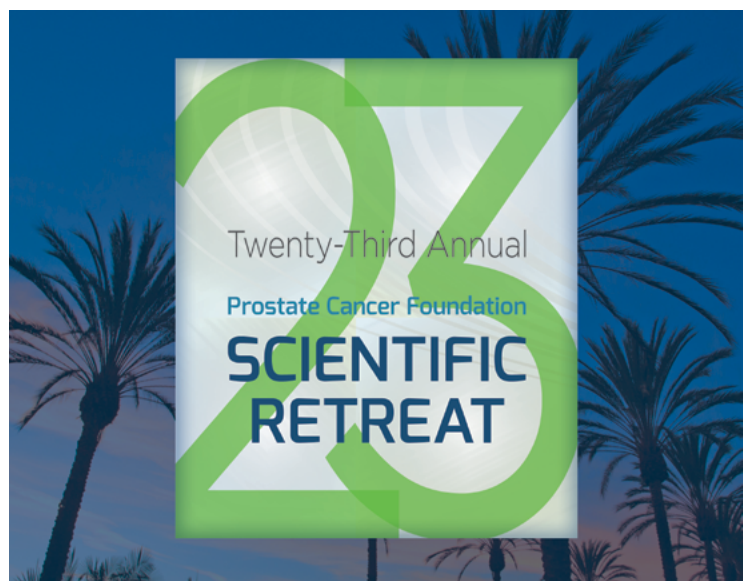


STATE OF THE SCIENCE REPORT

Highlights from
the 23rd Annual PCF
Scientific Retreat
October 2016



Provided with the compliments of
the Prostate Cancer Foundation



Prostate Cancer
Foundation
Curing Together.

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Introduction

The 23rd Annual Prostate Cancer Foundation (PCF) Scientific Retreat was held from October 27-29, 2016, at the La Costa Resort in Carlsbad, CA. The PCF Retreat started as an intimate gathering of PCF-funded researchers sharing their recent scientific progress. As the “PCF family” grew over the years, so did the Retreat, which is now the penultimate prostate cancer global knowledge exchange event in the world. Attendees hail from around the globe and include the world’s leaders in medicine, science, government, and business. The goal of the Retreat is to bring attention to the most exciting and impactful scientific advances of the year, in the hope that they may ultimately contribute to life-extending therapies for prostate cancer patients. These goals are met and built upon year after year, as attendance at the retreat continues to inspire new ideas and the formation of new collaborations.

The 23rd Annual PCF Scientific Retreat featured the following:

- 46 presentations in the Plenary Session plus a Precision Clinicopathologic Conference panel discussion
- 153 poster presentations
- 23 different scientific disciplines related to prostate cancer biology presented and discussed
- 65% of speakers presented first-in-field, unpublished data at a PCF Scientific Retreat for the first time
- Attendance by 539 participants from 16 countries, including 224 PhDs, 167 MDs, 90 MD PhDs, 5 PharmDs, 1 DMD, 1 DO, and 1 RN
- 102 academic institutions, 47 biopharmaceutical companies, 9 medical research foundations, and 7 other for-profit companies
- NIH, NCI, and Dept. of Defense research leaders
- Attendance by 146 PCF Young Investigators
- Attendance by 17 PCF Board of Director members and major donors, and 24 special guests

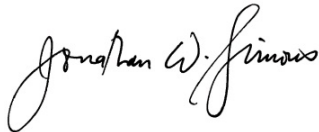
The PCF “Global Research Enterprise” now extends to 19 countries and funds a robust research portfolio. Since 1993, PCF has awarded \$435 million in innovative prostate cancer research projects, led by an estimated 1,100 prostate cancer researchers. This includes \$43.2

million awarded to 204 PCF Young Investigators since 2007 and nearly \$159 million to PCF Challenge Award teams since 2008.

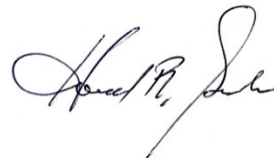
We thank the sponsors of the Retreat for their generous support: Sanofi Oncology, Astellas/Medivation, Bayer, Janssen, Clovis Oncology, Dendreon, Genentech, GenomeDx, and Bavarian Nordic.

The Prostate Cancer Foundation has prepared the 2016 State of Science Report, which summarizes each of the presentations given at the Retreat in order to disseminate this knowledge throughout the scientific community and the public. With this, we hope to further accelerate the development of new ideas, data exchange, and discourse, as well as stimulate support for scientific research. Please contact Dr. Andrea Miyahira at amiyahira@pcf.org if you have any specific questions.

Yours sincerely,



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



Howard R. Soule, PhD
Executive Vice President
Chief Science Officer



Session 1: New Treatments in Prostate Cancer

Biomarker-Driven Contexts of Use for a TROP2 Antibody-Drug Conjugate in Prostate Cancer

Joshua Lang, MD

University of Wisconsin Carbone Cancer Center

- Despite the recent development of several new therapeutics for the treatment of prostate cancer, cures remain elusive for men with advanced disease. New treatments and therapeutic strategies are necessary for extending and improving the lives of men with advanced prostate cancer.
- Dr. Joshua Lang is studying TROP-2 as a potential therapeutic target and biomarker in prostate cancer.
- TROP-2 is a protein expressed on the surface of epithelial cancer cells including those in prostate cancer. TROP-2 was previously found to mark prostate cancer cells that have the greatest proliferative potential and may identify a stem cell of origin for prostate cancer.
- IMMU-132 is a TROP-2-specific antibody that is conjugated to the topoisomerase-inhibiting drug SN-38, using a pH-sensitive linker. The antibody complex is internalized into cells that express TROP-2, and SN-38 is released to kill the cell.
- IMMU-132 was well-tolerated and demonstrated promising efficacy in a phase I/II clinical trial in solid tumors. The most common grade 3+ adverse events included neutropenia, anemia, diarrhea, leukopenia, and febrile neutropenia.
- In 58 patients with triple negative breast cancer who had received a median of 5 prior therapies, treatment with IMMU-132 resulted in 2 complete responses and 18 partial responses. 23 patients achieved stable disease, while 15 had progressive disease. Based on these data, IMMU-132 received FDA breakthrough status for triple negative breast cancer and registrational trials are ongoing.
- Dr. Lang is exploring whether IMMU-132 is active in the treatment of advanced prostate cancer.
- The expression of TROP-2 was assessed in prostate cancer tissue and circulating tumor cells (CTCs). TROP-2 was highly expressed in both primary and metastatic prostate cancer.
- In the Mayo Clinic PROMOTE trial, the expression of TROP-2 was assessed in metastatic tumor biopsies prior to treatment with abiraterone and 12 weeks post-abiraterone. Abiraterone treatment did not alter the expression of TROP-2, indicating that TROP-2 expression was persistent throughout the course of treatment.
- AR-V7 is an androgen receptor (AR) variant implicated in resistance to AR-targeted therapies such as abiraterone. AR-V7 was found to be expressed at high frequency in TROP-2-expressing prostate tumor cells, suggesting that TROP-2 may be a good therapeutic target in AR-V7-positive prostate cancer.

- VERSA is a micro-scale technology developed by Dr. Lang and colleagues to capture CTCs and assess phenotypic signatures, genomic alterations, and gene expression patterns in these rare cells.
- To determine the utility of TROP-2 as a prognostic or predictive biomarker in castrate resistant prostate cancer (CRPC), a VERSA assay was developed to capture and characterize TROP-2-expressing CTCs from CRPC patient blood specimens.
- Cells captured by the TROP-2 CTC VERSA assay were found to express the androgen receptor (AR), confirming these cells are of prostate origin (Figure).
- CTCs have been classically identified using the epithelial cell surface protein EpCAM. The phenotypes of CTCs isolated based on TROP-2 vs. EpCAM expression were concordant in 60% of prostate cancer patients analyzed.
- TROP-2 CTCs were found in ~73% of CRPC patients in the initial cohort. TROP-2 CTCs expressed markers of various prostate cancer phenotypes including AR and AR-variants, NKX3.1, and the neuroendocrine prostate cancer marker synaptophysin.
- Overall, these studies indicate that TROP-2 may be a relevant target for the treatment of prostate cancer at various disease stages including pre-chemotherapy or post-abiraterone/enzalutamide. Whether or not targeting TROP-2 may be beneficial in earlier settings such as in newly diagnosed patients, is also a question of interest.
- Clinical trials to examine the efficacy of targeting TROP-2 with IMMU-132 in prostate cancer are being planned.

Trop2 CTCs and the Androgen Receptor

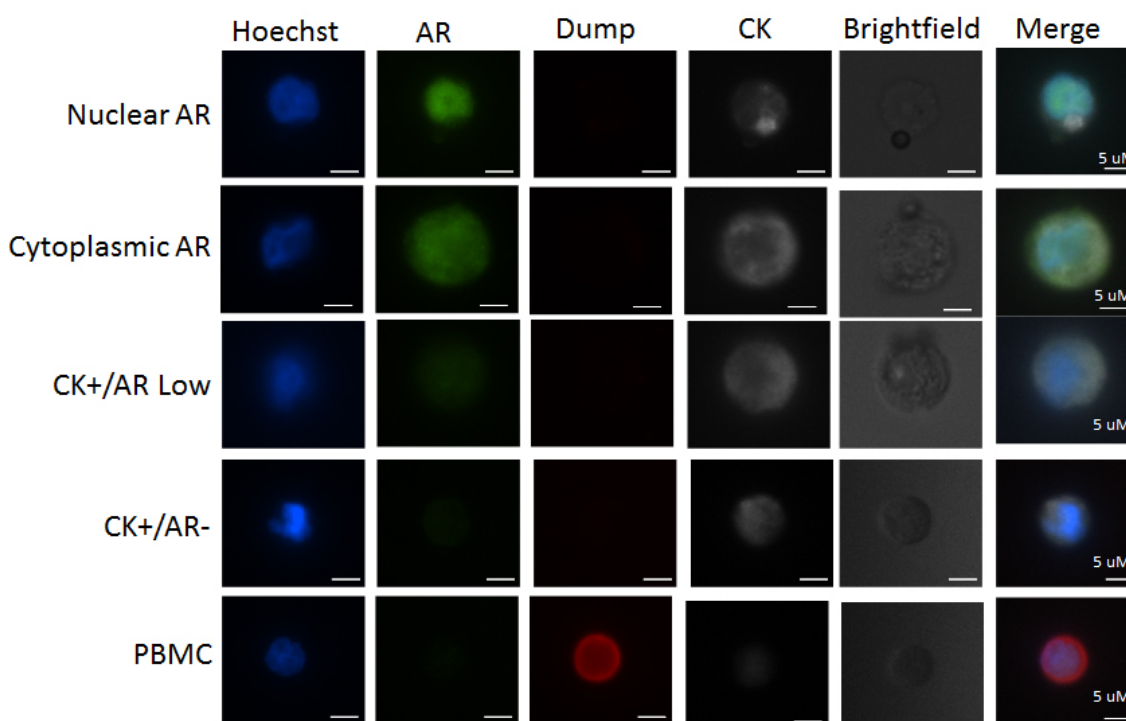


Figure: Cells captured by the TROP-2 CTC VERSA assay express various levels of prostate tumor markers androgen receptor (AR) and/or EpCAM (CK) but not blood cell markers ("dump"). Hoechst is a stain for DNA to identify all captured cells.

Understanding PREX2 and PTEN in Cancer

Ramon Parsons, MD, PhD

Icahn School of Medicine at Mount Sinai

- The tumor suppressor gene PTEN is commonly deleted in many cancers including prostate cancer. PTEN is a phosphatase which acts to deactivate the tumor-promoting PI3K pathway. Inactivation of PTEN unleashes the PI3K pathway, which results in altered cellular metabolism, allowing cells to become malignant.
- Dr. Ramon Parsons discussed the mechanisms and implications of interactions between PTEN and PREX2, a key protein in the PI3K pathway.
- PREX2 was found to interact with and inhibit the activity of PTEN.
- The expression of PREX2 is increased in cancer cells compared with normal cells (Figure).
- PREX2 is also frequently mutated or amplified in several cancer types, including in 25% of metastatic prostate cancers.

- PREX2 was found to promote PI3K pathway activity and the transformation of normal cells into cancer cells.
- PTEN reciprocally suppresses PREX2 activity. However, some tumor cells express mutated versions of PREX2 that are resistant to regulation by PTEN.
- The mechanism of PREX2 regulation was examined. PREX2 was found to be inactivated by PAK1, an enzyme in the PI3K-RAC pathway, in response to PI3K activating signals such as alterations in concentrations of cellular second messengers.
- These studies identify interactions and mechanisms of cross-regulation between the tumor suppressor gene PTEN and the tumor-promoting PI3K pathway.
- This knowledge will be used to develop therapeutic strategies that interfere with the ability of PREX2 to suppress PTEN and enhance PTEN activity for the treatment of prostate cancer.

