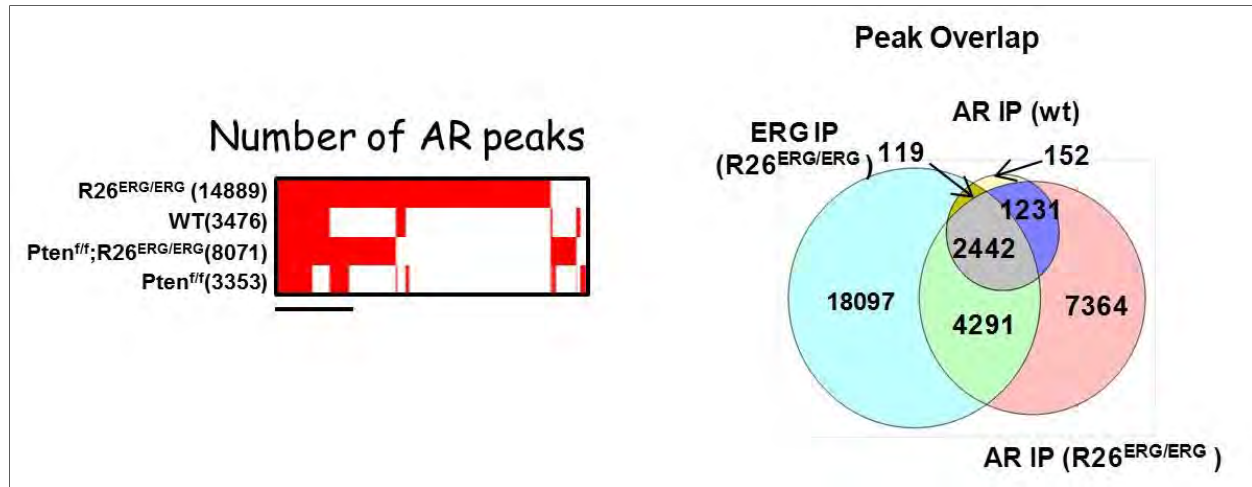
**PCF Young Investigator Award, 2011**

**Movember-PCF Challenge Award, 2013-2015**

- Chromosomal translocations that results in the fusion of TMPRSS2 and ERG (TMPRSS2:ERG), are a common event in prostate cancer. These translocations are found in the earliest form of pre-malignancy, prostatic intraepithelial neoplasia (PIN).
- The promoter of TMPRSS2 is androgen-regulated, and thus TMPRSS2:ERG fusions result in ERG overexpression in prostate cells. ERG is a transcription factor, and its common and early overexpression in prostate cancer indicates a role in cellular transformation.
- However, expression of TMPRSS2:ERG in mice did not lead to prostate cancer progression beyond the PIN stage, suggesting additional factors are needed for the development of carcinomas.
- Dr. Yu Chen discussed studies examining this context-dependent role of ERG in the development of prostate cancer.
- Genetic analysis of prostate cancer patient tumors revealed that loss of the tumor-suppressor gene PTEN commonly co-occurs with TMPRSS2:ERG fusions. A mouse model was developed in which TMPRSS2:ERG could be conditionally expressed in prostate cells of normal mice versus mice genetically lacking PTEN.
- TMPRSS2:ERG expression led to mild PINs in normal mice upon reaching advanced age. However, in mice lacking PTEN, TMPRSS2:ERG caused high grade, aggressive tumors that developed earlier and resulted in significantly reduced survival times.
- Normal and cancerous prostate cells require expression of genes regulated by the androgen receptor (AR) transcription factor for growth and survival. PTEN is a suppressor of AR. Thus the relationships between AR, ERG, and PTEN in prostate cancer were explored.
- Using an assay which detects the regions of DNA bound by transcription factors, the regions bound by ERG and AR were assessed in prostate cells from normal mice, mice lacking PTEN or expressing TMPRSS2:ERG, and mice lacking PTEN and expressing TMPRSS2:ERG. Significantly more binding of AR to genomic sites occurred in the presence of TMPRSS2:ERG expression, and a subset of unique sites was bound when PTEN deletion was combined with TMPRSS2:ERG

expression. ERG and AR were also found to have many overlapping site interactions.

- Thus, ERG appears to enhance the transcriptional activity of AR, and both maintains AR activity and promotes unique functions of AR when PTEN is deleted.



**Figure 27:** Left: A map and number of genomic regions bound by AR (red) in prostate cells from normal mice (WT), mice lacking PTEN (Pten<sup>f/f</sup>), mice expressing TMPRSS2:ERG (R26<sup>ERG/ERG</sup>), and mice both lacking PTEN and expressing TMPRSS2:ERG (Pten<sup>f/f</sup>;R26<sup>ERG/ERG</sup>). Significantly more binding of AR to genomic sites occurred in the presence of TMPRSS2:ERG expression, and a subset of unique sites was bound when PTEN deletion was combined with TMPRSS2:ERG expression. Right: An overlay of shared and unique genomic regions bound by AR (AR IP) in normal mice and mice expressing TMPRSS2:ERG, and sites bound by ERG (ERG IP) in mice expressing TMPRSS2:ERG.

## ERG Influences Cell Fate Decisions in Prostate Cancer

Ray Pagliarini, PhD

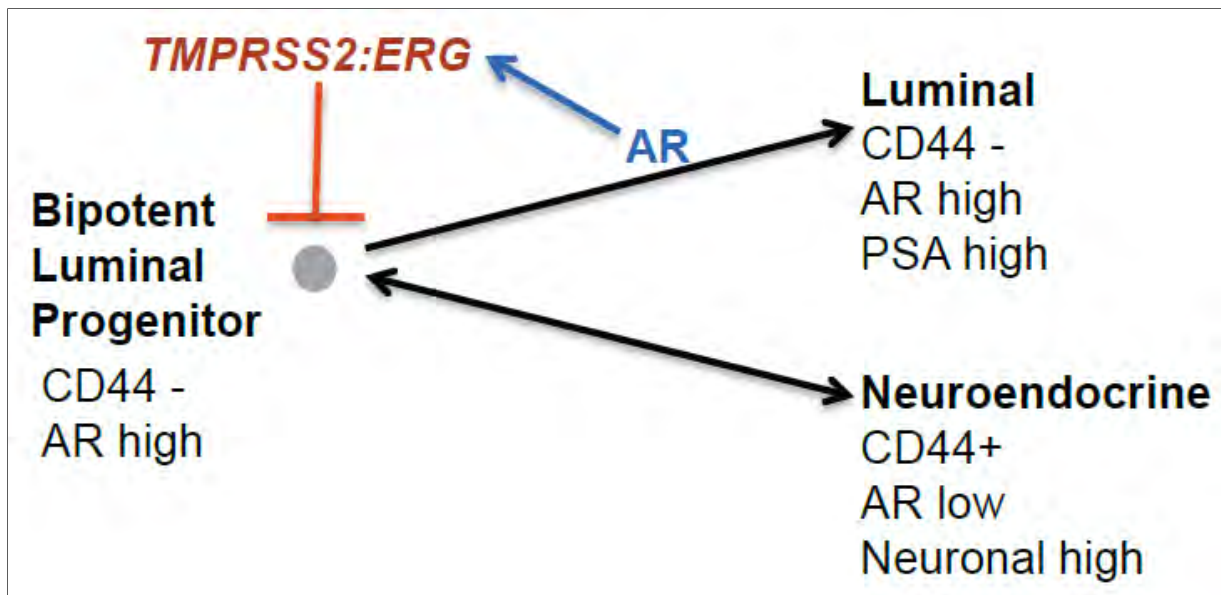
Novartis Institutes for BioMedical Research

- A fusion between the TMPRSS2 and ERG genes (TMPRSS2:ERG) due to chromosomal translocations, are a common and early event in prostate cancer. TMPRSS2:ERG fusions result in overexpression of the ERG transcription factor in prostate cells due to its now being under control of the TMPRSS2 promoter, which is regulated by the androgen receptor (AR). AR is the main transcription factor driving growth and survival of normal and cancerous prostate cells.
- Prostate cancers from different individuals can be classified into subtypes based on molecular and phenotypic characteristics. Most prostate tumors are of the luminal adenocarcinoma subtype. Some aggressive late-stage tumors that arise

following the development of resistance to androgen-deprivation therapy are of a neuroendocrine subtype, in which the prostate cancer cells have taken on the features of neuron tissue.

- Dr. Ray Pagliarini discussed his studies on understanding how the TMPRSS2:ERG fusion contributes to cell-fate decisions in prostate cancer.
- TMPRSS2:ERG expression in mouse prostate tissue lead to development of a hyperplastic phenotype with disorganized epithelial morphology. However, this genetic aberration alone was unable to promote progression to malignancies.
- Suppression of ERG expression in VCaP cells, a human prostate cancer cell line that harbors the TMPRSS2:ERG fusion, lead to a reduction in cell proliferation.
- To determine the effects of ERG overexpression, the genes regulated by TMPRSS2:ERG were examined in both human prostate cancer cell lines and mice.
- In both species, ERG repressed expression of neuronal genes. ERG was able to bind to the regulatory genomic regions of these genes, while AR could not. This indicates that ERG directly suppresses the expression of neuroendocrine genes in prostate cancer cells.
- As the TMPRSS2:ERG promoter is regulated by AR, inhibition of AR reduced the ability of ERG to suppress the expression of neuroendocrine genes. Thus, by functioning to promote TMPRSS2:ERG activity, AR may indirectly inhibit neuronal gene expression.
- ERG also blocked expression of genes characteristic of luminal cells, which are transcriptionally regulated by AR.
- Inhibition of ERG expression in VCaP cells led to differentiation of these cells into both luminal and neuroendocrine populations.
- Thus, ERG appears to arrest prostate cancer cells in a bi-potent progenitor state. When ERG is suppressed, luminal cells and neuroendocrine differentiation can occur.
- Thus, the decision made by prostate cancer cells to differentiate into luminal or neuroendocrine phenotypes depends on activities mediated by TMPRSS2:ERG and AR.
- Implications for how this function of ERG may affect responses to anti-androgen therapy were discussed.





**Figure 28:** ERG overexpression (via the TMPRSS2:ERG fusion) arrests prostate cancer cells in a bi-potent luminal progenitor phenotype by inhibiting differentiation into luminal and neuroendocrine phenotypes. When ERG is suppressed, AR promotes differentiation into luminal cells, while in the absence of both AR and ERG, neuroendocrine differentiation can occur.

## Session 9 – What This Means for Patients:

The discovery of the androgen-regulated TMPRSS2:ERG gene fusion in prostate cancer by Tomlins et al., (*Science*, 2005;310(5748):644-8). has led to an explosion of a data on this topic. Each of these talks describes a unique function of the gene fusion for the progression of prostate cancer. Investigations such as these have credentialed the ERG transcription factor as a therapeutic target for the treatment of advanced prostate cancer. We look forward to studies in the near future on this new class of medicines.

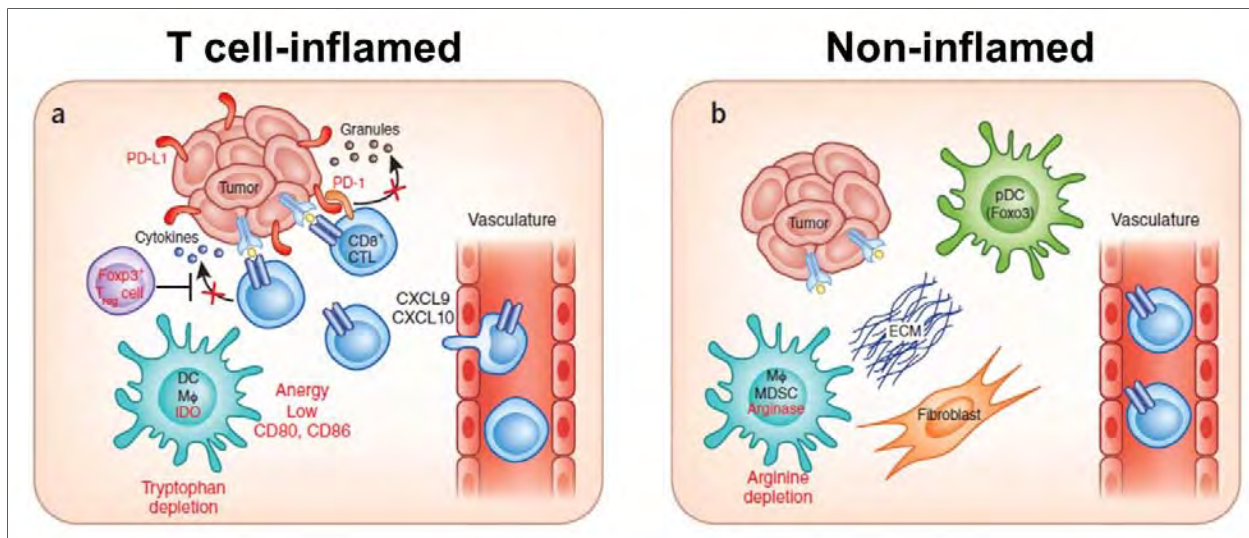
# Special Lecture: Immunotherapy for Solid Tumors with Implications for Prostate Cancer

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**Thomas Gajewski, MD, PhD**  
**University of Chicago**

- Cancer cells live within a microenvironment composed not only of cancer cells, but supportive endothelial and stromal cells of various lineages as well as tumor-promoting and tumor-inhibiting immune cell populations.
- In several cancer types, the presence and absence of certain immune populations is highly prognostic. Generally, CD8 T cells are beneficial tumor-killing cells, while negative regulatory CD4 T cells (Tregs), which are functionally different from other CD4 T cells, are considered detrimental to immune-mediated tumor control.
- Multiple mechanisms can turn on or off anti-tumor immune cell functions, many of which are being assessed in clinical trials. “Immune checkpoint inhibitors,” target a group of signaling pathways that turn off the activity of T cells, and include the PD1 pathway.
- Multiple immunotherapy clinical trials using antibody drugs that block the PD1 molecule on T cells or PD-L1 (the PD1 activating ligand) expressed on other cells in the tumor microenvironment, have shown significant clinical benefit in multiple cancer types. However, not all patients benefit from these or other immunotherapies.
- Dr. Thomas Gajewski discussed studies in which melanoma patients who responded vs. did not respond to immunotherapies were assessed for gene expression profiling of the tumor microenvironment.
- In patients with CD8 T cells in their tumor, the mechanism of immunotherapy failure was associated with immune inhibition through mechanisms including enhancement in PD-L1, Tregs, and IDO – an enzyme which degrades tryptophan required for T cell functions, and the induction of a state of T cell non-responsiveness (anergy or exhaustion).
- In patients with tumors lacking CD8 T cells, immune cell exclusion from the tumor appeared to be the cause of immunotherapy failure.
- Interestingly, the presence of CD8 T cells in tumors correlated with the presence of Tregs, as well as expression of PD-L1 and IDO. In mice, CD8 T cells were required for expression of PD-L1 and IDO in the tumor and were responsible for the recruitment of Tregs.

