

# STATE OF THE SCIENCE REPORT

Highlights from  
the 19<sup>th</sup> Annual PCF  
Scientific Retreat  
October 2012



Provided with the compliments of  
the Prostate Cancer Foundation



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## Introduction

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For the nineteenth consecutive year, the Prostate Cancer Foundation brought together the world's top prostate cancer physicians and scientists in a collaborative forum to share new data and concepts. The 19th Annual PCF Scientific Retreat was the best meeting ever organized by PCF as measured by the novelty of findings, breadth of attendees, the quality and topics of the presentations, and attendee and Board member feedback.

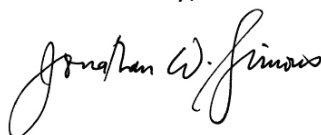
The overarching goal of all research discussed at the Retreat was to accelerate the end of death and suffering for men with prostate cancer. With a prime focus on scientific presentations, knowledge exchange and collaborative discussions, the Scientific Retreat featured the following:

- 55 scientific presentations and panels
- 91 poster presentations
- 18 different scientific disciplines related to prostate cancer biology presented and discussed
- 27 speakers (49%) presented first-in-field data at a PCF Scientific Retreat for the first time
- Attendance by 465 participants from 14 countries
- 107 academic institutions, 41 biopharmaceutical companies and 6 medical research foundations represented
- Attendance by 172 PhDs, 149 MDs, 86 MD PhDs, 3 PharmDs, and 1 DMD
- Attendance by 81 PCF Young Investigators
- Attendance by 9 PCF Board of Directors and 4 major donors

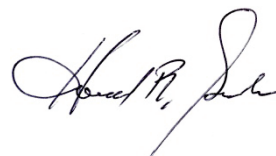
The PCF "Research Enterprise" is expanding globally with 200 research projects in 15 countries. PCF currently funds a robust portfolio that totals \$54 million in innovative prostate cancer research projects. This includes \$16.9 million to PCF Challenge Award Teams, including the Honorable A. David Mazzone Challenge Awards made possible by the disbursement of \$5 million of unclaimed settlement funds for a class-action lawsuit in the state of Massachusetts, \$12.5 million to fund two new Dream Teams, co-sponsored by Movember and Stand Up 2 Cancer, \$9.75 million to fulfill our goal of funding 100 PCF Young Investigator Awards, and \$3.2 million to 9 centers in the PCF-DoD Therapy Consortium (<http://pcctc.org>), \$3.65 million to Creativity Award projects, \$3.04 million to support awards generously sponsored by major PCF donors, and \$5 million to complete a special \$10 million Challenge Award at the University of Michigan that is being matched dollar for dollar by the University.

This PCF 2012 State of the Science Report summarizes each presentation individually. The highlights of each session provide a brief overview of the scientific discipline and a summary of the latest findings that impact prostate cancer diagnosis, prognosis or treatment. PCF aims to translate these new findings, as rapidly as possible, into clinical investigation. Toward that end, we hope this report will be useful to you and will stimulate further dialogue, data exchange, and questioning. If you have specific questions, please contact Dr. Guneet Walia at [gwalia@pcf.org](mailto:gwalia@pcf.org).

Yours sincerely,



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



Howard R. Soule, PhD  
Chief Science Officer

# Special Lecture: Tumor Heterogeneity: Clonality and Consequences

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**Charles Swanton, MD, PhD**

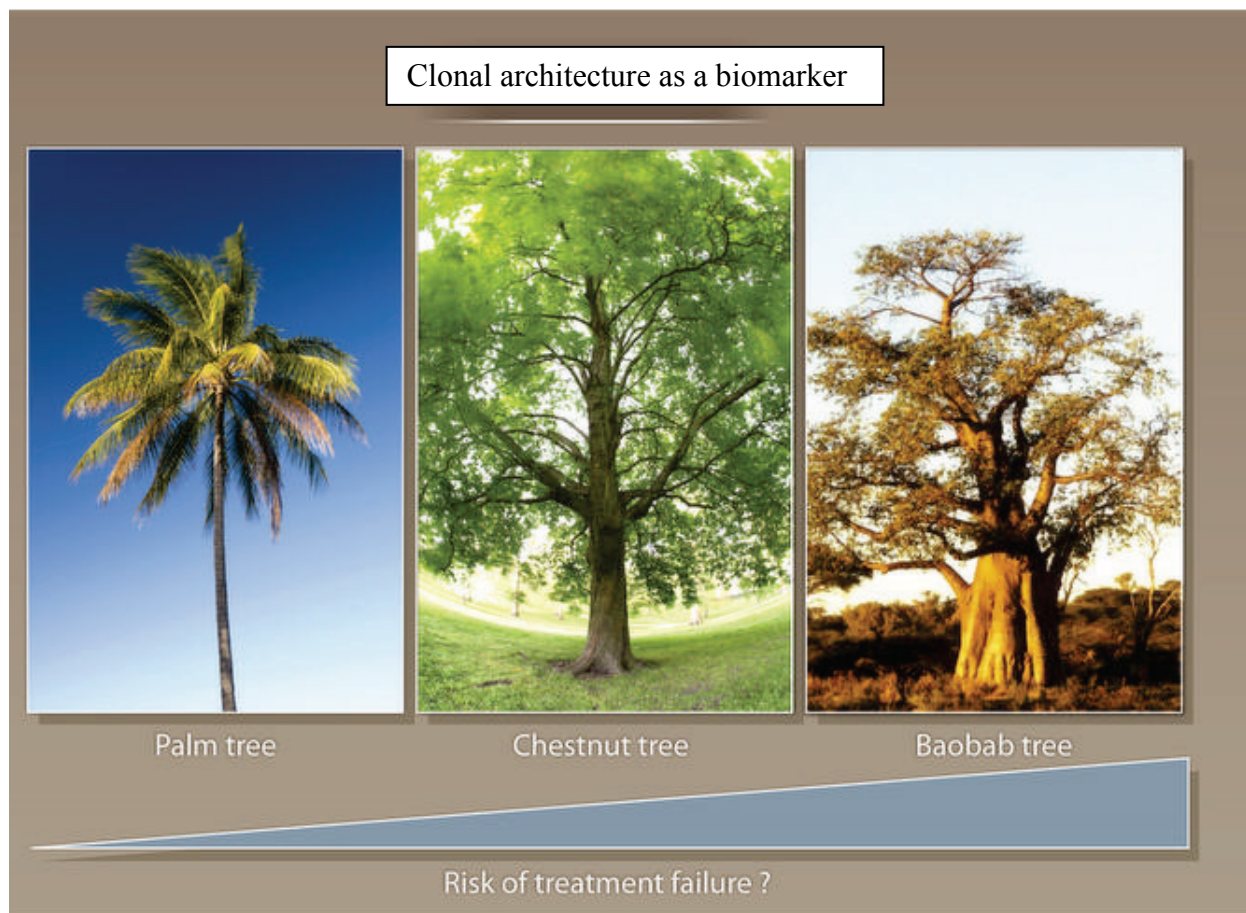
**Cancer Research UK, London Research Institute & University College London Hospitals & Cancer Institute**

**Funded by a PCF Challenge Award**

- Most advanced solid cancers display multi-drug resistance and we still haven't understood the underlying causes for the emergence of this treatment resistance.
- Advanced cancers also display multifocality in the organ of origin. This is especially the case for prostate cancer where numerous foci of genetically distinct lesions that vary in size and grade are observed. The significance of this heterogeneity is not known but is thought to be a source of drug resistance. An understanding of this heterogeneity is an especially important factor in the new era of precision medicine where the genetic make-up of the patient's tumor will guide therapy.
- Biomarkers can serve as mechanism to report the underlying biology and behavior of an individual's cancer. However, despite an estimated 150,000 biomarkers being tested for various cancers, only a handful few (~100) actually make it to the clinic as reliable predictors of tumor response to therapy.
- Dr. Charles Swanton's studies on renal cell carcinoma have shown that intratumor heterogeneity may contribute to therapy resistance and difficulties in biomarker validation strategies, i.e. variations in the genetic makeup within a single tumor.
- His studies on core biopsies obtained from multiple spatially separated primary renal carcinoma tumors and associated metastatic sites revealed that 63 to 69% of all mutations are not detectable across every tumor region, i.e. the genetic landscape of each segment of a single tumor is variable and very different from each adjacent site of the same tumor. These paradigm-shifting results therefore suggest that genomic analyses from a single tumor biopsy, that is the current norm for disease diagnoses and monitoring, may not actually be reporting the full mutational burden of these widely heterogeneous tumors.
- Dr. Swanton's studies on renal cell carcinoma suggest that these differences in the genetic landscape stem from heterogeneous mutations as well as chromosomal instability.
- This intratumoral heterogeneity holds the potential to causes tumor evolution and 'adaptation' to therapy, i.e. development of treatment resistance, and therapy failure.

- These studies question the very foundation and current approach to personalized-medicine that prescribes treatments based on the evaluation of a single tumor-biopsy sample.
- If validated in prostate cancer, these results suggest that predicting therapeutic outcome should account for both, the polygenic nature of drug resistance and intratumor heterogeneity.
- Dr. Swanton compared the development of intratumor heterogeneity to a growing tree. The trunk of this tree harbors the founding driver mutations of a cancer that will eventually be ubiquitously present in every tumor and geographically distant region.
- The branches of the tree represent different geographically separated regions of the tumor or subclones present within single biopsies that carry heterogeneous mutations that are not present in every tumor cell or tumor region.
- Under distinct selection pressures such as treatment stresses that kill one subclone, these heterogeneous branch mutations hold the potential to become driver mutations, that allow the resistant subclone (due to its unique mutations) to grow and take over the tumor.
- Dr. Swanton's studies suggest that the ubiquitous genetic events present in the tree trunk will serve as better potential biomarkers and therapeutic targets than the heterogeneous branching mutational events.
- According to the 'growing tree model', the length of the tree trunk is analogous to the number of driver/ubiquitous mutations in a tumor, while the size of the tree branches is analogous to the number of heterogeneous mutations in the distant parts of a single tumor, or a single biopsy (Figure).
- Therefore "Palm tree-like" tumors that carry many more ubiquitous genetic events (trunk) than heterogeneous mutations (branches), may result in improved clinical risk profiles. In contrast, "Baobab tree-like" tumors in which the heterogeneous genetic events outnumber the ubiquitous mutations may result in poorer clinical outcomes and increased propensity for drug resistance. Meanwhile, the risk of treatment failure with "chestnut tree-like" tumors may lie between these two extremes (Yap et al., Science Translational Medicine, 2012).
- Drs. Charles Swanton, Johann DeBono and their teams have been awarded a 2012 PCF Challenge Award to investigate the role of intratumor heterogeneity in the progression to metastatic disease and treatment-resistance in prostate cancer patients.





**Figure:** A trunk-branch model of intratumor heterogeneity.

## Session 1

### Field Cancerization and the Tumor Microenvironment

**Mikala Egeblad, PhD**

**Cold Spring Harbor Laboratory**

**Contributions of the Tumor Microenvironment to Chemoresistance**

- Cancer cells develop resistance to therapies by multiple different mechanisms. Some of these are *intrinsic* i.e. originate within a cancer cell while others are *extrinsic*, i.e. arise in the tumor microenvironment.
- The tumor microenvironment is the collection of normal cells, molecules and blood vessels that surround and feed a tumor cell. The tissue is composed of the resident cellular components (fibroblasts, smooth muscle, neuroendocrine cells, endothelium, nerves); structural components (matrix); infiltrating/ non-resident cells (inflammatory and bone marrow derived cells) and molecular components

(nutrients, hormones, growth factors and cytokines). Previous studies have shown that there is crosstalk between the tumor and its surrounding normal cells.

- Dr. Mikala Egeblad is studying these extrinsic mechanisms of therapy resistance originating in the tumor microenvironment in breast cancer preclinical models of disease.
- She has developed impressive *in vivo* imaging techniques to study the dynamic interactions between a tumor and its surrounding stroma (the connective, supportive framework outside a cell) in live mice.
- Dr. Egeblad used this intravital microscopy to study drug distribution, cell death, and tumor-stroma interactions in chemotherapy-treated mouse mammary carcinomas.
- These real-time microscopic imaging studies on preclinical models of disease show that
  1. The tumor microenvironment is altered by chemotherapy.
  2. The tumor microenvironment contributes to treatment resistance by two mechanisms:
    - a) It regulates the permeability ('leakiness') of the blood vessels feeding tumors. Vascular permeability is regulated by the class of enzymes called matrix metalloproteases, specifically MMP9. Deleting/inhibiting MMP9 makes blood vessels leaky, thereby improving tumor response to chemotherapy with doxorubicin. Therefore, combining doxorubicin with therapies targeting MMP9 hold potential in improved patient outcomes.
    - b) Inflammatory cells such as the myeloid cells are an important component of the microenvironment. Myeloid cells are recruited to the site of the tumor after chemotherapy with doxorubicin by activation of signals through a receptor called CCR2. Dr. Egeblad's studies show that inhibiting this pro-cancer signaling pathway diminishes myeloid cell recruitment and improves tumor response to doxorubicin.
- In summary, Dr. Egeblad's studies demonstrate that the tumor microenvironment impacts tumor response to therapies via regulation of vascular permeability and innate immune cell infiltration. Further, the real-time live imaging techniques developed by Dr. Egeblad and colleagues hold potential in studying treatment response in situ.



**Neil Bhowmick, PhD**

**Cedars-Sinai Medical Center**

**Paracrine Contributors of Prostate Cancer Progression: A Response to Carcinogenesis**

**Funded by a PCF Challenge Award**

- Paracrine signaling is a form of signaling in which the signal originates from the surroundings of a cell ('para' meaning near), e.g. maintenance of epithelial tissues requires the stroma (the connective, supportive framework outside the lumen of the gland), with these two compartments of a gland being in constant cross-talk through paracrine signals.
- Prostate cancer is initiated by the transformation of the luminal epithelial cells that line the prostatic ducts. Studies have shown that the progression to adenocarcinoma is governed by the inherent genetic instability as well cues from the surrounding microenvironment composed of cells from the stroma.
- Changes in the stroma include alterations in the activity of the oncogenic cytokine, TGF- $\beta$  (transforming growth factor- $\beta$ ); loss of expression of the TGF- $\beta$  type II receptor (T $\beta$ RII) etc. These changes in the stromal compartment promote prostate cancer progression.
- A better understanding of the 'cancer stromal effect' i.e. stromal contributions to cancer progression is important to understand the combinatorial signals that support and promote tumor growth, dedifferentiation, invasion, and survival. These studies will allow the development of better therapeutics targeting the stroma, thereby preventing cancer progression.
- Dr. Neil Bhowmick is working towards understanding the role of these stromal factors in prostate tumorigenesis.
- His studies have identified *heterogeneity* in the cells of the stromal compartment. These cells of the cancer-associated stromal compartment demonstrate a heterogeneous loss of TGF- $\beta$  signaling which promotes prostate epithelial transformation.
- Dr. Bhowmick's studies demonstrated that these unaltered cells of the cancer stroma are not merely bystanders. They also contribute to the generation of pro-carcinogenic signals in a cooperative fashion, establishing a cancer stromal field. This cooperativity of signaling within a heterogeneous stromal compartment can potentially explain the multifocal and polyclonal progression of prostate cancer.
- Downstream of this heterogeneous loss of TGF- $\beta$  signaling, Dr. Bhowmick's studies have identified a range of paracrine signaling factors released by the heterogeneous stromal compartment that promotes prostate tumorigenesis. These factors include pro-proliferative signaling factors (hepatocyte growth factor and Wnt proteins), pro-inflammatory cytokines (CCL2) and pro-invasion cytokines such as the protein SDF-1.

- Dr. Bhowmick also showed that these alterations in the stromal paracrine signaling not only mediate localized prostate cancer progression, but also mediate metastatic progression to the bone.
- Altered stromal paracrine signaling results in the upregulation of the protein Mcl-1 in the prostatic epithelium. Mcl-1 is known to antagonize the function of apoptosis (cell death) proteins, preventing prostate cancer cell death.
- Dr. Bhowmick and his team have identified the inhibitor Sabutoclax that targets Mcl-1 activity and mediates cell death both in treatment-resistant localized as well as bone metastatic prostate cancer cells.

## **Special Lecture: Cancer Interception & Metabolic Transformation**

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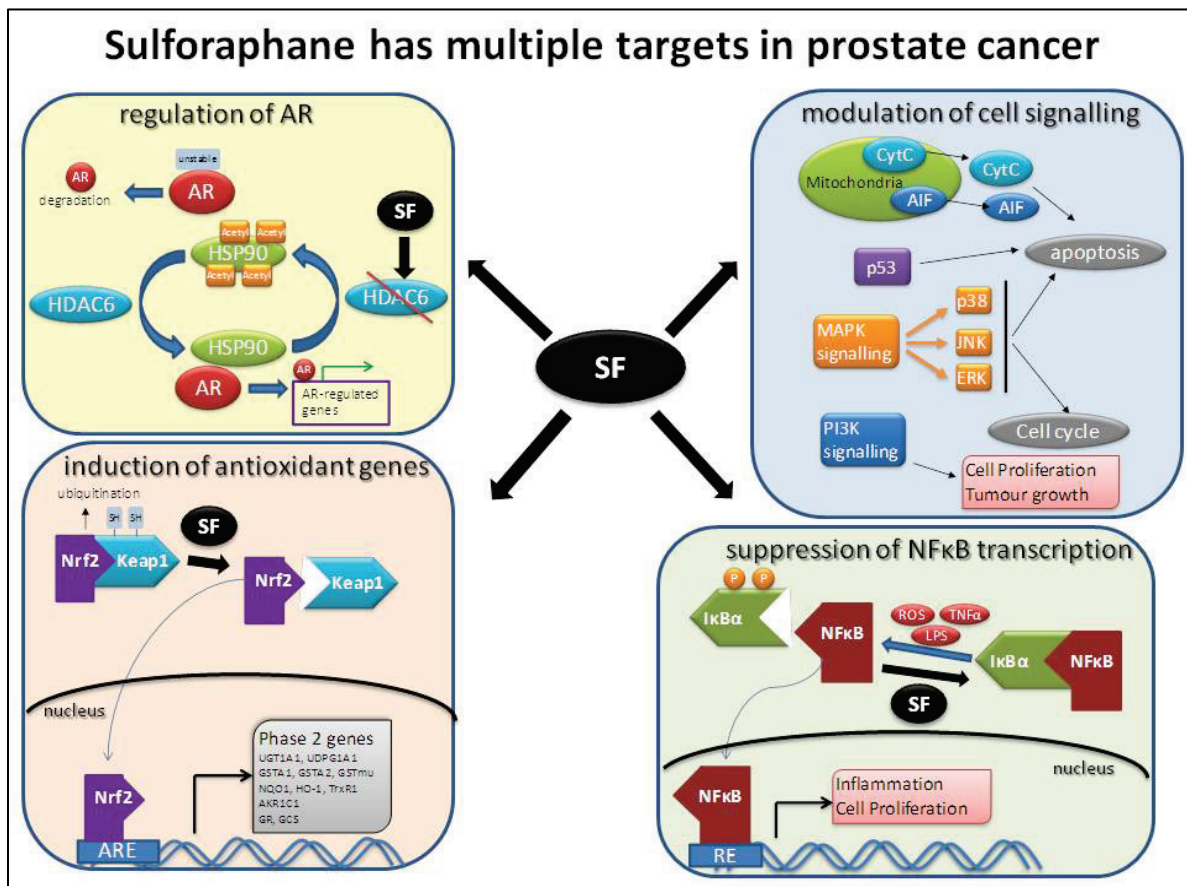
**Richard Mithen, PhD**

**Institute for Food Research, Norwich Research Park**

**Funded by a PCF Challenge Award**

- Dr. Richard Mithen, a leading plant scientist from the UK, discussed the concept of cancer interception i.e. 'to actively intercept a cancer development process before the damage is done, that is, before the full-blown advanced tumor presents in the clinic'.
- Prostate cancer follows a progression from localized prostate cancer to advanced metastatic disease that is accompanied by several alterations in normal metabolism such as deregulation of lipid and glucose metabolism, increase in oxidative stress and an increase in pro-inflammatory cytokines.
- Dr. Mithen proposed targeting the environment in which cancer proliferates to reduce the progression of localized cancer, and one means of doing this may be diets rich in sulforaphane.
- Broccoli contains the compound, 4-methylsulphinybutyl glucosinolate (glucoraphanin) which is converted by the enzyme myrosinase to the active ingredient 4-methylsulphinybutyl isothiocyanate, also called sulforaphane after consumption.
- The action of sulforaphane can be likened to a sponge that soaks up the harmful reactive oxygen species (ROS) and protects the body from molecular injury and diseases such as prostate cancer.
- Dr. Mithen has developed high glucoraphanin broccoli, commercialized as Beneforte™ broccoli and sold throughout the UK and US, as an effective vehicle for delivering sulforaphane to men with localized prostate cancer.

- In a pilot study to examine the effects of a high glucoraphanin broccoli on gene expression in prostate tissue, Dr Mithen and his team compared gene expression profiles from biopsy tissue from men with HGPIN before and after a 12 month dietary intervention study.
- They observed that while men on the control pea-rich diet showed relatively few changes in global gene expression, men on the broccoli-rich diet demonstrated significant changes in gene expression associated with several cell signaling pathways, including insulin, PI3K, transforming growth factor beta 1 (TGFβ1) and epidermal growth factor (EGF) gene signaling pathways in prostate tissue, consistent with some previously described effects of sulforaphane in animal studies.
- These cellular signaling pathways play critical roles in the initiation/development of prostate cancer. Further studies demonstrated that sulforaphane has multiple targets in prostate cancer such as AR regulation, antioxidant gene induction, cell proliferation inhibition etc. (Figure). By regulating cell signaling through critical pathways such as PI3K, TGFβ1 and AR, sulforaphane may prevent prostate cancer initiation and progression.



**Figure:** The multiple effects of sulforaphane on a prostate cell. By regulating cell signaling through critical pathways such as MAPK, PI3K and AR, sulforaphanes prevent prostate cancer initiation and progression.

- Further human intervention studies have shown that sulforaphane diets have significant effects on lipid metabolism, probably mediated by the cell signaling pathways previously identified. Analysis of the genetic variation amongst the volunteers in this study suggested that sulforaphane may be directly interacting with a G protein coupled receptor, which mediates the changes in cell signaling pathways, which then, in turn, modifies lipid metabolism.
- Dr. Mithen and his team, with funding from a PCF Challenge Award, propose to conduct a large, comprehensive, randomized double-blind parallel intervention study in prostate cancer patients on active surveillance. Patients will be divided into two groups, one on a high sulforaphane diet and the other on lower sulforaphane diet.
- The team will conduct global gene expression analysis, metabolomics (read out of the gene expression), 3T MRI and urine exosome analysis to study the effects of diet on prostate metabolism.

## **Session 2**

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### **Role of Growth Factor and Signal Transduction Alterations in Prostate Cancer Initiation, Progression and Therapy Resistance**

**Michael Ittmann, MD, PhD**  
**Baylor College of Medicine**

**Targeting Fibroblast Growth Factor Receptor Signaling Inhibits Prostate Cancer Progression**

**Funded by a PCF Donor-Directed Award**

- Dr. Michael Ittmann discussed the significance of targeting the Fibroblast Growth Factor (FGF) signaling pathway in prostate cancer.
- FGFs are a family of 18 proteins that function as growth factors and play critical roles in cellular proliferation and differentiation. FGFs are involved in a wide variety of physiological and pathological processes including development, angiogenesis and cancer, and are critical in prostate development. Endocrine FGFs circulate and play a role in energy, bile acid and calcium homeostasis.
- The expression of classical FGFs such as FGF2, FGF6, FGF9 and FGF17 is enhanced in prostate cancer and these growth factors promote cancer cell growth, inhibit cell death and enhance angiogenesis.
- FGF signaling is an excellent target in prostate cancer therapeutics because this pathway plays key roles not only inside the cancer cell, but also in the tumor

microenvironment that influences the growth of cancer cells and their response to therapy.

- FGFs mediate their activity through their receptors, the FGFRs. This FGF-FGFR complex, a signaling axis involving multiple FGF ligands and receptors, mediates tumor–stromal interactions and is one of the most commonly altered signaling pathways during prostate cancer progression.
- Dr. Ittmann presented data on a specific FGF receptor, FGFR-4, a particular mutation in which is associated with both prostate cancer incidence and its aggressive clinical course.
- This mutation, the FGFR-4 Arg388/Gly388 polymorphism, leads to the substitution of charged amino acid for uncharged amino acid in transmembrane domain of the receptor protein resulting in sustained activation of the receptor and its downstream signaling that results in enhanced activity of several oncogenic and pro-inflammatory factors such as ERK (Extracellular signal-Regulated Kinase) and SRF (Serum Response Factor).
- Dr. Ittmann's studies have shown that this mutation in the FGFR protein results in increased motility and invasiveness of prostate cancer cells.
- Dr. Ittmann presented preclinical data on two experimental compounds, AZ8010 and AZ4547, which are potent FGF receptor kinase inhibitors and target FGF signaling effectively, preventing prostate cancer cell invasion and tumor growth in experimental animals by inhibiting ERK activity.
- Dr. Ittmann proposed appropriate combination therapy with these FGFR inhibitors that can potentially lead to patient benefit.