Increased Expression of P-REX2 in Cancer vs. Normal (Oncomine)

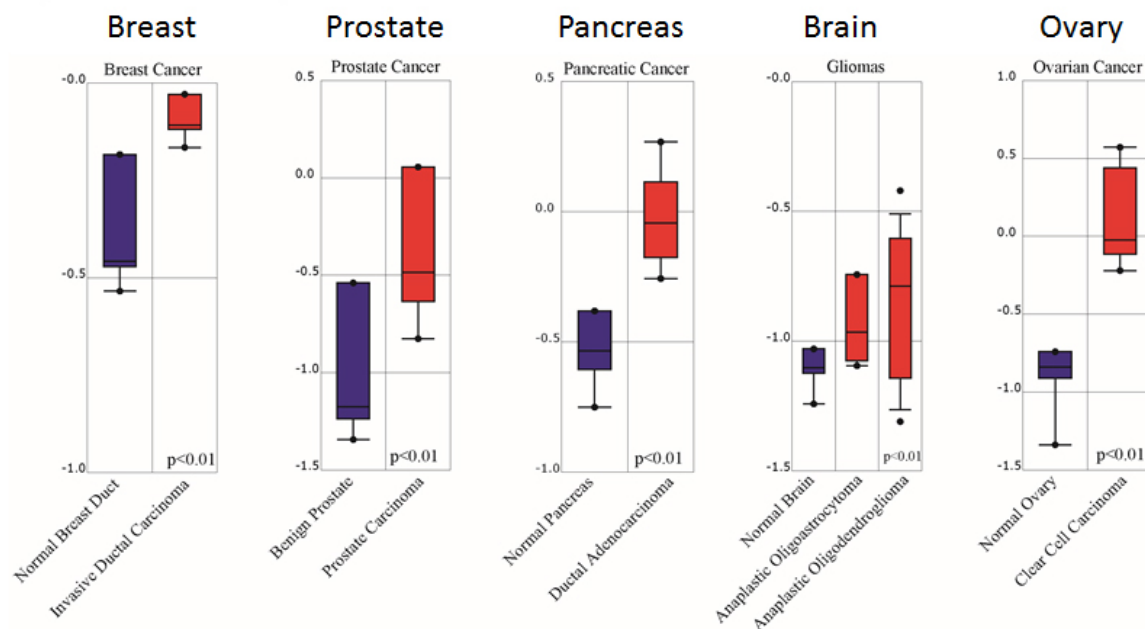


Figure: The expression of PREX2 is increased in cancer cells including prostate cancer, compared with normal cells. Source: Fine et al., *Science*, 2009

IRE1 Signaling Drives Prostate Cancer

Fahri Saatcioglu, PhD

University of Oslo

- The endoplasmic reticulum (ER) is an organelle with many functions within the cell, including protein production and processing, synthesis of lipids and organelle membranes, and storage of calcium ions. The ER also monitors the intracellular environment for any changes and alters its activities in reaction to cellular stresses such as glucose deprivation, low oxygen, or altered protein levels.
- The “unfolded protein response” (UPR) is a highly integrated stress response regulated by the ER that is associated with a number of pathologies including cancer.
- The UPR is activated by sensors on the ER that detect aberrant levels of unfolded or misfolded proteins, which can result from cell stress, and which could cause cellular crises if left unchecked.
- The UPR has both tumor-inhibitory and tumor-promoting properties, and can enable cell survival under unfavorable conditions or initiate cell death pathways.
- IRE1 is an unfolded protein sensor embedded in the ER membrane that activates the UPR and regulates ER functions.
- Dr. Fahri Saatcioglu discussed the role of IRE1 and the UPR in prostate cancer, and the therapeutic potential for targeting IRE1 for prostate cancer treatment.
- Whether the androgen receptor (AR), the primary driver of prostate cancer, can activate the UPR was explored. AR expression correlated with expression of genes regulated by the UPR in prostate cancer specimens.
- The expression of IRE1 as well as target genes of this pathway were enhanced by androgens through direct binding of AR to gene regulatory sites. Expression of IRE1 increased throughout prostate cancer progression.
- Whether IRE1 and the UPR act to promote or suppress prostate cancer was examined.
- Knockdown of IRE1 expression in prostate cancer cells resulted in reduced tumor cell growth in cell culture assays and preclinical prostate cancer animal models. This indicates that increased IRE1 and UPR activity in prostate cancer has a tumor-promoting function and not a tumor-suppressing response to aberrant cell growth.
- Toyocamycin is an antibiotic derivative that inhibits IRE1. Treatment of mice bearing human prostate tumors with toyocamycin significantly reduced tumor growth (Figure).
- Other clinical-grade IRE1 inhibitors are being explored for the treatment of prostate cancer.
- These results suggest that IRE1 may be a promising therapeutic target for prostate cancer and warrants further studies.

A small molecule inhibitor of IRE1 inhibits prostate cancer growth in vivo

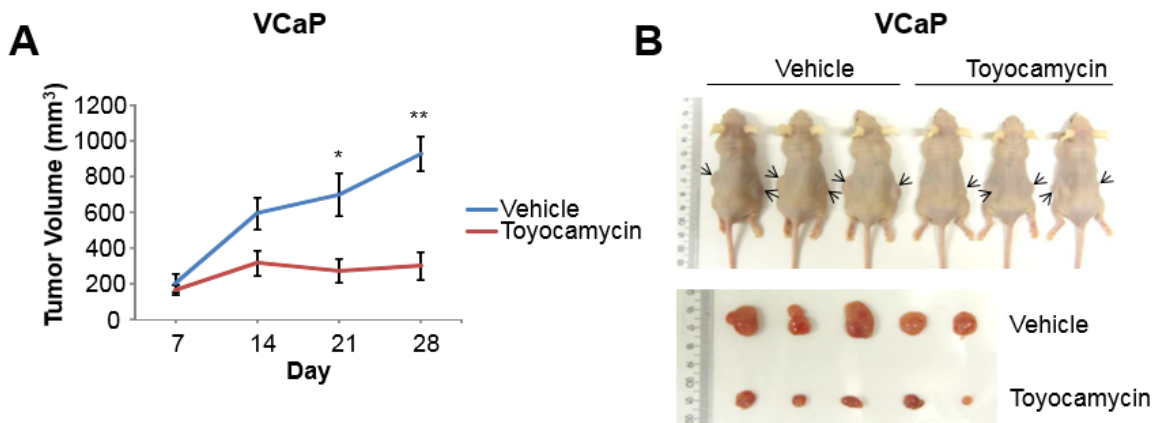


Figure: Treatment of mice bearing human prostate-tumors with the IRE1-inhibitor toyocamycin significantly reduced tumor growth, indicating that IRE1 may be a promising therapeutic target for prostate cancer. Sheng et al., *EMBO Mol Med*, 2015.

ROR- γ Nuclear Receptor as a New Therapeutic Target in Advanced CRPC

Hongwu Chen, PhD

University of California, Davis

- The androgen receptor (AR) is the primary driver of prostate cancer and is also the primary therapeutic target for treatment of advanced disease.
- AR belongs to a family of 48 proteins called nuclear hormone receptors. These receptors sense hormones or other molecules and initiate cellular responses by directly binding to DNA and activating gene expression programs.
- AR senses androgens and responds by activating the expression of genes involved in growth and survival of prostate cancer. Whether other nuclear hormone receptors may be good targets for the treatment of prostate cancer is important to understand.
- Dr. Hongwu Chen discussed studies on targeting ROR- γ , a member of the “orphan” subfamily of nuclear hormone receptors for which the activating signals have not yet been identified.
- ROR proteins have been implicated in various cellular functions and disorders including cancer, inflammatory diseases, and metabolic diseases.

- ROR- γ (also called ROR-C) was found to be highly expressed in prostate cancer, with highest expression in metastatic tumors. Knockdown of ROR- γ expression in prostate cancer cells reduced cell growth.
- The ROR- γ gene was amplified in tumors from a subset of patients with metastatic castrate resistant prostate cancer (mCRPC) ranging from 6% to 36% in different patient cohorts.
- A special algorithm was used to probe the 3D structure of ROR- γ and identify novel small molecule inhibitors.
- Several ROR- γ -inhibitors were tested and found to potently inhibit the growth and survival of prostate cancer cells. ROR- γ -inhibitors were effective against prostate cancer cells that depend on AR for growth, but were ineffective against AR-negative prostate cancer cells. Knockdown of ROR- γ expression in prostate cancer cells produced similar results.
- Treatment of prostate cancer cells with ROR- γ -inhibitors resulted in the suppression of classic AR-driven genes, resembling the effects of treatment with the AR-antagonist, enzalutamide.
- ROR- γ -inhibition was found to prevent the ability of AR to bind to DNA.
- These data suggest that ROR- γ -inhibition suppresses the activity of AR, and prompted studies into the underlying mechanisms.
- ROR- γ -inhibition was found to inhibit the expression of full-length AR and shorter variants of the AR protein. This indicates that ROR- γ promotes the expression of AR.
- ROR- γ was found to bind directly to a sequence within the AR gene and activate AR expression. Knockout of this AR gene sequence reduced AR expression and eliminated sensitivity of AR expression to ROR- γ -inhibitors.
- Treatment of prostate tumor-bearing mice with ROR- γ -inhibitors potently blocked tumor growth and activated cell death mechanisms (Figure).
- ROR- γ -inhibitors also blocked the growth of enzalutamide-resistant prostate tumors and were synergistic with enzalutamide.
- No overt toxicities were observed in mice treated with ROR- γ -inhibitors with the exception of a reduction of white adipose tissue. This is consistent with studies indicating a role for ROR- γ in lipid metabolism and adipose tissue growth. No effects were seen on other androgen-sensitive organs, including the testes and prostate.
- Ongoing work to develop orally bioavailable ROR- γ inhibitors that are more potent, have increased selectivity for ROR- γ , and have better pharmacodynamic profiles are in progress.
- Biomarkers that inform ROR- γ activity and that can be used to select patients most likely to respond to ROR- γ -inhibitors are also being discovered.
- These studies have identified ROR- γ as a critical regulator of AR and as a highly promising novel therapeutic target for the treatment of prostate cancer.

ROR γ antagonists effectively inhibit tumor growth and AR signaling in xenograft models

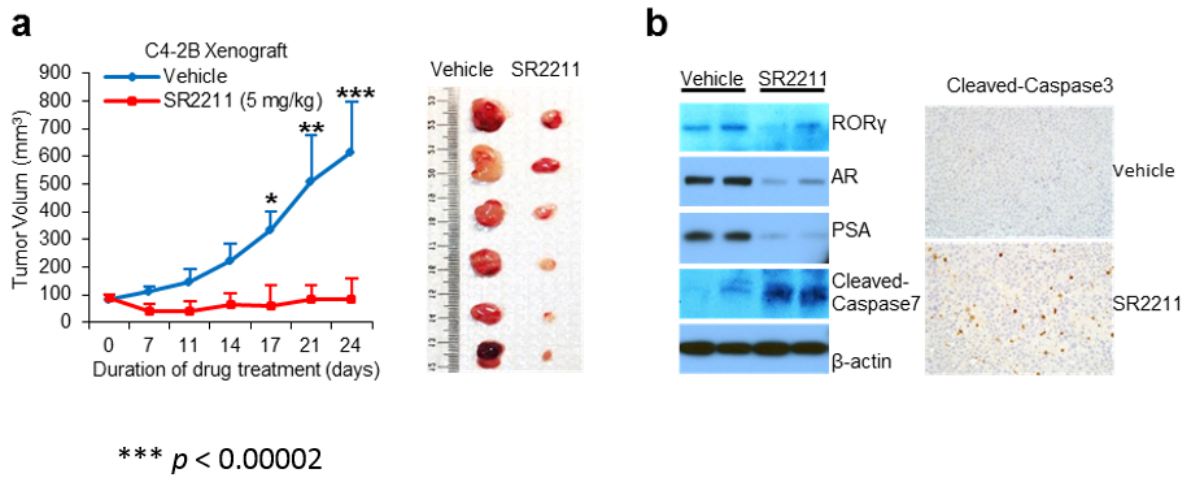


Figure: Treatment of prostate tumor-bearing mice with an ROR- γ -inhibitor (SR2211) strongly blocked tumor growth (a), and turned on cell death mechanisms (cleaved-caspase 3/7) (b).