- This suggests that although CD8 T cells are capable of killing tumor cells, they also activate negative feedback mechanisms for their own suppression, which contribute to the failure of immunotherapies.
- Thus, combining immunotherapeutic drugs that promote CD8 T cell activity with those that inhibit negative feedback mechanisms are likely to be highly synergistic.
- To explore this possibility, mice with tumors were given various combinations of immune-modulating therapies that target different aspects of immune activation and suppression. For example, inhibiting both PD-L1 and IDO synergistically inhibited tumor growth and improved CD8 T cell numbers and functions within the tumor.
- Many of these immunotherapeutic combinations are being tested in clinical trials of patients with various cancers.
- Striking clinical trial results demonstrated that combinatorial treatment with antibodies blocking immune checkpoint inhibitors PD-1 and CTLA-4, led to faster, more pronounced, and durable tumor regression compared with targeting either inhibitor alone.
- Dr. Gajewski is defining optimal conditions under which anti-tumor T cells can be activated, as well as the mechanisms by which tumors act to block this. A class of cytokines called interferons (IFN) is required for immune cell activation. IFNs are expressed by innate immune cells upon encountering certain pathogen or damage-associated molecules, including extracellular DNA.
- Radiation therapy leads to the release of DNA by dying tumor cells. A single dose of radiation treatment in melanoma tumor-bearing mice led to production of IFNs, T cell activation, and tumor control. Interestingly, the anti-tumor effect of radiation required IFNs, indicating that activation of anti-tumor immune cells is a primary mechanism of tumor control by radiation therapy.
- Of note, the immunophenotype of prostate cancer is relatively unstudied. CD8 T cells have been observed in primary prostate tumors. However, more studies are needed to characterize immunophenotypes of prostate cancer metastatic sites, including the bone.
- Overall, immunotherapy is a highly promising new avenue for cancer treatment. Synergy has been demonstrated with combinations of immunotherapeutics that target different elements of the immune system. Additionally, the effects of immunotherapies in combination with radiation therapy and chemotherapy are being studied in multiple cancer types including prostate cancer.



**Figure 29:** Model of the immune environment of T cell-infiltrated and non-infiltrated tumors: Left: In tumors infiltrated by CD8 T cells (blue), immunotherapy failure may be attributed to immune inhibition through enhancement in PD-L1 expression, Tregs, IDO, and the induction of T cell non-responsiveness (anergy or exhaustion). Right: In non-inflamed tumors, CD8 T cell exclusion from the tumor may be the cause of immunotherapy failure. Figure from: Gajewski et al., *Nature Immunology*, 2013.

## What This Means for Patients:

Prostate cancer appears to be less sensitive to treatment by immunotherapy than other human solid tumors such as melanoma and lung cancer. Dr. Thomas Gajewski has shown that in melanoma patients who failed to respond to immunotherapies, resistance is due to either immune cell inhibition or immune cell exclusion from the tumor. Combinatorial strategies to optimally activate immune cells while blocking immune-inhibitory responses, thereby enhancing the efficacy of immunotherapy, are being tested in clinical trials.

## **Session 10: Reports from PCF Scientific Working Groups**

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### **Scientific Working Group for the Neuroendocrine Prostate Cancer Pathology Workshop – Meeting held on July 31, 2013 at Weill Cornell Medical College**

**Mark Rubin, MD**  
**Weill Cornell Medical College**

**PCF Challenge Award, 2011**

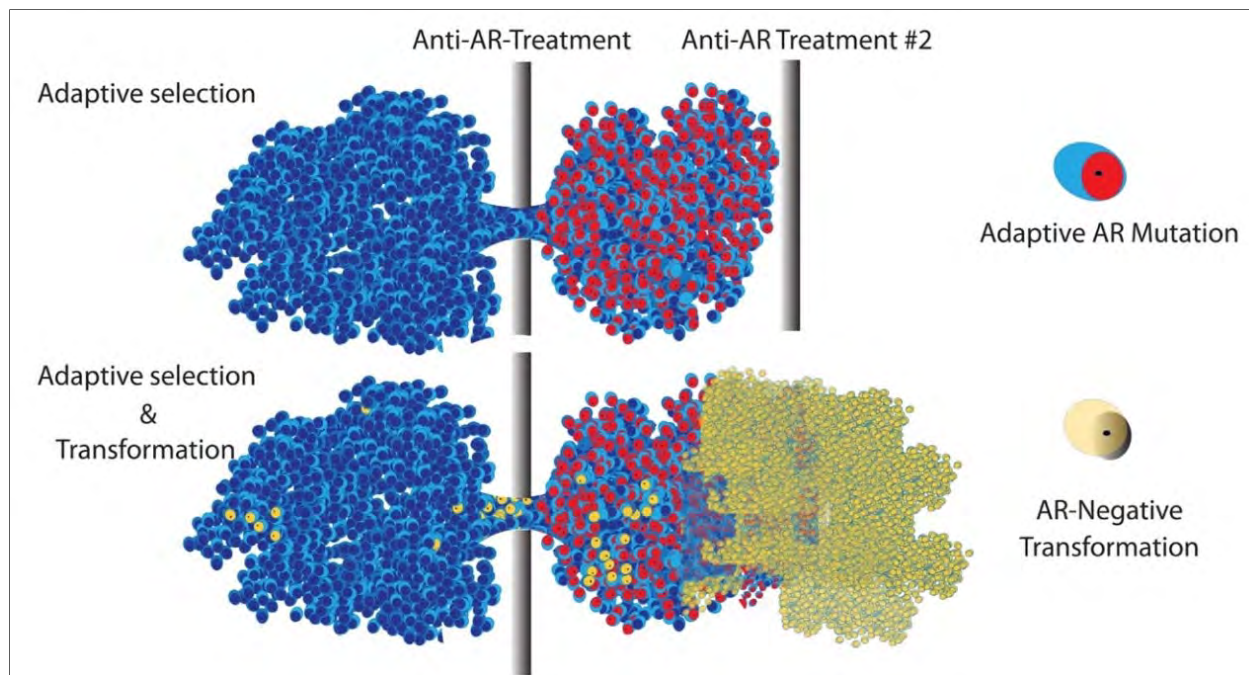
**Movember-PCF Challenge Award, 2012**

**PCF “Precision Therapy for Advanced Prostate Cancer” Dream Team, 2012**

- Dr. Mark Rubin gave an overview of the main points discussed at the Scientific Working Group for the Neuroendocrine Prostate Cancer Pathology Workshop Meeting that was held in July.
- Neuroendocrine prostate cancer (NEPC) is an aggressive molecular subtype of castrate resistant prostate cancer (CRPC), defined by neuronal features.
- However, the classification of a tumor as NEPC by surgical pathologists can be difficult, as expression of the markers of neuronal differentiation used to define NEPC are highly heterogeneous between patients.
- Targeting the androgen receptor (AR) pathway is the major treatment mechanism of prostate cancer. However, NEPCs are not AR-dependent, and furthermore, progression to NEPC appears to be promoted by androgen-deprivation therapy (ADT).
- The hypothesis of the Neuroendocrine Prostate Cancer Pathology Scientific Working Group, is that widespread use of ADT will lead to treatment-induced NEPC (t-NEPC).
- This workshop was designed to address the following for the treatment of NEPC patients:
  - How to better define NEPC?
  - Where does NEPC arise from?
  - Can patients who will develop NEPC be identified in advance?
  - What causes neuroendocrine resistance phenotypes?
  - How can we effectively treat NEPC?



- A new classification system for NEPC (t-NEPC) will be defined, published, and adopted, to enable pathologists to identify this subset of resistant patients.



**Figure 30:** Multiple anti-AR treatments are thought to promote development of NEPC (yellow).

## Scientific Working Group for Tumoroid (Avataroid) Biology: “Generation of Patient Derived *In Vitro* Models of Prostate Cancer” – Meeting held on April 25, 2013 at Memorial Sloan- Kettering Cancer Center

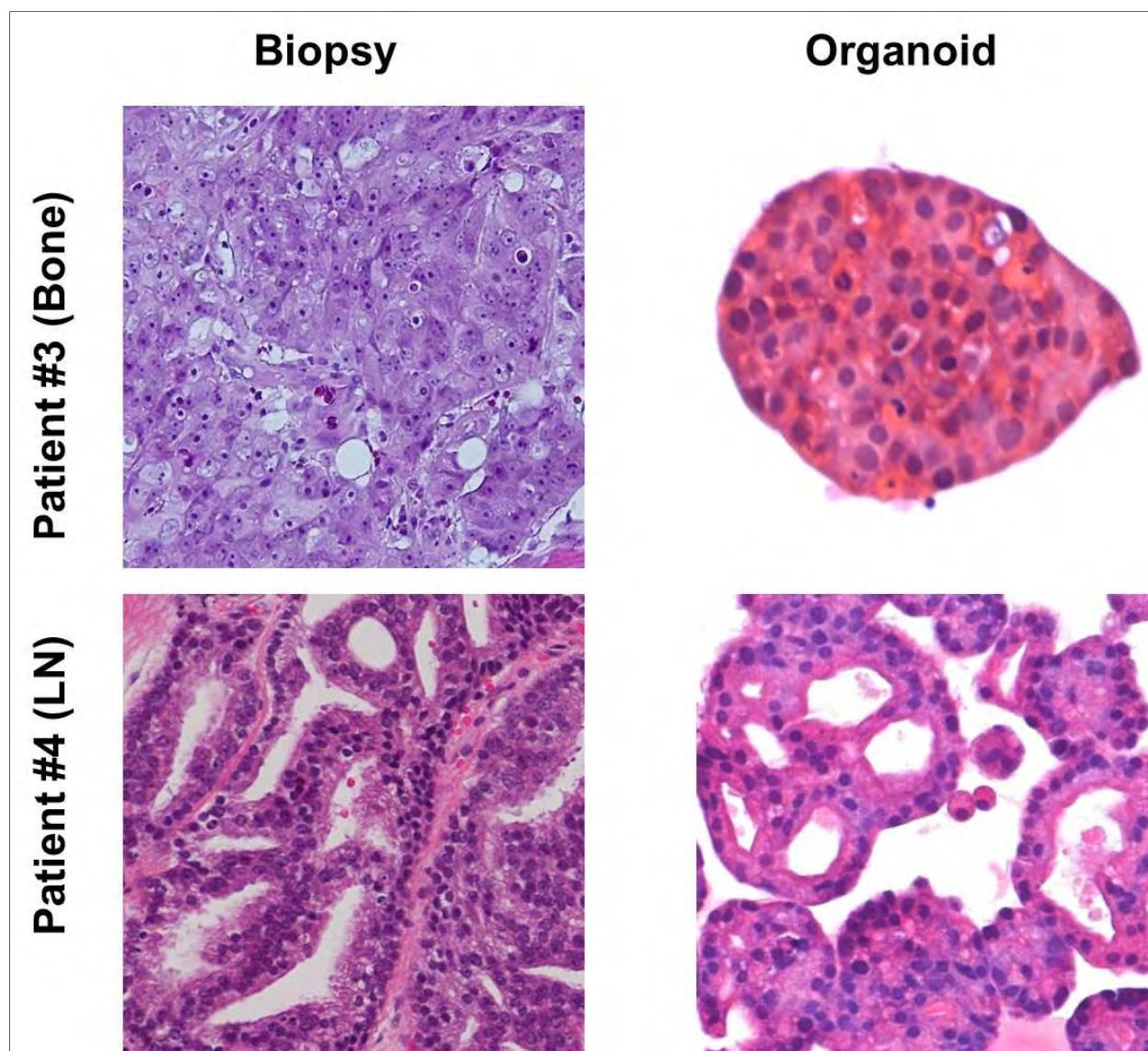
**Yu Chen, MD, PhD**  
**Memorial Sloan-Kettering Cancer Center**

**PCF Young Investigator Award, 2011**  
**Movember-PCF Challenge Award, 2013-2015**

- One of the most common methods to study the biology of any cell type is to grow the cells in the laboratory (*in vitro*) in “tissue culture” systems. This method generally employs regulated incubators where cells grow in specially coated plastic containers, in a liquid medium containing nutrients and other factors optimized to support growth of the particular cell type.
- However, the biology of cells grown *in vitro* can change substantially from their original site of growth in a patient (*in vivo*). Many methodologies have been

developed to more closely replicate *in vivo* biology using specialized *in vitro* conditions.

- One type of specialized *in vitro* culture condition generates what are known as “tumoroids” and can direct cells to grow in a 3D structural organization that resembles their tissue of origin. Studies are in process comparing the biological and genetic properties of tumoroids to the original patient tissue.
- The process to produce tumoroids is as follows:
  - Surgical removal of the tumor from a patient.
  - The tumor mass is “digested” with enzymes to create a single-cell suspension.
  - Single tumor cells are then grown in a specialized tumoroid-promoting medium and are incubated until they grow out as colonies of 3D cells.
  - Tumoroids can then be passaged to expand their number and frozen at ultra-low temperature to preserve them.
- The architectural structure, expression of tumor markers, and genetic alterations of prostate tumor tumoroids grown under these conditions were examined by histology and genomic methods, and found to resemble the original tumor.
- An important application for these tumoroids is testing tumor sensitivities to various drugs to aid in treatment decisions.
- Future directions of this technology include dissemination of the technology, incorporation of tumoroid culture on pre-treatment and post-treatment biopsies in precision medicine studies and other clinical trials, and determination of best methods for genomic and phenotypic assessments.
- Thus, “avataroid” technology will allow for many studies to be performed on patient tumors to gain insight into prostate cancer biology, and to support precision medicine decision making protocols.



**Figure 31:** Comparing histological features of patient prostate tumor biopsies versus organoids derived from the same tumor. Bone (top) and lymph node (LN, bottom) metastatic samples from different patients are shown.

## Session 11: New Treatments Beyond AR I

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### A Bispecific Small Molecule Antibody Conjugate for Hormone-Refractory Recurrent Prostate Cancer

**Chan Hyuk Kim**

**California Institute for Biomedical Research (Calibr)**

- T cells have the inordinate ability to kill target cells that express proteins recognized by the T cell receptor (TCR) as “foreign” or associated with abnormalities. While anti-tumor T cells can recognize specific tumor-associated antigens in cancer patients, it is not a simple task to define these antigens for the purposes of identifying or specifically activating and expanding these T cells.
- Bispecific antibodies are engineered molecules that link two antibodies that recognize different targets. Bispecific antibodies have been generated that recognize a tumor-associated antigen and CD3, a molecule on T cells that allows the targeted bispecific antibody to deliver killer T cells to prostate cancer. Thus, bispecific antibodies link a killer T cell and a tumor cell.
- Bispecific antibodies engage killer T cells with a tumor target cell in a way that activates the T cell to kill the target.
- Dr. Chan Hyuk Kim discussed the development of a bispecific antibody for prostate cancer.
- PSMA (Prostate Specific Membrane Antigen) is a protein that is upregulated on prostate cancers at all stages. The expression of PSMA is highly specific to prostate cancer, although low levels are expressed on kidney, brain, and colon cells. This specificity makes PSMA an ideal target for prostate cancer therapy.
- Small molecule binding partners of PSMA have been developed. These molecules can be conjugated to other molecules and thus have been used as tumor-targeting vehicles, delivering cytotoxic drugs and imaging agents. Due to small size, these molecules are better able to penetrate into the tumor than antibodies.
- Stable antigen-binding fragments (FAb) of antibodies which retain target recognition, yet have better tissue penetration than full-sized antibodies, can also be generated.
- As an alternate strategy that is similar in principle to bispecific antibodies, Dr. Kim has created a bispecific molecule using a CD3-targeting FAb ( $\alpha$ T cell FAb) conjugated to small molecule PSMA-binding partners.