## **Nora Navone, MD, PhD**

### **The University of Texas MD Anderson Cancer Center**

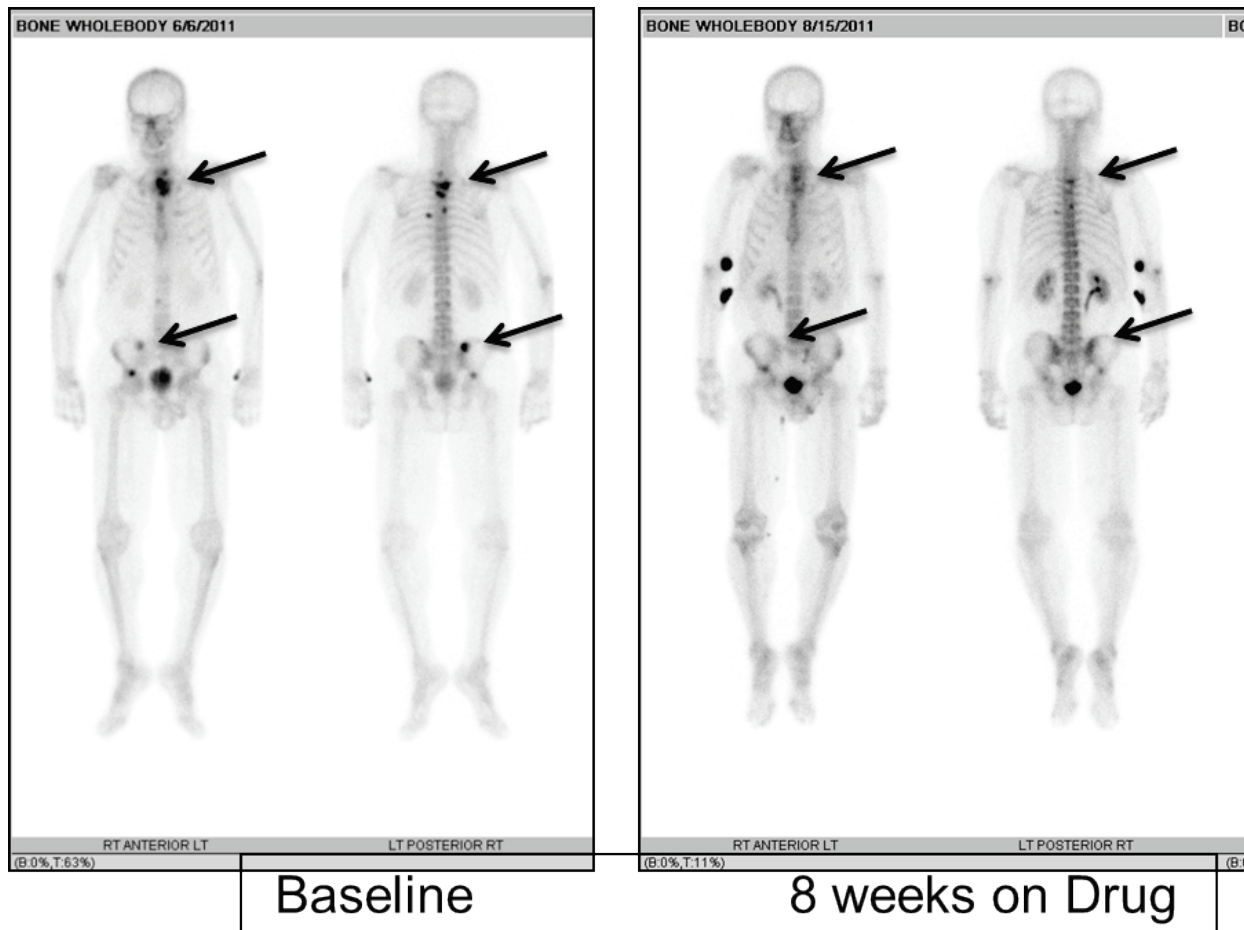
#### **Targeting FGF Signaling in Prostate Cancer Bone Metastases**

#### **Funded by a PCF Creativity Award**

- Signaling through the fibroblast growth factors (FGFs) and their concomitant receptors (FGFRs) is known to play critical roles in normal bone development and maintenance of homeostasis in the bone environment.
- One example is FGF9, an important skeletal growth and differentiation factor that is critical for normal bone development.
- This FGF-FGFR signaling axis is subverted by prostate cancer cells when they metastasize to the bone which is the primary site of treatment-resistant disease progression.
- Dr. Nora Navone's studies have shown that the growth factor FGF9 plays important roles in prostate cancer progression to the bone. Her studies have also

shown a crosstalk between prostate cancer cells that metastasize to the bone, and bone cells mediated by the FGF-FGFR axis.

- Therefore, inhibition of FGF-mediated signaling pathways will likely be therapeutically beneficial.
- All FGFs mediate their activity via FGF receptors which are proteins embedded in the cellular membrane that sense signals outside the cell and relay the signals inside.
- This signal relay is made possible by enzymatic (tyrosine kinase) activity of the FGFRs on the inside of the cell. Inhibiting this tyrosine kinase activity of the FGFRs holds potential in inhibiting its downstream pro-cancer signaling.
- Dovitinib is an experimental compound from Novartis Oncology that potently inhibits FGFR downstream signaling.
- Dr. Navone and her colleagues tested the activity of dovitinib in preclinical models of prostate cancer in bone.
- Results of her studies show that this compound inhibits prostate cancer progression to the bone in a mouse model. Dovitinib reduced FGFR1 expression in prostate cancer tumors growing in bone after 7 days of treatment.





- In these studies, dovitinib also exhibited anti-angiogenesis activity, i.e. the compound inhibited the generation of new blood vessels that are essential to provide nutrients to the growing cancer cell mass. Inhibition of angiogenesis can result in the death of growing tumors due to lack of nutrients and oxygen.
- Based on these preclinical results, Dr. P. Corn, in collaboration with Dr. Navone, tested dovitinib in a clinical study of metastatic castration-resistant prostate cancer patients.
- Bone scan and CT scan analysis taken before and after dovitinib therapy revealed tumor regression in bone and soft tissue metastases and progression-free survival (PFS) of greater than 20 weeks (mPFS 28.9 wks, range 20 to 35 wks) in a subset of patients with advanced disease (~25%).
- Dr. Navone and her team are currently working on developing methods to clinically identify the subset of likely responders.
- Their studies showed that this subset of patients demonstrated a decline in their bone specific alkaline phosphatase levels, which may serve as a potential biomarker to identify these patients.
- Dr. Navone also presented results of inhibition of FGFR signaling by the Astra Zeneca compound, AZD4547. Like dovitinib, AZD4547 also is a tyrosine kinase inhibitor and inhibits FGFR1, 2, 3 and 4 signaling.
- Preclinical studies with AZD4547 caused tumor volume reduction in the bone.
- In summary, Dr. Nora Navone demonstrated the clinical benefits of targeting the FGF-FGFR signaling axis for the treatment of prostate cancer bone metastases.

## **Pete Nelson, MD**

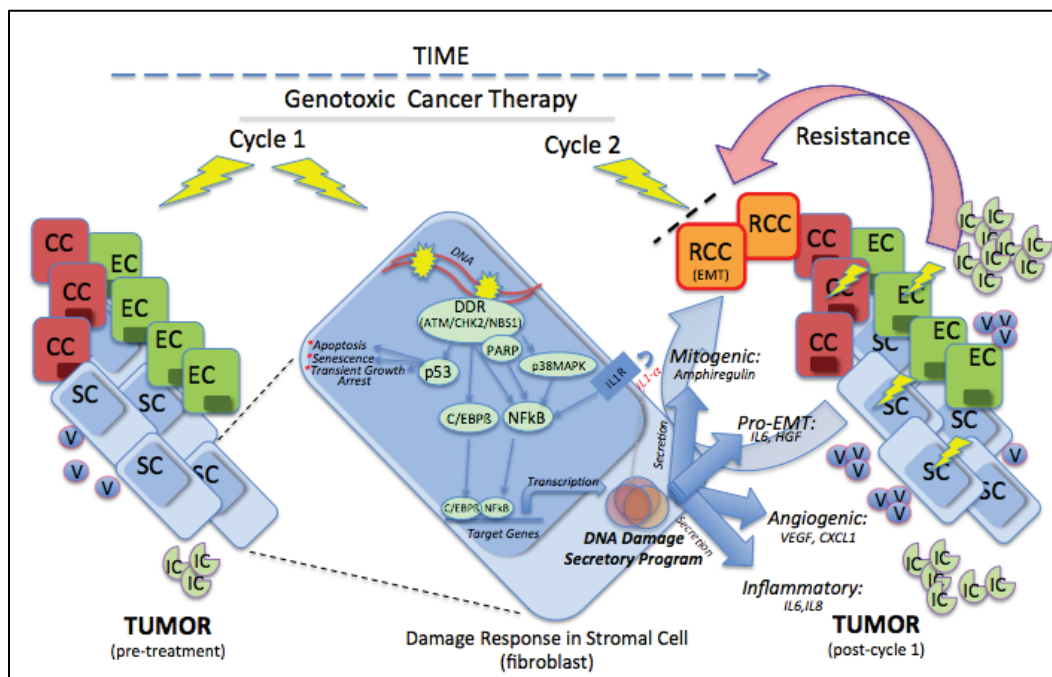
### **Fred Hutchinson Cancer Research Center**

#### **Damage Induced Growth Factor Responses in the Tumor Microenvironment Influence Therapy Resistance**

#### **Funded by PCF Creativity and Challenge Awards**

- An important barrier to effective cancer treatment with DNA-damaging chemotherapy and radiation is the near inevitability of tumor resistance to applied therapy. This acquired tumor resistance is the critical reason for treatment failure in over 90% patients with metastatic disease.
- Dr. Peter Nelson and his team have identified a mechanism for the development of acquired resistance by cancer cells, and a specific protein that can be targeted to overcome resistance.
- Their results show that the 'tumor microenvironment' plays an important role in the response of tumors to therapy, enabling the development of treatment resistance and cancer progression.

- The tumor microenvironment is the collection of normal cells, molecules and blood vessels that surround and feed a tumor cell. The tissue is composed of the resident cellular components (fibroblasts, smooth muscle, neuroendocrine cells, endothelium, nerves); structural components (matrix); infiltrating/ non-resident cells (inflammatory and bone marrow derived cells) and molecular components (nutrients, hormones, growth factors and cytokines). Previous studies have shown that there is crosstalk between the tumor and its surrounding normal cells.
- Dr. Nelson showed that prostate cancer treatments (such as chemotherapy, radiation etc.) induces DNA damage in the normal tissues surrounding the tumor comprising the tumor microenvironment.
- These treatment-induced alterations cause these normal cells to express a diverse set of growth factors and cytokines (such as the proteins WNT16B and SPINK1).
- This DNA Damage-associated Secretory Program (DDSP) includes several pro-inflammatory, angiogenic, neurogenic and matrix re-modeling factors which promote therapy resistance and subsequent tumor progression.



**Figure:** Model for therapy-resistance effects originating in the tumor microenvironment in response to cancer therapeutics. The initial round of therapy causes cell death in a subset of tumor cells and activates a DNA damage response (DDR) in the stromal cells (fibroblasts) comprising the tumor microenvironment. The DDR includes a spectrum of proteins that promote tumor repopulation through pro-growth signaling pathways in a subset of the cancer cells. These secretory components such as WNT16B promote resistance to subsequent cycles of cytotoxic therapy. CC, cancer cell; EC, epithelial cell; SC, stromal cell; RCC, resistant cancer cell; DDR, DNA-damage response (Sun, Y et al. Nature Medicine, 2012).

- One of the critical DDSP factors is the protein WNT16B (pronounced 'WINT16B') which is activated in the cells comprising the tumor microenvironment such as the fibroblasts.
- Dr. Nelson and his team observed a sixteen-fold increase in WNT16B expression in the tumor microenvironment upon DNA damage.
- Dr. Nelson presented the mechanism of action of oncogenic WNT16B signaling: DNA damage, genotoxic stress → activation of NF-κB signaling in tumor microenvironment → release of secretory proteins like WNT16B → effect  $\square$  catenin oncogenic signaling in tumor cells → promotes an epithelial to mesenchymal transition (EMT) in the prostate epithelium → enhances prostate cancer cell invasiveness and resistance to cytotoxic therapy → ineffective second round of treatment due to rise of resistant subsets of tumor cells (Figure).
- Dr. Nelson concluded that approaches targeting these alterations in the tumor microenvironment in addition to conventional cancer therapeutics may enhance treatment response.
- There are still several unanswered questions in understanding the precise role of the tumor microenvironment in therapy resistance, such as the key initiators and effectors of the DNA damage program, the variations in this DNA damage associated secretory response in different tissues and individuals.

## Session 3

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### Advances in Molecular Imaging of Prostate Cancer

**John Kurhanewicz, PhD**

**University of California, San Francisco**

**Hyperpolarized MR Molecular Imaging of Prostate Cancer - From Cells to Man  
Funded by a PCF Competitive Award**

- A significant unmet medical need for the effective therapy of localized prostate cancer is accurate staging of the disease to determine extent of progression beyond the gland.
- Current imaging technology is insufficiently sensitive to reliably stage the disease.
- One way to create more sensitive imaging technology is to exploit differences in cellular metabolism specific to tumor cells.
- Two critical factors for effective molecular imaging are, one, a high signal-to-noise ratio (SNR) which allows detection of metabolites even at extremely low physiological concentrations, and two, high resolution.

- Positron Emission Tomography (PET) can achieve high SNR detecting molecules at molar concentrations of  $10^{-11}$ , however, PET image resolution is poor.
- On the other hand, magnetic resonance (MR) imaging provides superior image resolution but is riddled with the limitation of detecting targets at molar concentrations lower than  $10^{-3}$ .
- Hyperpolarized MR molecular imaging combines the positives of PET and MR to generate high-resolution images with high specificity.
- MR performed with a hyperpolarized C-13 tracer in a 1.5T imaging magnet produces 25,000 times as much signal as standard proton imaging.
- $[1-^{13}\text{C}]$  Pyruvate has been the most widely studied substrate to date due to its central role in cellular metabolism.
- Pyruvate can be metabolized in a cell down two pathways, either to lactate by the enzyme lactate dehydrogenase or to alanine by the enzyme alanine transaminase. These reactions catalyzed by lactate dehydrogenase and alanine transaminase are known to be altered in cancer.
- Therefore, studying the metabolic rate of conversion of  $[1-^{13}\text{C}]$  pyruvate to lactate and alanine, and the proportion of each metabolite produced can help evaluate tissue viability, indicating if the tissue being studied is healthy or diseased.
- $[1-^{13}\text{C}]$  Pyruvate also has advantages such as the ease with which it can be hyperpolarized, its relatively long T 1 relaxation time, and its very rapid transport across the cell membrane and subsequent metabolism. Its high solubility in water is also advantageous as it allows a higher available concentration upon dissolution.
- Dr. John Kurhanewicz presented the results of a Phase I clinical trial to study safety and imaging feasibility in 31 men prior to therapy with biopsy-confirmed prostate cancer who had undergone a previous screening multiparametric  $^1\text{H}$  MR examination.
- 13 patients were studied at the MTD of 0.43mL/kg body weight which was used to optimize MR acquisition and assess biologic variability. Hyperpolarized pyruvate was taken up by cells within 20 seconds of injection, the maximal pyruvate signal emerged at 27s and maximum hyperpolarized lactate production was seen in 45s in cancer cells. Volumetric metabolic coverage of the entire prostate was seen in 17s.
- Dr. Kurhanewicz' data showed that hyperpolarized  $(^{13}\text{C})$  MR spectroscopic imaging demonstrates accuracy in measuring tumor location, volume and grade.
- The study demonstrated that very negligible hyperpolarized lactate is produced in the benign prostate while the hyperpolarized lactate/pyruvate ratio increases with pathologic grade, holding potential in discriminating aggressive from indolent prostate cancer.

- Dr. Kurhanewicz discussed the potential clinical applications of the hyperpolarized (13)C MR spectroscopic imaging technique which can:
  1. be used to lymph node and bone metastases,
  2. allow simultaneous imaging of tumor metabolism and microenvironment (e.g. using dihydroascorbate as a measure of the redox state of the cell, HP (13)C bicarbonate as a measure of the interstitial pH etc.),
  3. be used to study the response to androgen deprivation therapy and the development of treatment resistance,
  4. be used to study response to targeted therapy (PI3K signaling has direct effects on glucose metabolism through rapamycin (mTOR)–activated posttranscriptional control of hypoxia inducible factor-1 (HIF-1), which controls the expression of several glycolytic enzymes, including LDH. Therefore treatment with a PI3K-AKT-mTOR pathway inhibitor negatively modulates hyperpolarized lactate formation).
- In summary, Dr. Kurhanewicz demonstrated hyperpolarized (13)C magnetic resonance spectroscopic imaging as a novel *in vivo* metabolic imaging technique with potential for detecting/ diagnosing cancer as well as studying its response to treatment.
- A combination of various hyperpolarized probes has the potential to determine the complex interaction between cellular metabolism, perfusion, redox and pH to provide a more complete understanding of prostate cancer progression and therapy response.
- Dr. Kurhanewicz' studies showed that hyperpolarized (13)C MR spectroscopic imaging can potentially detect the molecular effect of various cell signaling inhibitors, thereby providing a radiation-free method to predict tumor response.

## **Jason Lewis, PhD**

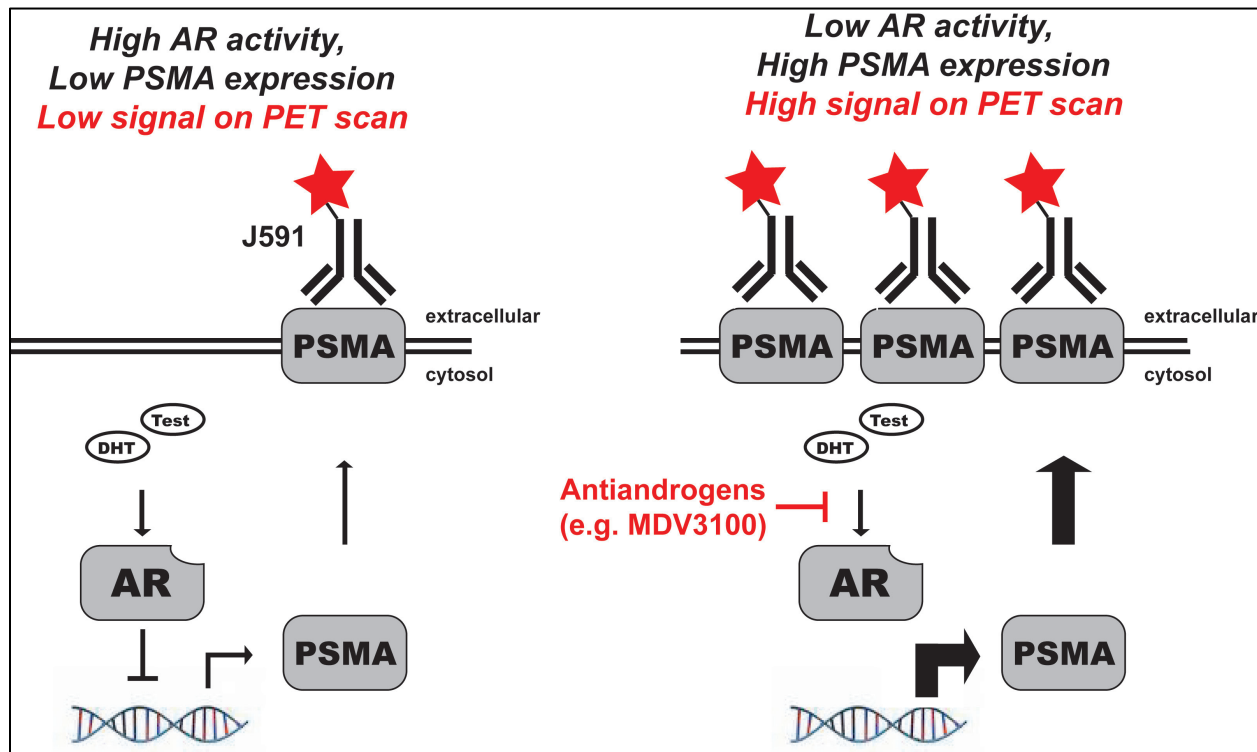
### **Memorial Sloan-Kettering Cancer Center**

#### **Imaging of Prostate Cancer Biomarkers by PET**

- There is an urgent need for effective and reliable imaging biomarkers that can systematically report the underlying changes in cell biology associated with disease progression and treatment response.
- Since treatment-resistant prostate cancer is fueled by the Androgen Receptor (AR), there is an unmet need to identify a biomarker of intratumoral AR signaling that can efficiently report AR activity and thereby the response to androgen deprivation therapy and therapy resistance.
- A potential reporter of AR activity is the protein PSMA (Prostate Specific Membrane Antigen) which is expressed nearly ubiquitously on prostate cancer,

especially in advanced disease and whose production is directly correlated to AR activity.

- PSMA can be detected in prostate cancer cells using a fully humanized monoclonal antibody that has been cleared for clinical use, J591.
- Dr. Jason Lewis and team hypothesized that serial PET scans with radiolabeled J591 ( $^{89}\text{Zr}$ -J591) could be an effective way to monitor the impact of AR-pathway directed therapies, with the expectation that an increase in PET signal would indicate effective therapeutic intervention (Figure).

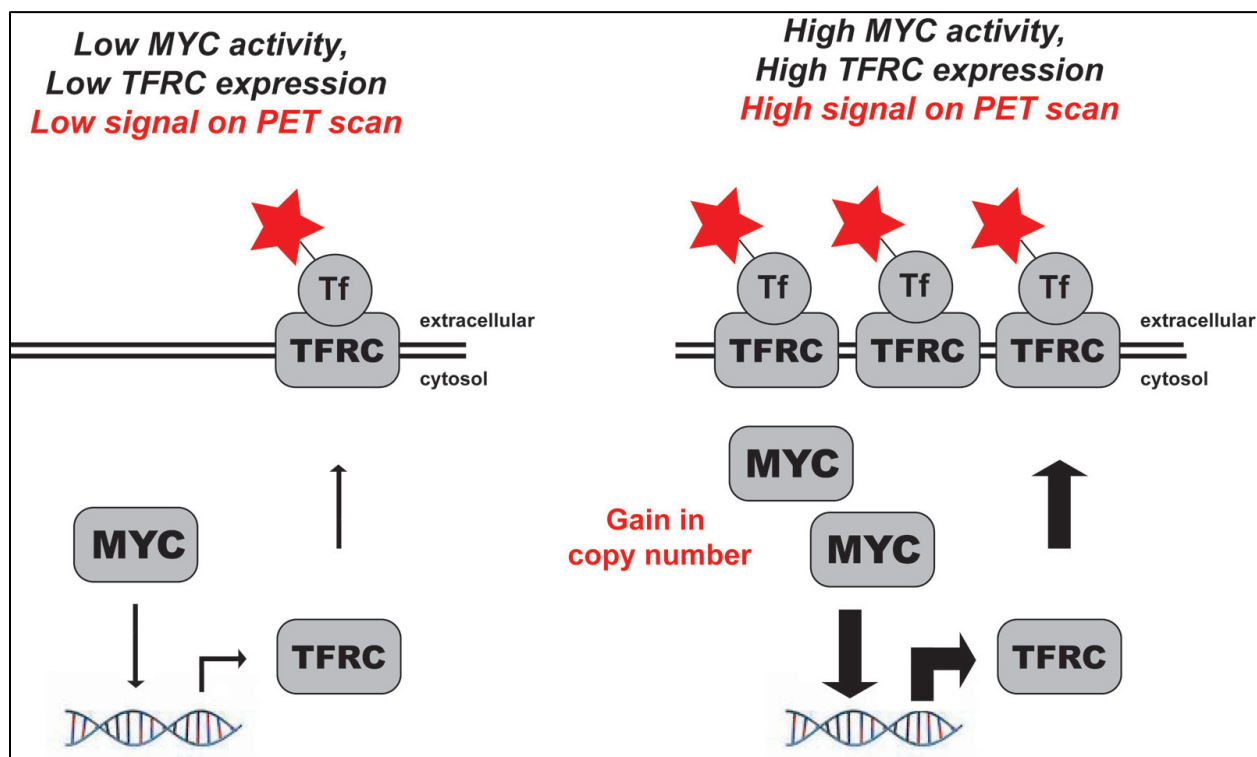


- This was validated in animal models of treatment-resistant disease when the largest increase in PET signals was associated with enzalutamide, the most effective anti-androgen used in the study.
- These studies therefore provide proof-of-principle that AR signaling can be quantified by PET imaging and pharmacologically triggered elevations in PSMA expression can be measured by  $^{89}\text{Zr}$ -J591 PET.
- This agent is currently being tested in human clinical trials at Memorial Sloan-Kettering Cancer Center.
- Dr. Lewis also presented data on a biomarker for Myc status in prostate and other cancers.
- The protein Myc is a transcription factor that is believed to regulate the expression of ~15% of all genes. It functions as an oncogene in several cancers,



in that mutations in the *Myc* gene result in the protein being overexpressed, leading to the consequent unregulated expression of several genes.

- Approximately 30% of all prostate cancer patients present with a gain in *Myc* copy number (i.e. overexpression) and these copy number alterations correlate with poor clinical outcome.
- Therefore, a sensitive, non-invasive reporter of oncogenic *Myc*-driven gene expression is critical to our understanding of the clinical progression of the disease, effective diagnoses and targeted therapy.
- To answer this unmet need, Dr. Lewis and team developed  $^{89}\text{Zr}$ -desferrioxamine-labeled transferrin ( $^{89}\text{Zr}$ -transferrin), a PET radiotracer that binds the transferrin receptor.
- The transferrin receptor is a validated *Myc* target gene, therefore relative changes in transferrin receptor expression can serve as a biomarker of *Myc* status in tumors such that high *Myc* levels  $\rightarrow$  high transferrin receptor levels  $\rightarrow$  more transferrin receptor available to bind radiolabeled transferrin  $\rightarrow$  better PET signal (Figure).