Session 2: Prostate Cancer Research in the Netherlands

Prostate Cancer Grading Beyond Gleason Score: 3D, Molecular and Clinical Perspective

Arno van Leenders, MD, PhD

Erasmus Medical Centre, The Netherlands

- The Gleason grading system has been used by pathologists to stratify the aggressiveness of primary prostate cancer since 1966.
- Gleason grading is based on tumor growth patterns and is a subjective method that partially accounts for tumor heterogeneity.
- However, it can be difficult to appropriately classify some tumors using only Gleason grading, which can lead to suboptimal patient staging and treatment.
- Dr. Arno van Leenders discussed strategies beyond Gleason grading that can be used to improve patient staging.
- Some tumors can have a “cribriform” morphology, in which tumor cells grow in a sieve-like pattern that resembles Swiss cheese.
- The presence of cribriform morphology in prostate tumors at radical prostatectomy and in diagnostic biopsies was found to associate with more aggressive tumors and significantly reduced metastasis free survival. This could be observed in all of the Gleason grade groups.
- When cribriform status was incorporated into Gleason grading, the absolute grade lost independent predictive value for patient outcome. This finding suggests that the predictive value of Gleason grading depends on the inclusion of cribriform growth status.
- To understand why cribriform growth has such a strong impact on patient outcome, prostate tumors were subjected to whole genome sequencing and were reclassified according to cribriform growth status. Genomic alterations associated with cribriform growth were identified.
- Genomic alterations associated with aggressive prostate cancer behavior were more frequent in tumors with cribriform growth. This suggests that cribriform growth is an indicator of genetic instability.
- The relationship between various prostate tumor growth patterns and patient outcomes was assessed using new microscopy techniques that allow tissues to be visualized in 3D as opposed to 2D (Figure).
- “Ill-formed” Gleason 4 tumors were found to exhibit small, branching, interconnected glands. Ill-formed glands were smaller and highly branched in comparison to the more glandular morphology of Gleason grade 3 tumors.
- In cribriform patterns, the glands were still branched and interconnected, but were further distorted and resembled a Swiss cheese-like pattern.

- Overall, these studies suggest that architectural growth patterns beyond Gleason grading, cribriform patterns especially, should be taken into consideration when grading prostate tumors. This will lead to improved treatment planning and potentially improved outcomes for patients.

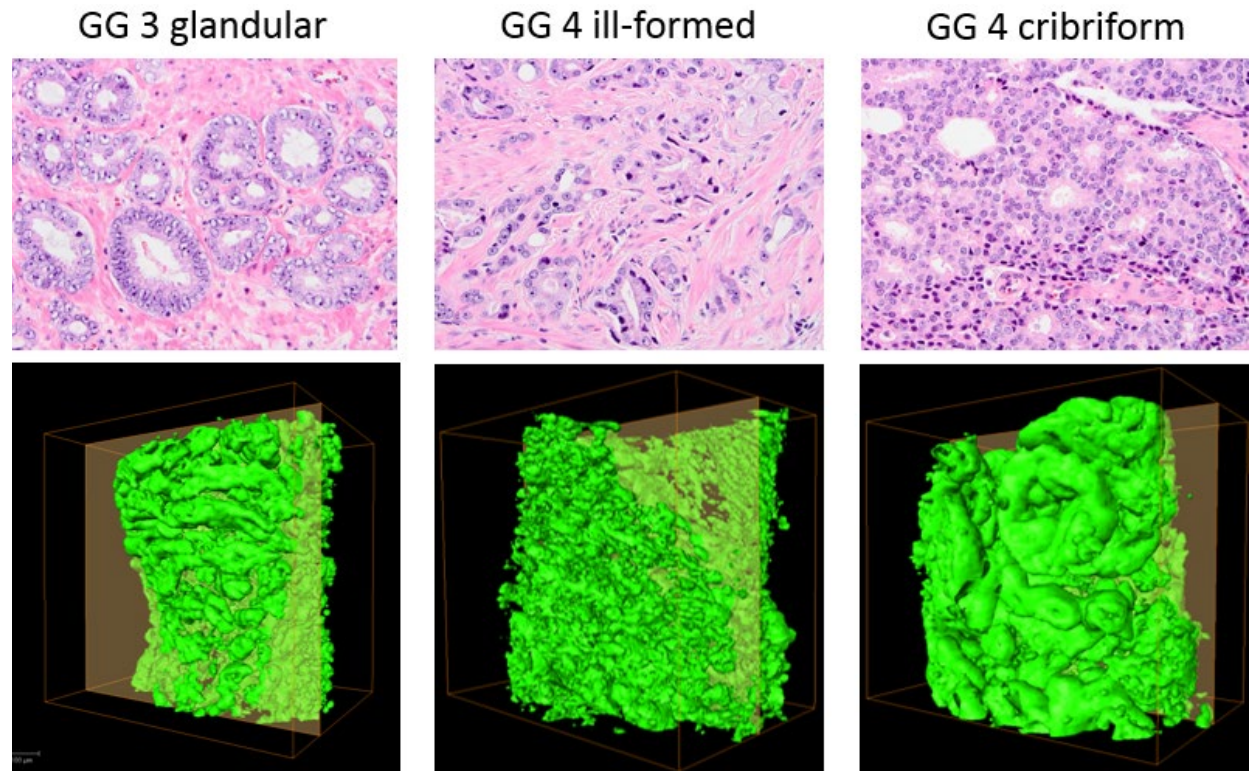


Figure: 2D and 3D images of Gleason grade 3 glandular, Gleason grade 4 ill-formed, and Gleason grade 4 cribriform tumors.

Session 3: Radium for CRPC

Radium-223: Is Bone Targeted Therapy Meeting its Full Potential?

Michael Morris, MD

Memorial Sloan Kettering Cancer Center

- Radium-223 is a radioactive element that behaves like calcium, which is incorporated into bone, and is FDA-approved for the treatment of bone-only metastatic prostate cancer.
- FDA approval of Radium-223 was based on the phase III Alsympca trial that demonstrated a 3.6 month improvement in median overall survival and reduced skeletal-related events.
- Many questions regarding the optimal use of Radium-223 remain. These include identifying the optimal dose and duration of therapy, identifying serum and imaging biomarkers that select patients who will most likely benefit from treatment, examining sequencing and combinations with other therapies, determining the optimal time in the disease history to administer Radium-223, and understanding the biology of how Radium-223 exerts its effects.
- Dr. Michael Morris discussed these questions, and efforts to improve the use of Radium-223 in the clinic.
- On September 8, 2016, the Prostate Cancer Foundation hosted a Scientific Working Group Meeting on Radium-223 to discuss issues surrounding the optimal clinical use of Radium-223 and defining the biology of response.
- The major topics discussed at the meeting were: biology of bone and investigations on the mechanism of action of Radium-223, adjuvant Radium-223 for immunotherapy, molecular imaging to understand Radium-223 therapy, and clinical investigations to extend the utility of Radium-223 therapy.
- Radium-223 works by being incorporated into sites of active bone growth (typically sites of tumor growth) in place of calcium and releasing radioactive alpha particles that damage and kill nearby cells. Alpha particles only penetrate tissues a few cells deep, minimizing damage to surrounding tissues and thereby minimizing side effects. However, alpha particles likely do not penetrate deeply into the tumor mass. Radium-223 cannot target all tumor cells, even in patients with bone-only disease. In addition, patients with bone-only metastases have ample numbers of circulating tumor cells, which are likely not affected by Radium-223.
- The original FDA approval of Radium-223 required two additional hypothesis-driven prospective clinical trials to address safety issues.
- A three-arm extended dosing trial is underway to test safety and efficacy of either increasing the dose or the duration of Radium-223 therapy in patients with bone metastatic castrate resistant prostate cancer (mCPRC).
- Two ongoing phase III prostate cancer trials are testing the efficacy of combining Radium-223 with abiraterone/prednisone (ERA223 trial) or enzalutamide (EORTC PEACE-III trial).

- The potential benefit of combining Radium-223 with docetaxel chemotherapy is being examined in an ongoing phase II clinical trial in mCRPC (Figure). Because of concerns about side effects, the dose of docetaxel was reduced in the docetaxel plus Radium-223 combination arm compared with the docetaxel alone arm. This resulted in reduced side effects for the combination, including reduced anemia, neutropenia, and leukopenia relative to docetaxel alone. Declines in PSA and in alkaline phosphatase, a measure of adverse bone activity, were greater in the combination arm. Time to biochemical relapse was also more favorable with Radium-223 plus docetaxel compared to docetaxel alone. This suggests that combining docetaxel with Radium-223 may improve the treatment of prostate cancer.
- Based on these data, two phase III trials testing the combination of docetaxel and Radium-223 are planned.
- One trial, to be conducted by the Prostate Cancer Clinical Trials Consortium (PCCTC), will examine the same regimen of docetaxel alone versus a lower dose of docetaxel in combination with Radium-223 in patients with mCRPC.
- A second trial, to be conducted by the Alliance and NRG Oncology groups, has been proposed in order to test the efficacy of docetaxel plus Radium-223 in castrate-sensitive metastatic disease. This is in response to the CHAARTED trial, which demonstrated increased efficacy for docetaxel when given to patients with castration-sensitive prostate cancer at the time of initiation of androgen deprivation therapy (ADT).
- Other novel radionuclide therapies are being tested in preclinical and clinical studies. Actinium-225 and Lutetium-177 molecularly targeted to prostate-specific membrane antigen (PSMA) are encouraging treatments for patients with very advanced prostate cancer but neither has yet been tested in randomized clinical trials.
- Ongoing and planned phase III clinical trials will address many of the unresolved questions surrounding the optimal use of radium-223, including duration of therapy, optimal dose, patterns of relapse, combinations with AR-targeted therapy and chemotherapy, and efficacy in castrate-sensitive and castrate-resistant metastatic prostate cancer. The efficacy of combining Radium-223 with other therapies such as immunotherapies, chemotherapy and PARP-inhibitors are also being explored.

Time to PSA Progression

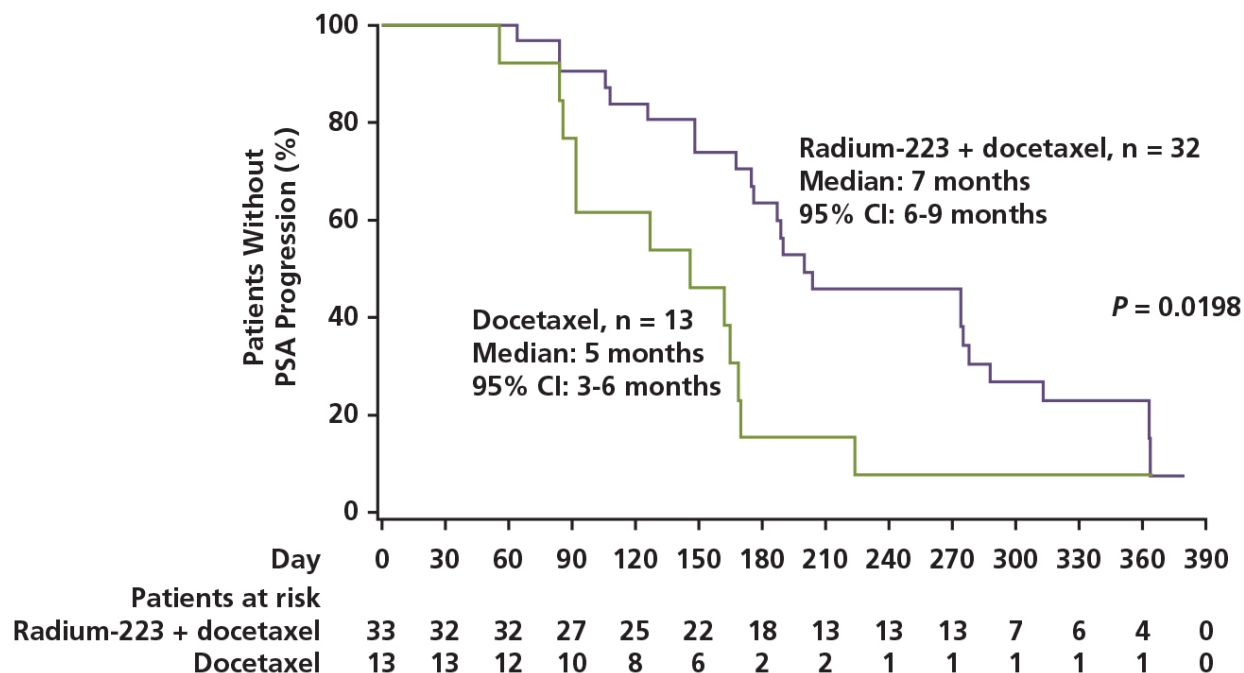


Figure: In an ongoing phase II clinical trial, time to PSA progression was more favorable with docetaxel plus Radium-223 compared with docetaxel alone.

Sept 8th, 2016 - PCF Scientific Working Group on Radium 223 @ MSKCC



Attendees at the Prostate Cancer Foundation Scientific Working Group Meeting on Radium-223, held on September 8, 2016 at Memorial Sloan Kettering Cancer Center.