- PSMA-targeting  
Bispecific Small Molecule  
Antibody Conjugate (P-SMAC)**

**Synthetic  
Ligands  
Target  
Binding**

**Fab  
Effector  
Function**

**aCD3**

**aPSMA (Prostate Specific  
Membrane Antigen)  
Small Molecule ligand**

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## A High Throughput Screen to Identify Small Molecule Inhibitors of the AR N-terminus

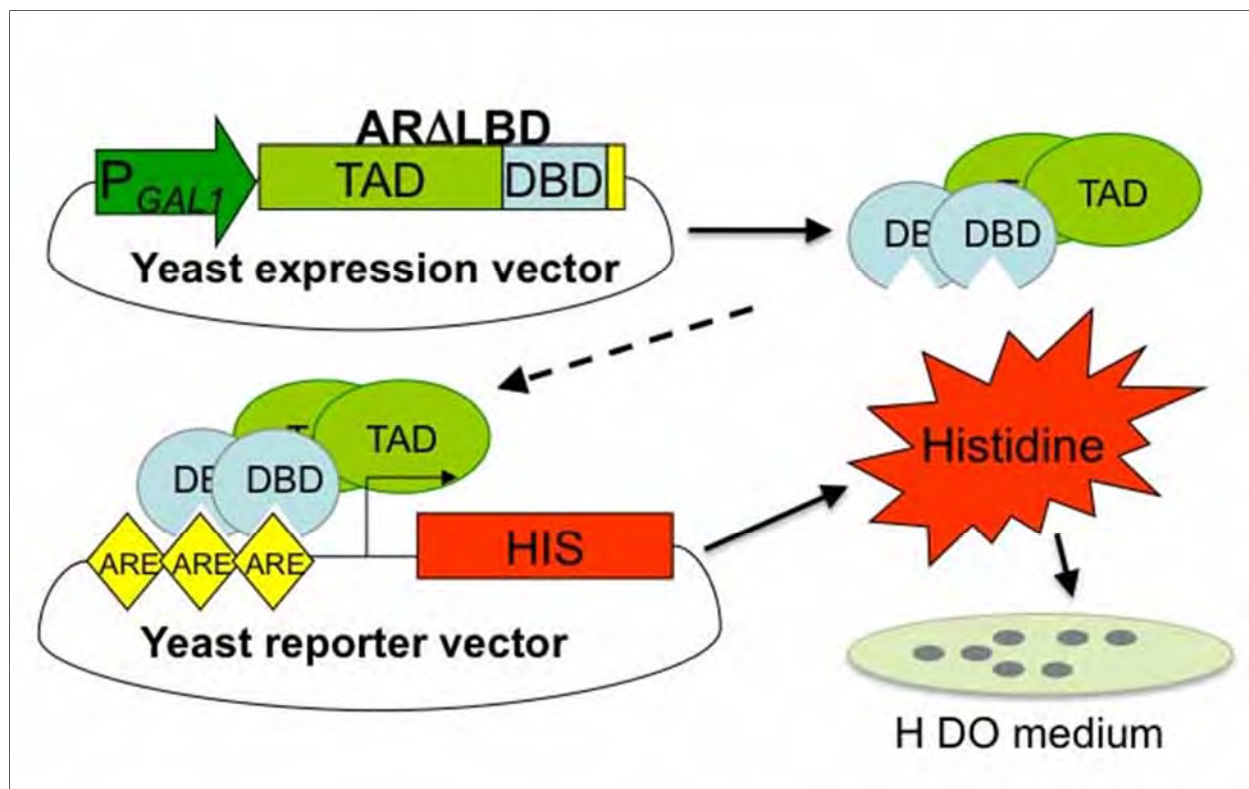
**Matthew Rettig, MD**

**David Geffen School of Medicine at UCLA**

**Movember-PCF Challenge Award, 2012**

- Prostate cancers are dependent on the expression of genes regulated by the androgen receptor (AR) transcription factor. However, progressive cancers that are resistant to anti-androgen drugs will eventually develop.
- The AR protein is composed of three major regions: a ligand-binding domain (LBD) which binds to androgens, a DNA-binding domain (DBD) and an N-terminal transcriptional activation domain (TAD).
- In the absence of androgen-binding, the LBD creates a molecular conformation that inhibits AR-transcriptional activity. When bound to androgens, the LBD changes conformation and transcription can ensue.
- Many drugs have been developed to target the ligand-binding domain of AR, which inhibits AR by blocking androgen binding (bicalutamide, enzalutamide, ARN509, etc.). However tumor cells can evolve mutations in AR, which allow activation without the need for androgens. These include AR-variants that lack the LBD (AR $\Delta$ LBD), generated either by mutations or alternate splicing, that enable constant, androgen-independent activity of AR.
- Dr. Matthew Rettig discussed the identification of small molecule inhibitors of the N-terminal domain of AR.
- Dr. Rettig devised a strategy using yeast cells to screen thousands of small molecules available from the NCI and UCLA for their ability to inhibit the transcriptional activity of AR $\Delta$ LBD-variants.
- Yeast cells were engineered to produce the AR $\Delta$ LBD-variant in a way that would inform inhibition of androgen signaling. Using a special robot, each small molecule drug was spotted onto such yeast cultures.
- From this screen, six small molecules were identified as potential AR $\Delta$ LBD-variant inhibiting drugs.
- Dr. Rettig presented data for several of these drugs, which were validated for their ability to specifically inhibit the activity of AR $\Delta$ LBD compared with the closely related transcription factor, the glucocorticoid receptor (GR).
- These drugs killed prostate cancer cell lines that express AR, but not those that have lost AR expression and survive by androgen receptor-independent mechanisms.

- Further characterizations of these compounds include the assessment of their effects and mechanisms of action on prostate cancer cells *in vitro* and *in vivo*. Candidates for the therapeutic treatment of prostate cancer will be identified. This work is particularly relevant for patients who have developed resistance to current anti-androgen drugs through the acquisition of constitutively active AR $\Delta$ LBD-variants.



**Figure 33:** Yeast system to detect small molecules that inhibit transcriptional activity of AR $\Delta$ LBD-variants. Yeast cells were transduced with a yeast expression vector that expresses the constitutively active AR $\Delta$ LBD-variant and a yeast reporter vector that encodes a histidine gene under the control of an ARE-promoter. The AR $\Delta$ LBD thus activates transcription of histidine, allowing yeast to grow on agar lacking histidine. However, if the AR $\Delta$ LBD is inhibited by drugs, histidine expression will not occur and the yeast will die.

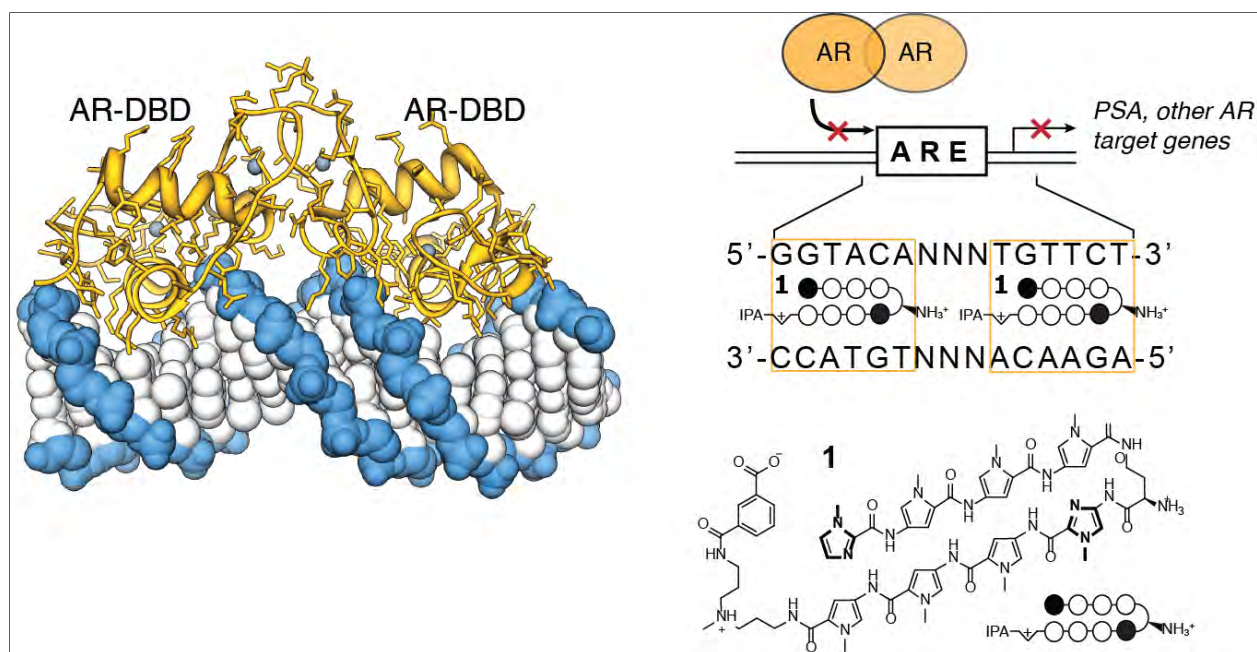
## Therapeutic Targeting of Transcription in Advanced Prostate Cancer

**Nicholas Nickols, MD, PhD**  
**University of California, Los Angeles**

**Laboratory of Peter Dervan, PhD**  
**California Institute of Technology**

- Dr. Nicholas Nickols discussed studies taking place in Peter Dervan's laboratory at the California Institute of Technology, regarding the development of a class of DNA-binding small molecules, called "polyamides," that offer a novel strategy to circumvent castration resistant prostate cancer (CRPC).
- The development of prostate cancer is primarily driven by the androgen receptor (AR), a testosterone-activated transcription factor that regulates the expression of genes required for the growth and survival of normal and cancerous prostate cells.
- The androgen response element (ARE) is a DNA sequence in regulatory regions of genes that is specifically recognized and bound by AR, in order to activate gene expression.
- The AR is a target of many forms of drugs that directly or indirectly inactivate AR through the inhibition of testosterone and AR interaction.
- However, existing therapeutics ultimately lead to a disease state where aberrant gene expression is no longer dependent on the testosterone-AR interaction, but is still dependent on the AR-ARE interaction. Therefore, direct interference with the AR-ARE interaction using a molecule that interacts with the ARE, might circumvent known resistance mechanisms of conventional AR-targeted therapeutics.
- Polyamides are a class of molecules developed in the Dervan lab that interact with DNA with sequence-specificity and without genotoxicity, which distinguishes them from conventional DNA-interacting drugs.
- "Polyamide 1" was designed to interact with the ARE sequence. Polyamide 1 was shown to inhibit AR binding to AREs on DNA in prostate cancer cells, and reduced the expression of genes regulated by AR, including prostate specific antigen (PSA).
- The repression of genes in prostate cancer cells by Polyamide 1 were compared with that of bicalutamide, a drug that inhibits androgen-activation of AR. Interestingly, many genes inhibited or induced by Polyamide 1 were distinct from those altered by bicalutamide, suggesting that Polyamide 1 has additional mechanisms of action.

- Polyamide 1 demonstrated favorable bioavailability in mice and significantly inhibited the growth of prostate tumors in mice, but at super-therapeutic doses, mice developed liver and kidney toxicities.
- Therefore, analogs of Polyamide 1 were developed and tested for efficacy and toxicity. A derivative called "Polyamide 3" was identified that maintained anti-tumor activity without toxicity at any measured dose.
- The glucocorticoid receptor (GR) is a transcription factor similar in structure to AR and binds similar DNA sequences.
- One mechanism of prostate cancer resistance to AR-targeting therapies is aberrant activation of GR, which substitutes for AR to induce ARE-regulated genes. Both Polyamides 1 and 3 were tested and found to inhibit the ability of GR to activate genes.
- Thus, this class of polyamide drugs may have significant therapeutic benefit and work to inhibit normally functioning AR as well as circumvent several mechanisms by which prostate cancers resist anti-AR drugs.
- These drugs are being further tested for pharmacokinetics with the goal of a first-in-man clinical trial.



**Figure 34:** Left: structure of the AR-DNA binding domain (DBD) bound to DNA. Right top: the ARE sequence in the promoters of AR-regulated genes that Compound 1 integrates within. Right bottom: Compound 1.

## Session 11 – What this Means for Patients:

Although significant advances have been made in the development of new medicines for the treatment of CRPC, patients inevitably become resistant, resulting in disease progression and death. We urgently need new medicines beyond what is available today. This session addressed that need.

- A bi-specific small molecule antibody conjugate for CRPC is being developed by Dr. Chan Hyuk Kim. This immuno-conjugate will deliver killer T cells directly to distant prostate cancer sites of metastasis, thereby controlling disease.
- Prostate tumor cells under extreme selective pressure during treatment with medicines such as abiraterone and enzalutamide respond by evolving androgen receptor (AR) variants that drive disease progression in the absence of androgens. Dr. Matthew Rettig and colleagues have developed a drug screening method for the selection of new experimental medicines that will inhibit these AR-variants.
- Most medicines that have been developed inhibit only a few types of molecules, such as receptors and enzymes, resulting in a therapeutic effect. Many more disease targets are considered undruggable due to their molecular complexity. Dr. Nicholas Nickols and colleagues are discovering and developing a new class of medicines that will inhibit an androgen receptor activity formerly considered undruggable. This would represent a new therapeutic approach for human prostate cancer.



## Session 12: Game-Changing Research—High-Achieving Young Investigators II

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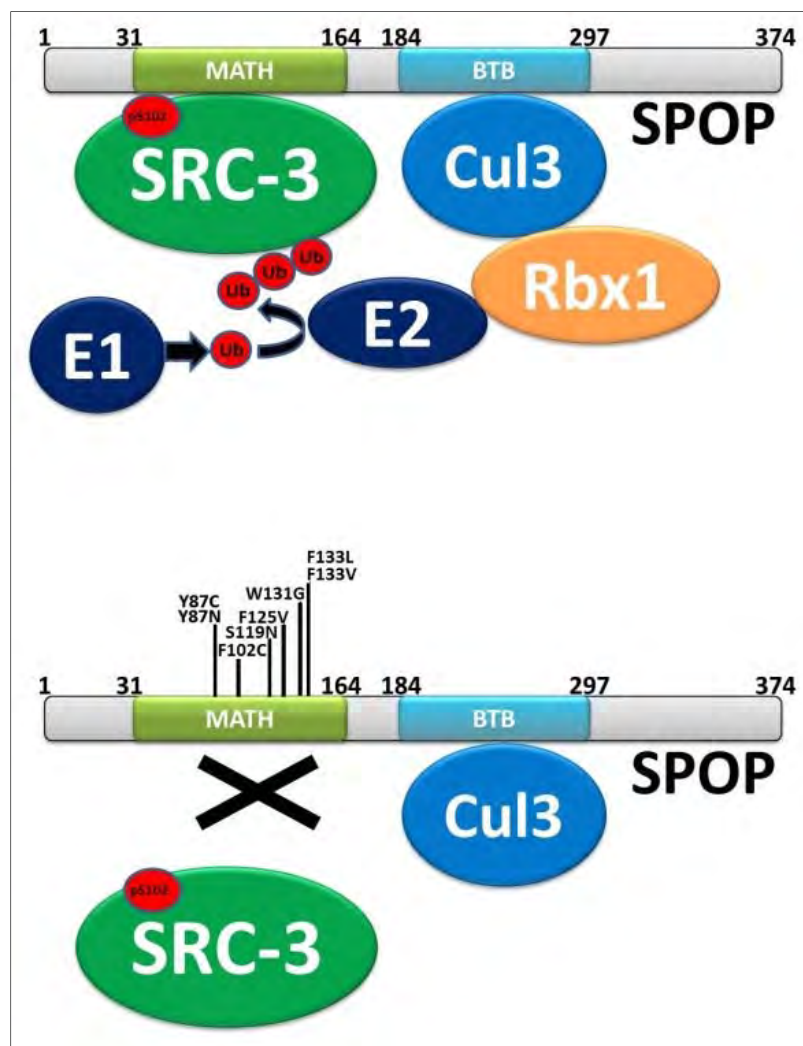
### Steroid Receptor Coactivators: Master Transcriptional Regulators in Prostate Cancer

**Nicholas Mitsiades MD, PhD**  
**Baylor College of Medicine**

**PCF Young Investigator Award, 2010**  
**PCF Challenge Award, 2012**

- The androgen receptor (AR) is a transcription factor that regulates the expression of genes required for the growth and survival of normal and cancerous prostate cells.
- AR is activated by binding to androgens. To induce gene transcription, AR needs help from additional co-factors that perform other functions in RNA transcription.
- The p160 steroid receptor co-activator proteins (SRCs) are “scaffolds” that act as transcriptional co-activators by binding to AR and necessary transcriptional co-factors, thereby forming the backbone of an active transcriptional complex.
- SRCs are highly expressed in prostate cancer, particularly in advanced disease stages, and are associated with resistance to therapy.
- Dr. Nicholas Mitsiades and colleagues are studying the effects of inhibiting SRCs in castrate-resistant prostate cancer (CRPC).
- An important protein that SRC-3 interacts with is Speckle-type POZ protein (SPOP).
- Interacting with SPOP causes SRC-3 to be degraded by cellular clearing pathways (Ubiquitin-ligase mediated). Less SRC-3 results in decreased AR-transcriptional activity.
- Mutations in the substrate-binding domain of SPOP are the most common amino acid-altering point mutations that occur in prostate cancer, found in up to 15% of patients.
- SPOP acts as a tumor suppressor factor in prostate cancer, and the effects of SPOP mutations in prostate cancer are under-addressed.
- Dr. Mitsiades found that SPOP mutants from prostate cancers were unable to bind SRC-3 and promote SRC-3 degradation. Inhibiting the expression of normal SPOP caused SRC-3 stabilization, and overexpressing normal SPOP decreased AR-transcriptional activity.