- The use of  $^{89}\text{Zr}$ -transferrin produces high-contrast PET images that quantitatively reflect treatment-induced changes in *Myc*-regulated transferrin receptor expression in a *Myc*-driven prostate cancer xenograft model.
- Dr. Lewis' studies show that  $^{89}\text{Zr}$ -transferrin imaging can detect the in situ development of prostate cancer in a transgenic *Myc* prostate cancer model, as

well as in prostatic intraepithelial neoplasia (PIN) before histological or anatomic evidence of invasive cancer.

- In summary, Dr. Jason Lewis presented the results on two promising biomarkers that can be monitored by immune-PET imaging and hold potential in effective prostate cancer diagnoses and response to targeted therapy.

## **Jamey Weichert, PhD**

### **University of Wisconsin**

#### **Phospholipid Analogs as Broad Spectrum Diagnostics**

- Dr. Jamey Weichert presented his studies on compounds or 'diagnostics' (diagnostic+therapeutic) that allow broad spectrum prostate cancer and prostate cancer stem cell detection and treatment.
- These compounds are fundamentally composed of radiolabeled phospholipid analogs. Phospholipids are a type of fats (lipids) that are a major component of cell membranes. Therefore employing phospholipids in the structure of these compounds allows easy uptake by tumor cells.
- Detailed structure-activity relationship studies allowed Dr. Jamey Weichert and colleagues to identify the exact size and chemical properties of these molecules for selective and maximal uptake only by tumor cells as well as rapid plasma clearance.
- In the course of their studies, Dr. Weichert and colleagues observed that conjugating these phospholipid analogs to radiolabeled iodine not only allowed PET imaging of tumors, but also molecular radiotherapy at higher doses of the compounds.
- Preclinical imaging with one of these compounds, the diagnostic CLR1404 radiopharmaceutical, has shown selective tumor uptake and prolonged retention in 52 out of 54 tumor types in mice.
- These diagnostics are not taken up or retained by pre-malignant or inflammatory lesions, and are highly selective for tumors and metastases. This tumor selectivity is mediated by the lipid rafts which are overexpressed in cancer cells compared to normal cells. The tumor-selective uptake is also independent of the anatomic location of cancer.
- Safety and pharmacological toxicity studies in preclinical cancer models such as rodents and non-human primates have shown an exceedingly high safety index even at >800 times the anticipated human dose.
- At the PCF Scientific Retreat, Dr. Weichert demonstrated videos that followed the route of these compounds in cancer mouse models. These imaging studies showed tumor uptake within 9-12 hrs of injection and body clearance in 24 hrs.

Dr. Weichert and team observed retention of 20 days in human adrenal xenograft models. They could monitor  $I^{124}$  in these animals for 10 days post single injection.

- The  $I^{124}$ -CLR1404 dipeptide has been progressed into Phase I/II clinical trials as a PET imaging agent for several cancers-lung, brain, prostate, breast, pancreatic, head and neck. Preliminary results show selective uptake and retention by primary tumors and metastases in advanced non-small cell lung and brain cancer patients.
- Preliminary radiotherapy results with  $I^{131}$ -CLR1404 in over 12 mouse models of cancer have shown significant life extension and this agent is currently being evaluated in Phase Ib trials.
- Dr. Weichert's studies with another dipeptide compound CLR1501 showed that this dipeptide also allows illuminating the margins of a tumor in real time during cancer surgery using a handheld Fluobeam<sup>TM</sup> microscope. This holds potential in enabling more complete and selective removal of malignant tissue and potentially improving patients' prognosis.
- In summary this dipeptide treatment paradigm developed by Dr. Jamey Weichert and colleagues will potentially allow both tumor detection and treatment.
- PET/CT using these agents will allow full body quantitative 4D mapping of the cancer as well as the *in vivo* bio-distribution of these compounds. PET/CT based dosimetry may also potentially predict the precise therapeutic dose for each individual patient depending on the imaging results, i.e. assessing tumor burden from the initial imaging dose will allow determining the second therapeutic dose.

## Breakthrough Lecture: Organoids: A Profound New Prostate Cancer Model System

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**Charles Sawyers, MD**

**Memorial Sloan-Kettering Cancer Center**

**Funded by 2012 PCF-SU2C Dream Team Award**

- Prostate cancer is a complex, heterogeneous mix of several poorly characterized molecular subtypes. As we move towards the era of precision medicine, it is important to molecularly characterize and define prostate cancer subtypes to guide appropriate treatment decisions and targeted therapy for patients.
- However, prostate cancer research is currently limited by a paucity of good preclinical animal models and cell lines that recapitulate human disease.

- Prostate cancer researchers currently have ~6 validated cell lines, very few xenograft models and a handful of genetically engineered mouse models (GEMMs).
- None of these models accurately represents the natural history of prostate cancer. An example of the challenges faced by researchers is the GEMM that has the PTEN gene deleted. It takes ~6-8 months to genetically engineer this mouse model and another 6-8 months for it to develop prostate cancer specific alterations, such as Prostatic Intraepithelial Neoplasia (PIN).
- As a solution to these challenges faced by prostate cancer researchers, Dr. Sawyers described an exciting new PLATFORM technology that he has developed in collaboration with Dr. Hans Clevers, a physician and developmental biologist from the Netherlands.
- Using a specific mixture of growth factors and cellular signaling compounds, Dr. Clevers has pioneered the technology of growing masses of human tissue or 'organoids' in the laboratory.
- These organoids are genetically indistinguishable from human tissues in terms of architecture, cell type composition and growth dynamics, and can be grown indefinitely, frozen to preserve, and shipped around the world.
- Dr. Clevers has successfully generated organoids from human intestinal, colon, gastric and esophageal tissues.
- A graduate student from Dr. Clevers' group, Walter Karthaus, adapted the technology to normal mouse and human prostate tissue as well as primary prostate cancer tissues, and repeated this work at MSKCC in the Sawyers lab.
- Joint experiments by these laboratories have shown that the human organoids and tumoroids display all hallmarks of normal and malignant human prostate tissue.
- These lab-generated tissues are sensitive to the male hormones such as DHT and shrink when deprived of androgens recapitulating the biology of hormonal response in man.
- Another promising aspect of this new technology is the fact that it takes much less time to generate organoid/tumoroid pairs than generating genetically engineered mouse models.
- This holds immense potential in developing personalized organoids/tumoroids for prostate cancer patients, studying their individual disease and screening medicines on these laboratory tissues to identify the ones with maximal benefit for the individual patient.
- One of the first examples of the advantages of using the organoid/tumoroid system to study prostate cancer was its ability to answer a pertinent question that has to date been difficult to answer conclusively.

- Prostate cancer is known to be driven by androgens (which mediate their activity through the androgen receptor (AR)). Therefore, the primary treatment modality for prostate cancer is androgen ablation. Unfortunately, despite androgen deprivation therapy with medications like Lupron and abiraterone, prostate cancer returns in most patients.
- This disease relapse is hypothesized to be a result of the very low levels of tumor-generated androgens that spur the activity of the androgen receptor. However, this has not been conclusively proven to date due to the impossibility of depleting 100% of androgen, especially in man. This question was addressed using the organoid technology. First, the size of prostate organoids and tumoroids was measured in growth medium containing DHT. When DHT was removed the cellular structures became smaller. But the remarkable finding was that the structures were further reduced in size when androgen receptor inhibitors like enzalutamide or ARN 509 were added to androgen-depleted cultures.
- For the first time it was shown that these direct androgen receptor inhibitors are active by a DHT ligand-independent mechanism arguing for discovery of more potent and selective direct androgen receptor antagonists.
- A good analogy of the system is a room light dimming switch. The current treatment modalities for prostate cancer such as enzalutamide and ARN509 allow the switch to be dimmed to its lowest intensity, but not completely switched off. There is, therefore, an urgent need to develop treatments that completely turn off the prostate cancer switch.
- The organoid technology holds immense promise in not only answering these fundamentally unanswered questions that further our understanding of disease mechanisms, but also providing a useful platform for drug discovery. In turn, selected drug candidates may be analyzed for activity in these cell systems to better predict activity in man.

## **PCF-StandUpToCancer Dream Team Overview 1**

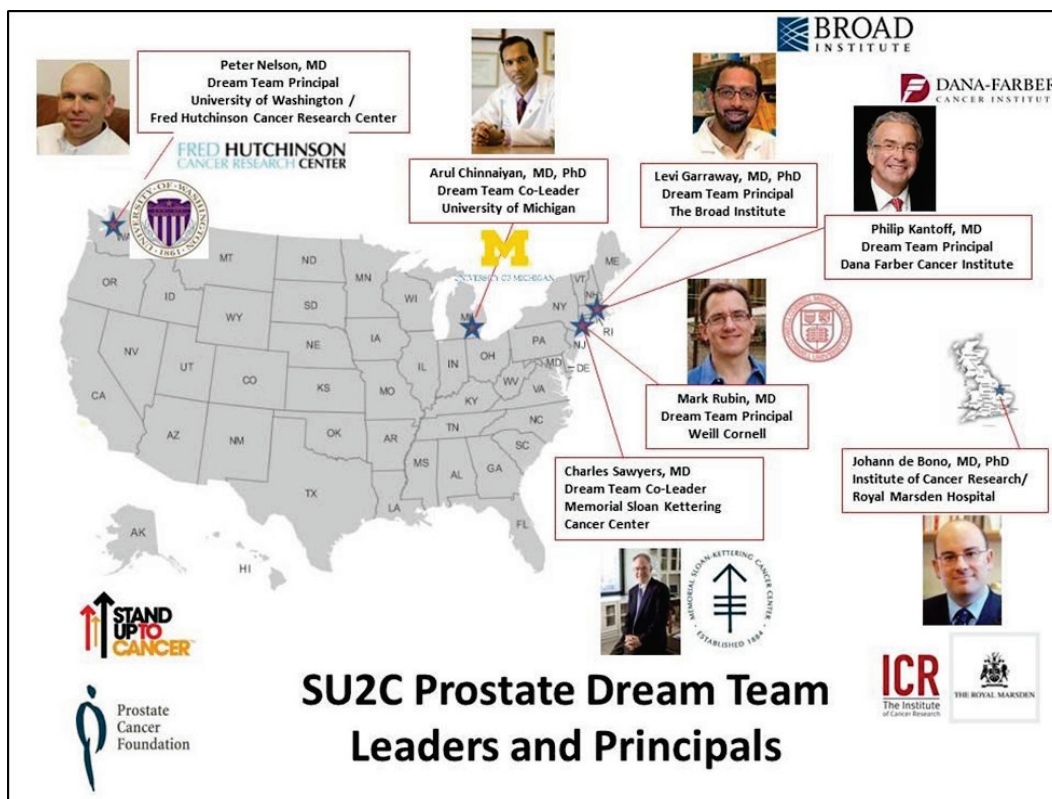
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**Arul Chinnaiyan, MD, PhD**  
**University of Michigan**

- PCF in association with Stand Up To Cancer (SU2C) and SU2C's scientific partner American Association for Cancer Research (AACR) has funded a cutting-edge, collaborative research (SU2C) Dream Team project to develop personalized treatments for advanced prostate cancer.
- This PCF-SU2C Prostate Dream Team Translational Cancer Research grant will provide \$10 million over three years for a seven-center project including both

clinical centers and two research infrastructure sites that will address therapeutic interventions for advanced prostate cancer, with special emphasis on metastatic disease, and deliver near-term patient benefit through investigation by a multidisciplinary, multi-institutional, synergistic Dream Team of expert investigators.

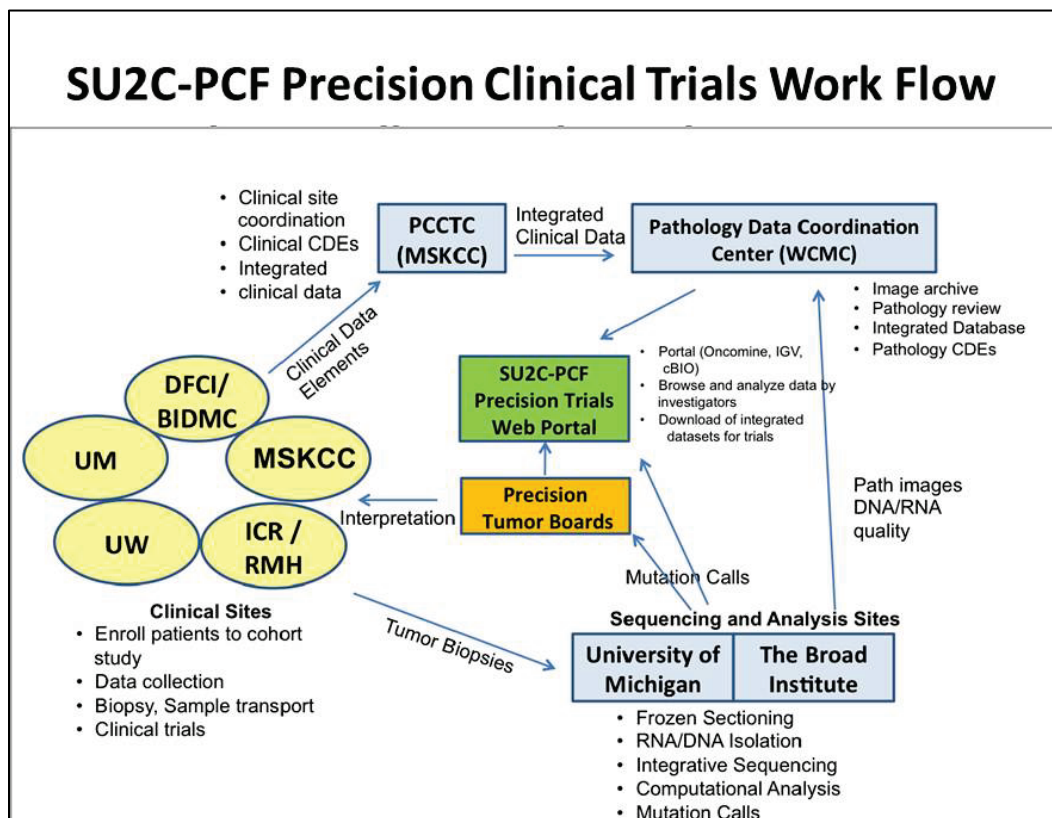
- The research project titled 'Precision Therapy for Advanced Prostate Cancer' is led by Dr. Arul Chinnaiyan from the University of Michigan Comprehensive Cancer Center and Dr. Charles Sawyers from Memorial Sloan-Kettering Cancer Center.



- Apart from these two principal investigators, this international Dream Team draws core leaders from four academic research institutes in the United States: University of Washington, Dr. Peter Nelson; Weill Cornell Medical College, Dr. Mark Rubin; Dana-Farber Cancer Institute, Dr. Phillip Kantoff and, one from the United Kingdom, Institute of Cancer Research/ Royal Marsden Hospital, Dr. Johann De Bono.
- The teams plan to do this by carrying out detailed high-throughput sequencing of the genomes of 500 treatment resistant prostate cancer patients and comparing the genomic sequences of their normal cells to those of the cancer cells, to identify underlying aberrations. These genetic aberrations can be informative for identifying rational targeted therapies either currently available (approved agents) or investigational agents in preclinical or clinical trials, for these patients.



- This strategy of directing patients towards the treatment most likely to have an effect on their tumor, often referred to as 'personalized medicine', will lead to more effective and lasting treatments, and potentially spare patients from unnecessary non-targeted, highly toxic therapies.
- The results of these studies (whole-genome sequencing of the tumor, targeted whole-exome sequencing of tumor and normal DNA, and transcriptome sequencing (RNA-Seq)) will be evaluated by a multi-disciplinary, multi-institutional Precision Tumor Board comprised of clinical oncologists, clinical geneticists, bioethicists, pathologists, informaticians and patient advocates.
- These initiatives will help in the identification of rare "actionable" mutations in the cancer of these patients with advanced prostate cancer, and provide them with rational clinical trial options based on the underlying biology of their tumors.
- Detailed studies like these enable the identification of resistance mechanisms and sensitivity biomarkers for new therapies.
- The work of the PCF-SU2C Dream Team will also establish the use of Precision Tumor Boards to guide the management of advanced prostate cancer.



- The studies on the 500 treatment resistant prostate cancer patients by the PCF-SU2C team, or the CRPC500 studies will help establish a 'Rosetta stone' resource of mutation profiles for advanced prostate cancer which will serve as an informational source for both researchers and patients.

- These efforts by the Dream Team principals will establish advanced prostate cancer as a model tumor type for the precision medicine paradigm.
- In the first 6 months, the Dream Team plans to a) establish regulatory approval for the CRPC 500 cohort study protocol at all 5 clinical sites, b) establish intra- and multi-institutional Precision Medicine Tumor Boards, c) establish Standard Operating Procedures at all sites for sample and data collection, d) adapt the Caisus database for data management, e) establish sequencing analysis pipelines at the University of Michigan and the Broad Institute, and f) have active enrollment and biopsy collection from 4 CRPC clinical trials.
- By the end of year 1, the Dream Team expects to have a) enrolled and biopsied 100 patients in the CRPC study, b) carried out sequence analysis of all successful biopsies, c) established a web portal for data sharing and viewing, and d) completed one pre-clinical xenograft study.

## PCF-StandUpToCancer Dream Team Overview 2

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### Eric Small, MD University of California, San Francisco

- PCF, AACR and Stand Up To Cancer announced a second Prostate Cancer Dream Team Translational Cancer Research Grant in October, 2012.
- This research project titled “Targeting Adaptive Pathways in Metastatic Treatment-Resistant Prostate Cancer” is led by Dr. Eric Small, University of California, San Francisco and Dr. Owen Witte, University of California, Los Angeles.
- The Dream Team is comprised of six world-class institutions, five from the United States: four campuses of the University of California (San Francisco, Los Angeles, Santa Cruz and Davis), Oregon Health and Science University, and one from Canada, the University of British Columbia (Prostate Center).
- In addition to the PIs, Drs. Eric Small and Owen Witte, the other research teams are led by Dr. Tomasz Beer, Oregon Health & Science University; Dr. Hsing-Jien Kung, PhD, University of California, Davis; Dr. Joshua Stuart, PhD, University of California, Santa Cruz, and Dr. Martin Gleave, University of British Columbia. This Dream Team is colloquially referred to as the West Coast Dream Team as all investigators are on the west coast of North America.
- The research proposal by this Dream Team is based on the hypothesis that cancer cells use a set of ‘adaptive pathways’ to develop resistance to treatment. Molecular characterization of these adaptive responses will lead to the identification of appropriate targets that will improve patient outcomes with additional precision medicine-selected agents.

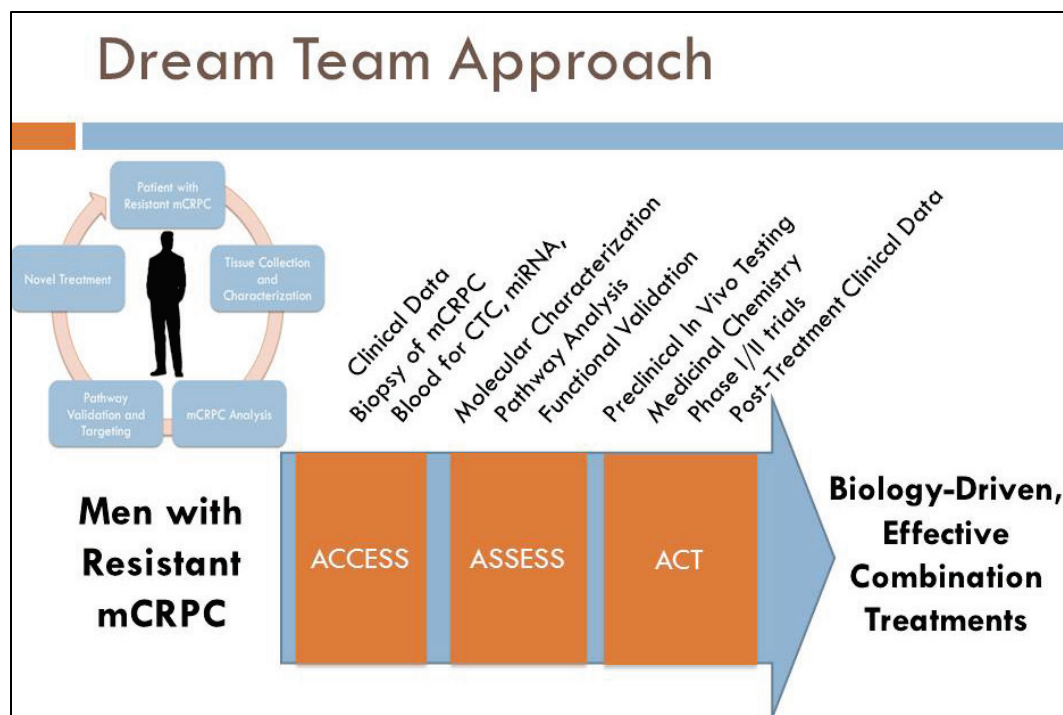
## West Coast Dream Team Locations



- The overall goal of this world-class Dream Team is to understand those adaptive pathways most commonly used by metastatic treatment resistant prostate cancer for resistance to emerging therapies, functionally validate these pathways, develop co-targeting therapeutic strategies, and improve care by rapidly developing biologically relevant co-targeting approaches.
- The West Coast Dream Team is organized around five working groups (WG)- tumor characterization WG, pathway targeting WG, therapeutics development WG, functional validation WG and clinical WG)- all of which will integrate expertise from each of the six centers.
- A sixth “knowledge-exchange” working group will coordinate and harmonize activities of the Dream Team, which will address therapeutic interventions for advanced prostate cancer with special emphasis on metastatic disease and delivering near-term patient benefit.
- The team will systematically subject patient biopsies (fixed, frozen and fresh tissue) and blood samples to a comprehensive molecular assessment and pathway-based analysis to determine the activity level of known and novel pathways.
- Once the pathways activated in treatment resistant prostate cancer are identified, the Dream Team will devise co-targeting approaches that they will first validate in preclinical models. After validation they will implement molecularly guided clinical trials to test novel therapeutic combinations that co-target adaptive pathways associated with resistance. By combining established

therapies with new treatments that co-target adaptive pathways, the Dream Team hopes to dramatically improve outcomes for men with advanced prostate cancer.

- The Dream Team also proposes to centralize and integrate the considerable amount of data generated by their work into MedBook, which will use a simple social media concept to support information exchange and discussion. The centralized information will be updated continuously based on new data, and contribute to the development of molecular disease models that codify the most current clinically actionable adaptive pathways in metastatic treatment resistant disease. This information will be instrumental to the Dream Team's Clinical Working Group for recruiting patients to specific trials.



## Exceptional Progress Report – PCF Young Investigator

### Integrated Molecular Analysis of Circulating Tumor Cells: The Microfluidic VeriFAST Platform Funded by a PCF Young Investigator Award

- Prostate circulating tumor cells are often found in the bloodstream of men with cancers that have spread throughout the body.

- Current technology allows scientists to capture these cells; they can then be studied for how they react (in real time in some cases) to drug treatments as well as their unique genetic composition, which may ultimately lead to tailored drug treatments.
- Circulated tumor cells (CTCs) can also act as prognostic factors or to alert doctors that metastatic disease is progressing.
- VerlFAST is an integrated, microfluidic platform used for molecular analysis of CTCs.
- It does this in four steps: CTC capture and purification, live cell staining, protein analysis, nucleic acid extraction, all from the same sample.
- Dr. Lang spoke to the audience about the platform and its uses—including its capture and purification potential, providing 80-90 percent cell isolation efficiency. The platform capture is so sensitive; it can capture one cell out of 20 million blood cells.
- Moving beyond simply capturing cells, he spoke about the need to analyze CTCs for their unique mechanisms of drug resistance, variations between CTCs and tumor cells in the prostate and in metastatic regions like bone.
- Advanced CTC platform characteristics provide both high sensitivity and high specificity in tumor analysis.
- As Dr. Lang advances the VerlFAST technology, he hopes to move beyond enumeration.
- Ultimate goals include understanding the mechanism of treatment resistance, creating a prospective trial of androgen receptor CTC technology for patients on androgen receptor targeting therapy, assessing CTCs for therapeutic targets and understanding the heterogeneity between CTCs and primary or metastatic lesions.