Radiation Induced Immunogenic Modulation: Insights for Combination Therapy

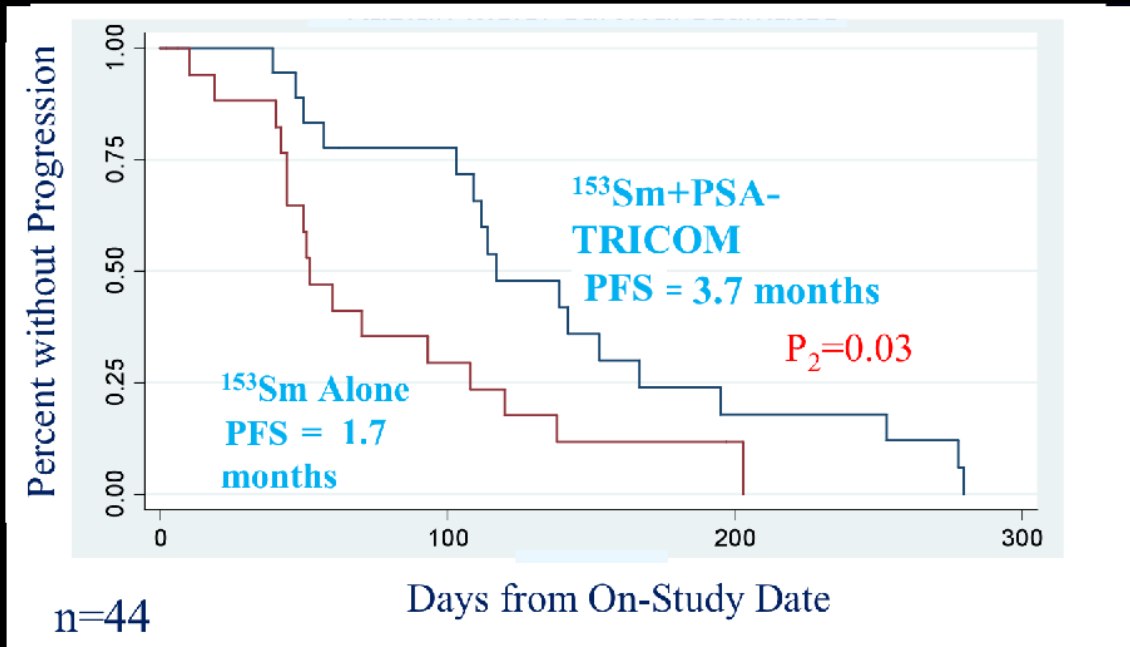
James Gulley, MD, PhD
National Cancer Institute

- The immune response can be activated during the death of tumor cells. The immune system can perceive tumor cell death as a threat and activate tumor-killing immune responses that can have a therapeutic benefit. This process is known as immunogenic cell death.
- Radiation therapy can induce immunogenic cell death and may enhance the effects of immunotherapy. Trials testing combinations of radiation therapies and immunotherapies for cancer treatment are warranted.
- Dr. James Gulley discussed preclinical and clinical studies testing the combination of radiation therapeutics with immunotherapies.
- High doses of radiation can lead to immunogenic tumor cell death. However, lower doses of radiation that do not overtly kill tumor cells can also activate anti-tumor immune responses by modulating the expression of proteins that allow immune cells to recognize and target tumor cells (immunogenic modulation).
- Sm-153-EDTMP (Quadramet) is a bone-targeting radiopharmaceutical therapy that is FDA approved for the treatment of bone metastases in multiple tumor types.
- Treatment of prostate cancer cells with palliative levels of Sm-153 upregulated the expression of genes that promote immune recognition and targeting, including MHC-I, Fas, ICAM-1, and tumor associated antigens. Tumor cells treated with Sm-153 had enhanced sensitivity to immune cell killing. These studies indicate that Sm-153 treatment may potentiate the effects of immunotherapy.
- A phase II clinical trial was conducted to test the combination of Sm-153 with a prostate cancer vaccine (PSA-TRICOM) that activates immune cells to target and kill cells that express the prostate tumor associated protein, PSA (Figure).
- The combination extended progression-free survival by 2 months over Sm-153 alone and a higher number of patients that received the combination therapy experienced PSA declines compared with patients who received SM-153 alone. This suggests that combining immunotherapy with a radiopharmaceutical may result in clinical benefit.
- Immune responses to PSA were observed in 25% of patients who received Sm-153 alone, indicating that Sm-153 can induce an anti-tumor immune response. However, 60% of patients who received Sm-153 + PSA-TRICOM exhibited anti-PSA immune responses
- Various immune cell parameters were examined in patients prior to therapy in order to identify a predictive biomarker for patients who might benefit from therapy.
- Several parameters associated with immune function and immune cell maturation were associated with survival. Immune biomarkers were consolidated into an “immunoscore” that could be used to stratify patients that did versus did not benefit from the therapeutic combination.

- Radium-223 is a bone-targeting radiopharmaceutical agent that has been approved by the FDA for the treatment of prostate cancer patients with bone-only metastases. As a calcium mimetic, Radium-223 is incorporated into bone in regions of regeneration which is often adjacent to tumor. Dr. Gulley is interested in whether Radium-223 might facilitate an immune response in bone.
- Preclinical studies were conducted to examine the potential therapeutic benefit of combining Radium-223 with immunotherapy.
- In cell culture studies, Radium-223 slowed the growth of prostate cancer cells, though cells largely remained alive. However, the addition of anti-tumor t-cells to Radium-223-treated prostate cancer cells led to significantly enhanced tumor cell death.
- Lower doses of Radium-223 that do not elicit cell death were found to induce cell stress responses in tumor cells. This included upregulation of proteins involved in immune cell targeting of tumor cells such as calreticulin.
- Blocking calreticulin abrogated immune cell killing of tumor cells, indicating that calreticulin acts to promote anti-tumor immunity and may be a useful predictive biomarker of response to immunotherapy.
- Treatment of tumor-bearing mice with radiation therapy upregulated the expression of negative regulatory immune checkpoint molecules PD-1 and PD-L1 on tumor cells. The combination of anti-PD-L1 immunotherapy with radiation therapy exhibited synergy in preventing tumor growth. Clinical trials to test this combination are being planned.
- Overall, these studies suggest that radiation can induce anti-tumor immune responses by inducing immunogenic cell death or by modulating tumor cells to enhance recognition and killing by immune cells. Radiation therapies are candidate therapeutic partners for various types of immunotherapies including tumor vaccines and checkpoint inhibitors.
- Clinical trials continue to test whether combinations of radiation therapy and immunotherapy will result in better outcomes for patients.

^{153}Sm +/- PSA-TRICOM

- Final data of n = 44 patients with mCRPC
- 1st endpoint: PFS
- Progression defined by utilizing PCWG, but not PSA criteria



Heery, Madan, Stadler, Stein, Dahut...Schlom, Gulley, *Oncotarget*, 2016
 PCWG = Prostate Cancer Working Group response criteria

10

Figure: In a phase II trial, the combination of Sm-153 with PSA-TRICOM extended progression-free survival by 2 months compared Sm-153 alone. This suggests that combining immunotherapy with a radiopharmaceutical may have a clinical benefit.

Targeting the Bone Compartment in Metastatic Prostate Cancer

Nora Navone, MD, PhD

The University of Texas MD Anderson Cancer Center

- The bone is the primary site of prostate cancer metastasis. Bone metastases are present in ~85-90% of patients with metastatic prostate cancer.
- In a phase III clinical trial, treatment with the bone-targeting radiopharmaceutical Radium-223 was found to provide a median survival benefit of 3.6 months. This finding led the FDA to approve Radium-223 for the treatment of prostate cancer patients with bone-only metastases.
- The development of biomarkers to identify patients that will benefit from bone targeting agents as well as combination treatment strategies that integrate bone-targeting therapy are urgently needed.

- Dr. Nora Navone discussed how prostate cancer interacts with bone in order to promote disease progression. Studies on the efficacy and mechanisms of bone-targeting therapies in prostate cancer were also discussed.
- An experimental system was developed to study the biology and mechanisms of bone metastatic human prostate cancer. Patient specimens were obtained and used to generate cell lines or were grown as tumors in mice. Studies on metastatic prostate cancer including examination of gene expression, functional aspects, and therapeutic sensitivities were performed.
- When a patient-derived castrate-resistant prostate cancer (CRPC) cell line was injected into the bones of mice, osteoblastic (bone-building) bone metastases developed. When prostate cancer cells were injected under the skin of mice, new bone began to develop at that site. This indicates that prostate cancer cells promote bone growth, which may underlie the predilection of prostate cancer to metastasize to bone.
- The FGF pathway mediates interactions between prostate cancer cells and bone and is a potential therapeutic target for the treatment of bone metastatic prostate cancer.
- FGF9, an activator of the FGF pathway, was found to be highly expressed by prostate cancer cells. Blocking FGF9 reduced the ability of prostate cancer cells to form bone metastases (Figure). These results indicate that the FGF pathway facilitates bone metastases, and that FGF9 in particular, may be a relevant therapeutic target.
- Based on these studies, a clinical trial was initiated to test the FGF-targeting agent dovitinib in metastatic CRPC (mCRPC). Approximately 30% of patients treated with dovitinib exhibited tumor shrinkage on bone scans.
- PSA declines occurred in some patients, but did not strongly correlate with clinical benefit from dovitinib. Declines in bone-specific alkaline phosphatase, a measure of bone cell death and growth, strongly correlated with clinical benefit.
- These studies indicate that targeting the FGF pathway may have therapeutic benefit for the treatment of bone metastatic prostate cancer.
- Alkaline phosphatase may be a better biomarker for bone-targeted therapies compared to PSA. Clinical trials of Radium-223 and cabozantinib, which also target the bone microenvironment, have shown similar utility for alkaline phosphatase but not PSA as a biomarker of therapeutic benefit.
- Cabozantinib is an experimental therapy that was thought to target c-Met and has activity against prostate cancer bone metastases. However, when residual tumor tissue was analyzed from patients treated with cabozantinib, c-Met activity was not inhibited. This indicates that cabozantinib is not actually targeting c-Met. Studies in mice confirmed that effects of cabozantinib were not related to inhibition of c-Met activity.
- Preclinical studies are ongoing to examine the effects and mechanisms of Radium-223 on prostate cancer bone metastases.
- Preliminary results indicate that Radium-223 exhibited a dose-dependent inhibition of a prostate cancer patient-derived tumor grown in the bones of mice.
- Radium-223 treatment increased bone mass in mice both with and without prostate cancer.

- Radium-223 also reduced the numbers of bone-degrading cells (osteoclasts) in the bones of mice without prostate cancer, although the effects of Radium-223 on osteoclasts in mice with prostate cancer are still under study.
- Ongoing studies aim to identify factors secreted by bone and tumor cells that mediate the effects of Radium-223.
- These studies will enable the development of predictive biomarkers of therapeutic response to bone-targeting therapies and inform the design of clinical trials to test therapeutic combinations or drug sequencing using bone-targeted agents in prostate cancer.

FGF9 Implicated in PCa Bone Progression

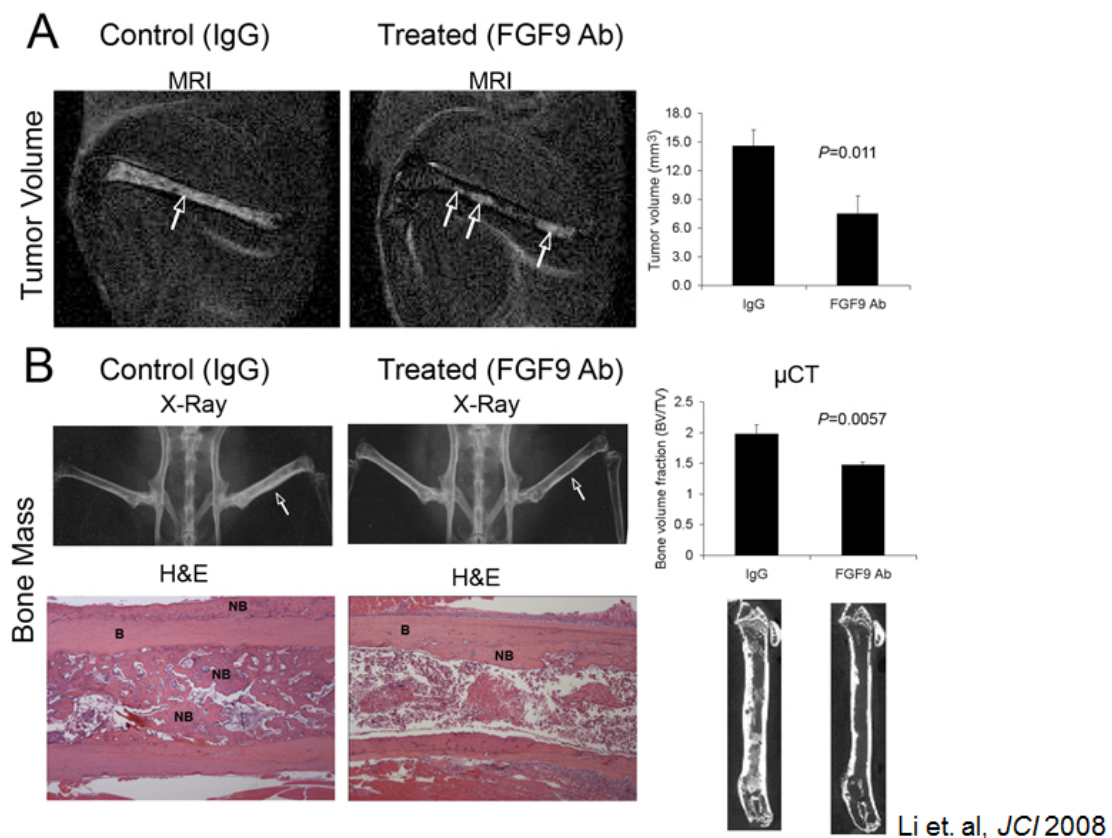


Figure: Blocking FGF9, an activator of the FGF pathway, reduced the ability of prostate cancer cells to form bone metastases.