- The effect of SPOP on tumor growth was evaluated by implanting immune-deficient mice with human tumor cells that express normal vs. mutant SPOP. Tumors with SPOP-mutants grew significantly faster than tumors expressing normal SPOP.
- Overall, these studies demonstrate that SPOP acts as a tumor suppressor gene by promoting SRC-3 degradation and thereby inhibiting the transcriptional activity of AR.
- SPOP is frequently mutated in prostate cancers, indicating that tumors benefit significantly from uninhibited SRC-3.
- SRC-3 represents an important therapeutic target in prostate cancer.



**Figure 35:** Top: normal SPOP proteins bind to SRC-3 to cause ubiquitination and degradation of SRC-3. Bottom: in prostate tumors, SPOP frequently has mutations in the MATH domain that binds SRC-3, leading to enhanced levels of SRC-3.

## What This Means for Patients:

Steroid receptor cofactors (SRC) are molecules that complex with the androgen receptor and promote the activity of this engine for prostate cancer progression and survival. Dr. Mitsiades and team have discovered that mutations in SPOP proteins, which are common in prostate cancers, cause uninhibited activity of a specific steroid receptor cofactor, SRC-3. Medicines that target SRC-3 will represent a novel approach in the treatment of CRPC.

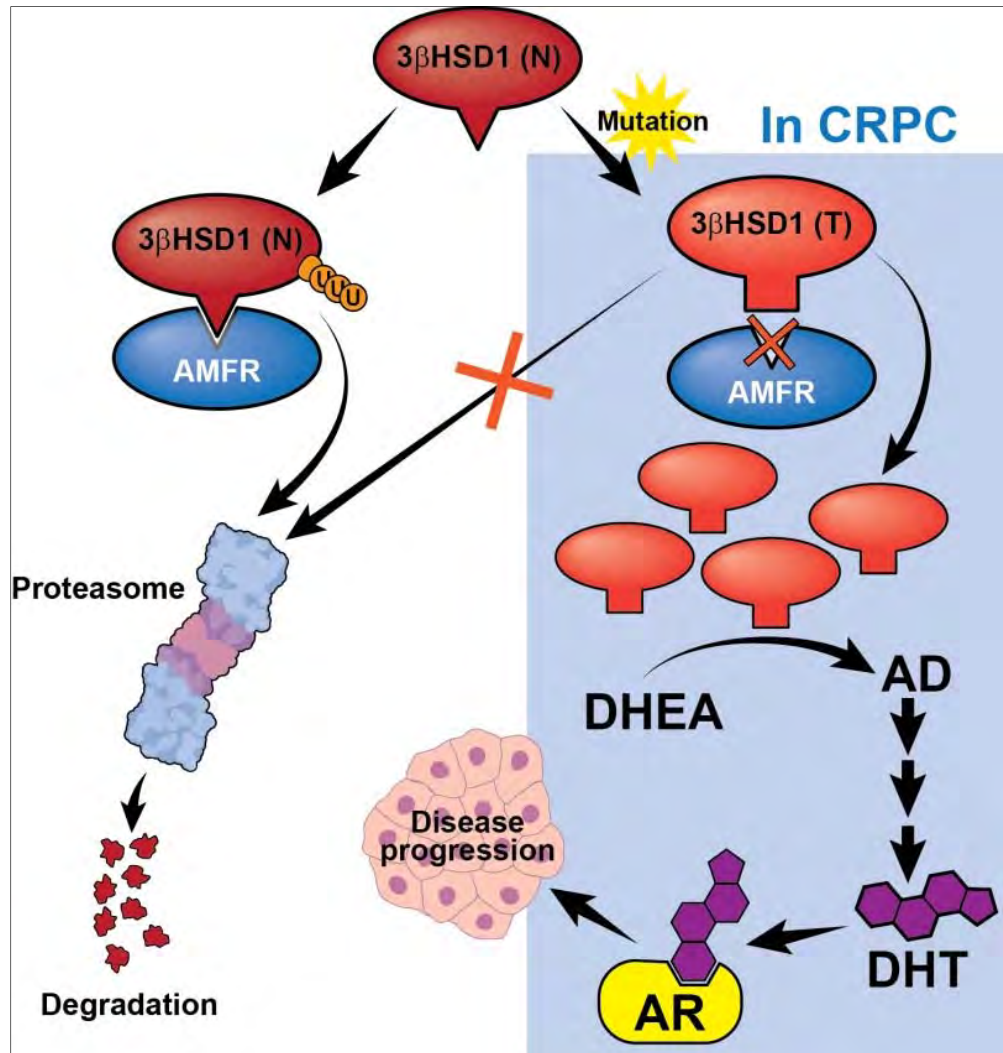
## A 3 $\beta$ HSD1 Mutation Elicits DHT Synthesis and Castration-Resistance

**Nima Sharifi, MD**  
**Cleveland Clinic**

### PCF Young Investigator Award, 2008

- The androgen receptor (AR) is a steroid hormone-activated transcription factor that regulates the expression of genes required for prostate cancer cell growth and survival.
- Dihydrotestosterone (DHT) is the most potent activator of AR.
- DHT is primarily synthesized in the prostate gland from testosterone, which originates in the testes, but can also be generated from dehydroepiandrosterone (DHEA) made in the adrenal glands.
- Dr. Nima Sharifi discussed his studies on 3 $\beta$ HSD1, a critical enzyme in the biosynthesis pathway of DHEA to DHT.
- A germ-line (inherited) mutation in 3 $\beta$ HSD1 that increases its enzymatic activity was found to occur in 38% of people.
- Spontaneous mutations in 3 $\beta$ HSD1 and loss of the normal 3 $\beta$ HSD1 gene in carriers were found in prostate cancer tumors. This indicates that the mutation in 3 $\beta$ HSD1 enhances its activity and promotes prostate cancer.
- Dr. Sharifi found that inhibiting expression of 3 $\beta$ HSD1 blocked the synthesis of DHT, and reduced AR-regulated gene expression. Prostate tumor cells without 3 $\beta$ HSD1 grew slower *in vitro* and were slower to develop tumors *in vivo*. Prostate cancer cells harboring the mutation produced tumors faster *in vivo*.
- Ubiquitination is a process of degrading proteins within a cell.
- Normal 3 $\beta$ HSD1 is ubiquitinated, which induces 3 $\beta$ HSD1 degradation. However, the mutant bypasses this degradation process and becomes stable.

- Thus, mutations in 3 $\beta$ HSD1 increase the stability of this enzyme and promote the synthesis of DHT from DHEA. This in turn, drives tumor growth in the absence of testosterone.
- 3 $\beta$ HSD1 acts downstream of the CYP17 enzyme in the cholesterol to DHT synthesis pathway. Thus, the mutation may also contribute to resistance to the CYP17 inhibitor, abiraterone acetate (AA) in CRPC.
- These data, taken together, credential 3 $\beta$ HSD1 as a therapeutic target for the treatment of CRPC.



**Figure 36:** Normal 3 $\beta$ HSD1 (N) is ubiquitinated (U) by AMFR, which induces degradation by the proteasome. The A1245C-mutant (T) is unable to be ubiquitinated and is stabilized, resulting in increased synthesis of DHT and enhanced AR activity, supporting CRPC disease progression.

## What This Means for Patients:

The normal metabolic pathway fueling prostate cancer progression and survival is activated by a molecule designated dihydrotestosterone (DHT). Normally DHT is synthesized from testosterone. Dr. Sharifi has identified a mutation in an enzyme that promotes production of DHT by an alternate pathway. Medicines against this enzyme are being discovered and will represent a new therapeutic modality for the treatment of CRPC.

## Glucocorticoid Receptor Confers Resistance to Anti-Androgens by Bypassing Androgen Receptor Blockade

**Vivek Arora, MD, PhD**  
**Memorial Sloan-Kettering Cancer Center**

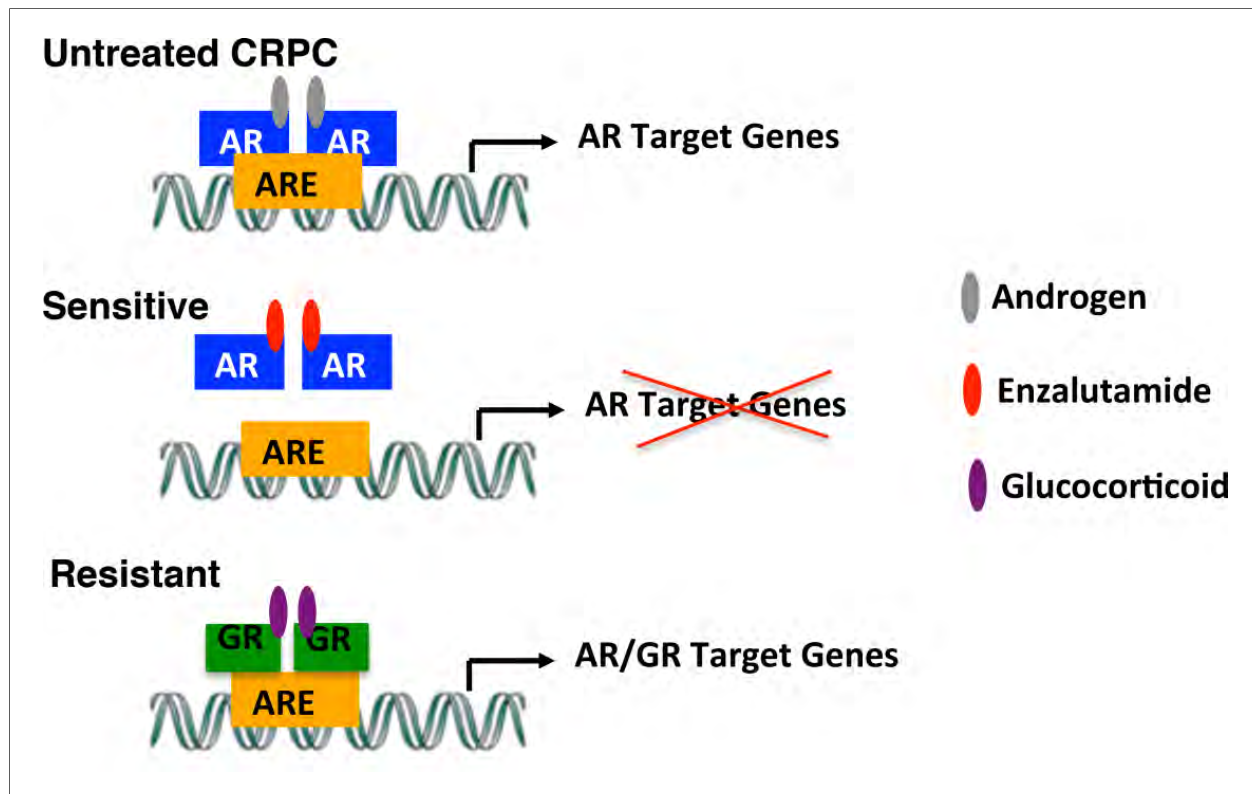
### PCF Young Investigator Award, 2013

- The androgen receptor (AR) is a master transcription factor that induces the expression of genes required for prostate cancer cell growth and survival, and is therefore therapeutically targeted in prostate cancer treatment.
- Resistance to anti-androgen therapeutics including enzalutamide can occur via multiple mechanisms, a profound clinical challenge in the treatment of prostate cancer.
- Dr. Vivek Arora discussed his discovery of one enzalutamide resistance mechanism.
- To study the mechanisms of anti-androgen drug resistance, cancer cells engineered to highly express AR were grown as tumors in mice, and then treated with enzalutamide or another anti-androgen drug ARN-509. In this model, treatment causes tumors to first regress, but then regrow, as resistance develops.
- The genes expressed in resistant tumors were analyzed and GR was found to be highly upregulated.
- Additionally, in one enzalutamide-resistant sub-cell line, LREX', GR expression was found to be required for resistance.
- The genes expressed in LREX' cells were analyzed. It was found that expression of a subset of AR-regulated genes was being reactivated by GR.
- GR and AR have similar DNA-binding domains and can bind to the same DNA sequences, although other factors contribute to the specificity of the genes they



activate. In LREX' cells, GR bound to promoter sites in DNA that are normally sites of AR binding.

- In another cell line, VCAP, stimulation with the GR-activator dexamethasone allowed continued tumor growth in the presence of enzalutamide.
- To determine if aberrant GR activity is a mechanism of enzalutamide resistance, tumors from patients with metastatic CRPC who were treated with enzalutamide were analyzed for GR expression. Tumors from patients with a poor response to enzalutamide were found to have enhanced expression levels of GR following enzalutamide treatment, compared to patients with better responses.
- Thus, overexpression of GR can occur in prostate cancer cells and contributes to resistance to anti-androgen therapeutics and CRPC development by reactivating expression of a subset of AR-target genes.
- In these patients, inhibition of GR may hold significant therapeutic promise.



**Figure 37:** In untreated prostate cancer, AR induces expression of necessary target genes. Enzalutamide treatment inhibits AR activity in enzalutamide-sensitive patients. However, in some patients, GR can become aberrantly activated and induce expression of important AR-genes, thereby contributing to enzalutamide-resistant CRPC.

## **What This Means for Patients:**

Pathways of resistance to new medicines such as enzalutamide for the treatment of CRPC are of paramount importance. Dr. Arora is studying one such pathway of resistance that involves an alternate nuclear hormone receptor named glucocorticoid receptor (GR). Since GR has the ability to promote prostate cancer progression and survival in place of AR, GR inhibitors are one way to overcome CRPC resistance to treatments such as enzalutamide.

## **Session 13: Measure Twice, Cut Once—What is the Proper Role for Surgery in Prostate Cancer 2013?**

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**Moderator: Stuart Holden, MD**  
**Prostate Cancer Foundation Medical Director**  
**& Cedars-Sinai Medical Center**

### **Panelists**

Eric Klein, MD  
Cleveland Clinic Foundation

Philip Kantoff, MD  
Dana-Farber Cancer Institute

Chris Logothetis, MD  
University of Texas MD Anderson Cancer Center

Patrick Walsh, MD  
Johns Hopkins University

This session included a panel of renowned urologists and medical oncologists who discussed a most significant issue regarding the role of surgery in the treatment of prostate cancer patients.

The key topics discussed by panel members included: 1) which patients should be treated with surgery, and 2) how should surgery be integrated with other treatment strategies?