## Session 4

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### Clinical Trial Advances in Metastatic Castration-Resistant Prostate Cancer

**Martin Gleave, MD**

**University of British Columbia**

**Randomized Phase II Trial of the Hsp27 Inhibitor, OGX-427**

**Plus Prednisone vs. Prednisone Alone in Patients with Metastatic Castrate Resistant Prostate Cancer**

**Funded by PCF-SU2C Dream Team Award**

- Prostate, bladder, pancreas, breast and non-small cell lung cancer cells overexpress the cell-survival protein, Heat Shock Protein 27 (Hsp27), whose production is induced by cellular stresses such as cytotoxic chemotherapy, radiation therapy, and hormone therapy.
- Hsp27 regulates cell signaling and survival pathways implicated in cancer progression.
- In prostate cancer models, Hsp27 interacts with the androgen receptor (AR) and enhances the dysregulated expression of AR-regulated genes.
- Previous work in Dr. Martin Gleave's laboratory has shown that Hsp27 inhibits cancer cell death and facilitates protein folding and function of pro-cancer proteins.
- Overexpression of Hsp27 in patient samples correlates with broad spectrum treatment resistance and is associated with negative clinical outcomes in patients with various tumor types. Therefore, Hsp27 is a good drug target.
- Dr. Martin Gleave and his team developed the investigational drug OGX-427 which is a second-generation antisense oligonucleotide i.e. a single strand of DNA that binds the *Hsp27* gene and prevents the production of the Hsp27 protein.
- Preclinical and Phase I studies with OGX427 have shown *in vitro* and *in vivo* efficacy in inhibiting the production of Hsp27, and the agent was well tolerated.
- At the PCF Scientific Retreat, Dr. Gleave presented the Phase II clinical trial results of OGX-427 + prednisone (31 patients) vs. prednisone (P) alone (33 patients) in the treatment of chemotherapy-naïve men with metastatic treatment-resistant prostate cancer (study PI was Dr. Kim Chi).
- Chemotherapy-naïve patients with no/minimal symptoms were randomized to receive OGX-427 600 mg IV x 3 loading doses then 1000 mg IV weekly with P 5 mg PO BID or P only.



- Primary endpoint was the proportion of patients progression free (PPF) at 12 weeks (PCWG2 criteria). Secondary endpoints included PSA decline, measurable disease response, and circulating tumor cell (CTC) enumeration/evaluation pre- and post-study treatment.
- In the OGX-427 plus prednisone arm, 71% of patients were progression-free at 12 weeks, compared to 48% in the prednisone alone arm. Forty-seven percent of patients who received OGX-427 plus prednisone experienced a >50% decline in PSA, versus 21% of patients who received prednisone alone. There was 1 complete response in the OGX-427 plus prednisone arm.
- Circulating tumor cell declines from greater than or equal to 5 to <5 occurred in 52% of patients receiving OGX-427 plus prednisone compared to 41% of patients receiving prednisone alone. Dr. Gleave also presented some preliminary data on cross-over patients (N=15).
- OncoGenex Pharmaceuticals plans to initiate investigator-initiated, randomized, controlled Phase II study of OGX-427 in combination with abiraterone in mCRPC, and OGX-427 in combination with gemcitabine-cisplatin for patients with metastatic bladder cancer.
- In summary, Dr. Gleave's results provide the first clinical evidence for Hsp27 as a therapeutic target in treatment-resistant prostate cancer. The experimental compound, OGX-427 demonstrated anti-tumor activity with objective responses, PSA declines, and delay in progression-free survival. OGX-427 treatment was well tolerated with predominant adverse events being infusion related and grade 1-2.

## **Richard Heyman, PhD**

### **Aragon Pharmaceuticals**

#### **ARN-509, A Next Generation Anti-Androgen: From Discovery to Clinical Development**

- Treatment-resistant prostate cancer (often referred to as CRPC) is a hallmark of prostate cancer after initial androgen deprivation therapy (ADT). This treatment resistance is known to be driven by the overexpression and activity of the androgen receptor (AR).
- While several AR signaling pathway-targeted therapies have shown promise, treatment-related side effects pose the need for newer and better targeted agents, e.g. in the treatment-resistant disease setting of AR overexpression, the first-generation anti-androgen bicalutamide shifts from an antagonist to an agonist role, stimulating AR activity, instead of inhibiting it, thereby promoting tumor growth.

- Treatment with abiraterone requires the co-administration of low-dose prednisone to ameliorate side effects such as hypertension, hypokalemia and fluid overload. Longer term usage of prednisone can be potentially harmful due to side effects such as diabetes, weight gain, Cushing syndrome and osteoporosis.
- Enzalutamide, another potent drug recently approved by the FDA, belongs to a class of anti-androgens that carry seizure risk, likely mediated via antagonism of the central nervous system-based GABAA receptor.
- Dr. Charles Sawyers (MSKCC), in collaboration with Dr. Michael Jung (UCLA; co-founders of Aragon Pharmaceuticals), devised a path to identify compounds with anti-androgenic activity in treatment resistant prostate cancer.
- This approach yielded ARN-509, a potent novel small molecule AR antagonist that impairs AR nuclear translocation and binding to DNA, inhibiting tumor growth and promoting cancer cell death (apoptosis).
- At the PCF Scientific Retreat, Dr. Rich Heyman described the preclinical development and Phase I and II clinical trials of ARN-509.
- Preclinical studies have shown that ARN-509 binds the androgen receptor with a 5-fold greater affinity than bicalutamide (Casodex), and induces tumor regression in hormone-sensitive and treatment-resistant animal models of disease.
- ARN-509 has shown similar *in vitro* activity to enzalutamide but greater *in vivo* activity in the treatment of CRPC animal models.
- In contrast to bicalutamide, ARN-509 lacks significant agonist activity and is well tolerated. In preclinical models of treatment of CRPC, ARN-509 shows maximal therapeutic response at a minimum effective dose of less than 30 mg/kg/day of ARN-509, compared to 100 mg/kg/day of enzalutamide.
- Due to the improved efficacy at lower concentrations, ARN-509 has lower plasma and brain exposure and therefore a better therapeutic index related to risk of seizures.
- Phase I studies of ARN-509, conducted by the PCF-funded Prostate Cancer Clinical Trials Consortium (PCCTC) were done in 30 men with metastatic CRPC. Doses (30 to 480 mg/day) were tested using standard 3x3 dose escalation criteria to assess safety, pharmacokinetics, and determine the recommended Phase 2 dose. Preliminary anti-tumor activity was assessed by PSA kinetics, radiographic responses, circulating tumor cells, and FDHT-PET imaging.
- The most common Grade 1-2 treatment-related adverse events were fatigue, nausea, and abdominal pain. 48% of the patients had  $\geq 50\%$  PSA declines.
- FDHT-PET imaging demonstrated robust AR blockade after 4 weeks across multiple dose levels. Based on preclinical assessment of maximum efficacious dose, PK, FDHT-PET demonstrating maximal AR blockade, and promising activity across all doses, 240 mg was selected as the recommended Phase II dose.

- An interim analysis of a Phase II study was conducted with ARN-509 in three CRPC patient cohorts who have been on study for at least 12 weeks: non-metastatic (N=46), treatment-naïve (N=25) and post-abiraterone metastatic (N=14): the data showed that ARN-509 treatment was very well tolerated, resulted in PSA declines in all three cohorts, with 91% and 88% declines at 12-week of >50% from baseline in the non-metastatic and treatment-naïve cohorts, respectively. Five out of eight patients (63% ORR) with measurable disease at baseline in the treatment-naïve metastatic CRPC cohort achieved a partial response according to RECIST. No seizures have been seen in the ARN-509 clinical program in more than 120 patients treated so far with dose-escalation up to 480 mg.
- Dr. Rich Heyman concluded his presentation by summarizing that these preclinical and clinical data provide multiple options for the developments of ARN-509 both as monotherapy and as combination therapy for advanced prostate cancer.

## Session 5

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### Messenger RNA Translation in Cancer

**Nahum Sonenberg, PhD**

**McGill University**

**eIF4E and Cancer**

**Funded by a PCF Competitive Award**

- In a normal cell, eIF4E (eukaryotic translation initiation factor 4E) plays a very critical role in protein synthesis. The production of almost all cellular proteins requires the presence of eIF4E as the latter recruits the large and complex molecular machine called ribosome that serves as the primary site of biological protein synthesis.
- The activity of eIF4E is dysregulated in several cancers and this protein is overexpressed (upto 3-10 fold) in cancers of the breast, colon, prostate and pancreas. Previous studies have shown that eIF4E overexpression in prostate cancer correlates with reduced overall survival.
- Dr. Nahum Sonenberg discovered the eIF4E protein ~30 years ago and has been working on understanding its complex mechanism of action in normal and tumor cells. His studies and those of several others have shown that the eIF4E protein sits as a downstream effector of several cellular cues such as those from insulin, growth factors, oxygen availability status or the lack of it, energy status, protein building block amino acid availability etc. All these cues transmit messages from outside the cell to eIF4E through complicated signal transduction pathways (such

as mTOR) and eIF4E regulates cell growth, proliferation and death in response to these signals.

- Based on its oncogenic role in several cancers, Eli Lilly is currently testing an inhibitor of eIF4E in Phase II clinical trials in several cancer patients, including prostate cancer.
- Dr. Sonenberg's detailed investigations revealed that phosphorylation of eIF4E, i.e. the enzymatic addition of a phosphate moiety to a specific site on the eIF4E protein (Serine 209) is very critical for its oncogenic activity.
- This phosphorylated eIF4E has been demonstrated to promote migration and invasion in preclinical models of advanced prostate cancer.
- Studies by Dr. Sonenberg and colleagues show that this phosphorylation is mediated by the proteins Mnk1 and Mnk2 (pronounced as 'Mink', MAP kinase-interacting kinase 1 and 2), which may be effective therapeutic targets to prevent prostate cancer progression.
- Based on these studies, Dr. Sonenberg proposes combination therapy targeting Mnk1/2 and mTOR activity for prostate cancer therapy.

## **Kevan Shokat, PhD**

### **University of California, San Francisco**

#### **Chemical Genetic Analysis of Oncogenic Signaling Cascades**

- Dr. Kevan Shokat's research is focused on identifying small molecule inhibitors of kinases, with unusual therapeutic index to treat cancer and other diseases.
- Kinases are a class of enzymes that play crucial roles in cellular metabolism and cell signaling. More than 518 kinases are known to function in normal cell signaling and several of these are either oncogenic themselves, or are immediately downstream of oncogenes and mediate their pro-cancer signaling.
- Being enzymes, kinases are eminently druggable; however targeting just one oncogenic driver will not be sufficient to provide maximal and sustained therapeutic benefit. Combinatorial therapy or polypharmacology is the way to go to obtain maximum therapeutic index. Polypharmacology, focuses on designing therapeutics to target multiple receptors, and has emerged as a new paradigm in drug discovery.
- Dr. Shokat's approach to drug discovery is different from typical drug discovery and development strategies in two ways:
  1. Typically, preclinical models of disease such as cell lines are used to screen for anti-cancer drugs. However in this study, Dr. Shokat and his colleagues used fly (*Drosophila*) cancer models to screen large chemical libraries for novel drug leads that shrank tumors. Flies are good surrogates for drug

testing because they tolerate molecules with modest pharmacokinetic properties early on in the discovery process. Based on the initial fly studies, and employing a combination of classical fly genetics with chemical modeling Dr. Shokat and colleagues developed second-generation drugs to better hit specific targets. Their studies show that screening in *Drosophila* cancer models holds potential in the identification of inhibitors against an optimized combination of kinase targets.

2. Many targeted cancer drugs often inhibit a spectrum of kinases, demonstrating off-target activity and therefore toxicity. However, the drug discovery principles employed by Dr. Shokat and team aim to hit multiple targets purposefully. By using fruit fly genetics they have identified, step-by-step, the specific targets that allow maximal therapeutic benefit and the 'anti-targets' whose inhibition contributes to toxicity.
- Dr. Shokat and colleagues studied a *Drosophila* model of multiple endocrine neoplasia type 2 (MEN2), a genetic disorder that is characterized by an enhanced propensity to develop medullary thyroid carcinoma. MEN2 is driven by mutations in the receptor tyrosine kinase RET.
  - The team screened a library of over 1000 chemical compounds with multi-kinase inhibitor activity in MEN2 fly models. These screens identified compounds that inhibited RET and some its downstream effectors. Dr. Shokat and his team tweaked the chemical structures of these compounds and found that some analogs very highly toxic as they inhibited not only RET but also some essential cell signaling pathways. These undesirable targets are what the team labeled as 'anti-targets', the inhibition of which led to toxic side effects.
  - Therefore, a rational polypharmacologic drug should potentially target an ideal spectrum of tumor-relevant kinases while avoiding anti-targets. Dr. Shokat synthesized such derivatives of the originally identified compounds that spared toxic side effects. These new chemical entities inhibited tumor growth and improved both in the fly model as well as the human MEN2 xenograft cells.
  - In summary, this innovative novel approach to drug development demonstrates that rational polypharmacology employing a combination of synthetic medicinal chemistry, kinome profiling and a *Drosophila*-based phenotypic screen can potentially identify inhibitory lead compounds with optimal potency and minimal toxicity.

## Session 6

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### Novel Therapeutic Approaches to Cure Advanced Prostate Cancer

**Bruce Zetter, PhD**

**Harvard Medical School**

**New Class of Therapies Targeting Lethal Prostate Cancer**

**Funded by a PCF Creativity Award**

- Treatment-resistant progression and metastases are the two hallmarks of advanced prostate cancer, both leading causes of prostate cancer associated mortality.
- Traditional chemotherapeutic approaches have failed to successfully treat metastatic disease. This is potentially because metastatic tumor cells differ in their responsiveness to particular agents.
- Dr. Bruce Zetter presented strategies for the identification of potent drugs to treat established metastases.
- He suggested screening and testing FDA-approved drugs for other indications in prostate cancer. These drugs have proven tolerability in patients that saves a substantial amount of time in taking these into the clinic, once preclinical evidence supports efficacy in prostate cancer.
- The next step would be to screen drugs that *selectively* kill highly metastatic tumor cells. This can be tested by comparing the effects of these drugs on the survival of at least two prostate cancer preclinical models of disease (e.g. cell lines) with differing metastatic potential.
- Other important points raised by Dr. Zetter during his presentation at the PCF Scientific Retreat included the fact that to make preclinical studies in mice more representative of the human disease it is important to allow tumors to develop until widespread metastases have formed in the animals. Drug screening and testing on these mice models should only be done *after* metastases are in place, to allow better recapitulation of human disease.
- Dr. Zetter presented an example of this strategy from his own studies on fenbendazole, an FDA-approved anti-parasitic agent, which has shown potent anti-tumor activity in models of metastatic treatment resistant prostate cancer.
- Fenbendazole belongs to a family of chemical compounds known as benzimidazoles that are relatively non-toxic to humans and have been shown to destabilize microtubules in cells.



- Microtubules are long polymeric tubes that are important for maintaining cellular structure and provide platforms for intracellular transport. The class of drugs called taxanes such as paclitaxel, work by targeting cellular microtubules preventing proper cell division, leading to cell death. However, cancer cells develop resistance to paclitaxel.
- Dr. Zetter and colleagues tested three benzimidazoles- fenbendazole, albendazole and mebendazole- on preclinical models of paclitaxel-resistant prostate cancer.
- Their studies have shown that benzimidazoles reduce tumor burden and increase the survival of mice models of metastatic prostate cancer.
- These benzimidazoles inhibit the growth of metastatic cancer cells in the bone as well as cancer cells that are resistant to paclitaxel.

## **Andrew Mazar, PhD**

### **Northwestern University**

#### **Advancing Therapies against the uPA Axis in Advanced Prostate Cancer Funded by the Koch-PCF Young Investigator Award**

- uPAR or the urokinase Plasminogen Activator Receptor is a multidomain protein tethered to the membranes of both tumor and tumor stromal cells. It is rarely detected in normal quiescent tissues.
- uPAR interacts with its ligand, uPA (urokinase Plasminogen Activator), and the uPA-uPAR complex plays crucial roles in cell adhesion, cell cycle regulation, cell migration, angiogenesis (formation of new blood vessels) and tissue remodeling.
- uPAR is transiently expressed in normal cells during development and wound healing.
- The uPA-uPAR axis is hijacked by tumor cells to promote tumor progression and metastases. uPAR expression is upregulated on tumor cells and higher plasma or serum levels of uPA correlate with tumor progression and poor prognoses in aggressive breast cancer, bladder cancer, gastric cancer, and prostate cancer.
- Elevated uPA levels have been shown to be associated with biochemical disease relapse after radical prostatectomy.
- uPA overexpression has been detected in 53% of primary prostate cancer tissues and more than 90% of lymph node metastases.
- Studies have shown that 76% of uPA-positive tumors are Gleason score 7 or higher.

- All this evidence suggests that, one, the uPA/uPAR axis is a cancer therapeutic target and two, uPA and soluble uPAR can serve as biomarkers of tumor progression in clinical specimens.
- Dr. Mazar presented the development of two novel agents targeting the uPA axis:
  1. ATN-291, a monoclonal antibody that targets uPA and exhibits robust anti-tumor activity in xenograft models. Dr. Mazar and team have formulated ATN-291 in novel stealth liposomes termed nanobins (NB) that deliver the antibody selectively only to cancer cells overexpressing the cognate receptor uPAR.
  2. ATN-658, a first-in-class humanized monoclonal antibody to uPAR that modulates numerous mediators of cancer cell signaling pathways and inhibits growth of metastatic lesions in bone and in viscera.
- ATN-658 blocks prostate cancer invasion, migration, growth, and experimental skeletal metastasis *in vitro* and *in vivo*.
- In preclinical animal studies, ATN-658 caused a significant decrease in tumor volume and a marked reduction in skeletal lesions as determined by Faxitron x-ray and micro-computed tomography. Immunohistochemical analysis of subcutaneous and tibial tumors showed a marked decrease in the levels of expression of pAKT, pMAPK, and pFAK.
- Humanized ATN-658 is ready to enter Phase I clinical trials in metastatic prostate cancer patients. Once advanced into human cancer clinical trials, ATN-658 will be the first uPAR targeted therapy to be evaluated in patients.

**Kent Kirshenbaum, PhD**

**New York University**

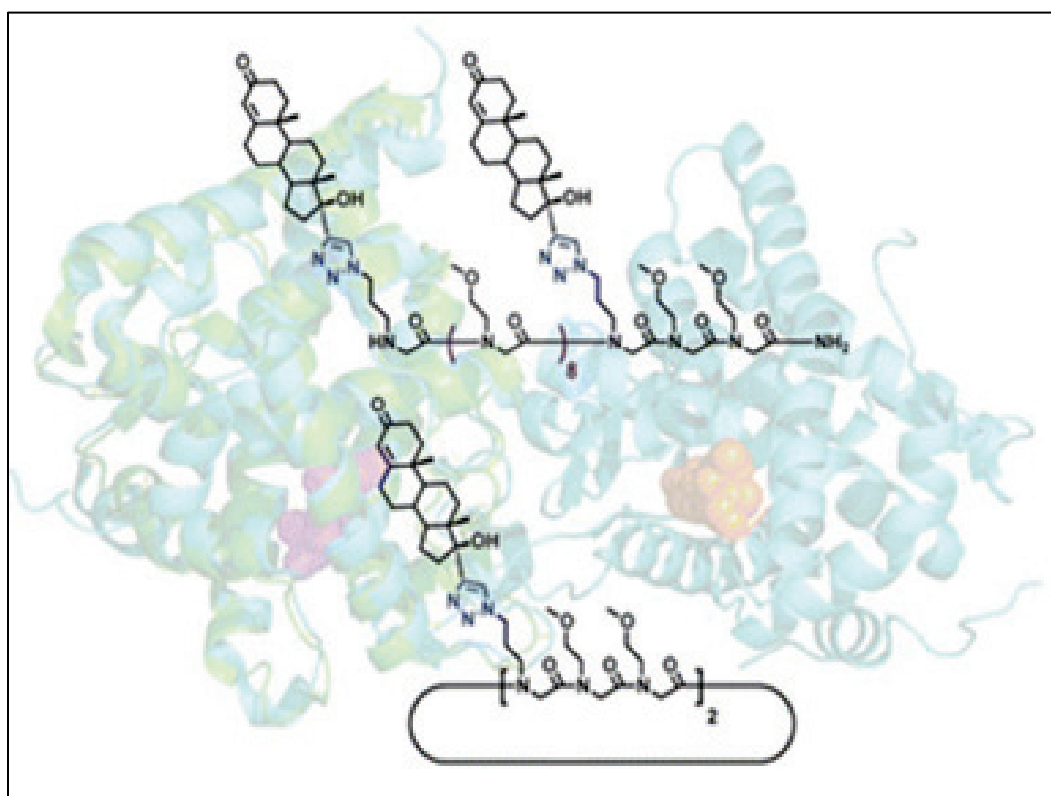
**Michael Garabedian, PhD**

**New York University School of Medicine**

**Peptoids on Steroids: Targeting AR-dependent Prostate Cancer with Multivalent Ethisterone Conjugates**

- The male hormones, androgens are the fuel for prostate cancer progression. The primary treatment for advanced prostate cancer is androgen depletion or ADT (Androgen Deprivation Therapy).
- Androgens mediate their activity through the androgen receptor that binds to DNA and regulates pro-cancer gene expression.
- Inevitably the tumor becomes resistant to ADT and regrowth occurs. This can arise due to mutation and overexpression of the androgen receptor (AR), which acquires capabilities to drive prostate cancer even in the absence of androgens.

- Therefore, an effective strategy to treat this androgen-independent, castration-resistant, and often lethal variety of prostate cancer is to target the faulty androgen receptors in new ways.
- Drs. Kent Kirshenbaum, Michael Garabedian and their team at New York University are working on this. They have rationally designed, synthesized and characterized a variety of chemical compounds that specifically target and bind the androgen receptor, and modulate its activity.
- Using clever tricks of peptide chemistry, Dr. Kirshenbaum and his team have devised methods to conjugate several such chemical entities into a single targeted multivalent compound that is much more potent than a monovalent compound.
- In their studies on prostate cancer cell lines, two of these compounds have shown potent anti-cancer activity against treatment-resistant prostate cancer.



- While both of these compounds share the same basic chemical backbone, one of these chemicals is linear in structure while the other one is cyclic (in picture above). Despite their structural similarities, these compounds show very different effects on androgen receptor (AR) activity.
- To mediate its activity, the androgen receptor moves from the outside to the inside of the nucleus, the part of the cell that harbors the genome (DNA).

- The novel linear anti-AR chemical compound blocks AR action by deterring its translocation into the nucleus, thereby preventing its ability to activate a pro-cancer gene signature. Since the androgen receptor is unable to activate pro-cancer gene expression, the team observed that this compound inhibited the proliferation of treatment-resistant prostate cancer.
- In contrast, the cyclic chemical compound allows AR nuclear localization, but alters the cell cycle, causing cancer cell death.
- The team then tested these compounds in animal models of prostate cancer. Both compounds were well tolerated by mice, exhibiting potent anti-proliferative activity in treatment-resistant prostate cancer cells.
- Drs. Kirshenbaum, Garabedian and their team also studied changes in gene expression, upon administration of these experimental androgen receptor inhibitors.
- Their results show that both compounds affect cancer cell gene expression differently, but both genetic signatures are consistent with anti-proliferative activity. The team has recently begun using animal models to test the compounds.
- These studies show that these two experimental compounds exhibit promise as potential therapeutic agents for treatment-resistant prostate cancer.
- The team is currently evaluating the *in vivo* activity and pharmacodynamics of these compounds in animal models before they progress these into clinical trials in prostate cancer patients.

## **Marc Diamond, MD**

### **Washington University in St. Louis**

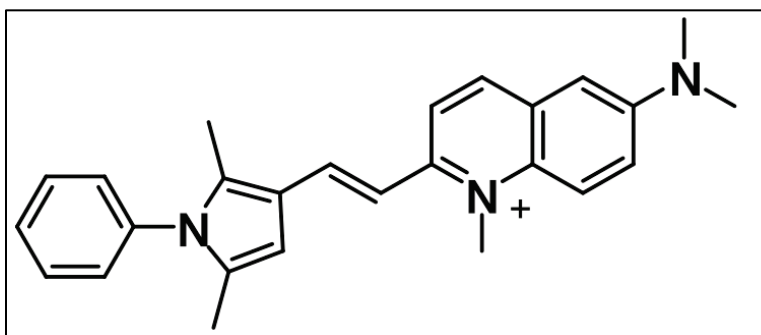
#### **A Novel AR Inhibitor: Targeting AR Conformational Change and Venturing into the Wnt Pathway**

#### **Funded by a PCF Competitive Award**

- Dr. Marc Diamond is a neurologist who works on nuclear hormone receptors, such as the androgen receptor (AR).
- Dr. Diamond is a past recipient of CaP CURE and PCF competitive research awards to study novel anti-androgens.
- Since the androgen receptor (AR) controls the expression of genes that promote prostate cancer initiation and progression, AR was Dr. Diamond's chosen drug target.
- However, foregoing conventional strategies of blocking androgen access to the receptor, Dr. Diamond designed a new strategy to target AR in prostate cancer. The receptor undergoes structural/conformational changes upon binding

androgens that are necessary for its activity. Dr. Diamond targeted these conformational changes in AR for the design of an inhibitory chemical compound that would freeze AR in an inactive state.

- To identify the compound that could prevent AR conformational changes, Dr. Diamond and his team at UCSF screened a library of 7000 compounds that included FDA-approved drugs, natural products etc.
- The team attached fluorescent tags to AR that reported even subtle conformational changes, to study the effects of these chemical compounds. This chemical screen helped in the identification of pyrvinium, a drug originally used to treat intestinal parasites, as a potent inhibitor of AR conformational changes.
- Pyrvinium is 50-times more potent in blocking AR activity than the prototype AR antagonist, bicalutamide (Casodex).