Radium-223: A Novel Approach to Prevention and Early Treatment of Bone Metastases from Prostate Cancer

Robert Coleman, MD

University of Sheffield, UK

- Prostate cancer has an overwhelming predilection for metastasizing to the bone. Identifying the sites in bone that prostate cancer cells are attracted to is critical for developing appropriate bone microenvironment-targeting therapies to prevent or treat bone metastases.
- Dr. Robert Coleman discussed identification of sites in bone where tumor cells home. Approaches to prevent early bone metastases by targeting single cancer cells within bone that have not yet formed detectable tumors (dormant tumor cells) were addressed.
- Using a specialized assay in which non-dividing tumor cells are marked with a red dye (the dye is lost if the cells divide), dormant tumor cells were found to home to sites close to the bone surface.
- Bone cell types are not evenly distributed over bone surfaces. More osteoblasts (bone building cells) are located along the lateral surface of bone while more osteoclasts (bone degrading cells) are located along the medial surface. Prostate tumor cells homed to and remained at osteoblast-rich areas of bone (lateral surface).
- These findings support the hypothesis that prostate cancer cells preferentially occupy the endosteal niche within the bone marrow, a site at the border between the bone and bone marrow that contains hematopoietic stem cells, osteoblasts and mesenchymal progenitor cells.
- Upon arrival in bone, prostate cancer cells are thought to become dormant and can exist there for years before developing into progressing tumors.
- In a study in which dormant and actively dividing prostate cancer cells were sorted and injected into mice, dormant prostate cancer cells were found to preferentially develop bone metastases compared with actively dividing cells.
- Homing of prostate cancer cells to areas of osteoblasts in bone suggests that osteoblasts may support prostate cancer growth.
- The effect of modulating osteoblast activity on prostate tumor cells was examined. Mice were treated with parathyroid hormone (PTH), a hormone which increases osteoblast activity, prior to tumor cell injection (Figure). A short schedule of PTH treatment significantly increased tumor growth, especially in bone sites that previously exhibited low levels of osteoblast activity. Conversely, PTH treatment resulted in reduced numbers of circulating tumor cells, implying that increasing osteoblast activity led to a high level of tumor cell recruitment from the circulation and retention in bone.
- Castration increases osteoblast activity and bone turnover. Castration of mice also increased the development of prostate cancer metastases in bone.

- Bisphosphonates such as zoledronic acid block the activity of osteoblasts and osteoclasts and prevent bone breakdown and remodeling. Zoledronic acid is an FDA-approved therapy for the treatment of prostate cancer bone metastases.
- Treatment of mice with zoledronic acid blocked castration-induced enhancement of prostate cancer bone metastases. These results suggest that combining androgen-targeted therapies with bisphosphonates may have a clinical benefit in treating and preventing bone metastases in prostate cancer patients.
- The optimal time to treat bone metastases is most likely at the time following establishment of dormant tumor cells in bone or during development into quiescent micrometastases. T-CELLGoals for such therapy include dormant tumor cell elimination or the induction or maintenance of tumor cell dormancy.
- In breast cancer clinical trials, treatment with adjuvant bisphosphonates significantly reduced tumor recurrences in bone but not in other sites. Nevertheless, this translated into a reduction in breast cancer mortality. Benefits were restricted to postmenopausal women.
- To date, randomized clinical trials of adjuvant bisphosphonates in prostate cancer have not been able to demonstrate significant effects on metastases prevention.
- The osteoclast-targeting agent denosumab delayed the onset of bone metastases in castrate-resistant prostate cancer patients by a median of 7 months, and may have prevented bone metastases in some patients, though longer follow-up on the trial is needed before conclusions can be made.
- Radium-223 is an FDA-approved bone-targeting radiopharmaceutical agent that improves median overall survival of bone-metastatic prostate cancer patients.
- Radium-223 has been shown to prevent the development of prostate cancer metastases in mice. This suggests that Radium-223 can eliminate dormant tumor cells.
- In addition to this preclinical data, other rationale support earlier administration of Radium-223 in order to prevent and treat bone metastases. Radium-223 targets the proper bone location, has powerful localized anticancer activity, and inhibits osteoblasts. Radium-223 exhibits low toxicity and is likely safe for administration in the early, high risk clinical setting.
- Clinical trials testing Radium-223 in combination with immunotherapy are warranted.
- Prostate cancer patients who may benefit from Radium-223 treatment include those with high-risk disease or biochemical relapse. Improved biomarkers to select patients most likely to benefit would be ideal.
- These studies demonstrate that prostate tumor cells home to osteoblast-rich sites in the bone and establish dormancy. Factors such as osteoblast activation can then drive dormant cells to develop into progressive metastases. Ongoing and future clinical trials are testing the efficacy of bone-targeting agents in the prevention and treatment of bone metastases.

Tumour Growth After PTH Pre-treatment in Bony Sites With Prior Low Bone Turnover

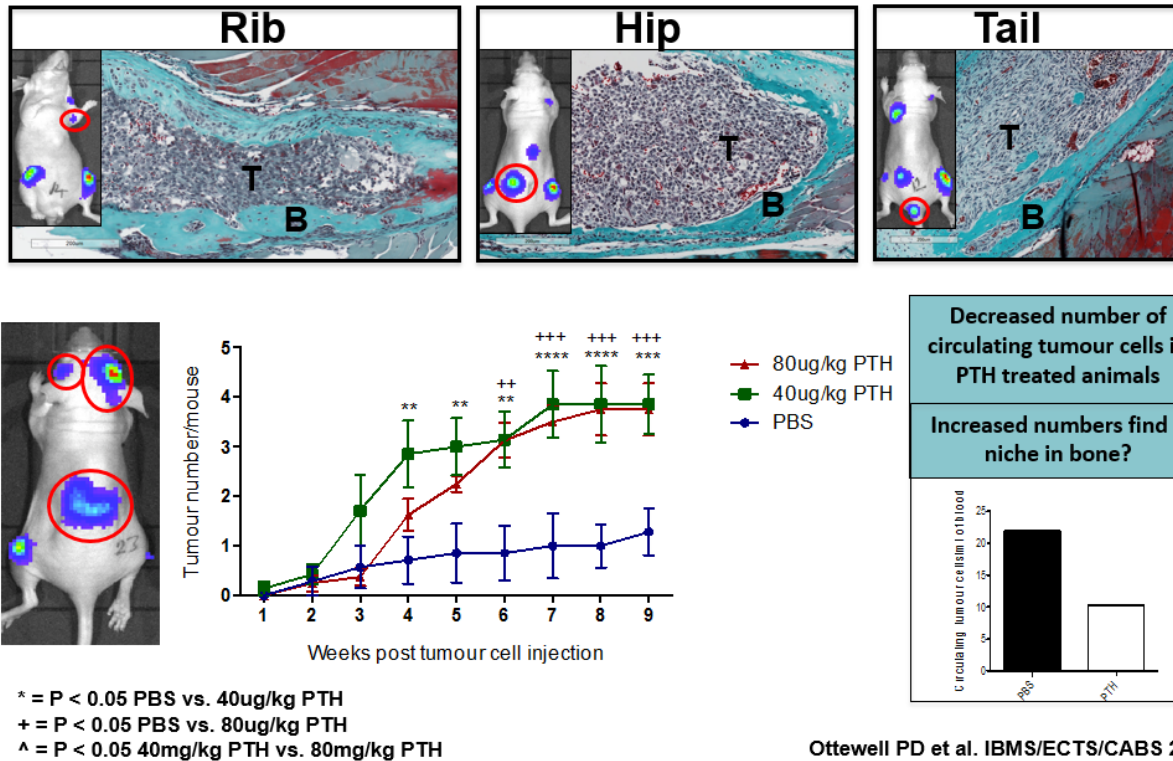


Figure: Treatment of mice with parathyroid hormone (PTH) prior to tumor cell injection exacerbated tumor growth, while lowering numbers of circulating tumor cells.

Session 4: Cancer Immunotherapy: Overcoming T-cell Exhaustion in the Tumor Microenvironment

Immune Suppression in the Tumor Microenvironment of Pancreatic Cancer

Sunil R. Hingorani, MD, PhD

Fred Hutchinson Cancer Research Center

- Immunotherapy has elicited significant responses in several types of cancer, but has not yet demonstrated similar efficacy in prostate and pancreas cancer. Understanding the barriers to effective immunotherapy is critical for harnessing this powerful therapeutic strategy.
- Dr. Sunil Hingorani discussed therapeutic strategies to activate effective anti-tumor immune responses in pancreas cancer models and patients.
- Pancreas tumors are characterized by an intense infiltration of fibroblasts and immune cells, while tumor cells represent a minor fraction. Targeting the non-tumor cell compartments within pancreas tumors is of high interest.
- Immune checkpoint therapy works by reawakening T-cells that are able to recognize and kill tumor cells but have been rendered inactive or exhausted by tumors. These inhibitors have been largely ineffective in pancreas cancer due to barriers that may include low numbers of neoantigens, which are tumor cell mutations that can activate anti-tumor immune responses, as well as other immune suppressive features of the tumor microenvironment.
- The KPC mouse pancreas tumor model uses mutations in the tumor-driving gene K-Ras and the tumor suppressor gene p53 to drive the development of tumors that resemble human pancreas cancer and have similar metastatic patterns.
- Using this model, various aspects of anti-tumor immunity were examined at different phases of tumor initiation and progression. At all stages, no effective anti-tumor immune responses were observed. During the development of pre-invasive disease, tumors became infiltrated by macrophages and regulatory T-cells, which function to suppress tumor-killing T-cell activity. In the subsequent transition from pre-invasive to invasive disease, tumors became highly infiltrated by granulocytic myeloid-derived suppressor cells (Gr-MDSCs), another class of immune cells that suppress tumor-killing T-cells.
- Treatment of KPC mice with an antibody that depletes Gr-MDSCs resulted in the reactivation of endogenous tumor-killing T-cells, with a dramatic influx of activated CD8 T-cells into tumors, and increased tumor cell death. Removal of this critical cellular barrier awakened endogenous anti-tumor immunity. In addition, Gr-MDSC depletion resulted in an altered tumor stromal architecture, including increases in tumor blood vessels, which may enhance the delivery of therapeutics.
- Another potential strategy to overcome immune suppression in pancreas cancer is adoptive immunotherapy which involves the administration of T-cells that target specific tumor-associated antigens (protein fragments).

- The pancreas tumor-associated protein mesothelin (MSLN) is highly expressed at all stages of pancreas cancer and therefore represents a promising target for T-cell immunotherapeutic strategies.
- To identify if any regions of the MSLN protein are ideal targets for T-cells, different epitopes of MSLN were injected into normal and MSLN-deficient mice, and anti-MSLN T-cell responses were measured. One region of MSLN was found to activate a T-cell response in both types of mice, and was selected as a promising antigen for generating MSLN-specific T-cells. Modifications of this region of MSLN were further made in order to create MSLN-specific T-cells with enhanced ability to bind MSLN.
- Preclinical studies were conducted to test the impact of MSLN-specific T-cell immunotherapy in models of pancreas cancer. Treatment of pancreas tumor-bearing mice with the combination of MSLN-specific T-cells, a vaccine containing the optimized MSLN protein region, and a T-cell growth factor (IL-2), resulted in high levels of activated anti-MSLN T-cells. T-cells tracked to primary tumor sites and expressed markers of activation within several days of administration.
- However, after 28 days, anti-MSLN T-cells began to exhibit hallmarks of immune exhaustion, including expression of inhibitory immune checkpoint receptors PD-1, TIM3, LAG3, and 2B4, and reduced expression of the immune activation proteins IFN γ and TNF α . This indicates that multiple immune checkpoints are activated only after an anti-tumor effector T-cell response.
- Administration of a second infusion of MSLN-specific T-cells resulted in a second wave of tumor-killing T-cell activity (Figure). This strategy resulted in a large reduction in tumor burden and improved overall survival compared with mice treated with non-specific T-cells. Serial infusion of MSLN-specific T-cells is a promising immunotherapeutic strategy for treatment of pancreas cancer.
- Overall, these studies demonstrate that immune-suppressive and immune-silent features of pancreas cancer can be overcome by specific immunotherapeutic strategies. Disruption of the tumor stroma may be essential for the eradication of pancreas cancer.
- Application of similar paradigms in prostate cancer patients may result in the development of significantly more efficacious immunotherapeutic regimens.