## 1. Which patients should be treated with surgery?

- With the introduction of safer methods for anatomic radical prostatectomy along with PSA screening for prostate cancer patients in the early 1980s, the number of patients diagnosed with prostate cancer and undergoing prostatectomies ballooned in the 1980s-90s.
- Many patients who underwent surgery likely did not benefit. Overtreatment of low-grade prostate cancer has been an ongoing issue. This is expected to change with new knowledge from clinical studies and the advent of precision medicine to better select patients for watchful waiting vs. prescription of specific treatments.
- Surgery has been avoided in patients with locally advanced and metastatic disease, because urologists felt they could not be cured. However, practice indicates that patients with intermediate to advanced disease are the group that most benefit from surgery. For instance, in a Scandinavian monitoring trial, Gleason 8-10 patients gained the best mortality benefit from surgery, and surgery reduced the likelihood of later needing hormonal therapy. Radiation therapy and surgery have similar potency morbidity rates in patients with high-volume disease, so surgery for these patients should be considered.
- Patients who present with positive lymph nodes survived longer if they underwent surgery. These patients who historically had not been operated on should be considered for surgery.
- For patients with metastatic prostate cancer, those who were previously treated by surgery or radiation therapy of the primary tumor had improved responses to hormonal therapy. This could be due to loss of a “re-seeding” tumor bed. Initial treatment of patients with surgery extended the interval from time of metastatic presentation to prostate cancer-specific mortality, compared with radiation therapy.
- The issue of whether patients with local but aggressive disease should be treated with surgery vs. radiation therapy should be addressed in clinical trials.
- One clinical trial has started to determine optimum local control in metastatic patients, stratified by biology & disease burden.

## 2. Which patients should be treated with surgery?

- Prostate cancer has not followed the paradigms of other solid tumors, including in responses to chemotherapy. For instance, randomized trials in >300 patients showed similar survival curves for patients receiving hormones vs. hormones combined with chemotherapy. Chemotherapy given pre-operatively has little benefit.

- In high-grade/high-volume disease, the “cure rate” is only improved ~5% with surgery alone, but 18% when surgery is combined with radiation therapy. Thus, there is a need to discover which patients will benefit from this dual therapy.
- The advent of precision medicine combined with the recent influx of many new treatment options will lead to robust scientifically-driven procedures for matching patients with therapies. Multi-modal clinical trials using precision-medicine decisions that select patients for therapies and therapeutic combinations should be performed and include surgery and radiation therapy comparison arms.
- Optimizing the combination, sequencing, and duration of therapy needs to be performed. For instance, would neo-adjuvant treatment, followed by surgery, and then systemic therapy give better outcomes?
- Comments from the floor centered on the integration of surgery with other treatments and precision medicine. Points made by the audience included the consideration that cryoablation and radiation therapy as opposed to surgery may be optimal when followed by immunotherapies as these treatments promote immune-system sensitizing tumor cell death. Overall, the biology of the treatments must be considered when designing therapeutic combinations.
- Dr. Howard Sandler, a radiation oncologist, was asked to weigh in on the hypothesis that adding surgery to hormones and radiation could improve outcome for patients with locally advanced high-grade disease. He was not optimistic enough to recommend this be done, but agreed that we owe it to patients to determine what is best.
- Dr. Walsh responded that in all cohort studies, none show that patients with radiation fared better than surgery. Surgery generally gave a better outcome but may be due to patient selection. Radiation may not completely sterilize the area. Well-designed clinical trials are needed to make a true determination.

The panel concluded that prostate cancer paradigms need to be changed in regards to the role of surgery. This is particularly true in this new age of precision medicine. Most clinical trials with surgical arms have been poorly designed and new studies need to be performed to definitively prescribe the role for surgery.

# Special Lecture: Bone-Targeted Radionuclide Therapy—Forging a Path Through the Known Unknowns

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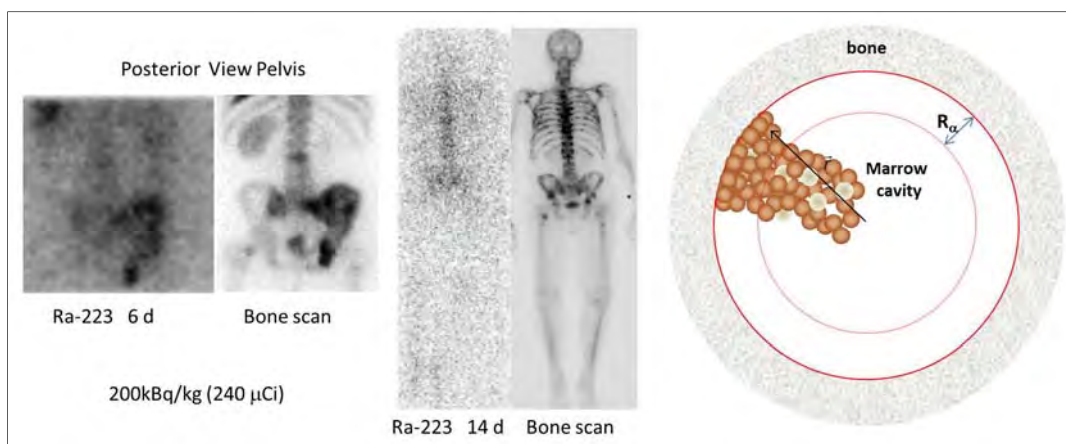
**Michael Morris, MD**  
**Memorial Sloan-Kettering Cancer Center**

## **A. David Mazzone-PCF Challenge Award, 2011**

- In May 2013, the FDA approved the use of Radium-223 (Xofigo), for the treatment of castrate resistant prostate cancer (CRPC) patients with symptomatic bone metastases and who do not have visceral metastases.
- Dr. Michael Morris discussed what is known and unknown about the mechanisms of Radium-223 therapy in prostate cancer patients, including questions regarding drug elimination, drug targets, and the putative mechanisms underlying clinical benefit.
- Radium-223 is a radionuclide with a half-life of 11.43 days. This isotope primarily emits alpha particles, which have a very short effective range and release a very high energy.
- Radium is in the same group in the periodic table as calcium, and therefore, due to similar chemical properties, is used by the body in place of calcium.
- Incorporation of Radium-223 into bones forms the basis for its use in treatment of prostate cancer bone metastases. Cancer cells stimulate a higher rate of bone turnover, and thus Radium-223 is preferentially incorporated into bone metastatic microenvironments.
- A randomized double-blind phase III clinical trial demonstrated a median overall survival benefit of 3.6 months for Radium-223 compared with placebo (14.9 months vs. 11.3 months), and extended the median time to first symptomatic skeletal event by 5.8 months.
- Radium-223 is highly tolerated with minimal hematologic toxicity. In phase I clinical trials, the maximum tolerated dose (MTD) was never reached. The optimal dose intensity, duration, density, interval between doses, and the safety of escalating doses or durations still remain to be determined.
- Radium-223 emits a series of alpha particles, beta particles, and gamma rays through a distinct decay chain until it stabilizes as lead (Pb). Several of these emission steps are imageable within different energy windows by molecular imaging technologies. Thus, the “parent” particle and its “daughters” can be visualized over time after drug administration to determine anatomic distributions and rates of metabolism.



- Parent and daughter Radium-223 particles were seen taken up and cleared in the small bowel, without re-locating to the kidney.
- In the blood, Radium-223 had a biphasic clearance rate, with a fast half-life of 11.4 minutes, and a slow half-life of ~4 days. It took approximately 7-8 days for whole body clearance of ~80% of parent and daughter Radium-223 particles.
- Radium-223 was notably retained in bones for an extended period of time, remaining imageable two weeks after administration.
- Autoradiographs of bone in pre-clinical models demonstrated that Radium-223 was localized to the bone surface and not to bone marrow, and was highly concentrated at sites of bone metastases. Thus, due to the short range of alpha-particles, there may be a relatively small effect of Radium-223 on hematopoietic cell populations located in the bone marrow cavity, and increasing the drug dose may not necessarily cause bone marrow toxicity.
- Questions that were not specifically addressed by the previous phase II and phase III clinical studies on Radium-223 include evaluating effects on pain, disease burden, distribution, and other benefits of treatment in addition to overall survival. The use of this drug in non-CRPC settings is unknown, as is optimizing its usage in combination with other drugs. Finally, questions regarding the mechanisms of clinical benefit remain to be addressed. For instance, does Radium-223 act by damaging the tumor cells themselves or the bone stromal microenvironment in which the tumor cells are located?
- Radium-223 (Xofigo) is one of the most recently FDA-approved treatments for prostate cancer patients with bone metastases, demonstrating a significant survival benefit with low toxicity. Expanding our knowledge on treatment modalities and mechanisms of action will further improve the benefits of this drug in prostate cancer patients.



**Figure 38:** Left/center: Images of Radium-223 compared with bone scans at 6 and 14 days post-drug administration. Right: Cross-section of bone demonstrating the relatively short radius of alpha-particle emission ( $R_\alpha$ ) extending from the bone surface into the marrow cavity. Figures are adapted from: Carrasquillo et al., *Eur J Nucl Med Mol Imaging*. 2013 Sep;40(9):1384-93 & Hobbs et al., *Phys Med Biol*. 2012 May 21;57(10):3207-22.

## What This Means for Patients:

Prostate cancer bone metastases are a great cause of morbidity in patients with CRPC. Radium-223 (Xofigo) represents the newest targeted treatment for bony disease. Dr. Michael Morris provided an overview and summarized the need for many clinical investigations to refine the dose and schedule and to determine rational combination therapies for this extremely promising new treatment that prolongs survival.

## Session 14: New Treatments Beyond AR II

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### Targeting the Neural Microenvironment and Neuronal Trans-Differentiation

**Gustavo Ayala, MD**

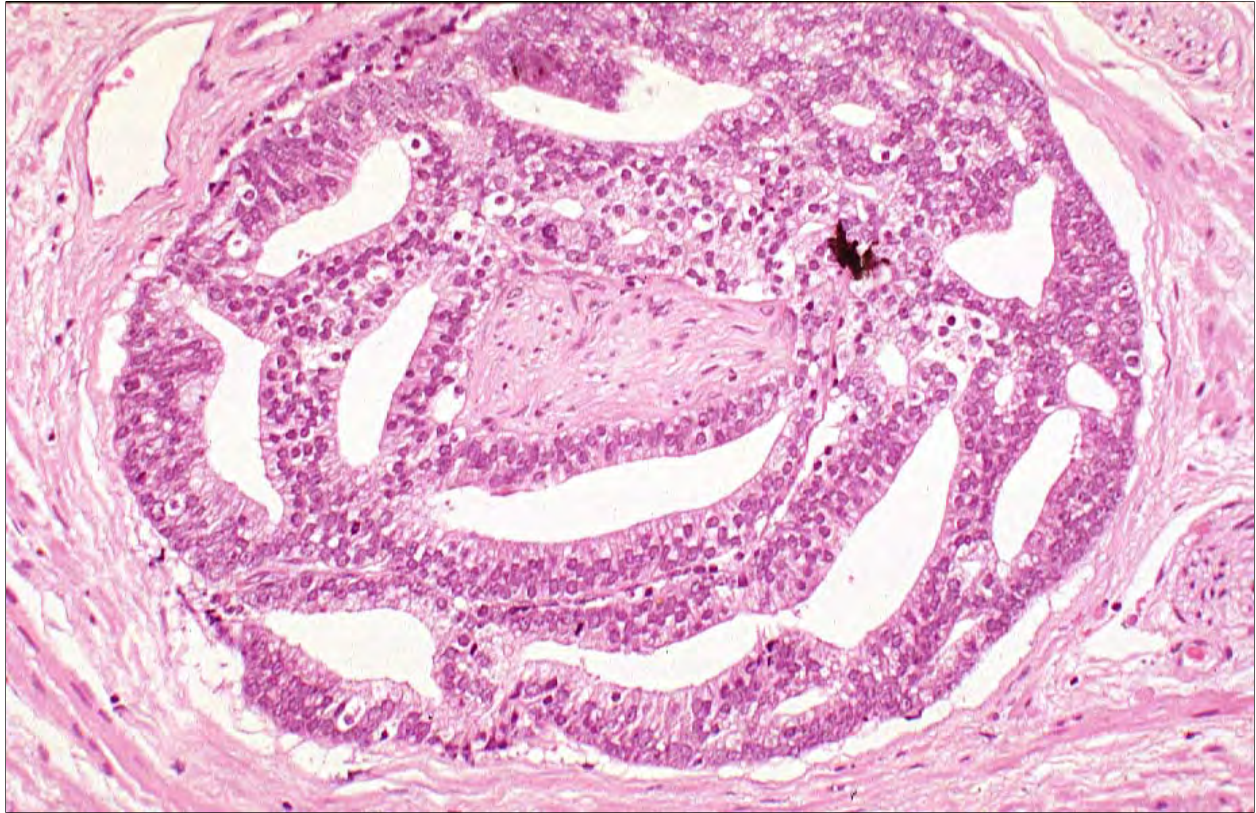
**University of Texas Health Science Center Medical School**

**Gordon Becker-PCF Creativity Award, 2010**

- The relationship between nerves and the development and progression of prostate cancer is an important field of study. Nerve tissue promotes prostate cancer activity and there is also an aggressive prostate cancer subtype characterized by the acquisition of neuronal tissue features.
- Dr. Gustavo Ayala discussed his studies on the role of nerves in the prostate epithelium and during prostate cancer development, and the therapeutic targeting of nerves for prostate cancer treatment.
- Botox is a neurotoxin. Injection of Botox into the prostate leads to loss of nerves (denervation) in the prostate followed by prostate atrophy. This indicates that nerves support the survival of prostate cells.
- To study the role of nerves in prostate cancer development, Botox was injected into the prostates of mice to induce denervation, followed by tumor cell implantation. A lower incidence of prostate cancer development was observed in Botox-treated mice.
- Analysis of the genes expressed in these tumors found that Botox treatment reduced expression of genes associated with translation and metabolism, and increased expression of inflammatory genes, stem cell features, and growth factors.
- To study denervation as a therapeutic approach in human prostate cancer, Dr. Ayala established a clinical trial in which Botox and saline are injected into

opposing sides of the prostate in men with bilateral prostate cancer. Four weeks later, patients undergo radical prostatectomies. Increased cell death and decreased nerve density in both the tumor and non-tumor regions occurred on the side of the prostate that received Botox compared with the side that received saline.

- Higher nerve density and expression of neuron development genes, were observed in tumors compared with non-tumor regions, indicating that tumors can induce *de novo* neurogenesis.
- Tumor nerve density was higher in patients with recurrent disease and was associated with poorer recurrence-free survival. Prostate cancer cells were observed to invade the protective sheath surrounding nerves, and the extent of this invasion was a significant predictor of prostate cancer-specific death. Thus, signals from nerves appear to support prostate cancer growth and disease progression.
- Importantly, following androgen-deprivation therapy (ADT), an aggressive prostate cancer subtype can arise, in which prostate cancer cells themselves begin to express high levels of neuronal genes and take on neuronal characteristics. This subtype is referred to as neuroendocrine prostate cancer (NEPC).
- Prostate cancer cells grown under stressed conditions and metastatic hormone-resistant prostate cancer tissues expressed many brain tissue genes with neurotransmitter, axon, glial, ion transport, and synapsis functions, and become elongated with dendrite-like structures.
- Thus, trans-differentiation into neuron-like phenotypes occurs in some prostate cancers to maintain their growth and survival.
- Overall, these studies indicate that nerve-functions play significant roles in prostate cancer development and progression by providing supportive signals to prostate cancer cells. Continued studies examining the therapeutic targeting of nerves for prostate cancer treatment is critical.