**Figure:** The structure of the lead compound, pyrvinium.

- Dr. Diamond and his team tested a combination of pyrvinium and bicalutamide in mice and combination treatment showed synergistic inhibitory effects on AR-mediated pro-cancer gene expression.
- However, pyrvinium is poorly absorbed orally and is difficult to administer to patients in its current form because it is poorly water-soluble. Therefore, with PCF and NIH funding, Dr. Diamond and his team of chemists at UCSF designed a modified version of pyrvinium that had similar properties of AR inhibition and synergy with bicalutamide but had better solubility. T
- his compound, tetrahydropyrvinium (THP) blocks AR signaling, AR-dependent tumor growth and induces prostate atrophy.
- In 2009, Dr. Diamond relocated to Washington University and launched a small biotech company, ARTA Bioscience in 2010. The next goal of his research on pyrvinium and its derivatives was to identify the precise mechanism of action of this class of AR antagonists.
- Further experiments revealed that pyrvinium and THP have no direct effects on either AR binding to androgens, AR translocation from the cytoplasm to the cellular nucleus or AR binding to DNA to effect gene expression.

- Around the same time an independent publication from a research group at Vanderbilt University demonstrated that pyrvinium activated an enzyme in a cellular signaling pathway. This pathway of Wnt (pronounced 'wint') signaling is normally involved in embryogenesis and physiological development. Many of the genes in this signaling network have been implicated in various cancers. Currently there are no inhibitors of Wnt signaling in clinical use.
- Further research by Dr. Diamond's team at Washington University confirmed the enzyme casein kinase 1 alpha (CSK1 $\alpha$ ) as the direct target of both pyrvinium and THP. THP activates CSK1 $\alpha$  which targets the protein,  $\beta$ -catenin for degradation.  $\beta$ -catenin is an established AR-cofactor, i.e.,  $\beta$ -catenin binds AR and activates it for pro-cancer gene expression. THP therefore inhibits the cascade: CSK1 $\alpha$   $\rightarrow$   $\beta$ -catenin  $\rightarrow$  AR  $\rightarrow$  gene expression.
- $\beta$ -catenin functions as a cancer-promoting gene (or an oncogene) in several cancers such as basal cell carcinoma, colorectal cancer, medulloblastoma and ovarian cancer.
- Therefore, THP and other pyrvinium derivatives hold potential to down-regulate oncogenic Wnt signaling in several solid tumors.
- Pyrvinium is a first-in-class Wnt signaling and  $\beta$ -catenin inhibitor. This class of compounds (pyrvinium and its derivatives) discovered by Dr. Diamond holds great promise for the treatment of drug-resistant prostate cancer as well as a number of other solid tumors, especially colon cancer.
- Dr. Marc Diamond and his team at ARTA Bioscience are currently formulating more potent, stable derivatives of pyrvinium. They discovered that THP is not as stable as pyrvinium in cell culture and are therefore working with medicinal chemists to create better compounds.
- They plan to study the pharmacokinetics (PK) and pharmacodynamics (PD) of these compounds in mouse models of prostate cancer. Xenograft studies are currently ongoing with THP.

## Special Lecture: Genetics of Prostate Cancer – Two Decades of Progress

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**Patrick Walsh, MD**  
**Johns Hopkins Medicine**  
**Funded by a PCF Donor-Directed Award**

- As the founder of prostate cancer genetic risk studies, Dr. Patrick Walsh presented an overview of the field of prostate cancer genetics and its evolution over the past two decades.



- He started his genetic research in the 1980s at Johns Hopkins Hospital by evaluating familial linkage of prostate cancer. These studies on family histories of 741 radical prostatectomy patients and their female companions as controls revealed that the relative risk for prostate cancer in men with one first degree relative increased by 2.2-fold, while two first degree relatives with prostate cancer increased a man's risk of the disease by 4.9-fold.
- However, Dr. Walsh and his team understood that family history does not distinguish the effect of inherited genetic factors from the influence of a shared environment. Therefore, in order to make this distinction, and understand the precise pattern of transmission between generations, they carried out segregation analysis. Segregation analysis is a genetic epidemiological technique that allows studying Mendelian inheritance. It provides concrete genetic evidence for the inheritance of a human trait, by identifying the factors responsible for heritability.
- This segregation analysis by Dr. Walsh and his team was the first to establish the genetic basis for prostate cancer. Their model predicted autosomal dominant inheritance of a rare (0.3%) high penetrance allele with early age of diagnosis and multiple affected family members. This model allowed defining the families that carried these rare genes (termed hereditary prostate cancer families-HPC) as families with 3 or more first degree relatives (father, son, brother) or 3 generations (grandfather, father, son) or in 2 first-degree relatives if both are less than 55 years old.
- Next, Dr. Walsh and his team set out to identify the precise genes that are responsible for this hereditary link to prostate cancer. Finding the particular genes from the genome responsible for a specific disease is akin to looking for a needle in a haystack. The best approach to this is by conducting genetic linkage analysis, i.e. by first finding the general region of the genome that is associated with disease inheritance. This is done by looking at known markers that flank this genomic region to home in on the candidate region of the genome linked to disease. Dr. Patrick Walsh and his team conducted the first linkage analysis for prostate cancer and identified a major susceptibility locus for prostate cancer on chromosome 1, which was later confirmed by several other independent studies. However, very few locations on the genome (loci) have been consistently identified by multiple investigators and none of the initially identified regions could account for a majority of the heritable associations of prostate cancer. Thus, despite strong evidence for a hereditary component to prostate cancer, identification of the genes involved has been elusive. One solution was provided by the advent of genome-wide association studies, GWAS, a method that examines genomes of large populations of cases (patients) and controls (non-patient for that particular disease) to discover SNPs (pronounced 'snip', Single Nucleotide Polymorphism). A SNP is a DNA sequence variation at a single nucleotide, i.e. at one building block of a DNA that introduces genetic variation among individuals. SNPs are beginning to be discovered and exploited for

disease detection in the personalized and predictive medicine era. GWAS studies have been successful in the identification of reproducible risk factors for many complex human diseases, many for the first time.

- GWAS studies have led to the successful identification of over 50 SNPs associated with prostate cancer risk. Although the relative increase in risk for any single SNP is small, the risk increases as the number of inherited risk SNPs increases.
- However, recent studies have shown that SNPs explain only about 25% of the hereditary risk.
- Only now with the advent of next-generation sequencing technologies is the field of prostate cancer genetics beginning to understand the contributions by other rare genetic variants towards disease heritability.
- One example of the advantages of next-generation sequencing analysis is the identification of a rare recurrent mutation in the gene *HOXB13* on chromosome 17, which appears to be a founder mutation originating in Nordic countries.
- This mutation results in the exchange of an amino acid in the resulting HoxB13 protein, and is associated with significant increases in prostate cancer risk, particularly in men with early age at onset and a positive family history. HoxB13 is important for the development of the prostate and high expression is maintained in the normal adult prostate. This recurrent mutation in HoxB13 maps to a conserved domain important for protein-protein interactions and may affect HOXB13 function.
- Although accounting for only a small fraction of all prostate cancer cases, this discovery may provide important clues about the pathogenesis of the disease and help to identify a subset of men who may benefit from additional or earlier screening.
- It is hoped that next-generation sequencing analyses in HPC families will allow the identification of other rare mutations in addition to HoxB13. Identification and improved understanding of more SNPs, newer gene-gene interactions and varied environmental influences will help us understand the genetic underpinnings of prostate cancer risk.

## Session 7

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### Epithelial Plasticity in Prostate Cancer: An Emerging Mechanism of Treatment Failure?

**Andrew Armstrong, MD**

**Duke University**

**Translation of EMT/MET Biology to Human Prostate Cancer**

**Funded by a PCF Young Investigator Award**

- The dictionary definition of plasticity is the state or capacity for being molded or altered in response to experience or varying conditions. Prostate cancer cells also display plasticity. For these cells, epithelial plasticity is defined as their ability to interconvert between different phenotypic states.
- Normal prostate cells have an epithelial phenotype, i.e. they are stationary, have tight cell-cell junctions and express cell-cell adhesion markers such as the protein E-cadherin. As cancer cells become invasive they undergo a phenotypic transition to the mesenchymal phenotype, i.e. they become motile, spindle shaped, lose cell-cell contacts and start expressing biomarkers such as N-cadherin, vimentin, fibronectin etc. This reversible process of transition from a stationary cell to a motile cell is termed as EMT or Epithelial Mesenchymal Transition. EMT allows cancer cells to become invasive and enter the bloodstream. EMT biomarker expression (E-cadherin, TGF- $\beta$ , SMAD4, Twist etc.) has been associated with disease recurrence, stem-cell like properties, metastasis and a poor prognosis.
- These cells circulating in the bloodstream have the potential to establish new sites of metastatic lesions. When they leave the bloodstream and settle at a new site, these circulating cancer cells undergo MET, i.e. Mesenchymal Epithelial Transition, a reversible process that confers stationary properties to these cells, allowing them to form new tumors.
- It is challenging to actually visualize this plasticity in human prostate cancer patients as this is a very dynamic process that is context dependent. It has traditionally been difficult to obtain metastatic biopsies, e.g. from the bone, and imaging of plasticity in bone, viscera, lymph nodes has lagged. The field also needs validated circulating biomarkers such as CTCs, RNA, microRNA to track plasticity.
- These plasticity biomarkers hold potential in predicting disease recurrence post-surgery, e.g. a switch from E $\rightarrow$ N cadherin expression (i.e. change from epithelial to a mesenchymal phenotype) in prostate cancer cells after radical prostatectomy is associated with an independent risk of metastasis and death.

- PCF Young Investigator Dr. Andrew Armstrong is investigating the role of epithelial plasticity in the promotion of metastasis in advanced prostate cancer through the interrogation of biomarkers in primary disease, metastases, and circulating tumor cells (CTCs).
- Published studies have shown that therapies such as androgen deprivation therapy (ADT) actually induce plasticity, by inducing EMT and stem-cell like properties in prostate cancer preclinical models. In these experiments, addition of androgens reversed this phenotype.
- Studies from Dr. Peter Nelson's group and others have shown that induction of this EMT phenotype in the tumor microenvironment by radiation or chemotherapy is responsible for the emergence of chemotherapy- and radiotherapy-resistance.
- Dr. Andrew Armstrong and his team studied circulating tumor cells (CTCs) from patients with progressive, metastatic, treatment-resistant prostate. Their results unequivocally demonstrated that a majority (80%) of CTCs from these men co-expressed both epithelial (cytokeratin, EpCAM, E-cadherin) and mesenchymal (N- and O-cadherin, vimentin) proteins.
- The current FDA-approved method for the detection of CTCs uses the protein EpCAM to capture these cells from a blood sample. However, EpCAM is a marker of epithelial cells alone. To work around this caveat, Dr. Andrew Armstrong and his team developed a novel CTC-capture ferrofluid to identify non-epithelial CTCs from the blood of men with CRPC. They used the markers  $\beta$ -catenin and O-cadherin to capture and characterize CTCs, excluding leukocytes. O-cadherin is an adhesion molecule expressed by normal osteoblasts. Treatment-resistant prostate cancer cells as well as circulating cells have been demonstrated to express O-cadherin in as a mechanism of osteo-mimicry that allows prostate cancer cells to home to the bone to seed metastases.
- In summary, Dr. Armstrong and his team have identified reversible transitional phenotypes in preclinical models of prostate cancer as well as prostate cancer patients that may contribute to metastasis, prostate cancer aggressiveness and treatment resistance. They have identified markers of plasticity in CTCs from prostate cancer patients, suggesting that these transitional cells are important for metastasis.

## Session 8

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### Mechanisms of Primary & Acquired Resistance to Androgen Axis Inhibitors

**Elahe Mostaghel, MD, PhD**

**Fred Hutchinson Cancer Research Center**

**Response and Resistance to Targeting Steroid Hormone Metabolism**

**Funded by a PCF Career Development Award**

- Prostate cancers are driven by the androgen-AR signaling axis throughout the course of the disease.
- The premise for both hormone-naïve and treatment-resistant prostate cancer therapy is that more potent elimination of tissue androgens will result in more effective inhibition of AR signaling and thereby improve clinical efficacy of hormone therapy.
- However, not all patients respond to these treatments, and not all patient responses are durable. Further, patients ultimately develop resistance.
- This de novo or acquired treatment-resistance can be attributed to three factors:
  1. AR-related resistance mechanisms such as mutations in the androgen receptor, amplification of the receptor, promiscuous binding of AR to other ligands etc.
  2. Ligand (androgen)-related resistance mechanisms such as residual androgens in the tumor despite therapy, or androgens produced by the aberrant induction of steroidogenesis etc.
  3. Agent-related resistance mechanisms, such as a pharmacogenetic variation in drug response, induction of drug export from cancer cells etc.
- Dr. Elahe Mostaghel is studying the development of these mechanisms of treatment resistance. In her presentation at the Retreat she presented her research on primary mechanisms of resistance to androgen deprivation therapy in hormone-naïve prostate cancer.
- In hormone-naïve localized prostate cancer, complete responses to androgen deprivation therapy are uncommon. This has generally been presumed to be due to inadequate suppression of the male hormones, androgens in these tissues.
- However, two recent clinical trials that tested androgen inhibitors in the neoadjuvant setting have shown that better tissue androgen suppression *is* possible.

- One trial evaluated men with intermediate/high risk clinically localized prostate cancer treated for three months prior to prostatectomy with zoladex and 1) avodart 3.5 mg QD; 2) avodart and casodex 50 mg QD; or 3) casodex, avodart and ketoconazole 200 mg TID. A second trial, also in men with high risk localized cancer, evaluated Lupron combined with the FDA-approved agent abiraterone for up to six months prior to prostatectomy.
- In both studies, patients in the multi-targeted treatment arms showed better suppression of serum and tissue androgen levels (compared to zoladex or Lupron alone), with lower PSA nadirs in those with better androgen suppression.
- In the first study, benign prostate tissue from men receiving multi-targeted therapy showed evidence of strongly decreased androgen receptor (AR) and PSA expression. However, these markers were not equivalently suppressed in tumor prostate tissue, and there was no direct correlation between better lower PSA nadirs and lower residual tumor volumes.
- In the second trial, PSA and androgen levels were even more dramatically suppressed; however the number of men achieving a complete prostate response (total eradication of cancer) was not increased compared to the first study.
- Notably, in both studies there was an increase in the number of patients showing only small/minimal areas of residual disease, although if these patients do better in the long run has not been established.
- These data suggest that if androgen levels mediate resistance, there is evidence that more potently suppressing tissue androgens does not impact prostate tumor AR activity as dramatically as expected.
- However, the increased number of men achieving minimal residual disease in these two studies suggests that including additional agents directly targeting activation of the AR may achieve the combined potency needed to maximally inhibit tumor AR activity and thereby effectively potentiate complete tumor eradication.

**Steve Balk, MD, PhD**

**Beth Israel Deaconess Medical Center**

**Androgen Receptor Functions in Advanced Castration-Resistant Prostate Cancer**

**Funded by a PCF Challenge Award**

- Dr. Steve Balk presented the new interacting partners of the androgen receptor (AR) that assist its role in prostate cancer development and progression.
- In a normal cell, through a negative feedback regulation loop the androgen receptor down-regulates its own expression, i.e. the AR protein represses its own



production. Dr. Balk's studies show that the androgen receptor protein does this by recruiting proteins such as LSD1 to the *AR* gene that repress gene activity and thereby AR production.

- To maintain homeostasis in normal cells, the AR represses genes involved in DNA replication and cell division, and activates those that participate in lipid and protein synthesis.
- This regulation is altered during treatment resistant prostate cancer when the upregulation of both intra-tumoral androgens and the androgen receptor leads to hyper-activation of pathways involved in DNA, lipid and protein syntheses, thereby contributing to tumor growth.
- Understanding the distinct mechanisms that mediate AR-induced repression and activation of these oncogenic pathways will lay the foundation for the development of targeted therapies.
- Dr. Balk and his team conducted detailed investigations to identify the protein players that help the AR in inducing and repressing several genes, both in normal development and in prostate cancer.
- These studies highlighted the protein LSD1 which acts as a gene on/off switch. LSD1 recruits the AR and large multi-protein complexes to the DNA to molecularly turn a gene on or off.
- These large multi-protein complexes such as CoREST (RE1-silencing transcription factor) involve interactions between AR, LSD1 and FOXA1 in prostate cancer cells.
- Dr. Balk's studies revealed that knockdown of/depleting Co-REST proteins decreases AR and LSD1 binding to both AR-induced and repressed genes and could hold potential in developing targeted therapies.
- In the second part of his presentation at the PCF Scientific Retreat, Dr. Balk presented his studies on another protein that is oncogenic in prostate cancer. Approximately 50% of all prostate cancers overexpress the protein ERG which mediates enhanced prostate cancer cell invasiveness and proliferation. However, the precise mechanism of its action is not currently known.
- Dr. Balk and his team identified the protein Sox9 that is a downstream effector of ERG in preclinical prostate cancer models.
- Sox9 belongs to a family of proteins called transcription factors like the AR. Transcription factors regulate expression of several genes. Dr. Balk's studies have shown that Sox9 promotes tumor proliferation, angiogenesis and invasiveness in prostate cancer xenograft models. Inhibiting Sox9 activity significantly reduces tumor volume in these models.
- These studies validate Sox9 as a potential therapeutic target in primary and treatment resistant prostate cancer.

**Amina Zoubeidi, PhD**  
**The Vancouver Prostate Centre**  
**Persistent Androgen Receptor Activation in MDV3100 Resistant Prostate Cancer**  
**Funded by the PCF Young Investigator Award**

- The androgen receptor is known to drive prostate cancer progression and treatment resistance by translocation from the cell cytoplasm into the nucleus where a genetic program is initiated that drives the disease.
- In August 2012 the FDA approved enzalutamide (formerly MDV3100) for the treatment of docetaxel chemotherapy-resistant prostate cancer. Enzalutamide works by binding the androgen receptor (AR) and preventing its nuclear translocation. It also inhibits AR interaction with DNA inside the nucleus.
- However, initial responders to enzalutamide therapy will ultimately develop resistance to the therapy.
- PCF Young Investigator Dr. Amina Zoubeidi is working on understanding the molecular basis of this enzalutamide resistance.
- Dr. Zoubeidi and her team developed enzalutamide-resistant prostate cancer cell lines and demonstrated that cell proliferation in these resistant cell lines is mediated by 1) upregulation of AR expression, and 2) an active AR signaling pathway.
- Dr. Zoubeidi presented data at the PCF Scientific Retreat demonstrating that the AR is localized in the cell nucleus and binds to DNA in enzalutamide-resistant cell lines.
- To target this aberrant activity of the androgen receptor, Dr. Zoubeidi and colleagues developed an anti-sense oligonucleotide (ASO), i.e. a DNA molecule that binds to the *AR* gene and prevents the production of the AR protein.
- Upon testing in enzalutamide-resistant prostate cancer xenograft models, this AR-ASO significantly delays tumor growth.
- During these studies on enzalutamide resistance, Dr. Zoubeidi also observed an enhanced expression of several intermediates of the androgen synthesis pathway. To target these androgens she tested another inhibitory anti-androgen on preclinical disease models. This investigational drug, named Compound 30 for now, inhibits the growth of enzalutamide-resistant cells, causing significant reduction in tumor volumes and serum PSA levels.
- Dr. Zoubeidi next looked into the actual mechanisms by which enzalutamide resistance developed. Her studies show that enzalutamide activates the protein Her-2.
- Her-2 (Human Epidermal Growth Factor Receptor 2) is a protein known to promote cell proliferation and inhibit cell death (apoptosis). Amplification of the

*ERBB2* gene that codes for the Her-2 protein is known to occur in 30% of all breast cancers, being prognostic for disease recurrence and poor outcome.

- Dr. Zoubeidi's studies show that Her-2 also plays important roles in enzalutamide resistance, by activating important oncogenic cell signaling pathways such as the MAPK AND AKT pathways.
- Dr. Zoubeidi tested combination therapy with enzalutamide and a Her-2 inhibitor, Lapatinib. This enzalutamide+Her-2 therapy showed promising inhibition in preclinical models of disease and delayed the onset of enzalutamide resistance.
- These studies, therefore, provide proof-of-concept that AR co-targeting is a promising strategy to control therapy resistance.

## **Scott Dehm, PhD**

### **Masonic Cancer Center, University of Minnesota**

#### **AR Gene Rearrangements and Splicing Variants: Implications for AR-Targeted Therapy**

#### **Funded by the PCF Young Investigator Award**

- Treatment resistant progression is a hallmark of advanced prostate cancer, e.g. despite an increase in overall survival with the recently FDA-approved agent enzalutamide, resistance has been seen to develop rapidly even in initial responders, while some prostate cancer patients present with *de novo* resistance.
- This treatment-resistant disease progression is spurred by the aberrant activity of the master regulator androgen receptor (AR) that mediates the activity of the male hormones, androgens. As the disease progresses, AR becomes androgen-independent and continues to fuel oncogenic cell signaling leading to disease spread.
- These changes in the androgen receptor from androgen-dependence to independence are caused by mutations and rearrangements in the *AR* gene and amplification of the AR protein.
- The AR protein has a modular structure with four main domains arranged linearly as: N-terminal domain (NTD)—DNA binding domain (DBD)—Ligand binding domain (LBD)—C-terminal domain (CTD).
- Alterations in the biological process of AR gene expression (splicing) results in truncated versions of the androgen receptor which can be produced in large quantities during advanced disease.
- Most of these truncated AR proteins called AR Splice Variants (ARVs) lose the LBD and CTD i.e. the domains necessary for AR interaction with androgens,

making these ARVs androgen-independent and free to constitutively activate cell signaling even in the absence of androgens.

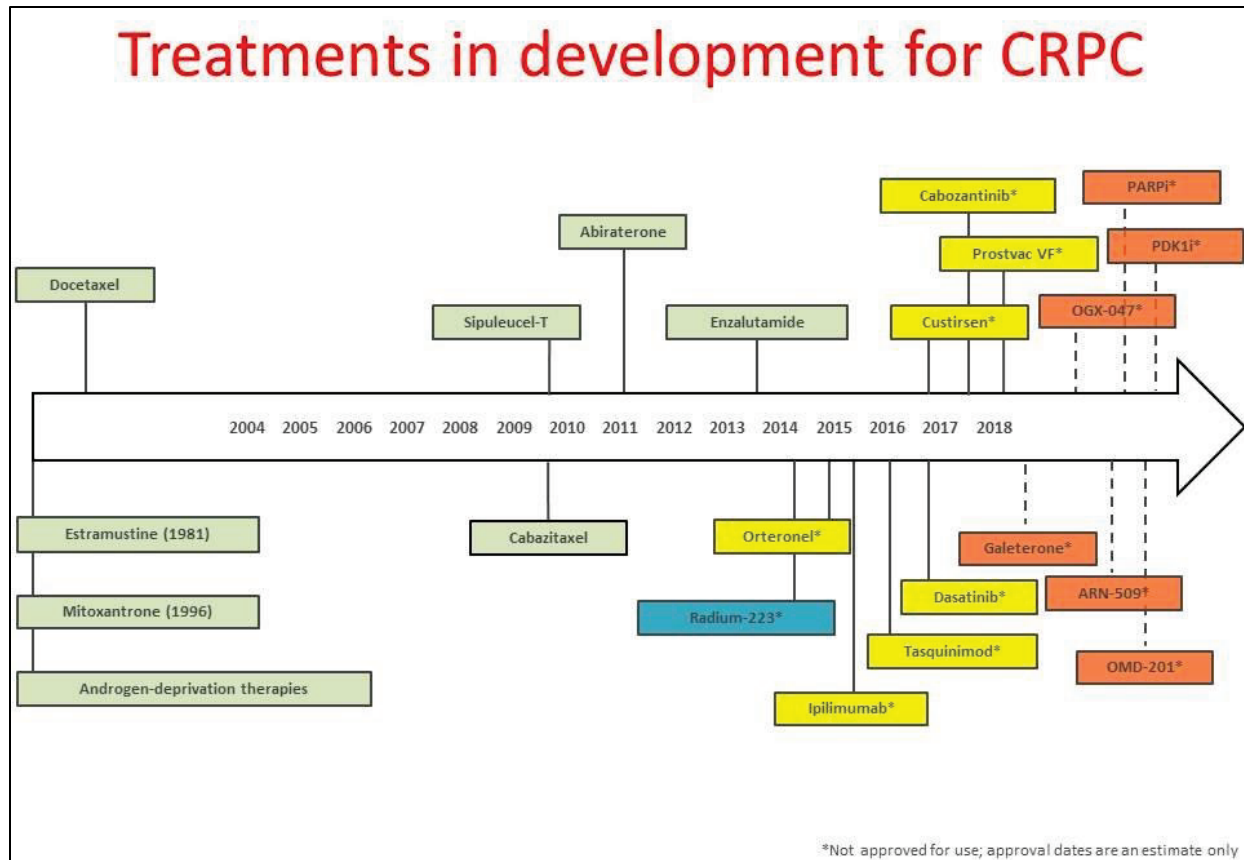
- ARVs have been postulated to cause treatment resistance, prostate cancer bone metastases and may be prognostic for shorter overall survival.
- Some ARVs have been shown to depend on the full length AR for their activity; therefore depleting the full-length protein by targeted therapy can eliminate the activity of the splice variants as well. However, recent evidence points to other AR splice variants that do not require the full length protein for their activity.
- Funded by a PCF Young Investigator award, Dr. Scott Dehm evaluated the roles of full length AR and the ARVs in supporting treatment-resistance and mediating responsiveness to enzalutamide.
- Prostate cancer patients resistant to enzalutamide therapy mostly present with rising PSA. The *PSA* gene is a downstream target of the *AR* gene i.e. an active AR activates the *PSA* gene leading to greater PSA production. This suggests that enzalutamide-resistant tumors in advanced treatment resistant disease are still progressing by persistent AR/ARV signaling.
- Dr. Dehm's studies with treatment resistant preclinical models of prostate cancer have shown that ARVs are sufficient to drive resistance to enzalutamide.
- Enzalutamide resistance is mediated by the activities of ARVs as independent effectors of oncogenic cell signaling (i.e. not requiring the full length protein), driving persistent/constitutive activation of a large subset of AR target genes to support cell proliferation.
- These studies provide proof-of-concept for targeting ARV expression and/or activity to reverse enzalutamide resistance.