Serial TCR₁₀₄₅ infusion increase OR and OS in KPC PDA

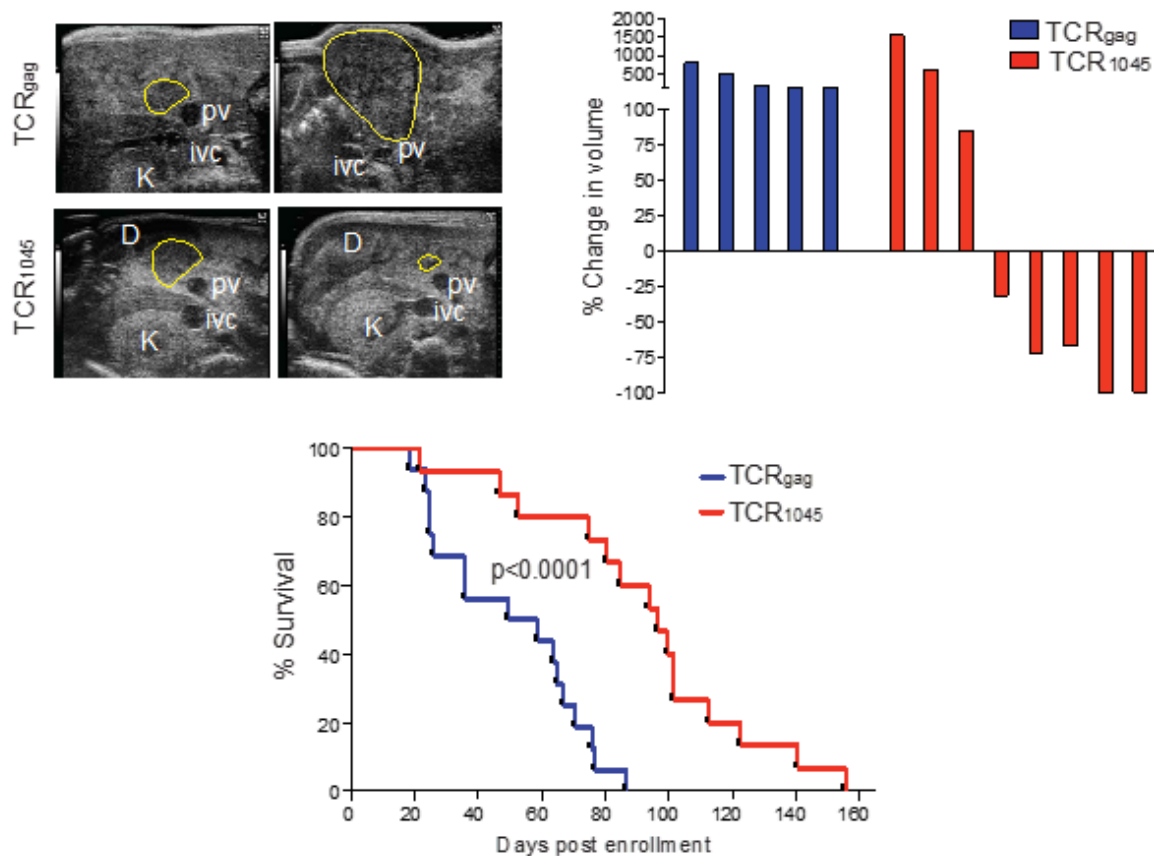


Figure: Serial infusion of MSLN-specific T-cells (TCR-1045) resulted in a large reduction in tumor burden and improved overall survival compared with mice treated with non-specific T-cells (TCR-gag). Source: *Cancer Cell* 28:638-652, 2015.

Overcoming T-cell Exhaustion in Prostate Cancer

Haydn Kissick, PhD

Emory University School of Medicine

- Anti-tumor immune responses are primarily driven by CD8 T-cells. CD8 T-cells are specialized immune cells that recognize and kill cells expressing antigens (small parts of proteins) associated with danger, such as pathogen infections or cancer.
- Every newly born T-cell has a genetically unique “T-cell receptor” (TCR) that recognizes a different antigen. T-cells that recognize normal self-antigens are eliminated in the T-cell maturation process (to avoid autoimmunity), while those that are released into the circulation recognize never-before-encountered antigens, which could be expressed by pathogen-infected or tumor cells.

- If a “naïve” CD8 T-cell encounters its cognate antigen, it becomes activated, kills the offending cell, and multiplies extensively to create an army of “effector” T-cell clones that defend the host against any cell expressing that antigen.
- In this way, individuals develop long term immunity to the antigen. However, tumors have robust mechanisms to deactivate tumor-targeting T-cells. Understanding how tumors modulate T-cell activities will enable the development of better immunotherapies.
- Dr. Haydn Kissick presented studies characterizing T-cells from prostate tumors in order to identify strategies to reactivate effective anti-tumor T-cell activity.
- T-cell activation actually requires two signals. The antigen recognized by the TCR must be presented on the surface of a cell being convased, but the T-cell will not respond unless a second, “co-stimulatory” signal is supplied.
- The immune system also has a built in off-switch, in which negative regulatory “checkpoint” signals can feedback on T-cells to turn off activity. T-cells in the off-state are termed “exhausted,” and express high levels of checkpoint molecules such as PD1.
- To understand the mechanisms regulating anti-tumor T-cell responses, Dr. Kissick examined the phenotypes of T-cells from cancer patients.
- In tumors from patients with renal cell carcinoma (RCC), several populations of CD8 T-cells were observed (Figure). “Standard effector” T-cells exhibited normal expression of costimulatory molecules (CD28) and low expression of checkpoint molecules (PD1). “Terminally differentiated” T-cells exhibited low expression of both costimulatory molecules and checkpoint molecules. “Highly active/exhausted” T-cells expressed very high levels of checkpoint molecules (PD1, TIM3 and TIGIT) and markers of having recently proliferated (Ki67).
- Many T-cells in RCC tumors were of the highly active/exhausted phenotype. The numbers of cells with this phenotype correlated with the number of T-cells within tumors, indicating that these T-cells are activated and proliferating.
- To study the functions of different CD8 T-cell phenotypes in tumors, T-cells were isolated from tumors and sorted into three separate populations representing these unique phenotypes.
- When the different populations of T-cells were stimulated with normal activating signals (TCR plus co-stimulation), only the standard effector T-cells became activated and proliferated. With each round of cell division, expression of negative regulatory checkpoint molecules (PD1, TIM3) increased on these cells, until they resembled the highly active/exhausted phenotype and ceased to proliferate. This indicates that standard effector T-cells are stem cell-like precursors to highly active/exhausted T-cells found in tumors.
- A similar “stem-exhaustion” T-cell pattern was observed in tumors from patients with kidney, bladder, and lung cancer (Figure).
- However, in prostate tumors, only the terminally differentiated phenotype of CD8 T-cells was observed (low expression of both costimulatory molecules and checkpoint molecules) (Figure). These cells are likely unable to sense and respond to signals in their surrounding environment, and represent a unique state of T-cell dysfunction. Similar populations of T-

cells have been observed in elderly patients, often against chronic viral infections such as Epstein - Barr virus.

- Prostate tumors were found to have no or very low levels of proliferating T-cells, while up to 30% of T-cells from kidney and bladder tumors were proliferative.
- In addition, significantly fewer numbers of T-cells were observed in prostate tumors compared with kidney and bladder tumors.
- Overall, these studies demonstrate that tumor infiltrating T-cells in prostate cancer are uniquely different from other cancers and are characterized by non-responsiveness to classical co-stimulatory molecules. Understanding how to reactivate these T-cells will enable the development of new immunotherapeutic approaches that can elicit effective anti-tumor immune responses in prostate cancer patients.

The CD8 T-cell Response to Cancer is Made Up of Diverse Cellular Subsets

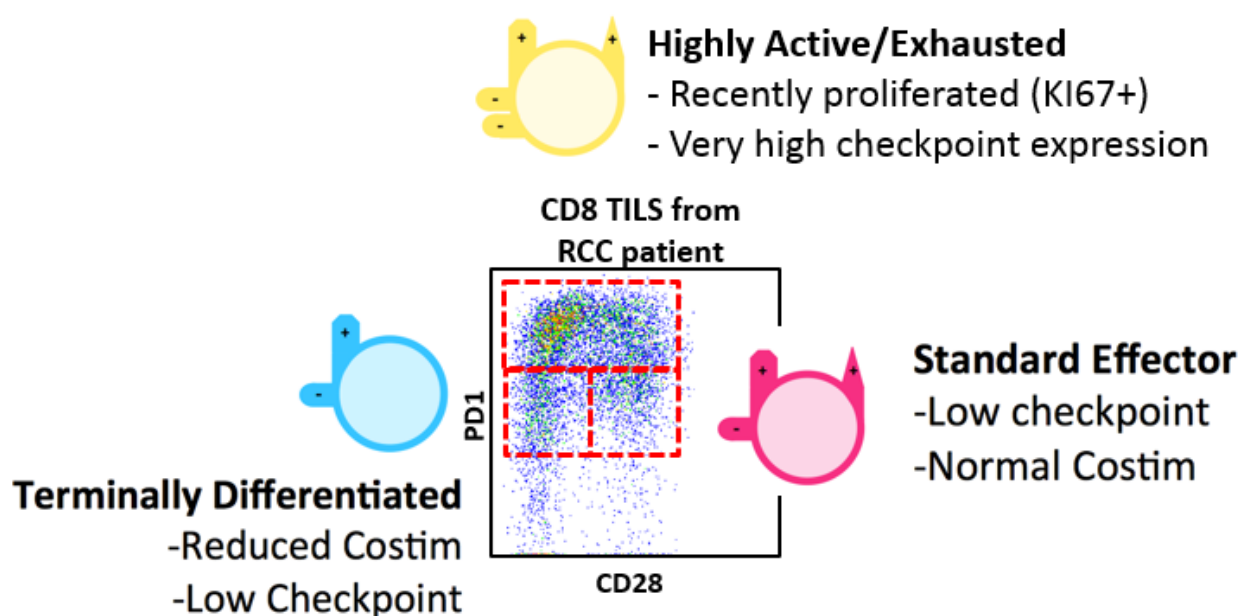


Figure: In tumors from cancer patients, several populations of CD8 T-cells can be observed. “Standard effector” T-cells exhibit normal expression of costimulatory molecules (CD28) and low expression of checkpoint molecules (PD1). “Terminally differentiated” T-cells exhibit low expression of both costimulatory molecules and checkpoint molecules. “Highly active/exhausted” T-cells express very high levels of checkpoint molecules (PD1, TIM3 and TIGIT) and markers of having recently proliferated (Ki67).

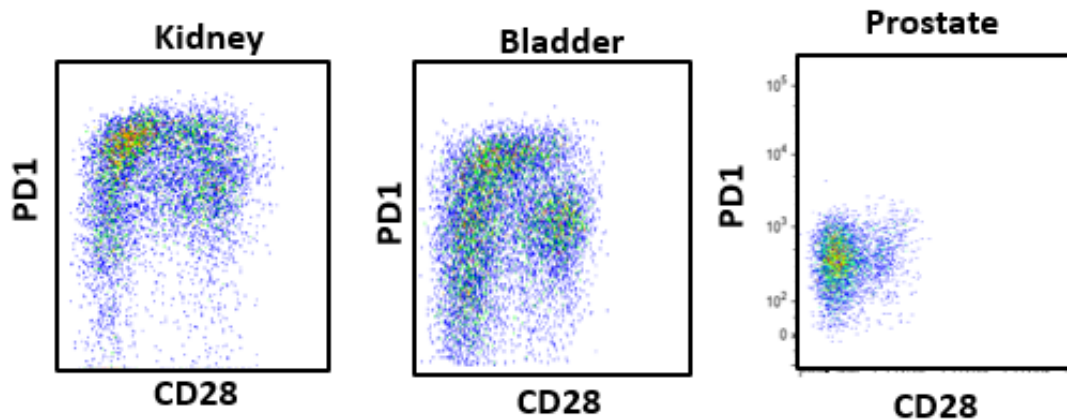


Figure: T-cells from kidney and bladder tumors include several populations not observed in prostate tumors: “standard effector” T-cells (CD28-high, PD1-low), and “highly active/exhausted” T-cells (PD1-high, CD28-positive/negative). Only the “terminally differentiated” T-cell phenotype was seen in prostate tumors (CD28-low, PD1-low).