**Figure 39:** Prostate cancer cells (dark pink) growing around a nerve (center).

## Protein Phosphatase 2A as a Target for Prostate Cancer Therapy

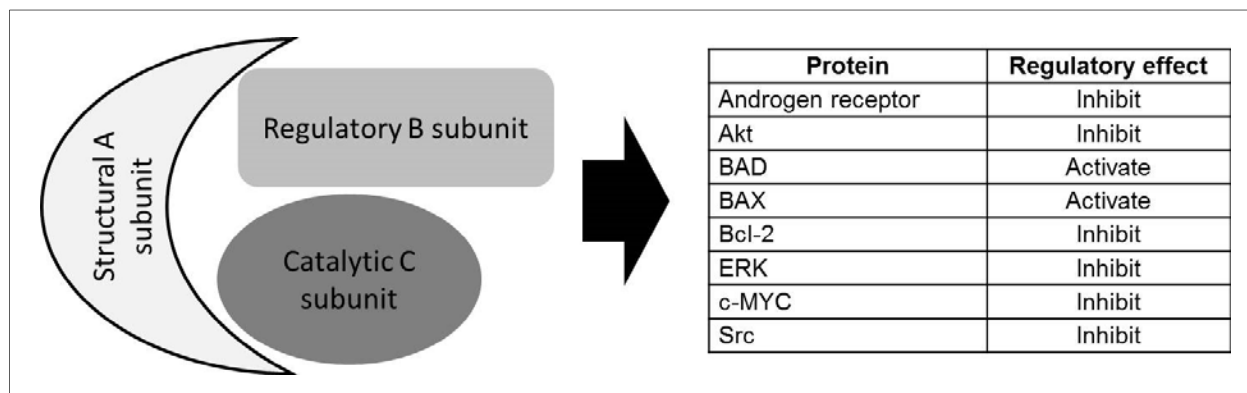
**Matthew Galsky, MD**  
**Mount Sinai School of Medicine**

**PCF Young Investigator Award, 2012**

- Phosphorylation, the addition of phosphate groups ( $\text{PO}_4^{3-}$ ) to a protein, is a major mechanism regulating protein function. Phosphate groups are added to proteins by kinases and removed by phosphatases.
- The androgen receptor (AR) is the major transcription factor regulating expression of prostate cancer growth and survival genes. AR is activated by binding to androgens but is also regulated by phosphorylation.
- Protein Phosphatase 2A (PP2A) is a serine-threonine phosphatase that dephosphorylates AR at multiple sites, as well as dephosphorylates multiple other oncogenic proteins, including c-MYC, Akt, and Src.



- Inhibition of PP2A in prostate cancer cells enhances AR activity and increases expression of AR and AR-target genes including prostate specific antigen (PSA).
- PP2A is considered a tumor suppressor gene, and can be mutated or decreased in multiple tumor types including prostate cancer. Decreased PP2A expression has been associated with poorer survival in prostate cancer patients with localized disease.
- Dr. Matthew Galsky discussed his studies exploring the use of tricyclic neuroleptic derivatives to activate PP2A in prostate cancer.
- Tricyclic neuroleptics have anti-cancer effects at high doses. However, the use of these compounds as therapeutic agents is limited by central nervous system (CNS) toxicity.
- Dr. Galsky and his colleagues Drs. Goutham Narla and Michael Ohlmeyer have generated novel tricyclic neuroleptic derivatives that retain anti-cancer effects without inducing CNS toxicity.
- TRC-794 is one derivative, and was found to directly bind and activate PP2A. This is a first-in-class small molecule activator of PP2A.
- Treatment of prostate cancer cell lines with TRC-794 inhibited growth and induced cell death.
- TRC-794 reduced the expression of AR by increasing AR protein degradation. Expression of AR-regulated genes was also reduced by TRC-794 treatment.
- TRC-794 was shown to have anti-tumor effects in mouse models of prostate cancer.
- In summary, PP2A dephosphorylates AR, causing AR destabilization and degradation, among other effects on critical oncogenic proteins, thereby inhibiting tumor growth.
- PP2A activation by the tricyclic neuroleptic derivative TRC-794 may be a promising new avenue for prostate cancer therapeutics.



**Figure 40:** PP2A is a serine-threonine phosphatase that dephosphorylates multiple oncogenic proteins including AR, c-MYC, Akt, and Src, leading to their inhibition.



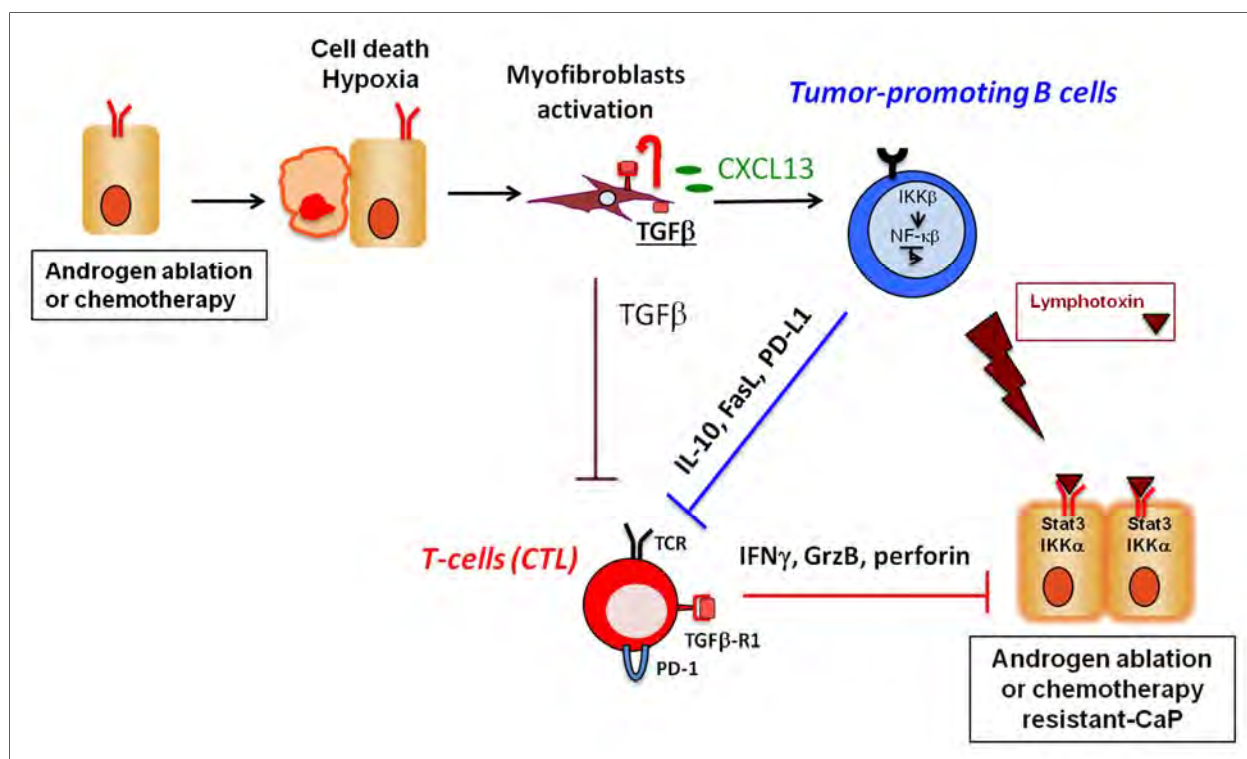
## Role of B and T Lymphocytes in the Development of Castrate Resistant Prostate Cancer and the Response to Chemotherapy

Michael Karin, PhD

University of California, San Diego

- Both positive and negative effects of the immune system on cancer have been described.
- Immune cell types including T cells and NK cells are known for their strong cancer cell-killing abilities.
- Inflammation, a process during which immune cells are recruited to a damaged tissue site in response to infection or injury, has some tumor-promoting effects, including the release of growth factors for tissue regeneration that tumor cells exploit.
- Immune cells are also recruited to tumors, and the balance between tumor-promoting and tumor-inhibiting immune properties impacts cancer patient outcome.
- Understanding the positive and negative effects of the immune system on tumors will enable the development of the most effective immune system-targeting therapies for cancer treatments.
- In patients with prostate cancer, androgen-deprivation therapy (ADT) causes initial tumor regression. However, castrate-resistant prostate cancer (CRPC) then develops.
- ADT can induce inflammation in patients.
- Dr. Michael Karin has been studying the relationships between inflammation and cancer.
- In mice, CRPC development was promoted by inflammation induced by ADT (castration).
- Dr. Karin's group explored the role of NF- $\kappa$ B, an inflammation-related transcription factor, in the emergence of CRPC after castration. Without IKK $\alpha$ , an activator of NF- $\kappa$ B, tumor re-growth after castration was much slower.
- The expression of BMI1, a component of the oncogenic Polycomb Repressive Complex 1 (PRC1), was found to be increased in malignant prostate tissue from patients, and its expression correlated with IKK $\alpha$  activity.
- In mice, IKK $\alpha$  was required for the activation of E2F1, a BMI1 expression-inducing transcription factor. IKK $\alpha$  was also associated with enhanced BMI1/PRC1 activities: repressing expression of cell-cycle inhibitors and tumor suppressor genes including p16ARF and p19INK4a.

- Thus, one pro-tumorigenic effect of IKK $\alpha$  is to promote the activity of E2F1, which activates expression of BMI1 and thus PRC1 activity. BMI1/PRC1 then exerts oncogenic activity by suppressing the expression of cell cycle inhibitor genes.
- Higher numbers of B cells are found in human prostate cancer tumors compared with normal prostate tissue, particularly in patients with high risk and recurrent disease.
- Dr. Karin hypothesized that injury to prostate cancer cells caused by ADT induces the production of chemokines that recruit cancer-promoting B-cells to the tumor. These “bad” B-cells then release factors that promote progression to CRPC.
- One B-cell recruiting chemokine is CXCL13, which was found to be produced by myofibroblast cells in the tumor stroma following castration of mice. Depleting myofibroblasts reduced CXCL13 and delayed tumor re-growth following castration.
- Prostate cancer patients also have higher serum levels of CXCL13, which associates with disease severity.
- Interestingly, B cells promoted resistance to chemotherapeutics. Following oxaliplatin treatment, mice without B cells had greater tumor inhibition and recruited higher numbers of T cells to tumors. These T cells highly expressed proteins that lyse tumor-cells, and promote anti-tumor immunity.
- In the presence of B cells, T cell functions were perturbed, as oxaliplatin treatment caused B cells to express T cell inhibitory genes (PD-L1 and Fas-L).
- Thus, ADT and chemotherapy induce prostate tissue injury and activation of myofibroblasts. Activated myofibroblasts produce CXCL13 to recruit B cells, which promote prostate cancer progression through mechanisms including the inhibition of anti-tumor T cell functions and activation of IKK $\alpha$ .
- B cells also express lymphotoxin, a protein that activates survival and proliferation gene programs in prostate cancer cells through its effects on IKK $\alpha$ .
- B cells, CXCL13, and inflammatory factors such as IKK $\alpha$  and NF- $\kappa$ B may therefore be valid therapeutic targets, particularly in CRPC. Understanding the mechanisms underlying pro- and anti-tumor immune functions under various conditions, including inflammation-promoting therapies, are important for maximizing therapeutic effects and designing optimal immune-modulating strategies. For instance, a drug named Rituxan normally used to treat B cell lymphoma, will be tested in prostate cancer patients to eliminate the pro-oncogenic activity associated with B cell infiltration.



**Figure 41:** ADT and chemotherapy induce prostate tissue injury and activation of myofibroblasts. Activated myofibroblasts and damaged prostate cancer cells then produce CXCL13 to recruit B cells, which promote prostate cancer progression through mechanisms including the inhibition of tumor-killing T cell functions and expression of lymphotoxin to activate survival and proliferation gene programs in tumor cells.

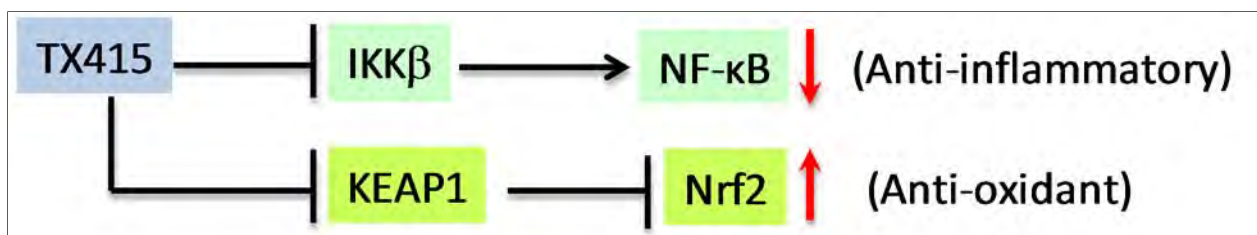
## Anti-Inflammatory Modulators—Targeting Prostate Cancer While Protecting Normal Tissues

Ulrich Rodeck, MD, PhD  
Thomas Jefferson University

Ben Franklin-PCF Creativity Award, 2011

- Approximately 60% of cancer patients receive radiation therapy as part of their treatment regimen. However, the benefits of radiation therapy are limited by 'collateral' damage to surrounding normal tissues. In prostate cancer patients, the bowel and bladder are particularly sensitive to side effects of radiation therapy.
- Dr. Ulrich Rodeck discussed his efforts to identify new drugs that selectively protect normal tissues from radiation damage.

- Inhibitors of NF- $\kappa$ B, an inflammation mediator, have provided protection from radiation damage in zebrafish and mice. Thus, NF- $\kappa$ B inhibition might be one such strategy.
- TX415 is a cysteine-modifying triterpenoid compound that inhibits the NF- $\kappa$ B activator, IKK $\beta$ . TX415 also leads to enhanced Nrf2-dependent transcription of multiple genes encoding antioxidant enzymes. Thus, TX415 might play dual protective roles if administered with radiation treatment: inhibition of inflammation and protection from oxidative stress.
- To determine if TX415 protected mice from radiation-induced tissue damage, TX415 was administered following radiation treatment. Radiation-induced death of mice is attributed to destruction of the intestinal epithelium or failure of hematopoietic system regeneration. TX415 treatment preserved the integrity of the gastrointestinal tract and the skin associated with reduced radiation-associated apoptosis. 100% of mice that received TX415 survived long-term after a lethal dose of radiation. Thus, both epithelial and hematopoietic damage were attenuated by TX415.
- By contrast, TX415 treatment markedly inhibited the growth of established prostate tumors in mice and induced prostate cancer cell death *in vitro* and in prostate tumors transplanted in mice. TX415 also inhibited tumor growth when combined with radiation therapy.
- TX415 treatment of prostate cancer cells reduced NF- $\kappa$ B activity *in vitro* and following radiotherapy *in vivo*. Activation of STAT5, a proliferation and survival promoting transcription factor, was also inhibited by TX415 treatment in select prostate cancer cells. The relative contribution of these and other molecular activities on the anti-tumor effects and normal tissue protection of TX415 is under investigation.
- In conclusion, TX415 directly inhibits prostate cancer cell growth alone and in combination with radiation therapy. At the same time, TX415 protects normal epithelial tissues from radiation damage. TX415 is a drug candidate for improving the therapeutic window of radiation therapy in prostate cancer treatment.



**Figure 42:** TX415 affects at least two pathways which govern the cell stress response. Blocking the NF- $\kappa$ B activator, IKK $\beta$ , inhibits inflammation associated with cell damage *in vivo*. Inhibiting KEAP1, which inhibits the transcription factor Nrf2, bolsters antioxidant responses.