# Special Lecture: Treatments in Development in the Post-Abiraterone Setting: Are we through with hormone therapy?

Gerhardt Attard, MD, PhD

The Institute of Cancer Research & Royal Marsden Hospital

Funded by a PCF Young Investigator Award



PCF Young Investigator, Dr. Gerhardt Attard presented an overview of the clinical pharmacologic strategies currently under evaluation for the treatment of treatment-resistant prostate cancer, which he classified into three categories:

1. Drugs targeting the androgen receptor: These treatment strategies are based on the hypothesis that AR-mediated signaling plays a critical role in a significant proportion of patients that develop resistance to new anti-androgens such as abiraterone and enzalutamide.
  - a) AR is a multi-domain protein and targeting its androgen-binding domain or the LBD (Ligand Binding Domain) will remain an effective treatment strategy.

- b) Another important criteria for successful Phase III clinical trials is efficient patient selection, i.e. only after understanding the underlying biology of each patient's disease can effective targeted therapies be employed.
  - c) Dr. Attard discussed two new AR antagonists, ODM-201 and AZD3514, the latter causes AR degradation and is being evaluated at the Royal Marsden in Phase I clinical trials in treatment resistant prostate cancer patients.
  - d) A critical factor for the use of abiraterone in patients is the fact that it requires administration with prednisone to minimize the risk of side-effects like hypokalemia, hypertension, fluid retention etc. which arise due to abiraterone-induced excesses in mineralocorticoid levels. Dr. Attard's studies have shown that these co-administered exogenous glucocorticoids can cause mutations in the androgen receptor, making it over-active. Dr. Attard presented preliminary data on an experimental compound VT-464 which is almost 60-fold more specific for the drug target CYP17A lyase than abiraterone, and obviates the need for exogenous glucocorticoid (prednisone) administration.
2. Drug combinations with/of effective drugs such as abiraterone, enzalutamide:
- a) Combinations targeting independent pathways in the cancer cell such as Rad223 + Abiraterone/ Enzalutamide.
  - b) A combination of OGX-011 with docetaxel was discussed.
  - c) Combinations targeting pathways that work together to cause disease resistance such as PI3K/Akt + AR will be an effective strategy for treating patients.
  - d) Combinations of agents targeting the androgen receptor will be effective for prolonged AR inhibition. Dr. Attard's studies have shown that abiraterone does not completely rid prostate cancer cells of androgens. Residual urinary androgens and estrogens were detected in urine samples of abiraterone-treated patients. Therefore better inhibition of androgen synthesis will be achieved by using combinations of targeted agents which can potentially reverse treatment resistance and increase patient response rates.
  - e) Results from Dr. Attard's studies show that abiraterone, in addition to its activity as an anti-androgen, also acts as an antagonist of the androgen receptor at higher doses. Therefore, administration of abiraterone at higher doses can serve 2 roles: androgen suppression and AR inhibition.
3. Drugs with uncertain or multiple mechanisms of action:
- a) Cabozantinib: induces tumor cell death at bone metastatic lesions and causes significant radiological regression of measurable soft tissue lesions.
  - b) PARP inhibition: Poly (ADP-ribose) polymerase (PARP) is a family of proteins that detects single-strand breaks in DNA and signals for their repair. A significant proportion of treatment resistant prostate cancer cells have



underlying DNA damage repair defects and will be selectively sensitive to therapies inhibiting PARP function. PARP inhibition is being evaluated under a 2012 PCF Challenge Award to Drs. Johann De Bono, Karen Knudsen, Felix Feng and Gerhardt Attard.

## Session 9

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### The Good, the Bad and the Ugly of Preclinical and Observational Research

**C. Glenn Begley, PhD**

**TetraLogic**

**(Former head of Hematology/Oncology Research at Amgen)**

**Raising the Bar for Preclinical Cancer Research**

- In a paper published in the journal *Nature*, titled 'Drug development: Raise standards for preclinical cancer research', authors Drs. Glenn Begley and Lee Ellis analyzed the low number of cancer-research studies that have been converted into clinical success, and concluded that a major factor was the overall poor quality of published preclinical data.
- Dr. Glenn Begley began his presentation at the PCF Scientific Retreat by stating that "We get what we incentivize".
- He stated that the pharmaceutical and drug development industry relies heavily upon targets and pathways identified by academic groups, and validation of these targets is critical as discovering published data is flawed later in development can be too costly.
- He presented the results of a 10-year long experiment during his tenure at Amgen as the head of hematology and oncology research. Dr. Begley and his team attempted to replicate the results of 53 landmark/ 'seminal' research studies but were successful in replicating only six of these, i.e. results claimed in only 11% of 53 studies stood up to the original assertions. Most of these 53 studies were preclinical, describing novel interacting proteins, new oncogenes, intracellular signaling pathways, novel functions for known proteins or new uses for established therapeutics.
- The Amgen scientists approached the papers' original authors to discuss findings and sometimes borrowed materials to repeat the experiments. In some cases, those authors required them to sign an agreement that they would not disclose their findings about specific papers. In effect, Drs. Begley and Ellis were not free to identify the irreproducible papers.

- In some cases the Amgen team observed that part of, or all of the data could not be reproduced even by the same investigator using the same reagents from the same lab that originally published the results.
- Some of the common features of these irreproducible studies were:
  1. These studies were not blinded. A blinded experiment is one in which identities of some information is hidden from the investigators/participants involved to avoid the conscious/subconscious introduction of bias, and result invalidation.
  2. All results were not shown in the publication, with only a few representative sets of data being shared.
  3. Typically in most of these studies experiments were not repeated to achieve statistical significance or establish conclusively the stated results.
  4. The experiments were not performed with positive and negative controls.
  5. The reagents used in the experiments were not validated.
  6. Appropriate statistical tests were not employed for data assessment.
- These discrepancies in scientific result reproducibility by the scientists at Amgen were also observed by the research teams at Novartis Oncology and Astra Zeneca who also suggested that “....only in approximately 20-25% of projects were the relevant published data completely in line with our in-house findings....in most cases (this) resulted in termination of the projects because the evidence....was insufficient to justify further investments into these projects”.
- These irreproducible studies have substantial impact in terms of wasted efforts of multiple investigators, multiple companies, wasted money and time. It is interesting and unfortunate to note that some of these 53 papers have spawned whole fields of new investigations, being followed by hundreds of secondary publications and initiation of multiple clinical trials.
- In their *Nature* paper, Drs. Begley and Ellis propose that methods of scientific pursuit and incentives for publication must change if patients are to benefit.
- Dr. Begley proposed that journal editorial boards need to stop ‘tolerating poor quality science’ and must raise standards for publication by encouraging the publication of confirmatory data, and rewarding and recognizing the value of findings that refute ‘high-profile’ studies.
- Dr. Begley’s recommendations are summarized in the Figure below:

## Recommendation:

Investigators, Institutions, and Reviewers, Editors,  
Funding Agencies, Consumers, Advocates, Press  
demand:

- 1) Studies are blinded
- 2) All results are shown
- 3) Experiments are repeated
- 4) Positive and negative controls are shown
- 5) Reagents are validated
- 6) Appropriate statistical tests are applied
- 7) Titles and Abstracts are not over-reaching
- 8) References are accurately cited

### **S. Stanley Young, PhD** **National Institute of Statistical Sciences** **Voodoo Statistics and Trust Me Science**

- Dr. Stanley Young suggests that the current observational study paradigm is “no correction for multiple testing, or multiple modeling and no sharing of data sets” i.e. “Voodoo Statistics” and “Trust Me Science”. He presented evidence of a false discovery rate for observational studies of >90%.
- Dr. Young began his discussion with an overview of the “players” in the field, including the *workers*-scientists and epidemiologists; the *communicators*-PR people, bloggers, reporters, science writers; the *consumers*-public, regulatory agencies, trial lawyers; and the *management*-funding agencies and journal editors.
- Dr. Young invoked the principles of Edwards Deming, the pioneer statistician credited with launching the Total Quality Management movement that lays

downs principles to improve design, service, product quality, testing, and sales through various methods, including the application of statistical methods.

- He contrasted the control of an observational study with that of a production process, and gave technical explanations for how the high false discover rate happens in observational studies and Deming reasons for why it continues. For example in an observational study production process, workers, i.e. study researchers conduct data collection, data cleaning, statistical analysis, interpretation and writing a report/publication, all without managerial control at each step of the process.
- Dr. Young suggested that the current system for conducting and analyzing observational studies is out of control. He invoked two principles proposed by Edwards Deming:
  1. A system that is out of control is not the fault of the workers; it is the fault of the managers that designed and run the system.
  2. It is the responsibility of managers to fix the system.
- Dr. Young suggests that that we need to stop blaming the workers as the *workers*, i.e. researchers and epidemiologists who conduct and analyze observational studies are responding to current incentives, publications and grants. Therefore, the *managers*, i.e. funding agencies and journal editors, need to redesign the rewards system.
- Dr. Young emphasizes that claims of observational studies should not be taken seriously unless these are replicated and data is public.
- Some of Dr. Young's suggestions for the effective management of observational studies are as follows:
  1. The study protocol should be publically posted before initiation of the experiments/studies.
  2. The data set produced from the studies should be publically posted
  3. The precise questions under consideration by the observational study should be clearly stated
  4. All observational studies must conform to 'Reproducible Research' guidelines.
  5. Any and all claims of observational studies must be independently replicated.

## Session 10

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### Immunotherapy of Prostate Cancer: 2012 Update

**Charles Drake, MD, PhD**

**Johns Hopkins Medicine**

**Immune Checkpoint Blockade to Treat Cancer: 2012 and Beyond?**

**Funded by a PCF Challenge Award**

- In recent years, immune therapies to treat cancer are proving effective. The idea is to boost the body's immune reaction in order to fight cancer. This can be done in two main ways: either amp up the body's immune reaction in general or "teach" our immune cells to "see" cancer cells more clearly so they can attack them more readily.
- Cancer vaccines can act to jump start a stalled anti-cancer immune cell response as tumor cells craft ways to evade the natural T-cell onslaught mounted against them.
- Immune checkpoint blockade is a type of immunotherapy that blocks the body's checks and balances on the immune system reaction. It is often described as taking the brakes off the immune response.
- Dr. Charles Drake and colleagues are working on therapies that amp up the body's natural immune response. Their studies have demonstrated that depleting androgen levels via hormone therapy in men with prostate cancer boosts a cancer vaccine's effectiveness. Additionally, PCF-funding to the Drake lab bore fruit demonstrating that adding low-dose chemotherapy also enhances the response of anti-cancer vaccines.
- Dr. Drake will soon be starting a clinical trial using combination therapies consisting of cancer vaccine, low-dose chemotherapy and androgen-lowering drugs in men with medium to high-risk prostate cancer prior to surgery and comparing those results to men receiving only androgen-lowering drugs prior to surgery to remove their prostate gland.
- This past summer Drake was an author on a study published in the New England Journal of Medicine that looked at Phase I data from an immunotherapy known as PD-1 blockade.
- The PD-1 protein is found on activated killer T-cells--the attack dogs of the immune system responsible for killing off molecules or cells perceived as a threat to the host. However, another protein (PD-L1) can bind to the PD-1 protein, killing off the activated T-cells and thus tamping down the body's natural immune response against cancer cells.

- Blockade of PD-1 using an experimental monoclonal antibody that prevents PD-L1 from binding to PD-1 restores T-cell function and demonstrated anti-tumor activity in patients with kidney, lung and skin cancers, as described in the NEJM paper.
- Dr. Drake's lab was among the first to show that T-cells sent to fight prostate cancer tumors have the PD-1 protein on their surfaces, making PD-1 blockade a reasonable avenue of investigation for prostate cancer. (In the NEJM article, no objective response was shown in prostate cancers, perhaps because combination therapies are needed to treat this cancer.)
- During Dr. Drake's presentation today at the PCF Scientific Retreat he presented first-in-field data showing that certain prostate cancer cells can express the PD-L1 protein—likely as a defense against immune system attack . (It's been generally thought PD-L1 is not expressed in prostate cancer.)
- This may be of substantial importance because PD-1 blockade seems to work best in cancer that express PD-L1. Ultimately Drake says he plans to conduct clinical trials in men with prostate cancer using PD-1 blockade antibodies that will take the brakes off the immune response, anti-androgens and cancer vaccines to prime the immune system in a more general fashion.
- Overall said Dr. Drake: "We often use the analogy of the brakes of a car to describe PD-1. In some patients, the gas pedal is being pressed (the immune response is revved up), but the car can't go because PD-1 is expressed (i.e. the brakes are on). That's why blocking PD-1 (taking off the brakes) is good enough for an immune response in some kinds of cancer, like kidney cancer. In prostate cancer, though, the gas is not on. We need to both turn the gas ON by using a vaccine (in combination with androgen-ablation), and to release the brakes by blocking PD-1. That's what we hope to do in the second trial.

**William Redmond, PhD**  
**Providence Portland Medical Center**  
**OX40 Agonists and Prostate Cancer Immunotherapy**  
**Funded by a PCF Young Investigator Award**

- OX40 receptor is a protein expressed on the surface of a kind of immune cells called T-cells. T-cells recognize foreign agents like infection and cancer, and launch an attack to rid the body of invaders.
- The OX40 receptor plays a critical role in the maintenance of this T-cell driven immune response beyond the first few days of foreign attack; enhances antigen-specific immune responses, and generates a 'memory' of the response.



- The OX40 receptor is activated upon its binding to its cognate ligand OX40L expressed on the surface of antigen presenting cells (i.e. cells that internalize tumor antigens or parts of tumor cells and present these to the immune system).
- Studies have shown that the engagement of the OX40R (either by its cognate ligand or an agonist) enhances antitumor immunity. This engagement of OX40R is therefore a practical approach for expanding tumor-reactive T cells to improve tumor immunotherapy in cancer patients.
- Dr. Redmond and colleagues have been studying a monoclonal antibody that specifically binds to and activates OX40R (engages the receptor as an agonist and activates its downstream signaling) leading to effective anti-tumor responses.
- In a Phase I clinical trial, the agonist anti-OX40R antibody was well tolerated and enhanced T-cell proliferation. Interestingly, maximal T-cell proliferation was observed in the three prostate cancer patients on the trial, in comparison to patients with other cancers.
- With funding by a PCF Creativity Award, Dr. Brendan Curti and colleagues at the Earle A. Chiles Research Institute, Providence Portland Medical Center evaluated this antibody in a Phase I clinical trial in combination with radiation therapy and cyclophosphamide chemotherapy, in metastatic prostate cancer patients who failed prior therapy. Their studies show that the addition of chemo and radiation therapies does not disrupt the ability of the anti-OX40 antibody to promote T cell proliferation and mount an anti-tumor immune response.
- In the third segment of his talk, Dr. Redmond described another combination approach that holds immense potential in augmenting anti-tumor immunotherapy.
- CTLA-4 is a protein expressed on T-cells that acts as an 'off' switch for the immune system i.e. it down-regulates the immune response. Blocking CTLA-4 holds potential in immunotherapy as it releases the brakes on immune system tolerance to tumors, allowing effective cancer cell killing. This has been demonstrated in metastatic melanoma by the FDA-approved agent ipilimumab which blocks CTLA-4 action and demonstrates durable responses in patients.
- Dr. Redmond presented results of preclinical studies evaluating a combination of these two approaches, namely, anti-OX40 + anti-CTLA-4 antibodies.
- As an analogy, engaging OX40R is like giving gas to the engine (immune system), while blocking CTLA-4 is analogous to releasing the brakes on the engine, spurring the immune system to aggressively attack cancer cells.
- Indeed these preclinical studies on animal models of advanced prostate cancer showed that combinatorial anti-OX40 therapy plus CTLA-4 blockade elicits potent anti-tumor immunity.

- Based on these promising preclinical data, Dr. Redmond and colleagues plan to test this combination therapy in prostate cancer patients with advanced disease.

## **Brian Olson, PhD**

### **University of Wisconsin Carbone Cancer Center**

#### **Tumor Antigen-Specific Regulatory T Cells to Predict and Monitor Vaccine Efficacy**

- Prostatic acid phosphatase (PAP) is an enzyme that plays an important role in the liquefaction of semen. It was one of the first proteins found elevated in the serum of prostate cancer patients. PAP was used as prostate cancer biomarker before the common application of the PSA test. PAP expression is limited to normal and malignant prostate tissue and immunohistochemical staining for PAP is still a very common test to conclusively establish the origin of metastatic cancer lesions (e.g. to distinguish metastases of prostate or bladder cancer).
- Since PAP is a prostate-specific tumor antigen, it is being investigated as the target of several anti-tumor vaccines in clinical trials. Anti-tumor vaccines, or active immunotherapies, seek to elicit immune responses within the host that recognize tumor-associated antigens to eliminate tumor cells expressing these antigens.
- Following vaccination with these tumor antigens, the host immune system usually responds with the generation of a certain population of white blood cells called the antigen-specific effector T cells (such as the CD8+ T-lymphocytes) which recognize cancer cells expressing the specific antigen, and kill these cells.
- Dr. Brian Olson and colleagues have generated a DNA vaccine targeting PAP which has been evaluated in a Phase I clinical trial in 22 biochemically recurrent prostate cancer patients. Eight out of the 22 patients had long-term immune responses to PAP; however, three of these eight responders did not demonstrate immediate immune responses following immunization.
- Their studies into these patient responses uncovered the role of antigen-specific regulatory T cells or Tregs in modulating immune responses in these patients. Tregs are a category of blood cells that are immunosuppressive, and play a critical role in immune tolerance, i.e. preventing the body from mounting an immune response against itself.
- Dr. Olson described the trans-vivo Delayed Type Hypersensitivity (tv-DTH) test in which patient peripheral blood cells are injected into the footpads of mouse models of prostate cancer, along with the tumor antigen PAP. Inflammation at the site of injection is measured 24 hrs later to evaluate antigen-specific immunity.

- Using these tvDTH tests and immune monitoring analyses, Dr. Olson and colleagues observed that in the three responders to PAP vaccination who did not demonstrate immediate responses to immunization, PAP-specific regulatory T cells were suppressing effector immune responses. These antigen-specific *regulatory* responses precluded the detection of *effector* responses.
- Dr. Olson and colleagues identified a novel population of regulatory T-cells in these patients. These Tregs were PAP-specific, CD8+CTLA-4+ and secreted the cytokine IL-35 which plays a role in immune suppression.
- Further studies revealed that this immune-suppressive response pre-existed in ~40% of all patients on this study prior to immunization and precluded the detection of effector responses post-immunization.
- Dr. Olson and colleagues propose that the presence of these novel, PAP-specific Tregs can serve as a biomarker to identify patients likely to respond immunologically to vaccination, i.e. patient selection for clinical trials. These Tregs can also help decide the best suited vaccines for patients depending on their underlying immune biology.
- Further, patients presenting with the novel CTLA4+ Tregs can be best treated with combinatorial therapy using both the PAP-DNA vaccine and ipilimumab, the FDA-approved therapy that targets CTLA-4.

## Haydn Kissick, PhD

### Beth Israel Deaconess Medical Center

#### Preclinical Development of a Prostate Cancer Vaccine Targeting the Transcription Factor ERG

- Peptide vaccination is the technique of exposing the immune system to short proteins called peptides normally expressed by tumors, to stimulate the immune system to mount a durable response against cancer cells expressing those peptides.
- Because these peptides are synthetic, there pose no risk of mutation. Further, chemical manipulation of the peptide structure before administration increases stability and decreases unwanted side effects. Peptide vaccines also offer the advantage of "exposing" parts of the tumor proteins that are not seen by the immune system
- Dr. Haydn Kissick presented a 5-point prostate cancer peptide vaccine development strategy:
  1. Identification of novel prostate cancer specific proteins that can serve as suitable antigens to activate the immune system.
  2. *In silico* design of immunogenic peptides that serve as the vaccine.

3. Preclinical studies to validate that human prostate tumor cells are targets of the peptide vaccine.
  4. Identify the presence of peptide reactive T-cells in prostate cancer patient blood to evaluate activation of the immune system.
  5. Evaluate peptide delivery platforms for potential clinical trials.
- Dr. Kissick described the application of the above strategy to the preclinical development of a peptide vaccine targeting the protein ERG in prostate cancer.
  - ERG belongs to a family of proteins called transcription factors that regulate the expression of several genes. Approximately 50% of all prostate cancer patients present with ERG overexpression which is driven by an aberrant gene rearrangement that results in the fusion of the *ERG* gene with the oncogene *TMPRSS2*. PCF-funded studies have shown that this ERG overexpression is associated with a disruption of the androgen receptor signaling pathway and the development of androgen-independent disease.
  - The ERG295 peptide vaccine developed by Dr. Kissick and team was immunogenic in humanized mice models and activates immune cells in the blood of prostate cancer patients.
  - Dr. Kissick and his team are currently evaluating the correlation of patient ERG expression status and reactivity to the ERG295 vaccine. They are also investigating various formulations to improve peptide loading and *in vivo* release profile of nanoparticles.

## **Exceptional Progress Report – PCF Young Investigator**

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### **Development of Radio-Tracers Targeting Prostate Specific Kallikreins for In Vivo Detection of Disseminated Disease and Measurement of Androgen Signaling**

**David Ulmert, MD, PhD**

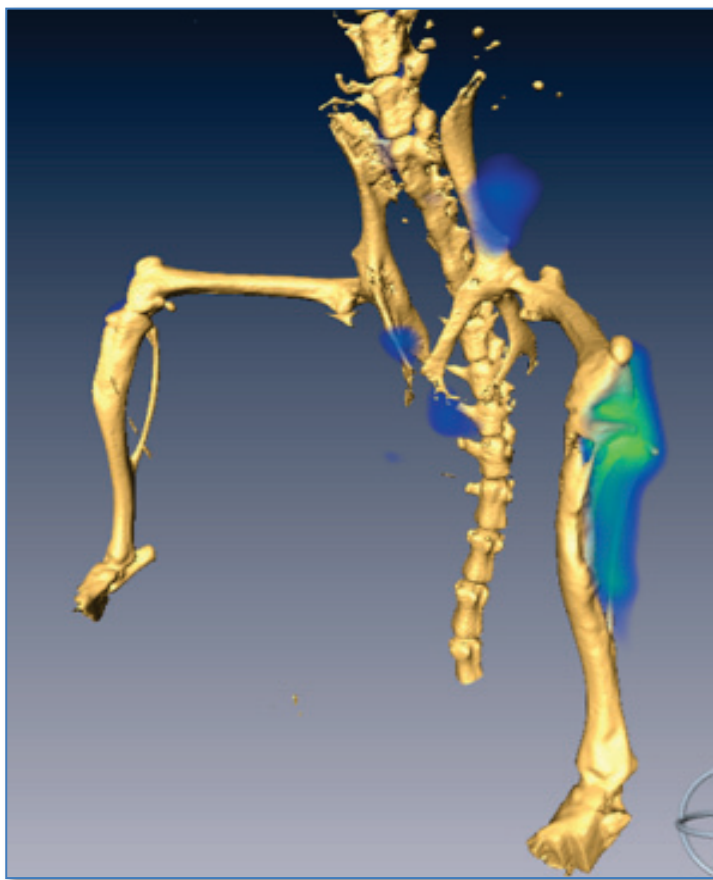
**Memorial Sloan-Kettering Cancer Center**

**Funded by PCF Young Investigator Award**

- Kallikreins are a class of enzymes called the serine proteases that are critical for several physiological functions. Some examples of kallikreins important in prostate development are human kallikrein 2 (hk2) and PSA (Prostate Specific Antigen).
- PSA is an enzyme whose production is governed by the male hormones, androgens through the Androgen Receptor (AR). Levels of PSA reflect the activity

of AR and can serve as an effective biomarker for prostate cancer detection and progression.