MMR Mutations and Microsatellite Instability in Prostate Cancer

Colin Pritchard, MD, PhD
University of Washington

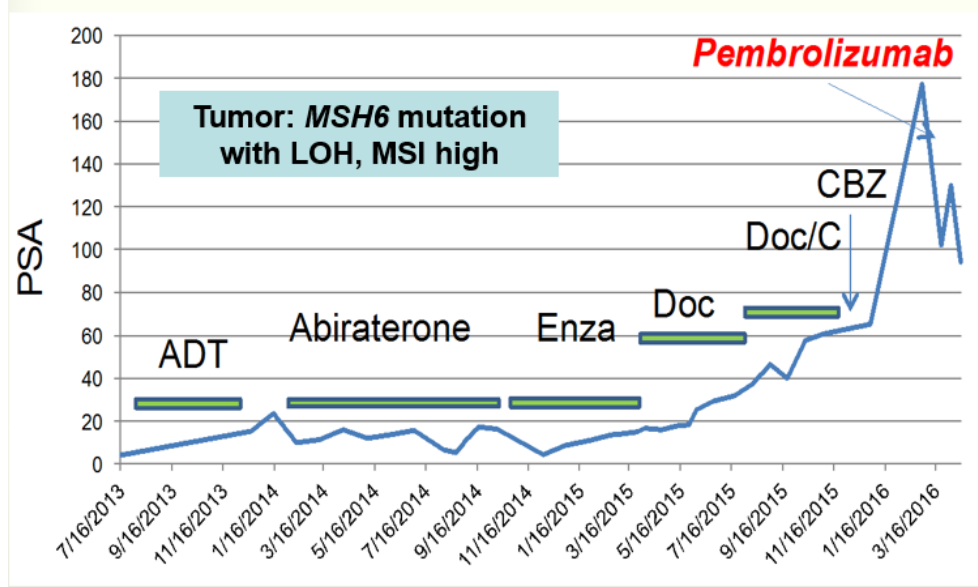
- For cells to divide, they must first replicate their entire genome. DNA replication enzymes sometimes make mistakes and incorporate incorrect nucleotides into the new strand of DNA. DNA mismatch repair (MMR) is a complex of proteins that scans newly synthesized DNA for mistakes and fixes them, to avoid creating mutations in newly formed cells.
- Cancer cells sometimes lose or mutate MMR genes. This can lead to a “hypermuted phenotype,” in which large numbers of mutations are acquired.
- Microsatellites are short sequences of DNA that are repeated many times in a row. The number of repeats for a given microsatellite sequence is highly variable across the population and a major factor studied in genetic linkage studies.
- One of the outcomes of MMR mutations is microsatellite instability, in which the number of microsatellite repeats is altered due to the relative ease by which DNA replication enzymes can make mistakes in these “slippery” regions.
- The role of MMR mutations and hypermutated phenotypes in prostate cancer have been relatively unstudied.
- Dr. Colin Pritchard hypothesized that hypermutated prostate cancer is due to MMR mutations and is associated with microsatellite instability.
- There are four key MMR genes that participate in DNA mismatch repair: MLH1, MSH2, MSH6, and PMS2.
- Hypermuted phenotypes are commonly observed in colorectal (16%) and endometrial (22%) cancers, and are primarily driven by MMR mutations (predominantly via genomic

silencing of MLH1), although mutations in DNA replication enzymes (POLE) can contribute to “ultra-mutated” phenotypes. It is now recommended that all patients with colorectal cancer, regardless of age or family history, are tested for inherited and tumor-acquired alterations in MMR genes.

- Inheritance of mutations in MMR genes causes Lynch syndrome, a condition which predisposes individuals to a very high chance of developing cancer, particularly colorectal cancer.
- A clinical assay was used to sequence a panel of cancer-associated genes including MMR genes in tumors from advanced prostate cancer patients. A total of 10 tumors carrying MMR mutations were identified from 103 patients (9.7%). In two of these patients, the mutations were inherited (Lynch syndrome).
- In a second cohort of 150 patients with metastatic castrate resistant prostate cancer (mCRPC), the prevalence of MMR mutations was 3%.
- In a third cohort of advanced prostate cancer, 6 of 98 patients (6%) had MMR-mutated tumors.
- More studies are needed to ascertain the true prevalence of MMR mutations in prostate cancer patients.
- The most commonly mutated MMR genes in advanced prostate tumors were MSH2 and MSH6. Mutations on one copy of the gene were typically accompanied by a loss or mutation of the other copy of the gene, leading to complete loss of gene activity.
- A bioinformatics approach was developed to detect microsatellite instability by next generation sequencing. All of the prostate cancer patients with hypermutated phenotypes also exhibited microsatellite instability.
- More efficient and cost-effective tests to detect the microsatellite instability phenotype in prostate tumors are being developed, in order to more easily identify patients with hypermutated tumors.
- Together, these data demonstrate that hypermutation in prostate cancer is largely due to MMR defects, which are present in ~3-10% of patients.
- Whether prostate cancer is part of the Lynch syndrome spectrum was previously unclear. However, a recent study found that the incidence of prostate cancer is 5-times higher in Lynch patients, indicating that Lynch syndrome is likely to predispose to prostate cancer.
- Lynch syndrome patients that develop prostate cancer are predominantly MSH2 and MSH6 mutation carriers, which is different from Lynch syndrome patients that develop colorectal cancer.
- In a cohort of advanced prostate cancer, patients, 4 of 692 patients (0.6%) had inherited mutations in MMR genes (Lynch syndrome). Inherited mutations were in the MSH2, MSH6, and PMS2 genes.
- In a cohort of 499 primary prostate cancer patients, 3 had Lynch syndrome (0.6%).

- Larger studies are needed to determine the true prevalence of inherited MMR mutations in prostate cancer patients and whether the prevalence differs between patients with primary and metastatic disease.
- The clinical courses of patients with MMR mutations in prostate tumors are being assessed.
- The presence of MMR mutations have been associated with clinical responses to checkpoint immunotherapy in colorectal cancer. Checkpoint immunotherapy has not yet proven effective in prostate cancer patients.
- In a clinical trial conducted at Oregon Health and Science University, 3 of 10 mCRPC patients were found to respond to pembrolizumab, an inhibitor of the negative regulatory immune checkpoint molecule, PD1. Genomic mutations were analyzed in the tumors from two of these patients, one of which was found to harbor MMR mutations.
- At the University of Washington, a heavily pre-treated mCRPC patient with tumor mutations in MSH6 exhibited an exceptional response to pembrolizumab (Figure). Unfortunately, the patient had to discontinue treatment due to treatment-related side effects.
- These observations indicate that MMR mutations may also mark prostate cancer patients that may benefit from treatment with anti-PD1 checkpoint immunotherapy.
- Future clinical trials will confirm whether MMR mutations are a biomarker of prostate tumor sensitivity to various checkpoint immunotherapies.

Hypermuted CRPC Case at UW: Evidence of PD-1 inhibitor Response



Thanks to
Bruce
Montgomery



Figure: At the University of Washington, a heavily pre-treated mCRPC patient with tumor MMR mutations (MSH6) exhibited an exceptional response to pembrolizumab, as measured by PSA. From: Schweizer *et al.*, *Oncotarget*. 2016 Oct 15.

Panel Discussion:

***Precision Clinicopathologic Conference (CPC):
Hype vs. Hope vs. Hypothesis***

Moderator: Johann de Bono, MD, PhD
Royal Marsden Hospital, UK

Felix Feng, MD (University of California, San Francisco)

Maha Hussain, MD (Northwestern University)

Christopher Logothetis, MD (University of Texas MD Anderson Cancer Center)

Silke Gillesen, MD (Kantonsspital, Switzerland)

William Nelson, MD, PhD (Johns Hopkins School of Medicine)

Todd Morgan, MD (University of Michigan)

Scott Tomlins, MD, PhD (University of Michigan)

*The panel discussion can be viewed in full at PCF.org:
<https://www.pcf.org/c/23rd-annual-scientific-retreat>*

Session 5: Immunotherapy for Prostate Cancer

Improving PSMA Specific Eradication of Metastatic Prostate Cancer Using TGF β Resistant CAR T-cells

Christopher Kloss, PhD
University of Pennsylvania

- T-cells are a type of immune cell that have the capacity to identify and destroy tumor cells in virtually any site in the body. Directing and activating T-cells to metastatic sites of tumor is a goal of most immunotherapeutic strategies and is being investigated by various approaches.
- Dr. Christopher Kloss discussed the generation of a genetically engineered “CAR” T-cell therapy that specifically targets and kills prostate cancer cells.
- CAR T-cells are produced by inserting a gene encoding a chimeric antigen receptor (CAR) into a patient’s T-cells. This genetically engineered protein is composed of two domains. The extracellular portion of the CAR molecule is derived from an antibody, and targets a tumor-associated molecule which provides targeting of the killer T-cells to tumor. The intracellular portion is composed of the signaling machinery that activates T-cell killing activity. The binding of a CAR to its target antigen will trigger the T-cell to become activated and kill the cell expressing the target.
- A CAR that targets the PSMA (prostate specific membrane antigen) protein was developed. PSMA is a protein specifically expressed by prostate and prostate tumor cells.
- TGF β is a molecule that promotes tumor growth and inhibits immune cell function. Dr. Kloss hypothesized that blocking the effects of TGF β in the tumor microenvironment would enhance the anti-tumor activity of CAR T-cells.
- A gene that can render T-cells resistant to signals from TGF β was constructed. In mouse models of cancer and autoimmunity, CAR T-cells expressing the TGF β -resistance gene were more potent and lived longer.
- Human PSMA-CAR T-cells have been developed and tested in phase I clinical trials at Memorial Sloan Kettering Cancer Center.
- T-cells transformed with the TGF β -resistance gene have also been tested in phase I clinical trials at the University of Pennsylvania. For safety testing of the TGF β -resistance gene, T-cells that target Epstein - Barr virus (EBV) were used.
- Human T-cells co-expressing the PSMA-CAR and the TGF β -resistance gene were generated and tested in preclinical studies.
- TGF β -resistant/PSMA-CAR T-cells were resistant to immunosuppressive signals from TGF β and specifically killed PSMA-expressing tumor cells in laboratory studies.
- The PC3 prostate cancer cell line secretes TGF β . TGF β -resistant/PSMA-CAR T-cells exhibited a greater ability to proliferate in the presence of PC3 cells than PSMA-CAR T-cells

without the TGF β -resistance gene, which is an indicator of how well the cells become activated. The mechanisms by which TGF β improves T-cell proliferation and tumor cell killing activity are being studied.

- The efficacy of TGF β -resistant/PSMA-CAR T-cells was tested in preclinical animal tumor models. Mice bearing human PSMA-expressing prostate tumors were injected with human CAR T-cells and analyzed for tumor growth and CAR T-cell activity.
- While PSMA-CAR T-cells (non TGF β -resistant) exhibited anti-tumor efficacy in mice, TGF β -resistant/PSMA-CAR T-cells were even more potent. At lower doses of CAR T-cells, only the TGF β -resistant/PSMA-CAR T-cells caused complete tumor regressions.
- Both types of CAR T-cells exhibited long term survival in mice, though even more T-cells were maintained when resistant to TGF β .
- Concurrently with tumor eradication, mice administered TGF β -resistant/PSMA-CAR T-cells lost a significant amount of weight. At higher doses of TGF β -resistant/PSMA-CAR T-cells, mice died from treatment-associated toxicity, while mice administered lower doses recovered. The treatment-associated toxicity is being investigated.
- These studies demonstrate that addition of a TGF β -resistance gene to PSMA-CAR T-cells renders them significantly more effective against prostate cancer. This is a promising novel therapy for the treatment of prostate cancer patients.
- Phase I trials to test the safety of TGF β -resistant/PSMA-CAR T-cells are scheduled to begin in 2017.