## Ingenious Delivery of an Old Drug

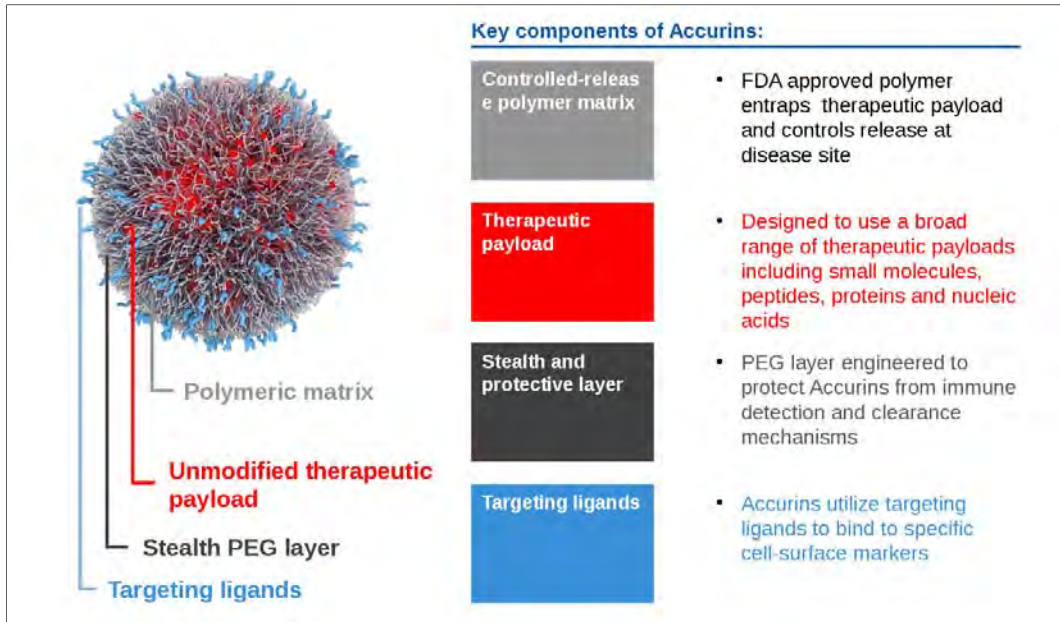
**Christopher Sweeney, MBBS**  
**Dana-Farber Cancer Institute**

### PCF Challenge Award, 2011

- Standard delivery of chemotherapeutic agents has the risk of causing significant toxicity from off-target damage to normal tissues.
- Dr. Christopher Sweeney discussed the collaborative efforts he has participated in with the inventors Dr. Omid Farokhzad and Dr. Robert Langer, and the team at BIND Therapeutics, to decrease bystander cell toxicity by using cancer cell-targeting Accurin nanoparticles to deliver drugs for prostate cancer treatment.
- The David Koch-PCF Program in Cancer Nanotherapeutics has provided key support that extended from the academic work through its translation to the clinic with BIND.
- Accurins are nanoparticles made from the only currently FDA-approved polymer matrix.
- Accurins are coated with a PEG layer to avoid clearance and immune detection, and can be engineered to incorporate tumor-targeting molecules.
- These nanoparticles are designed to carry a therapeutic payload and release it at a high concentration in a controlled manner, preferentially to the tumor.
- BIND-014 is an Accurin nanoparticle that targets prostate-specific membrane antigen (PSMA), a molecule specifically expressed on prostate cancer cells, and on the vasculature of most non-prostate solid tumors. Each BIND-014 particle carries  $10^3$ - $10^4$  molecules of the chemotherapeutic agent, docetaxel.
- In mice with tumors, BIND-014 treatment deposited a higher concentration of docetaxel at the tumor site and more significantly inhibited tumor growth compared with standard docetaxel administration.
- In an accelerated phase I clinical trial of BIND-014 treatment in 30 solid tumor patients, 4 patients with different cancer types exhibited responses, with 1 complete and 3 partial responders.
- At the maximum tolerated dose, BIND-014 toxicities were similar to those associated with standard solvent-based docetaxel administration, including grade 3-4 neutropenia, leucopenia, anemia, and fatigue.
- A longer half-life in the circulation was observed for BIND-014 particles compared with standard docetaxel.
- Evaluation of these patients and BIND-014 pharmacology is ongoing.



- Thus, tumor-targeting nanoparticles such as Accurins are a promising new avenue for targeted delivery of therapeutic agents, with the net effect of increasing tumor cell killing at an overall lower dose of agent, while minimizing non-specific toxicity to normal tissues.
- In addition to docetaxel, Accurins can carry a variety of other cancer therapeutics that are under development and validation.



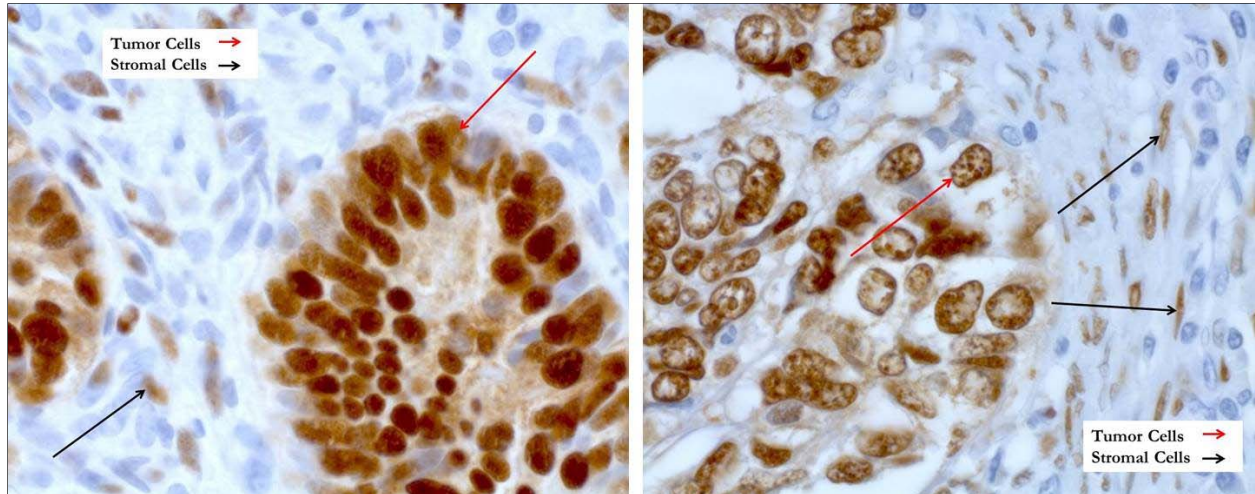
**Figure 43:** The composition of Accurin nanoparticles: an FDA-approved polymer matrix, stealth PEG layer to avoid clearance and immune detection, tumor-targeting ligands, and the ability to carry a therapeutic payload.

## Progesterone Receptor, a Potential Mechanism of Resistance and Target in AIPC

**Alexander Zukiwski, MD**  
**Arno Therapeutics**

- The androgen receptor (AR) is the master oncogenic transcription factor driving prostate cancer.
- The progesterone receptor (PR) transcription factor is highly homologous to AR, particularly within the domain that binds to specific DNA target sequences to activate gene expression, where there is 83% identity. Thus, PR and AR can induce expression of many of the same genes.
- PR is activated by progestin ligands, whereas AR is activated by androgens.

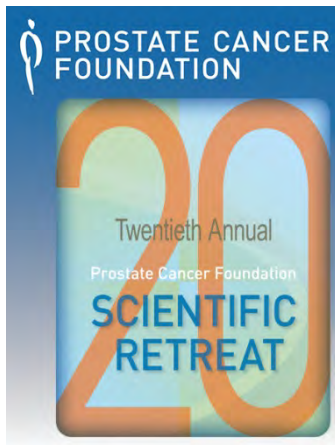
- PR expression has been reported to be expressed in some prostate cancer tumors, and is substantially increased in recurrent and metastatic disease, indicating a potential role for PR in prostate cancer progression.
- Dr. Alexander Zukiwski discussed the development of an immunohistochemical diagnostic method for identifying the activated form of PR, and its role in prostate cancer.
- PR exists in two observable states in the nucleus. In its unactivated state, PR is observed in an evenly distributed (diffuse) nuclear pattern. In its transcriptionally active state, PR forms aggregated foci.
- Using an antibody against PR, an immunohistochemical technique was developed which can differentiate diffuse from aggregated nuclear patterns of PR in cells and tissue samples by microscopy.
- Activated forms of PR were observed in endometrial tissue during the luteal phase of the menstrual cycle when there are high levels of circulating progesterone, and in endometrial and breast carcinomas.
- Castrate-resistant prostate cancer (CRPC) is an aggressive form of prostate cancer that arises following androgen-deprivation therapy (ADT), and no longer relies on physiological levels of androgens to grow.
- Understanding the mechanisms causing CRPC is critical for developing new and improved treatments.
- To determine if PR has a role in CRPC, prostate cancer specimens were examined for the nuclear staining patterns of PR as well as AR and the estrogen receptor (ER).
- Expression of AR but not PR or ER was observed in primary prostate cancer biopsy specimens. However, in several prostate cancer specimens from patients who had undergone ADT, preliminary analysis demonstrated expression of PR, and in some cases, PR nuclear aggregates were seen.
- Thus, these studies indicate that activation of PR occurs in prostate cancers following ADT and may be a mechanism of AR-independent growth and survival in CRPC by substituting for AR in transcription of cancer-promoting genes.
- A phase I clinical trial with the anti-progestin onapristone is planned, and will be tested in patients who have failed to respond or progressed on abiraterone or enzalutamide treatment, beginning in 2014.



**Figure 44:** Immunohistochemical PR (brown color) staining technique demonstrating diffuse unactivated PR (left) and focal activated PR (right) in the nuclei of tumor and stromal cells from endometrial cancer patient tissues.

## Session 14 — What This Means to Patients:

Castration resistant prostate cancer (CRPC) continues to be a clinical challenge for patients facing this most significant form of the disease. In this session, investigators presented multiple shots-on-goal for unique new therapies. Dr. Gustavo Ayala has identified therapeutic targets emanating from nerve cells that promote the metastasis of prostate cancer. He has demonstrated that destruction of nerves associated with prostatic tumors can slow the progression of disease. Dr. Matthew Galsky presented the discovery of a novel compound that reduces the expression of AR by increasing its degradation. Dr. Michael Karin demonstrated factors from B cells infiltrating prostate tumors that cause disease progression. He will target B cells with an existing FDA-approved drug that targets and eliminates B cells. Dr. Ulrich Rodeck has discovered an anti-inflammatory compound that attenuates radiation damage to normal tissues which could be a promising treatment for the reduction of side effects with delivery of external beam radiation. Dr. Christopher Sweeney presented early clinical data on the delivery of chemotherapy in a tumor-targeted nanoparticle. This system holds promise for delivering higher concentrations of medicine to tumors while sparing systemic side-effects. Dr. Alexander Zukiwsky presented data implicating the progesterone receptor as an escape mechanism for current treatments for prostate cancer. The targeting of the progesterone receptor and androgen receptor holds promise for the treatment of CRPC.



## ***20<sup>th</sup> Annual Prostate Cancer Foundation Scientific Retreat***

***October 24 - 26, 2013  
Gaylord National Hotel & Convention Center  
National Harbor, Maryland***

### **Program Committee:**

Program Committee Chair: Howard Soule, PhD (Prostate Cancer Foundation)

Jonathan Simons, MD (Prostate Cancer Foundation, Ex Officio)

Stuart Holden, MD (Prostate Cancer Foundation Medical Director & Cedars-Sinai Medical Center)

Jeffrey Karp, PhD (Brigham and Women's Hospital)

Owen Witte, MD (University of California, Los Angeles)

Lorelei Mucci, ScD (Harvard School of Public Health)

Felix Feng, MD (University of Michigan)

Vasan Yegnashubramanian, MD, PhD (Johns Hopkins University)

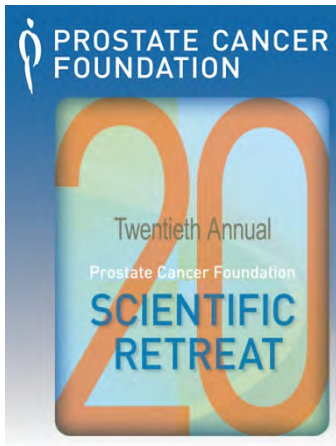
Howard Scher, MD (Memorial Sloan-Kettering Cancer Center)

David Rickman, PhD (Weill Cornell Medical College)

Nima Sharifi, MD (Cleveland Clinic)

Julie Rosenberg, MD (Bayer HealthCare Pharmaceuticals, LLC)

Matthew Galsky, MD (Mount Sinai School of Medicine)



# AGENDA

Thursday, October 24, 2013

## GENERAL SESSIONS

Location: Maryland Ballroom

### Welcome and Introduction

2:00PM - 2:10PM

Jonathan Simons, MD & Howard Soule, PhD  
Prostate Cancer Foundation

### Session 1: Convergence of Engineering and Oncology

2:10PM – 3:30PM

**Moderator: Jeffrey Karp, PhD**  
Brigham and Women's Hospital

2:10PM – 2:30PM

**Opening the Bioengineering Kimono**  
Jeffrey Karp, PhD  
Brigham and Women's Hospital

2:30PM – 2:45PM

**Micro Scale Engineering for Modeling the Microenvironment and Rare Cell Analysis**  
David Beebe, PhD  
University of Wisconsin

2:45PM – 2:50PM

Discussion

2:50PM – 3:05PM

**Engineering Cooperative Nanosystems for Cancer Diagnostics And Therapy**  
Sangeeta Bhatia, MD, PhD  
Massachusetts Institute of Technology

3:05PM – 3:10PM

Discussion

3:10PM – 3:25PM

**Stretchy Electronics and Sensors That Can Dissolve in Your Body**  
John Rogers, PhD  
University of Illinois

3:25PM – 3:30PM

Discussion



Thursday, October 24, 2013

**SPECIAL LECTURE**

**Experimental Scientific Integrity in the New Millennium**

3:30PM – 3:40PM

**Promoting a Culture of Reproducible Research**

**Elizabeth Iorns, PhD**

Science Exchange

3:40PM – 3:50PM

**Best Practices in Cell Line Authentication**

**Howard Soule, PhD**

Prostate Cancer Foundation

3:50PM – 3:55PM

**Discussion**

3:55PM – 4:10PM

**SPECIAL LECTURE**

**THE DATA SPHERE**

**The Project Data Sphere Initiative: Advancing Data Sharing in Cancer Research**

**Charles Hugh-Jones, MD, MCRP**

Member Life Science Consortium, CEO Roundtable on Cancer

**Introduction by Howard Soule, PhD**

Prostate Cancer Foundation

**4:10PM – 4:15PM Discussion**

**Session 2: Stem Cells & "Stemness" in Prostate Cancer**

**4:15PM – 5:15PM**

**Moderator: Owen Witte, MD**

University of California, Los Angeles

4:15PM – 4:30PM

**Signaling Pathways for Prostate Stem Cells**

Owen Witte, MD

University of California, Los Angeles

4:30PM – 4:35PM

**Discussion**

4:35PM – 4:50PM

**Cancer Stem Cells in Brain Tumors: What Are They and Where Do They Come From?**

Luis F. Parada, PhD

The University of Texas Southwestern Medical Center

4:50PM – 4:55PM

**Discussion**

Thursday, October 24, 2013

4:55PM – 5:10PM

**Prostate Epithelial Lineage Hierarchy and Cells of Origin for Prostate Cancer**

Li Xin, PhD  
Baylor College of Medicine  
Discussion

5:10PM – 5:15PM

**Special Lecture: Lessons in Leadership**

5:15PM – 5:30PM

**PCF Presidential Nanospeech 2013**

**Jonathan Simons, MD**

Prostate Cancer Foundation  
President & Chief Executive Officer

5:30PM – 5:50PM

**Perspective on Leadership Attributes for PCF Scientists in a Borderless, Digital Economy**

**Peter Grauer**

Bloomberg, LP

5:50PM – 6:00PM

**Discussion**

**Session 3: The Patho-Epidemiology of Prostate Cancer: Translating Population Science to Prevention and Treatment of Advanced Prostate Cancer**