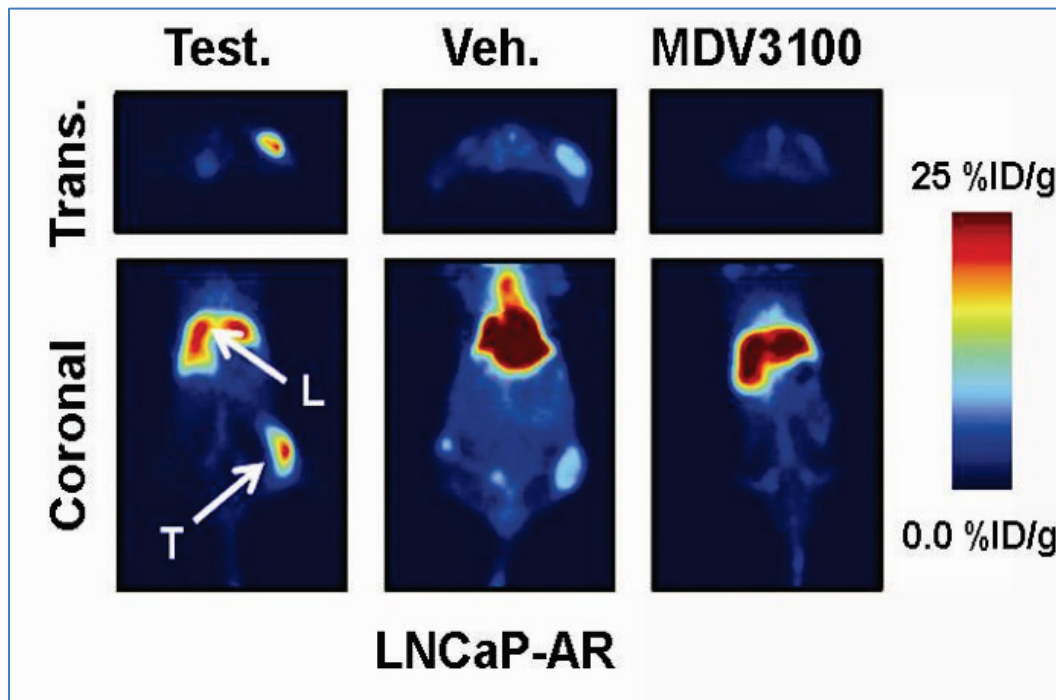
- The current methodologies for detecting PSA for prostate cancer diagnosis and monitoring measure PSA levels in the bloodstream and not those in prostate tumors or bone metastases.
- This PSA in the bloodstream is 'bound' PSA (i.e. PSA protein that is complexed to other proteins) which is not always a true measure of the actual levels of PSA in tumors.
- This is due to the small percentage of intra-tumoral PSA that is secreted into the serum. Also, the rate-limiting step of PSA transport to the bloodstream is poorly understood.
- Therefore, there is an urgent need for a non-invasive tool that measures tumor-associated PSA expression to more accurately reflect AR activity as well as prostate cancer progression and response to therapy with androgen axis inhibitors such as Abiraterone, MDV3100, TAK700, Aragon 509 and others.
- PCF Young Investigator Dr. David Ulmert, Dr. Hans Lilja and their teams at Lund University, Sweden, Turku University, Finland and Memorial Sloan-Kettering Cancer Center have developed a PET imaging method to detect 'free' PSA in prostate tumors and bone metastases, using 89Zr-5A10, a monoclonal antibody that specifically detects 'free' PSA. 89Zr-5A10 specifically binds to PSA in prostate tumors in soft tissue and in bone metastases.
- This uptake into tumors reflects an AR-regulated increase in PSA protein expression. PET imaging using the 89Zr-5A10 radiotracer in animal models of prostate cancer has allowed detection of prostate cancer throughout the animal as well as monitoring a therapeutic response to an androgen signaling inhibitor.
- Once validated in human patients, 89Zr-5A10-PET imaging holds potential to enable better prostate cancer detection and disease response monitoring.
- This radiotracer employs the antibody 5A10 (developed by Dr. Lilja in collaboration with a team at Turku University, Finland 20 years ago) in conjunction with radioactive 89Zr.



**Figure:** PET/CT image shows that  $^{89}\text{Zr}$ -5A10 localizes to an osseous LNCaP-AR graft located in the left tibia of an intact male mouse. The image is a 3-dimensional volume rendering of PET data overlaid onto surface-rendered CT data. To generate this image, a weighted average of the intensities along projections through the PET is computed and this in turn is blended with the CT-rendered image. The PET data are represented with a semi-quantitative blue (low)–green (high) color scale and show a region of contrast in the animal's left (tumor-bearing) tibia compared with the right (normal) tibia. (Ulmert, D. et al. Cancer Discovery, 2012).

- This  $^{89}\text{Zr}$ -5A10 antibody specifically recognizes and binds PSA on the region of the molecule that is exposed only when it is in the 'free' form, prior to its secretion into blood. Therefore, PET imaging using this radiotracer specifically detects 'free' PSA in tumors and bone metastases (Figure above).
- Dr. Ulmert described the better efficacy of  $^{89}\text{Zr}$ -5A10-PET imaging for prostate cancer detection compared to technetium-99 bone scans or fluoride-PET scans. The latter detect all metabolically active bone lesions, in a non-prostate cancer specific manner, while,  $^{89}\text{Zr}$ -5A10-PET scans are unaffected by metabolic changes in the bone and specifically measure levels of AR signaling in the bone.
- The  $^{89}\text{Zr}$ -5A10 monoclonal antibody radiotracer has currently been evaluated in prostate cancer cell lines and animal models. Dr. Ulmert's studies have demonstrated the specificity of PSA detection of this antibody: PET scans of testosterone-treated mice (to stimulate AR activity) picking up high intensity signals as against the MDV3100-treated mice (who respond to therapy with a concomitant lowering of AR/PSA levels), whose scans picked up very low PET signals (Figure below).
- Dr. Ulmert also presented unpublished data on a radiotracer targeting the kallikrein, hk2 that specifically localized to prostate tumors and bone metastases, and quantifies responses to anti-androgen therapy.





**Figure:** representative transverse (Trans.) and coronal PET slices of intact male mice bearing LNCaP-AR xenografts on the right flank and imaged with  $^{89}\text{Zr}$ -5A10 24 hours post injection after 1. subcutaneous testosterone (Test.), 2. Control (Veh.) and, 3. MDV3100 (80 mg/kg) for 7 days. Clear visual differences in tumor-associated  $^{89}\text{Zr}$ -5A10 can be seen between the groups. Arrows indicate the position of the tumor (T) and the murine liver (L). (Ulmert, D. et al. Cancer Discovery, 2012).

- In summary, the two important aspects of these fPSA- and fhk2-targeting radiotracers are:
  1. This methodology employing  $^{89}\text{Zr}$ -5A10-PET imaging will provide a new imaging system for the detection of prostate cancer, and
  2.  $^{89}\text{Zr}$ -5A10-PET will allow efficient disease and therapeutic monitoring, e.g. it will enable clear identification of disease recurrence (tissues once 'hot' on PET images, i.e. with high PSA expression and therefore prostate cancer become 'cold' upon responding to therapy, and progressively over time become 'hot' again, due to biochemical disease recurrence).
- $^{89}\text{Zr}$ -5A10-PET will also be used to monitor patient response to androgen axis therapy.
- Drs. Ulmert and the teams at Lund University (Sweden), Turku University (Finland) and MSKCC are in the process of humanizing the 5A10 monoclonal antibody as the first step towards producing an agent suitable for PET testing in man.

## Session 11

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### Panel Discussion: The Challenge: Drug Discovery and Development in Academia

**Moderator:** Llew Keltner, MD, PhD (AgonOx, Inc.)

**Panelists:** Jeff Himawan, PhD (Essex Woodlands Health Ventures)  
Ganesh Raj, MD, PhD (University of Texas Southwestern  
Medical Center)  
Alan Auerbach (Puma Biotechnology)

**Problem:** Making the transition for promising prostate cancer drugs from benchtop or early clinical research to commercialization is often much more challenging than doing great science, but there is a lack of good tools to help young scientists make the leap.

**Solution:** The Panel Discussion was intended as a first step in a process of establishing a set of tools, within PCF, available to assist PCF-associated scientists with the transition from lab/early clinic to commercial reality.

The panel members each presented their perspectives on how a young scientist today could best realize commercial success. Major points noted were:

- The very, very long journey ahead and the need to accept the reality of the level of difficulty of drug development and to be prepared for the long haul.
- Need to seek partnership support from large biotech or pharma when a technology is hot, despite the stage of development - and the need to be patient and wait for the next cycle when something is not hot.
- Cash for support of development is available - but the form it takes changes with economic and industry cycles. Venture is slowly returning; novel venture or private equity backed new companies are more popular; pharma-associated venture funds are on the rise; and large pharma is increasingly relying on external development for new candidate drugs. Venture funds are beginning to build - and like building - larger "collections" of related technologies in a single company in order to decrease risk.
- If possible, also develop markers or diagnostics in parallel with drugs. Path to commercialization is far shorter and easier.

Discussion from the floor focused on how scientists can find and use tools and expertise for moving toward commercialization. The relative capabilities and deficits of institutional technology licensing entities were discussed, with a focus on the potential conflict between the needs of a technology licensing entity and the needs of the

scientist. Panel members, responding to a number of questions, strongly advised scientists to stick to their area of expertise, and to try to seek out and surround themselves with people with drug development, business management, and licensing experience.

Many participants expressed strong interest in PCF becoming more active in providing tools. A full seminar on Drug Development Basics was suggested for the next PCF Scientific Retreat, including sessions on Intellectual Property, Licensing, Dealing with Institutional Licensing Entities, Drug Development Realities, Early-Stage Corporate Structures, Financing Alternatives, and other issues related to drug development.

#### Recommendations:

- As noted above, a session at the next Scientific Retreat.
- Consideration of a Commercial Transition Grant Fund sponsored by PCF to provide small, very focused grants to PCF-associated scientists to solve specific transition problems.
- Consideration of an "Entrepreneur-In-Residence" position at PCF for full-time ad hoc assistance, fund raising for a Transition Fund, lobbying with large biotech and pharma for broad support, and coordination with other "mentorship" and transition efforts (Texas state fund; Case Western/Cleveland Clinic development initiative, others).

## Session 12

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### New Precision & Predictive Tests for Lethal Prostate Cancer

**Peter Kuhn, PhD**

**Scripps Physics Oncology Center  
Total Fluid Biopsy in Prostate Cancer**

- Circulating Tumor Cells (CTCs) are tumor cells that detach/slough off of primary tumors, enter the bloodstream and may seed new tumors at distant sites (metastases).
- Identification, enumeration and interrogation of CTCs as predictive biomarkers to drug response hold potential in disease monitoring, and their presence is being investigated as a strong prognostic marker for overall survival in metastatic prostate cancer patients receiving certain medications.

- The only FDA-approved test for CTC enumeration is the CellSearch™ assay from Veridex that uses antibodies against the protein EpCAM expressed on the surface of CTCs. The detection limit for the CellSearch™ CTC assay is one CTC per 7.5 mL of blood.
- At the PCF Scientific Retreat, Dr. Peter Kuhn described the development of a 'fluid biopsy' approach that identifies CTCs without employing cell surface protein-based enrichment.
- This fluid biopsy technique allows CTC identification in cancer patient blood samples in sufficiently high definition (HD) to satisfy diagnostic pathology image quality requirements.
- The fluid biopsy approach defines CTCs as cells that have an intact nucleus (DAPI+), express cytokeratin (tissue origin marker), do not express CD45 (leucocyte origin marker), are morphologically distinct from surrounding leucocytes, and are displayed in diagnostic pathology quality. The image analysis system developed by Dr. Kuhn distinguishes this CTC system from all others.
- This enrichment-free 'HD-CTC' assay detects >5 HD-CTCs per mL of blood in 80% of patients with metastatic prostate cancer (N= 20).
- Among the advantages of this assay, Dr. Kuhn demonstrated its utility for studies on pathology, CTC enumeration, protein and genomic characterization on CTCs.
- Dr. Kuhn and his team have been able to isolate individual CTCs from prostate cancer patients, amplify them, conduct single cell analyses and whole genome sequencing studies, and molecularly image for the presence of various protein biomarkers (PTEN, AR, PSA).
- All experiments from assay development to validation were done with cancer patient blood samples (and not experimental models such as cell lines of mice).
- This research was carried out under the Scripps Research Institute's Physics Oncology Center framework and was licensed to Epic Sciences for commercialization.
- The assay is currently being utilized in 17 clinical studies globally with studies been done on blood samples of 1600 cancer patients.
- In blood samples from prostate cancer patients, Dr. Kuhn and colleagues have studied the androgen receptor, its presence/absence, its sub-cellular localization, ratio of AR-positive to AR-negative cells in a sample etc.
- Dr. Kuhn presented a case study of a 60 year old prostate cancer patient, diagnosed with advanced metastatic prostate cancer with bone involvement. The patient was sequentially prescribed androgen deprivation therapy (Lupron), docetaxel-based chemotherapy, abiraterone and cabazitaxel by Dr. Mitchell Gross of USC.
- Dr. Kuhn's studies on the CTCs of this prostate cancer patient have revealed the evolution of cancer cells during treatment. These studies show that treatment

modalities such as ADT and abiraterone alter the expression of the androgen receptor.

- Detailed investigations revealed that prior to treatment with abiraterone the ratio of AR positive to AR negative cells was approximately equal, upon 3 weeks of treatment all AR positive cells were eliminated from circulation. However, upon 9 weeks of abiraterone treatment all cells were AR positive.
- Isolation and amplification of CTCs from this patient, followed by whole genome sequencing studies revealed all other genomic alterations that led to treatment resistance. The genomic architecture of the cancer cells changed by a great measure upon abiraterone administration, leading to the rise of mutant clones that propelled disease resistance.
- This fluid biopsy technology developed by Dr. Kuhn and colleagues holds potential in both, monitoring and predicting cancer progression, as well as evaluating patient response to therapy.

## **Ryan Dittamore**

### **Ventana Medical Systems, Inc.**

#### **Multiplexed Molecular Characterization of Circulating Tumor Cells- Investigating a Novel Automated Approach**

- As treatment options for prostate cancer patients increase and we move closer to an era of personalized therapy, it is important to guide patient management by optimizing the type and the sequence of future targeted therapies.
- These decisions will be driven by enhanced understanding of the specific molecular characteristics of patients' tumors.
- CTCs provide a window into understanding an individual patient's cancer. Molecular characterization of CTCs holds the potential to provide specifics on the alterations driving tumor burden.
- As described above the only FDA-approved test for CTC enumeration is the CellSearch™ assay from Veridex, a blood test that captures and assesses circulating tumor cells to determine the prognosis of patients with prostate cancer. The caveat with the CellSearch™ assay is that it is a quantitative CTC enumeration platform that does not provide detailed molecular information on the CTCs.
- Mr. Ryan Dittamore described the development of an automated platform amenable to standard clinical use that allows molecular characterization of patient CTCs.
- The technique isolates CTCs from patient blood samples, transfers them to a glass slide, stains these with specific agents to detect molecular alterations,

images them and uses sophisticated software to understand the underlying biology.

- Specifics of assay development: Mr. Dittamore and team isolated CTCs from metastatic treatment resistant prostate cancer patients for enumeration. This assay is adaptable to three independent methods of CTC isolation: the Veridex CellSearch™ system, the Epic Science CTC enumeration system described above (Dr. Peter Kuhn) and the CTC isolation system developed at Memorial Sloan Kettering Cancer Center.
- Following enumeration, these cells were transferred to glass slides and interrogated by employing automated staining techniques to probe for proteins that play critical roles in prostate cancer, such as ERG, PTEN etc.
- This assay employs the techniques of *multiplexed* immunofluorescence and in situ hybridization that allow high resolution imaging and detection of several disease biomarkers on the same glass slide.
- This automated CTC characterization assay will shortly be available for clinical validation experiments.
- The results from these studies support the hypothesis that understanding the biological characteristics of CTCs has both prognostic and predictive implications in improve risk prediction, monitoring disease response, and tailoring treatment strategies for patients with solid tumors.

## **Leileata Russo, PhD**

### **Exosome Diagnostics, Inc**

#### **The Use of Urinary Exosomes/Microvesicles for the Non-Invasive Diagnosis of Prostate Cancer**

- Exosomes are 30-90 nm vesicles secreted by many mammalian cells. These vesicles carry components of the parent cell they are derived from, such as high integrity DNA, RNA and proteins.
- Tumor cells also release exosomes into the blood and/or urine and these exosomes are beginning to be exploited for non-invasive diagnostic purposes as they carry cancer-associated biomarkers.
- Exosomes have been identified in the urine samples of prostate cancer patients and the release of exosomes into urine may be enhanced during cancer.
- Due to their unique stability (exosomes are stable at 4°C and -80°C) and the fact that they contain prostate-specific RNA, Exosome Diagnostics, is developing a urine-based molecular diagnostic test for use in personalized and non-invasive prostate cancer diagnoses.



- Studies by Dr. Russo and her team have shown that high quality RNA can be reproducibly extracted from urine exosomes of prostate cancer patients.
- This ease of sample accrual from a random urine sample (i.e. one that does not require a prostate massage), combined with Exosome Diagnostics' technique for the preparation of high purity RNA, allows for rapid biomarker analyses.
- Dr. Russo's studies show that RNA biomarkers expressed in the urine exosomes demonstrate a high level of concordance (81%) with the biomarkers expressed in prostate tumor tissue, suggesting that this exosome signature is a true representation of tumor biology.
- Dr. Russo and her team have developed this technology into an 'exosome biofluid RNA isolation kit' and plan to take it to the FDA for approval.
- This technology was evaluated in two multi-center clinical pilot studies (14 academic and community sites) across the United States.
- The first study compared random urine samples from 146 patients pre- and post-biopsy, and led to the identification of a four-gene signature (*ERG*, *AMACR*, *LR3*, *LR4*) that can help predict the presence of cancer.
- The second 112 patient study that compared pathology analysis from radical prostatectomy specimens with prostate cancer patient urine exosomes and suggested that biomarkers that allow identifying patients with clinically significant, aggressive disease from those with latent disease could be identified.
- Dr. Russo's studies identified the gene *ICAM1* as a potential biomarker to distinguish patients with Gleason grade  $\leq 7$  from the patients who had more aggressive, clinically significant disease with a Gleason grade  $\geq 8$ .
- Dr. Russo and colleagues are currently working on developing an algorithm using these gene signatures that can help in the identification of patients likely to present with a biopsy positive outcome. They will add aggressive markers to this algorithm which will help detect not only the presence of cancer, but also differentiate aggressive from indolent disease.
- In summary, Dr. Russo described a biofluid (urine)-based, non-invasive diagnostic test that can reliably determine the presence and nature of a prostate malignancy. This urine-based diagnostic test can mimic the results of other RNA-based tests, without the need for a digital rectal exam.

## **David Olmos, MD, PhD**

### **Spanish National Cancer Research Centre (CNIO)**

#### **Blood mRNA Expression Signatures to Stratify Castration Resistant Prostate Cancer Patients and Predict Poor Outcome**

- Prostate cancer is a complex heterogeneous disease with patients presenting a spectrum of symptoms varying from indolent to aggressive. Research into these disease subtypes still lags behind for want of suitable biomarkers that can help patient stratification.
- In order to over-diagnose and over-treat less, there is an urgent need to identify reliable biomarkers to dissect this heterogeneity of prostate cancer between patients to improve targeted treatment, distinguish lethal from indolent disease and accelerate drug development.
- Dr. David Olmos, his mentor, Dr. Johann DeBono and their team analyzed whole blood samples of prostate cancer patients to derive clinically relevant genetic signatures that allow risk stratification of patients and predict overall survival rates in patients with treatment-resistant prostate cancer.
- In the first stage of their studies, the team compared blood samples from 69 patients with castration-resistant prostate cancer (test set) with blood samples from 31 patients with prostate cancer selected for active surveillance (control set). All these patients were recruited from The Royal Marsden Hospital NHS Foundation Trust (Sutton, UK) and The Beatson West of Scotland Cancer Centre (Glasgow, UK).
- Based on their varying genetic patterns derived from statistical modeling experiments, patients on the test set were sub-divided into four groups. A 2.5 year follow-up revealed the sub-group that presented with the worst survival rate (10.7 months compared to an average of 25.6 months for the other three sub-groups).
- These studies led to the identification of a nine-gene signature that can stratify patients with treatment-resistant prostate cancer into distinct prognostic groups, as well as identify the ones with the worst clinical outcome.
- Most of the genes in this signature play critical roles in regulating the immune response to cancer.
- This nine-gene signature was validated in a treatment-resistant prostate cancer patient cohort (N=70) recruited from the Memorial Sloan-Kettering Cancer Center.
- It could accurately predict the subset of patients with aggressive disease and shorter overall survival, 9.2 months compared with 21.6 months for patients not expressing the nine-gene pattern.

- This blood test holds the potential to eventually for use alongside the existing PSA test, at diagnosis, to select patients with most aggressive disease that require immediate therapy.
- If validated in additional independent patient cohorts, this nine-gene signature can potentially provide important prognostic information to guide treatment and patient stratification for clinical trials of new therapeutics for treatment-resistant disease.

## Special Lecture: Transitioning from Drug to Therapy Development of Prostate Cancer

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**Chris Logothetis, MD**

**The University of Texas MD Anderson Cancer Center**

**Funded by a PCF Challenge Award**

How the central role the environment a tumor resides in can determine how aggressive a tumor may become and what tumor microenvironment factors sustain or refrain any tumor's growth.

The milestone that points to the development of non-lethal prostate to lethal prostate cancer is the transition from the endocrine to the paracrine state of signaling.

How the time-dependent heterogeneity of prostate cancer needs to be better understood and can ultimately be exploited to huge advantage.

The microenvironment-dependent phase of a tumor, which accounts for many of the clinical factors that drive this disease and determine response to therapy, needs to be better understood for the development of predictive markers.

Dr. Logothetis then said that for the 30 years he's been in cancer research the conceptual framework of cancer progression was that one cancer cells becomes two, four become eight and so on. An increase in cancer cell numbers coincided with an increase in heterogeneity and thus increasing resistance to therapy.

Thus, many years ago, we thought chemo would be the answer, he said. The thinking was: using early chemo with hormonal therapy to treat prostate cancer. But this butted up against the reality that earlier chemotherapy does not prolong survival in most prostate cancers.

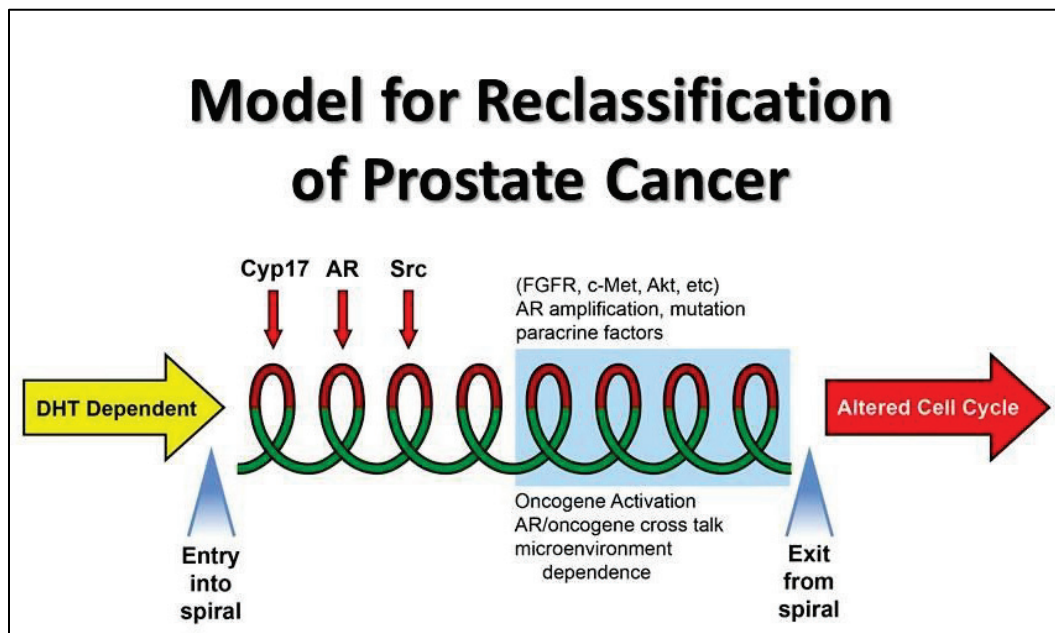
Dr. Logothetis then presented a slide titled "Model for Reclassification of Prostate Cancer." He said that there is much current data to support the model he presented which is: prostate cancer is unique in that in its development there is a period of time when tumors must depend upon prostate and bone development pathways for survival.

Slide credited to Dr. Nora Navone, et al, showing xenografts taken directly from patient tumors and placed under the skin of mice produce entire bone tissue mass. That bone tissue mass made in non-bony sites in mice is composed of both murine bone and human bone. In order to survive in a non-bone (hostile) environment this cancer graft has had to attract murine components of bone subcutaneously in this hostile territory to bone development.

This and other data has led us to the present updated model of prostate cancer progression: Many of the initial cancer cells are unable to engage the hostile microenvironment, and that inability probably distinguishes cancer cells with lethal potential from those without lethality in their blueprints. And that there's a subsequent phase unique to prostate cancer that involves a specific interaction with a specific microenvironment which accounts for dependency on AR signaling and dependence on the interaction with bone that accounts for cancer bone homing that dominates the initial clinical presentation of lethal prostate cancers.

Data now shows that you can target bone metastases and prolong survival.

This places bone at the center of treatment targeting.