6:00PM – 6:45PM

**Moderators: Lorelei Mucci, ScD**

Harvard School of Public Health

**Stephen Finn, MBBS, PhD**

University of Dublin, Trinity College

**Massimo Loda, MD**

Dana-Farber Cancer Institute

6:00PM – 6:04PM

**Patho-Epidemiology and its Promise to Reduce Suffering from Prostate Cancer**

Stephen Finn, MBBS, PhD  
University of Dublin, Trinity College

6:04PM – 6:19PM

**Digital Pathology and Tissue Imaging for Conducting Patho-Epidemiology Studies**

Peter Hamilton, PhD  
Queens University Belfast

**Thursday, October 24, 2013**

6:19PM – 6:35PM

**Examples of Successful Patho-Epidemiology Research Collaborations**

Jennifer Rider, ScD  
Harvard School of Public Health

Lorelei Mucci, ScD  
Harvard School of Public Health

Corinne Joshi, PhD  
Johns Hopkins University

Mark Pomerantz, MD  
Dana-Farber Cancer Institute

6:35PM – 6:45PM

**Future Directions in Patho-Epidemiology and Discussion**

Angelo De Marzo, MD, PhD  
Johns Hopkins University

Massimo Loda, MD  
Dana-Farber Cancer Institute

**Dinner & Awards**

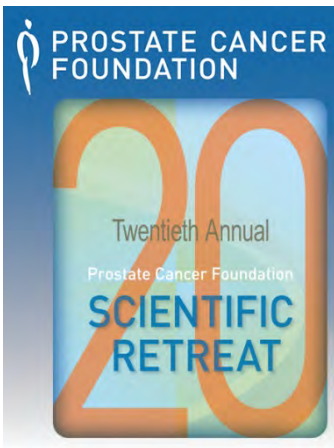
**7:30PM – 9:00PM**

**Poster Session and Dessert**

**9:00PM – 11:00PM**

*Dinner Location: Maryland Ballroom*

*Poster Session Location: Maryland Ballroom & Foyer*



# AGENDA

Friday, October 25, 2013

6:45AM – 7:45AM

Breakfast

Maryland Ballroom

7:45AM

Move to Session 4

## GENERAL SESSIONS

Location: Maryland Ballroom

### Session 4: The Role of Long Non-Coding RNAs in Prostate Cancer Progression

8:00AM – 9:00AM

**Moderator: Felix Feng, MD**

University of Michigan

8:00AM – 8:15AM

**Integrative Annotation and Functional Characterization of Long Non-Coding RNAs**

W. Lee Kraus, PhD

University of Texas Southwestern Medical Center at Dallas

8:15AM – 8:20AM

Discussion

8:20AM – 8:35AM

**Oncogenic and Tumor Suppressive Long Non-Coding RNAs in Prostate Cancer**

X. Shirley Liu, PhD

Dana-Farber Cancer Institute, Harvard School of Public Health

8:35AM – 8:40AM

Discussion

8:40AM – 8:55AM

**Identification of SchLAP1, an Unannotated Long Non-Coding RNA That Promotes Metastases in Prostate Cancer**

Felix Feng, MD

University of Michigan

8:55AM – 9:00AM

Discussion

9:00AM – 9:15AM

**SPECIAL PRESENTATION:**

**Prostate Cancer Foundation of Norway**

**Introduction by**

**Chris Evensen**

PCF Board Member

**Edward Schaeffer, MD, PhD**

Johns Hopkins University

**Stein Erik Hagen**

Chairman of the Board, Orkla ASA

**Session 5: Game Changing Research: PCF High-Achieving Young Investigators I**

9:15AM – 10:15AM

**Moderator: Daniel George, MD**

Duke University

9:15AM – 9:25AM

**Translational Modeling of Neuroendocrine Transdifferentiation**

Alexander Wyatt, DPhil

Vancouver Prostate Centre

9:25AM – 9:30AM

Discussion

9:30AM – 9:40AM

**Proteome-based Biomarker Discovery in Prostate Cancer;  
Building the Heme-oxygenase (HO-1) Interactome**

Geraldine Gueron, PhD

IQUIBICEN-CONICET, University of Buenos Aires

9:40AM – 9:45AM

Discussion

9:45AM – 9:55AM

**Functional and Genomic Characterization of Viable CTCs  
Enabled by Nanowells**

Atish Choudhury, MD, PhD

Dana-Farber Cancer Institute

9:55AM – 10:00AM

Discussion

10:00AM – 10:10AM

**Epithelial Progenitor Cells and Inflammation**

Andrew Goldstein, PhD

University of California, Los Angeles

10:10AM – 10:15AM

Discussion



Friday, October 25, 2013

**Session 6:**  
**Progress Reports from the Prostate Cancer Foundation Dream Teams: What We Have Learned So Far?**

**Moderator:**  
**Howard Soule, PhD**  
**Prostate Cancer Foundation**

10:15AM – 10:25AM	<b>West Coast Dream Team Presentation</b> Eric Small, MD University of California, San Francisco Owen Witte, MD University of California, Los Angeles
10:25AM – 10:30AM	<b>Discussion</b>
10:30AM – 10:40AM	<b>International Dream Team Presentation</b> Arul Chinnaiyan, MD, PhD University of Michigan Medical School
10:40AM – 10:45AM	<b>Discussion</b>

**KEYNOTE ADDRESS**

**11:00AM – 12:00PM**

**Michael Milken**  
Prostate Cancer Foundation  
Chairman & Founder

***Introduction by Stuart Holden, MD***  
PCF Medical Director & Cedars-Sinai Medical Center

**Lunch**

**12:00PM – 1:00PM**

***Location: Maryland Ballroom***

Friday, October 25, 2013

1:00PM

*Move to Session 7*

**Session 7: The Prostate Cancer Epigenome and Epigenomic Therapy**

1:15PM – 2:15PM

**Moderator: Vasan Yegnasubramanian, MD, PhD**  
Johns Hopkins University

1:15PM – 1:30PM

**Early Life Exposures (re) Program the Prostate Epigenome to Increase Prostate Cancer Risk**

Cheryl Lyn Walker, PhD  
Institute of Biosciences & Technology Texas A&M Health Science

1:30PM – 1:35PM

Discussion

1:35PM – 1:50PM

**Targeting Epigenetic Regulators in Prostate Cancer**

Myles Brown, MD  
Dana-Farber Cancer Institute

1:50PM – 1:55PM

Discussion

1:55PM – 2:10PM

**Targeting the Prostate Cancer Epigenome for Development of Biomarkers and Therapeutics**

Vasan Yegnasubramanian, MD, PhD  
Johns Hopkins University

2:10PM – 2:15PM

Discussion

**Session 8: Predictive Biomarkers for Prostate Cancer Progression and Survival**

2:15PM – 3:35PM

**Moderator: Howard Scher, MD**  
Memorial Sloan-Kettering Cancer Center

2:15PM – 2:30PM

**Enumeration of CTCs for Clinical Decision-Making**

Howard Scher, MD  
Memorial Sloan-Kettering Cancer Center

2:30PM – 2:35PM

Discussion

2:35PM – 2:50PM

**Personalized Genomic Biomarkers for Cancer Progression and Survival**

Victor Velculescu, MD, PhD  
Johns Hopkins Kimmel Cancer Center

2:50PM – 2:55PM

Discussion

2:55PM – 3:10PM

**Circulating DNA and MicroRNAs as Biomarkers in Cancer Patients**

Klaus Pantel, MD, PhD  
University Medical Center Hamburg

3:10PM – 3:15PM

Discussion

Friday, October 25, 2013

3:15PM – 3:30PM      **Circulating Large Oncosome Profiling and Prostate Cancer Progression**  
Dolores Di Vizio, MD, PhD  
Cedars-Sinai Medical Center

3:30PM – 3:35PM      Discussion

**3:35PM – 3:55PM**

**SPECIAL LECTURE**

*Prostate Epithelium: Why is it Androgen-Dependent?*

**Craig B. Thompson, MD**  
Memorial Sloan-Kettering Cancer Center

**3:55PM – 4:00PM    Discussion**

**Session 9: Understanding Mechanisms of Prostate Cancer Initiation and Progression**

**4:00PM – 5:00PM**

**Moderator: David Rickman, PhD**  
Weill Cornell Medical College

4:00PM – 4:15PM      **Implications of ERG-Mediated Alterations in Chromatin Conformation**  
David Rickman, PhD  
Weill Cornell Medical College

4:15PM – 4:20PM      Discussion

4:20PM – 4:35PM      **Context-Specific Oncogenesis of Aberrantly Expressed Transcription Factors in Prostate Cancer**  
Yu Chen, MD, PhD  
Memorial Sloan-Kettering Cancer Center

4:35PM – 4:40PM      Discussion

4:40PM – 4:55PM      **ERG Influences Cell Fate Decisions in Prostate Cancer**  
Ray Pagliarini, PhD  
Novartis Institutes for BioMedical Research

4:55PM – 5:00PM      Discussion

5:00PM – 5:20PM

**SPECIAL LECTURE**

*Immunotherapy for Solid Tumors with Implications for Prostate Cancer*

**Thomas Gajewski, MD, PhD**  
University of Chicago

*Introduction by Walter Stadler, MD*  
*University of Chicago*

5:20PM – 5:25PM Discussion

**Session 10: Reports from PCF Scientific Working Groups**

5:25PM – 5:55PM

**Moderator: Howard Soule, PhD**  
Prostate Cancer Foundation

5:25PM – 5:35PM

**Scientific Working Group for the Neuroendocrine Prostate Cancer Pathology Workshop** – *Meeting held on July 31, 2013 at Weill Cornell Medical College*

Mark Rubin, MD  
Weill Cornell Medical College

5:35PM – 5:40PM

Discussion

5:40PM – 5:50PM

**Scientific Working Group for Avataroid Biology: Generation of Patient Derived *In Vitro* Models of Prostate Cancer** – *Meeting held on April 25, 2013 at Memorial Sloan-Kettering Cancer Center*

Yu Chen, MD, PhD  
Memorial Sloan-Kettering Cancer Center

5:50PM – 5:55PM

Discussion

**Session 11: New Treatments Beyond AR I**

5:55PM – 7:15PM

**Moderator: Howard Soule, PhD**  
Prostate Cancer Foundation

5:55PM – 6:10PM

**ProMark™, An Automated Proteomics Prostate Cancer Prognostic Biopsy Test for Intact Tissue Slides**

Peter Blume-Jensen, MD, PhD  
Metamark

6:10PM – 6:15PM

Discussion

6:15PM – 6:30PM

**A Bispecific Small Molecule Antibody Conjugate for Hormone-Refractory Recurrent Prostate Cancer**

Chan Hyuk Kim, Ph.D.  
California Institute for Biomedical Research (Calibr)

6:30PM – 6:35PM

Discussion

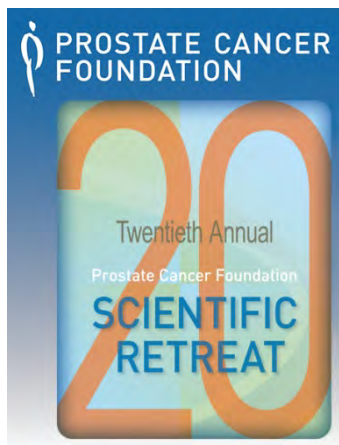
**Friday, October 25, 2013**

6:35PM – 6:50PM	<b>A High Throughput Screen to Identify Small Molecule Inhibitors of the AR N-terminus</b> Matthew Rettig, MD David Geffen School of Medicine at UCLA
6:50PM – 6:55PM	Discussion
6:55PM – 7:10PM	<b>Therapeutic Targeting of Transcription in Advanced Prostate Cancer</b> Nicholas Nickols, MD, PhD University of California, Los Angeles
7:10PM – 7:15PM	Discussion

**8:00PM – 10:00PM**

**Dinner, Movember Appreciation**  
**Entertainment**

***Location: Maryland Ballroom***



# AGENDA

Saturday, October 26, 2013

## GENERAL SESSIONS Location: Maryland Ballroom

6:45AM – 7:45AM      Breakfast      Maryland Ballroom

7:45AM      Move to Session 12

### Session 12: Game Changing Research: High - Achieving Young Investigators II

8:00AM – 8:45AM

**Moderator: Nima Sharifi, MD**  
Cleveland Clinic

8:00AM – 8:10AM

#### **Steroid Receptor Coactivators: Master Transcriptional Regulators in Prostate Cancer**

Nicholas Mitsiades MD, PhD  
Baylor College of Medicine

8:10AM – 8:15AM

Discussion

8:15AM – 8:25AM

#### **A 3βHSD1 Mutation Elicits DHT Synthesis and Castration-Resistance**

Nima Sharifi, MD  
Cleveland Clinic

8:25AM – 8:30AM

Discussion

8:30AM – 8:40AM

#### **Glucocorticoid Receptor Confers Resistance to Anti-Androgens by Bypassing Androgen Receptor Blockade**

Vivek Arora, MD, PhD  
Memorial Sloan-Kettering Cancer Center

8:40AM – 8:45AM

Discussion

8:45AM – 8:55AM

### **GLOBAL TREATMENT SCIENCE NETWORK LAUNCH**

**PHILIP KANTOFF, MD**

Dana-Farber Cancer Institute

*Introduction by Jonathan Simons, MD*

Prostate Cancer Foundation



Saturday, October 26, 2013

8:55AM – 9:10AM

**SPECIAL MEETING REPORT: Prouts Neck Meeting on Prostate Cancer Beyond AR**

**Ken Pienta, MD**  
Johns Hopkins University

9:10AM – 9:15AM      Discussion

9:15AM – 9:55AM

**PANEL DISCUSSION: Session 13:**

**Measure Twice, Cut Once:**  
**What is the Proper Role for Surgery in Prostate Cancer 2013?**

**Moderator: Stuart Holden, MD**  
Prostate Cancer Foundation Medical Director & Cedars-Sinai Medical Center

**Panelists:**  
**Eric Klein, MD**  
Cleveland Clinic Foundation

**Philip Kantoff, MD**  
Dana-Farber Cancer Institute

**Chris Logothetis, MD**  
University of Texas MD Anderson Cancer Center

**Patrick Walsh, MD**  
Johns Hopkins University

9:55AM – 10:00AM      Discussion

10:00 AM – 10:15AM

**Special Lecture**

**Bone-Targeted Radionuclide Therapy: Forging a Path Through the Known Unknowns**

**Michael Morris, MD**  
Memorial Sloan-Kettering Cancer Center

Introduction by Howard Soule, PhD  
Prostate Cancer Foundation

10:15AM – 10:20AM      Discussion

**Saturday, October 26, 2013**

**Session 14: New Treatments Beyond AR II**

**10:20AM – 12:20PM**

**Moderator: Matthew Galsky, MD**  
Mount Sinai School of Medicine

10:20AM – 10:35AM

**Targeting the Neural Microenvironment and Neuronal Trans-Differentiation**

Gustavo Ayala, MD  
University of Texas Health Science Center Medical School

10:35AM – 10:40AM

Discussion

10:40AM – 10:55AM

**Protein Phosphatase 2A as a Target for Prostate Cancer Therapy**

Matthew Galsky, MD  
Mount Sinai School of Medicine

10:55AM – 11:00AM

Discussion

11:00AM – 11:15AM

**Role of B and T Lymphocytes in the Development of Castrate Resistant Prostate Cancer and the Response to Chemotherapy**

Michael Karin, PhD  
University of California, San Diego

11:15AM – 11:20AM

Discussion

11:20AM – 11:35AM

**Anti-Inflammatory Modulators – Targeting Prostate Cancer While Protecting Normal Tissues**

Ulrich Rodeck, MD, PhD  
Thomas Jefferson University

11:35AM – 11:40AM

Discussion

11:40AM – 11:55AM

**Ingenious Delivery of an Old Drug**

Christopher Sweeney, MBBS  
Dana-Farber Cancer Institute

11:55AM – 12:00PM

Discussion

12:00PM – 12:15PM

**Progesterone Receptor, a Potential Mechanism of Resistance and Target in AIPC**

Alexander Zukiwski, MD  
Arno Therapeutics

12:15PM – 12:20PM

Discussion

**Meeting Adjourned**

*\*\* A boxed lunch will be provided \*\**

*If you are staying on Saturday evening, there will be an informal dinner at 6:30 pm at The Old Hickory Grill located inside of the Gaylord National Hotel. Participants will not need to RSVP to attend this dinner.*