Dr. Logothetis discussed the hypothesis of a new model of prostate cancer progression in scientific detail, talking about:

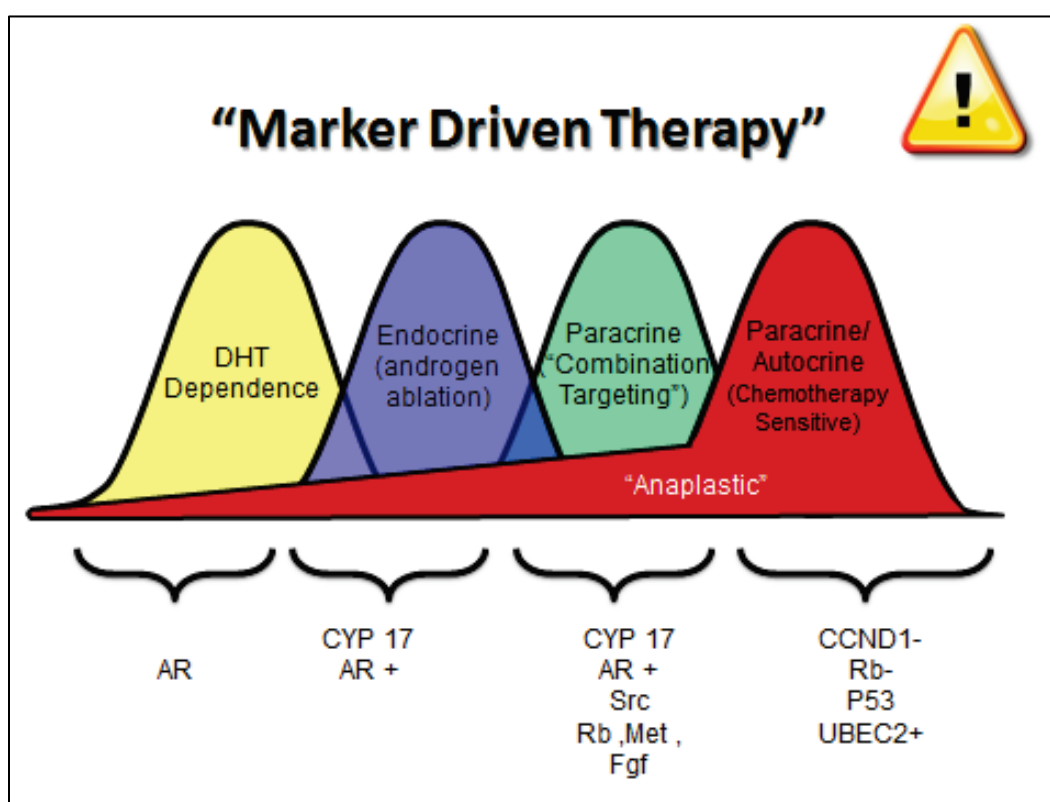
- The primary DHT dependent phase of the disease
- Disease must pass through all steps in this model
- A transition from endocrine to paracrine phase
- Under androgen ablation, Cyp17 is initially up-regulated, followed by alterations in AR, followed by more complex signaling pathways in this iterative progression spiral, depicted in the image above.

So instead of a single vicious cycle, it is a series of vicious cycles of disease progression. Every turn of spiral, is where researchers should look for predictive biomarkers. As cancer exits from the spiral (depicted above) these tumors represent the typical lethal prostate cancers that doctors see.

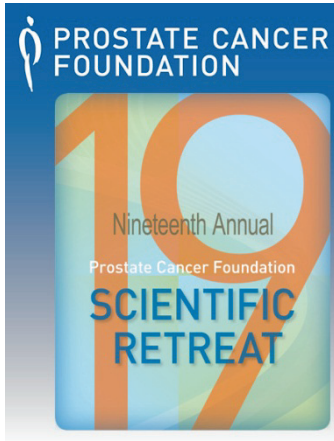
Dr. Logothetis then stated that they are looking at which patients respond best to abiraterone acetate and which to MDV3100 and what is the response when those drugs are given in

combination. But the ultimate goal is to banish the large randomized clinical trials and replace them with smaller trials, targeted via biomarker prediction tied to disease state /phase to patients most likely to benefit from a particular intervention at a particular time in the course of prostate cancer disease progression. He said they now think they have biomarkers for when patients enter the DHT dependent phase of prostate cancer progression and for when patients become endocrine dependent as well as other stages of cancer progression. He said evidence suggests that some patients may, in fact, benefit from early chemotherapy administration, based on their biomarker status for these phases of cancer progression.

Dr. Logothetis's final slide—"Marker Driven Therapy" dovetailed with his new model of the development of prostate cancer, showing what biomarkers are likely best as a predictive marker at each phase of the new progression model. This of course, opens the door to targeting treatments to the specific time in a tumor's lifecycle it is most vulnerable to a specific therapy or combination of therapies.



**What this means for patients:** By carefully dissecting each phase and type of prostate cancer and determining at what point in time in each tumor's lifecycle it is most vulnerable to a specific therapy or therapeutic agent, and understanding what biomarkers are present that show clinicians that point in time has or is about to occur, Dr. Logothetis's presentation further set the stage for a systemic and unique approach to overcoming lethal prostate cancers.



# AGENDA

Thursday, October 25, 2012

**12:00PM – 1:30PM**

**SPECIAL WORKSHOP**

**Invitation Only**

**Becoming an Author in a Nature Publication and Other 21<sup>st</sup> Century Tips  
on Publication in the Digital Age**

*Presented by Anna Saar, Publishing Manager for Academic Journals  
Nature Publishing Group, New York, NY*

*Location: Costa Del Sol A*

**GENERAL SESSIONS: Costa Del Sol Ballroom**

**Welcome and Introduction**

**2:00PM -2:10PM**

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

**2:10PM – 2:30PM**

**SPECIAL LECTURE**

**Tumor Heterogeneity: Clonality and Consequences**

**Charles Swanton, MD, PhD**

Cancer Research UK, London Research Institute & University College London Hospitals & Cancer  
Institute

*Introduction by Howard Soule, PhD*

Prostate Cancer Foundation

**2:30PM – 2:35PM Discussion**

**Session 1: Field Cancerization & the Tumor Microenvironment**

**2:35PM – 3:35PM**

**Moderators:** Leland Chung, PhD

Cedars-Sinai Medical Center

Yusuke Shiozawa, MD, PhD

University of Michigan



**Thursday, October 25, 2012**

2:35PM – 2:50PM	<b>Contributions of the Tumor Microenvironment to Chemoresistance</b> Mikala Egeblad, PhD Cold Spring Harbor Laboratory
2:50PM – 2:55PM	Discussion
2:55PM – 3:10PM	<b>Paracrine Contributors of Prostate Cancer Progression: A Response to Carcinogenesis</b> Neil Bhowmick, PhD Cedars-Sinai Medical Center
3:10PM – 3:15PM	Discussion
3:15PM – 3:30PM	<b>Premetastatic Niche Involving RANKL-RANK Signaling Contributes to Cancer Bone Metastasis</b> Leland Chung, PhD Cedars-Sinai Medical Center
3:30PM – 3:35PM	Discussion

**3:35PM – 3:50PM**

***SPECIAL LECTURE***

**Cancer Interception & Metabolic Transformation**

**Richard Mithen, PhD**

Institute for Food Research, Norwich Research Park

*Introduction by Jonathan Simons, MD*

Prostate Cancer Foundation

3:50PM – 3:55PM Discussion

**Session 2: Role of Growth Factor and Signal Transduction Alterations in Prostate Cancer Initiation, Progression and Therapy Resistance**

**3:55PM – 5:15PM**

**Moderators:** Michael Ittmann, MD, PhD  
Baylor College of Medicine

Brett Carver, MD  
Memorial Sloan-Kettering Cancer Center

3:55PM – 4:10PM	<b>Targeting Fibroblast Growth Factor Receptor Signaling Inhibits Prostate Cancer Progression</b> Michael Ittmann, MD, PhD Baylor College of Medicine
4:10PM – 4:15PM	Discussion

**Thursday, October 25, 2012**

4:15PM – 4:30PM	<b>Targeting FGF Signaling in Prostate Cancer Bone Metastases</b>  Nora Navone, MD, PhD The University of Texas MD Anderson Cancer Center
4:30PM – 4:35PM	Discussion
4:35PM – 4:50PM	<b>Tyrosine Kinase Signaling in Prostate Cancer</b> Owen Witte, MD University of California, Los Angeles
4:50PM – 4:55PM	Discussion
4:55PM – 5:10PM	<b>Damage Induced Growth Factor Responses in the Tumor Microenvironment Influence Therapy Resistance</b> Pete Nelson, MD Fred Hutchinson Cancer Research Center
5:10PM – 5:15PM	Discussion

**Session 3: Advances in Molecular Imaging of Prostate Cancer**

**5:15PM – 6:15PM**

**Moderators:**

Glenn Liu, MD  
University of Wisconsin Carbone Cancer Center

John Kurhanewicz, PhD  
University of California, San Francisco

5:15PM – 5:30PM	<b>Hyperpolarized MR Molecular Imaging of Prostate Cancer - From Cells to Man</b> John Kurhanewicz, PhD University of California, San Francisco
5:30PM – 5:35PM	Discussion
5:35PM – 5:50PM	<b>Imaging of Prostate Cancer Biomarkers by PET</b> Jason Lewis, PhD Memorial Sloan-Kettering Cancer Center
5:50PM – 5:55PM	Discussion
5:55PM – 6:10PM	<b>Phospholipid Analogs as Broad Spectrum Diagnostics</b> Jamey Weichert, PhD University of Wisconsin
6:10PM – 6:15PM	Discussion

**Thursday, October 25, 2012**

**PCF Presidential Nanospeech 2012**

**6:15PM – 6:30PM**

Jonathan Simons, MD

*Introduction by Howard Soule, PhD*

Prostate Cancer Foundation

**6:30PM – 6:40PM**

**BREAKTHROUGH LECTURE**

**Organoids: A Profound New Prostate Cancer Model System**

**Charles Sawyers, MD**

Memorial Sloan-Kettering Cancer Center

*Introduction by Jonathan Simons, MD*

Prostate Cancer Foundation

6:40PM – 6:50PM Discussion

**PCF Stand Up To Cancer Dream Team Overviews**

**6:50PM – 7:00PM**

**Arul Chinnaiyan, MD, PhD**

University of Michigan

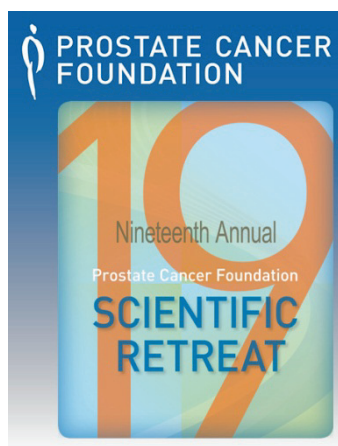
**7:00PM – 7:10PM**

**Eric Small, MD**

University of California, San Francisco

*Introductions by Jonathan Simons, MD*

Prostate Cancer Foundation



# AGENDA

**Friday, October 26, 2012**

## **GENERAL SESSIONS: Costa Del Sol Ballroom**

7:30AM – 7:40AM

**Exceptional Progress Report – PCF Young Investigator Integrated Molecular Analysis of Circulating Tumor Cells: The Microfluidic VeriFAST Platform**

Joshua Lang, MD

University of Wisconsin Carbone Cancer Center

7:40AM – 7:45AM

Discussion

### **Session 4: Clinical Trial Advances in Metastatic Castration-Resistant Prostate Cancer**

**7:45AM – 8:25AM**

#### **Moderators:**

Howard Scher, MD

Memorial Sloan-Kettering Cancer Center

Ana Aparicio, MD

University of Texas MD Anderson Cancer Center

7:45AM – 8:00AM

**Randomized Phase II Trial of the Hsp27 Inhibitor, OGX-427 Plus Prednisone vs. Prednisone Alone in Patients with Metastatic Castrate Resistant Prostate Cancer**

Martin Gleave, MD

University of British Columbia

8:00AM – 8:05AM

Discussion

8:05AM – 8:20AM

**ARN-509, A Next Generation Anti-Androgen: From Discovery to Clinical Development**

Richard Heyman, PhD

Aragon Pharmaceuticals

8:20AM – 8:25AM

Discussion

**Friday, October 26, 2012**

**Session 5: Messenger RNA Translation in Cancer**

**8:25AM – 10:05AM**

**Moderators:**

Davide Ruggero, PhD  
University of California, San Francisco

Andrew Hsieh, MD  
University of California, San Francisco

8:25AM – 8:40AM

**Decoding the Translational Landscape of the Prostate Cancer Genome: Functional Insights and Therapeutic Implications**

Davide Ruggero, PhD  
University of California, San Francisco

8:40AM – 8:45AM

Discussion

8:45AM – 9:00AM

**eIF4E and Cancer**

Nahum Sonenberg, PhD  
McGill University

9:00AM – 9:05AM

Discussion

9:05AM – 9:20AM

**Targeting the eIF4F Complex in Prostate Cancer**

Jeremy Graff, PhD  
Eli Lilly and Company

9:20AM – 9:25AM

Discussion

9:25AM – 9:40AM

**Regulation of Growth by the mTOR Pathway**

David Sabatini, MD, PhD  
Whitehead Institute

9:40AM – 9:45AM

Discussion

9:45AM – 10:00AM

**Chemical Genetic Analysis of Oncogenic Signaling Cascades**

Kevan Shokat, PhD  
University of California, San Francisco

10:00AM – 10:05AM

Discussion

**Session 6: Novel Therapeutic Approaches to Cure Advanced Prostate Cancer**

**10:05AM – 11:25AM**

**Moderators:** Bruce Zetter, PhD  
Harvard Medical School

Richard Lee, MD, PhD  
Massachusetts General Hospital Cancer Center, Harvard Medical School

**Friday, October 26, 2012**

10:05AM – 10:20AM	<b>New Class of Therapies Targeting Lethal Prostate Cancer</b> Bruce Zetter, PhD Harvard Medical School
10:20AM – 10:25AM	Discussion
10:25AM – 10:40AM	<b>Advancing Therapies Against the uPA Axis in Advanced Prostate Cancer</b> Andrew Mazar, PhD Northwestern University
10:40AM – 10:45AM	Discussion
10:45AM – 11:00AM	<b>Peptoids on Steroids: Targeting AR-dependent Prostate Cancer with Multivalent Ethisterone Conjugates</b> Kent Kirshenbaum, PhD New York University Michael Garabedian, PhD New York University
11:00AM – 11:05AM	Discussion
11:05AM – 11:20AM	<b>A Novel AR Inhibitor: Targeting AR Conformational Change and Venturing into the Wnt Pathway</b> Marc Diamond, MD Washington University in St. Louis
11:20AM – 11:25AM	Discussion

**11:25AM – 11:50AM**

**SPECIAL LECTURE**

**Genetics of Prostate Cancer – Two Decades of Progress**

**Patrick Walsh, MD**

Johns Hopkins Medicine

*Introduction by Donald S. Coffey, PhD*

John Hopkins Medicine

11:50AM – 11:55AM Discussion

**12:00PM – 1:00PM**

**KEYNOTE ADDRESS**

**Mike Milken**

Prostate Cancer Foundation

*Introduction by Stuart Holden, MD*

Prostate Cancer Foundation Medical Director & Cedars-Sinai Medical Center

**Friday, October 26, 2012**

**Session 7: Epithelial Plasticity in Prostate Cancer: An Emerging Mechanism of Treatment Failure?**

**2:30PM – 3:50PM**

**Moderators: Andrew Armstrong, MD, PhD**  
Duke University

**Amina Zoubeidi, PhD**  
The Vancouver Prostate Centre

2:30PM – 2:45PM

**EMT and Stem Cells in Cancer Progression**

Sendurai Mani, PhD  
The University of Texas MD Anderson Cancer Center

2:45PM – 2:50PM

Discussion

2:50PM – 3:05PM

**The Essential Role of Snail in EMT and Prostate Cancer Metastasis**

Hong Wu, MD, PhD  
University of California, Los Angeles

3:05PM – 3:10PM

Discussion

3:10PM – 3:25PM

**The Amoeboid Phenotype: Implications for Prostate Cancer Metastasis**

Michael Freeman, PhD  
Cedars-Sinai Medical Center

3:25PM – 3:30PM

Discussion

3:30PM – 3:45PM

**Translation of EMT/MET Biology to Human Prostate Cancer**

Andrew Armstrong, MD  
Duke University

3:45PM – 3:50PM

Discussion

**3:50PM – 4:05PM**

**SPECIAL LECTURE**

**EZH2 Oncogenic Activity in CRPC is Polycomb Independent**

**Myles Brown, MD**

Dana-Farber Cancer Institute

*Introduction by Philip Kantoff, MD*

Dana-Farber Cancer Institute

4:05PM – 4:10PM Discussion



**Friday, October 26, 2012**

**Session 8: Mechanisms of Primary & Acquired Resistance to Androgen Axis Inhibitors**

**4:10PM – 5:30PM**

**Moderators:**

Pete Nelson, MD  
Fred Hutchinson Cancer Research Center

Steve Balk, MD, PhD  
Beth Israel Deaconess Medical Center

4:10PM – 4:25PM

**Response and Resistance to Targeting Steroid Hormone Metabolism**

Elahe Mostaghel, MD, PhD  
Fred Hutchinson Cancer Research Center

4:25PM – 4:30PM

Discussion

4:30PM – 4:45PM

**Androgen Receptor Functions in Advanced Castration-Resistant Prostate Cancer**

Steve Balk, MD, PhD  
Beth Israel Deaconess Medical Center

4:45PM – 4:50PM

Discussion

4:50PM – 5:05PM

**Persistent Androgen Receptor Activation in MDV3100 Resistant Prostate Cancer**

Amina Zoubeydi, PhD  
The Vancouver Prostate Centre

5:05PM – 5:10PM

Discussion

5:10PM – 5:25PM

**AR Gene Rearrangements and Splicing Variants: Implications for AR-Targeted Therapy**

Scott Dehm, PhD  
Masonic Cancer Center, University of Minnesota

5:25PM – 5:30PM

Discussion

**5:30PM – 5:55PM**

**SPECIAL LECTURE**

**Treatments in Development in the Post-Abiraterone Setting**

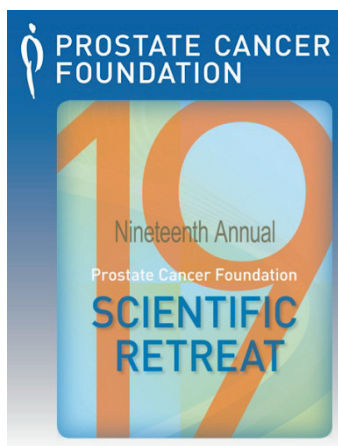
**Gerhardt Attard, MD, PhD**

The Institute of Cancer Research & Royal Marsden Hospital

*Introduction by Mary-Ellen Taplin, MD*

Dana-Farber Cancer Institute

5:55PM – 6:00PM Discussion



# AGENDA

**Saturday, October 27, 2012**

## **GENERAL SESSIONS: Costa Del Sol Ballroom**

7:30AM – 7:40AM

**Exceptional Progress Report – PCF Young Investigator:  
Identification of Diverse Modes of Androgen Action:  
Potential for Novel Forms of Androgen Deprivation Therapy?**

Hannelore Heemers, PhD  
Roswell Park Cancer Institute

7:40AM – 7:45AM

Discussion

### **Session 9: The Good, the Bad and the Ugly of Preclinical and Observational Research**

**7:45AM – 8:25AM**

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

7:45AM – 8:00AM

**Raising the Bar for Preclinical Cancer Research**

C. Glenn Begley, PhD  
TetraLogic

8:00AM – 8:05AM

Discussion

8:05AM – 8:20AM

**Voodoo Statistics and Trust Me Science**

S. Stanley Young, PhD  
National Institute of Statistical Sciences

8:20AM – 8:25AM

Discussion

### **Session 10: Immunotherapy of Prostate Cancer: 2012 Update**

**8:25AM – 10:00AM**

**Moderator:** Charles Drake, MD, PhD  
Johns Hopkins Medicine

## Saturday, October 27, 2012

8:25AM – 8:40AM	<b>Immune Checkpoint Blockade to Treat Cancer: 2012 and Beyond?</b> Charles Drake, MD, PhD Johns Hopkins Medicine
8:40AM – 8:45AM	Discussion
8:45AM – 9:00AM	<b>OX40 Agonists and Prostate Cancer Immunotherapy</b> William Redmond, PhD Providence Portland Medical Center
9:00AM – 9:05AM	Discussion
9:05AM – 9:20AM	<b>Tumor Antigen-Specific Regulatory T Cells to Predict and Monitor Vaccine Efficacy</b> Brian Olson, PhD University of Wisconsin Carbone Cancer Center
9:20AM – 9:25AM	Discussion
9:25AM – 9:40AM	<b>Preclinical Development of a Prostate Cancer Vaccine Targeting the Transcription Factor ERG</b> Haydn Kissick, PhD Beth Israel Deaconess Medical Center
9:40AM – 9:45AM	Discussion
9:45AM – 9:55AM	<b>Exceptional Progress Report – PCF Young Investigator: Development of Radio-Tracers Targeting Prostate Specific Kallikreins for In Vivo Detection of Disseminated Disease and Measurement of Androgen Signaling</b> David Ulmert, MD, PhD Memorial Sloan-Kettering Cancer Center
9:55AM – 10:00AM	Discussion

**Saturday, October 27, 2012**

**10:00AM – 10:45AM**

**PANEL DISCUSSION – Session #11**

**The Challenge: Drug Discovery and Development in Academia**

**Moderator: Llew Keltner, MD, PhD**

AgonOx, Inc.

**Panelists:**

**Jeff Himawan, PhD**

Essex Woodlands Health Ventures

**Charles Sawyers, MD**

Memorial Sloan-Kettering Cancer Center

**Alan Auerbach**

Puma Biotechnology

**Session 12: New Precision & Predictive Tests for Lethal Prostate Cancer**

**10:45AM – 12:20PM**

**Moderators:** Howard Soule, PhD

Prostate Cancer Foundation

Michael Haffner, MD

John Hopkins Medicine

10:45AM – 11:00AM

**Total Fluid Biopsy in Prostate Cancer**

Peter Kuhn, PhD

Scripps Physics Oncology Center

11:00AM – 11:05AM

Discussion

11:05AM – 11:20AM

**Multiplexed Molecular Characterization of Circulating Tumor Cells-Investigating a Novel Automated Approach**

Ryan Dittamore

Ventana Medical Systems, Inc.

11:20AM – 11:25AM

Discussion

11:25AM – 11:40AM

**Validation of the Genomic Prostate Score, a Standardized, Biopsy-Based Genomic Assay, for Pre-Operative Risk Assessment**

Peter Carroll, MD

University of California, San Francisco

11:40AM – 11:45AM

Discussion

**Saturday, October 27, 2012**

11:45AM – 12:00PM	<b>The Use of Urinary Exosomes/Microvesicles for the Non-Invasive Diagnosis of Prostate Cancer</b> Leileata Russo, PhD Exosome Diagnostics, Inc.
12:00PM – 12:05PM	Discussion
12:05PM – 12:15PM	<b>Blood mRNA Expression Signatures to Stratify Castration-Resistant Prostate Cancer Patients and Predict Poor Outcome</b> David Olmos, MD, PhD Spanish National Cancer Research Centre (CNIO)
12:15PM – 12:20PM	Discussion

**12:20PM – 12:50PM**

**SPECIAL LECTURE**

**Transitioning from Drug to Therapy Development of Prostate Cancer**

**Chris Logothetis, MD**

The University of Texas MD Anderson Cancer Center

*Introduction by Jonathan Simons, MD*

Prostate Cancer Foundation

12:50PM – 12:55PM Discussion

**\*\*\* Meeting Adjourned \*\*\***

## PCF-funding to Retreat Presenters

Name, Degree(s)	Most Recent Award	Past Award Years Funding
Charles Swanton, MD, PhD	2012 Challenge Award	
Neil Bhowmick, PhD	2012 Challenge Award	
Leland Chung, PhD	2011 Challenge Award	1993, 1994, 1996, 1997, 1998, 1999
Richard Mithen, PhD	2012 Challenge Award	
Michael Ittmann, MD, PhD	2012 Donor Directed Award: Golfers Against Cancer	2009, 2010, 2011
Nora Navone, MD, PhD	2010 Creativity Award	1996, 1998, 1999, 2000, 2001, 2003, 2004
Owen Witte, MD	2012 Stand Up 2 Cancer - Dream Team 2 2011 Creativity Award	1995, 1996, 1997, 1998, 1999, 2000, 2001, 2003, 2004, 2005, 2006, 2008
Peter Nelson, MD	2011 Creativity Award 2011 Challenge Award	1999, 2000, 2001, 2003, 2005, 2008, 2010
John Kurhanewicz, PhD	1995 Competitive Award	
Charles Sawyers, MD	2012 Stand Up 2 Cancer - Dream Team 1	1996, 1997, 1998, 1999, 2000, 2001, 2003
Arul Chinnaiyan, MD, PhD	2012 Stand Up 2 Cancer - Dream Team 1	2001, 2005, 2006, 2007, 2008, 2009
Eric Small, MD	2012 Stand Up 2 Cancer - Dream Team 2	1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011
Joshua Lang, MD	2010 Young Investigator Award	
Martin Gleave, MD	2012 Stand Up 2 Cancer - Dream Team 2	2003, 2006, 2007, 2009, 2010
Nahum Sonenberg, PhD	2001 Competitive Award	
Bruce Zetter, PhD	2011 Creativity Award	1994
Marc Diamond, MD	2006 Competitive Award	2003, 2005
Patrick Walsh, MD	2002 Donor Directed Award	1994, 1997, 1998, 1999, 2000
Hong Wu, MD, PhD	2008 Challenge Award	1999, 2000, 2003
Andrew Armstrong, MD, PhD	2008 Young Investigator Award	
Myles Brown, MD	2011 Challenge Award	1995, 1996
Elahe Mostaghel, MD, PhD	2006 Career Development Award	
Steven Balk, MD, PhD	2011 Challenge Award	2008
Amina Zoubeidi, PhD	2010 Young Investigator Award	
Scott Dehm, PhD	2008 Young Investigator Award	
Gerhardt Attard, PhD	2008 Young Investigator Award	
Hannelore Heemers, PhD	2010 Young Investigator Award	
Charles Drake, MD, PhD	2011 Challenge Award	2010
William Redmond, PhD	2008 Young Investigator Award	
David Ulmert, MD, PhD	2012 Young Investigator Award	
Peter Carroll, MD	2008 Donor Directed Award	1996, 1997, 1998, 1999, 2001, 2002, 2003, 2004, 2005, 2006, 2007
Christopher Logothetis, MD	2011 Challenge Award	1993, 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010

The table above identifies the speakers who are or have been funded by the Prostate Cancer Foundation.