Quantification of Tumor Burden

in vivo PC3 Tumor Eradication

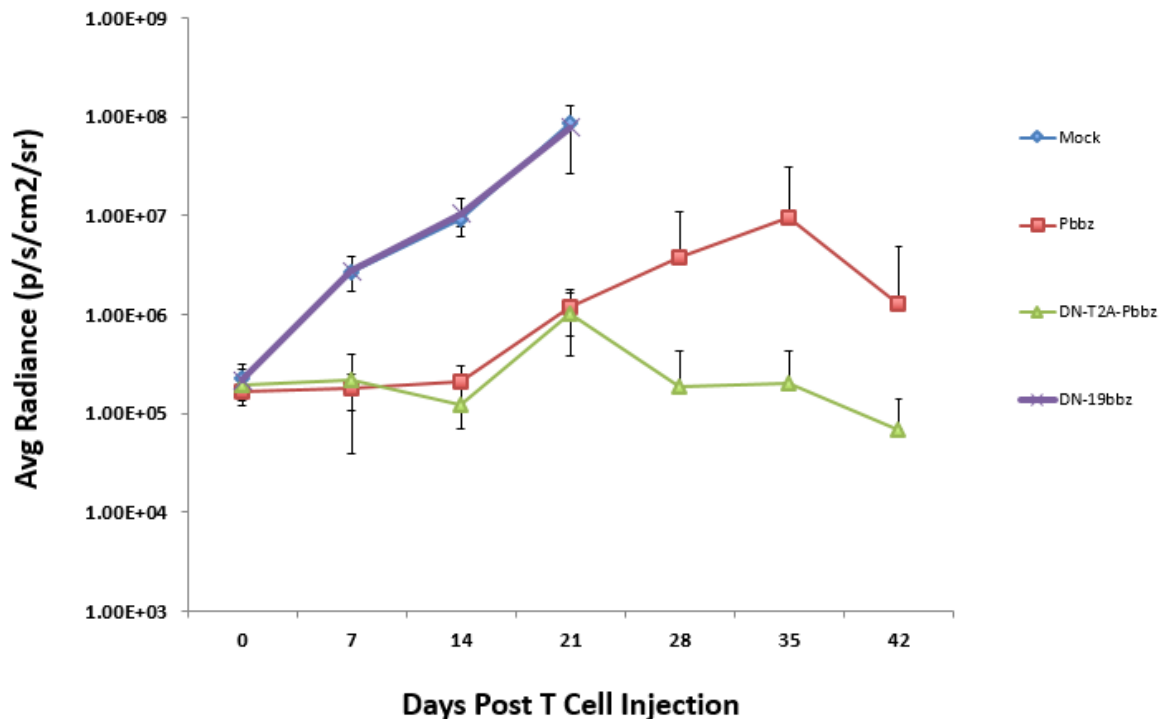


Figure: Mice given human PSMA-expressing prostate tumors were treated with CAR T-cells and analyzed for tumor growth (average radiance). While PSMA-CAR T-cells exhibited some anti-tumor efficacy in mice (Pbbz, red), TGF β -resistant/PSMA-CAR T-cells were even more effective at blocking tumor growth (DN-T2A-Pbbz, green), compared with untreated mice (mock, blue) or mice treated with CAR T-cells targeting a non-prostate tumor antigen (DN-19bbz, purple).

Marrow Infiltrating Lymphocytes: Their Biology and Clinical Implications

Ivan Borrello, MD

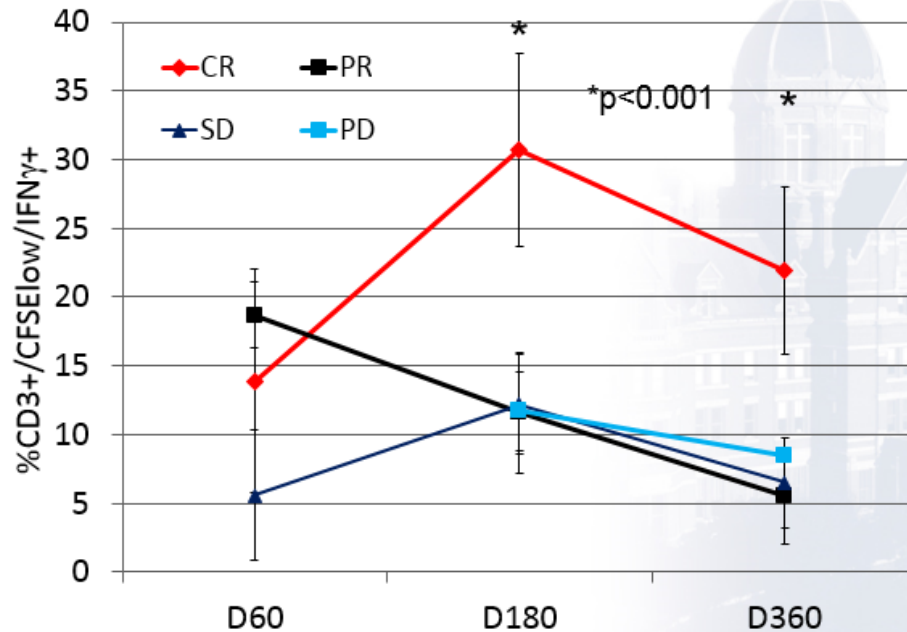
Johns Hopkins Sidney Kimmel Comprehensive Cancer Center

- T-cells are specialized immune cells that seek and kill cells expressing a target antigen that they have been trained to recognize. T-cells that have gained “memory” status can persist in the body for years, providing long-term immunity against their target. T-cells are ideal weapons against cancer.
- Numerous therapeutic strategies are being tested for the ability to mount effective anti-tumor T-cell responses, including tumor vaccines and adoptive immunotherapy.

- Adoptive immunotherapy is a strategy that involves delivering tumor-killing T-cells to patients.
- For adoptive immunotherapy to be effective, the administered T-cells must be able to recognize and kill tumor cells, traffic to tumor sites, and live for a long time. Ideally, the T-cells will be able to recognize a wide range of tumor antigens so that tumor cells cannot escape T-cell detection simply by turning off or mutating any single target antigen.
- Dr. Ivan Borrello discussed an adoptive immunotherapy strategy using T-cells obtained from a cancer patient's bone marrow.
- The bone marrow has unique immunological aspects that play a major role in maintaining an ideal population of T-cells. Bone marrow cells function in the initial activation of naïve T-cells into effector T-cells, and the maintenance of long-lived memory T-cells.
- Both virus-specific (influenza) and tumor-specific (breast cancer, melanoma, and multiple myeloma) memory T-cells have been found to reside at high levels in the bone marrow.
- These observations suggest that bone marrow derived T-cells, termed "marrow infiltrating lymphocytes" (MILs), may have therapeutic application in various disease settings.
- In myeloma, the bone marrow is a major site of disease. This is also true for metastatic prostate cancer.
- To determine if MILs would be effective against multiple myeloma, bone marrow cells were harvested from myeloma patients and activated in the laboratory against tumor cell antigens.
- Under these conditions, MILs proliferated to very high levels and exhibited significantly more potent tumor cell killing ability compared with T-cells harvested from blood.
- This indicates that MILs are composed of tumor-specific T-cells that have been turned off by immunosuppressive signals from tumors, but can be reactivated when removed from patients and expanded during optimal laboratory culturing.
- When activated MILs were administered to myeloma-bearing mice, they were able to traffic to tumor sites and eradicate tumors. MILs also remained alive within the mice for a long time.
- Compared with T-cells from blood, MILs expressed high levels of CXCR4. CXCR4 is the receptor for SDF-1, a signal that attracts cells to the bone marrow.
- The T-cell receptor (TCR) is a gene that is unique in sequence to each newly born T-cell, and is responsible for the ability of each T-cell to target a unique antigen.
- When the TCR sequences of MILs were analyzed, they were found to contain a more discrete subset of T-cell clones (copies of T-cells targeting the same antigen) compared with T-cells in the blood. This indicates that only certain antigen-specific T-cell clones take up residence in bone marrow and become MILs, whereas a more diverse population is present in the blood.
- A phase I/II clinical trial is in process to test the efficacy of MILs in multiple myeloma patients.

- MILs were collected from patients via bone marrow aspiration, expanded under laboratory conditions, and reinfused into 25 multiple myeloma patients, who were then monitored for tumor growth and immune cell parameters. MILs could be effectively expanded from over 95% of patients.
- Anti-myeloma T-cells were low in number at the time of MIL collection, but were highly increased in patients following MIL reinfusion.
- Myeloma patients with higher levels of anti-tumor MILs experienced improved clinical outcomes, indicating that MILs have promising therapeutic activity (Figure).
- T-cells from responding patients exhibited a memory phenotype, persisted for over one year, and trafficked to the bone marrow.
- One year post-MIL infusion, patients fell into three categories depending on if they retained high, medium, or low numbers of tumor-specific MILs in their bone marrow. High MIL retention was associated with high tumor-specificity of MILs, while low MIL retention was associated with low tumor-specificity of MILs.
- MILs isolated from patients with breast and lung cancer were also found to have high anti-tumor activity in preclinical studies.
- Compared with other adoptive immunotherapy strategies (such as CAR T-cells), MILs have several advantages – a relatively low toxicity profile, no genetic modification is needed, and an inherent ability to recognize a wide range of tumor antigens.
- Myeloid derived suppressor cells (MDSCs) are immunosuppressive immune cells found at high levels in tumors. MDSCs suppress T-cells by producing the T-cell deactivating signals, nitric oxide and arginase. Tadalafil (Cialis), a therapeutic used to treat erectile dysfunction and enlarged prostates, blocks these immunosuppressive functions in MDSCs.
- Treatment of myeloma and head and neck cancer patients with Tadalafil resulted in blockade of MDSC activity, reduction of MDSC numbers in tumors, and increased numbers of anti-tumor T-cells in tumors. This indicates that Tadalafil treatment may enhance the activity of MILs and deserves further study.
- These studies demonstrate that MILs contain a memory population of tumor-killing T-cells that can be obtained with relative ease, expanded, and used to treat patients with cancer. This novel adoptive immunotherapy approach is now being explored in prostate cancer.

Tumor-specific Response in the BM Correlates with Clinical Outcomes



(Noonan et al. STM 2015; 7:288)



Figure: In myeloma patients treated with MILs in a phase I/II clinical trial, higher levels of anti-tumor MIL activity (% CD3+/CFSE-low/IFNγ-high) were associated with improved clinical outcomes. CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease.

STATE OF THE SCIENCE 2016

Jonathan Simons, MD
Prostate Cancer Foundation

Introduced by Howard Soule, PhD
Prostate Cancer Foundation

This talk can be viewed in full at PCF.org:
<https://www.pcf.org/c/23rd-annual-scientific-retreat>

KEYNOTE ADDRESS

Michael Milken
Founder and Chairman
Prostate Cancer Foundation

Introduced by Stuart Holden, MD
Prostate Cancer Foundation

This talk can be viewed in full at PCF.org:
<https://www.pcf.org/c/23rd-annual-scientific-retreat/>

Session 6: Targeting DNA Repair Deficiency in Prostate Cancer

Special Lecture: Targeting DNA Repair in Cancer Therapy

Alan D'Andrea, MD

Harvard Medical School

- For complex multicellular organisms such as humans to grow and thrive, maintenance of the accuracy and integrity of the genome is critical. When a cell divides it must first replicate its entire genome. Mistakes made in the new copy of DNA are typically corrected by proteins that function in DNA damage repair (DDR) pathways. Deficiencies in DDR pathways lead to high rates of mutations and can result in the development of cancer.
- Human cells have at least 6 major DDR pathways that repair different types of DNA damage. Deficiencies in many of these pathways have been observed in malignant cells.
- Because at least some level of genomic integrity is required for cancer cells to survive, deficiencies in one DDR pathway result in dependencies on other DDR pathways. This renders the tumor cells more sensitive to therapies that either damage DNA (chemotherapy and radiation) or target the DDR pathway(s) they have become reliant on. This concept is referred to as “synthetic lethality.”
- Identifying the associations between DDR gene mutations and therapeutic sensitivities is critical for optimizing precision medicine treatment plans. For example, cancer cells harboring mutations in BRCA1 are likely to be sensitive to treatment with PARP-inhibitors.
- Dr. Alan D'Andrea discussed the relationships between DDR gene mutations and treatment sensitivities in ovarian cancer and the implications of these findings for prostate cancer.
- The Fanconi Anemia (FA) family of DDR genes is responsible for repairing cross-linked DNA.
- Fanconi Anemia (FA) is a rare autosomal recessive genetic disease caused by mutations in FA genes and results in developmental defects, bone marrow failure, and susceptibility to various types of cancer. Cells from FA patients have a very characteristic phenotype and are highly sensitive to agents that cause DNA crosslinking, such as cisplatin, and to PARP-inhibitors.
- Various types of cancer including ovarian, breast, and prostate, can also be driven by mutations in FA genes. These cancers are often sensitive to treatment with cisplatin and PARP-inhibitors.
- A camp was established for children with FA to provide support for patients, and advance research and advocacy. At the camp, it was found that parents of children with FA, who are heterozygous carriers of FA mutations, rarely developed ovarian, breast, and metastatic prostate cancer, but at a higher than expected frequency, compared to the general population. Fortunately the camp accelerated the surveillance of these individuals and resulted in earlier anti-cancer treatment.

- BRCA1 and BRCA2 are the most infamous FA proteins, known for their role in increasing susceptibility to breast, prostate and ovary cancer. One child in the camp was found to be a homozygous carrier of BRCA2 mutations. A careful family history analysis revealed early onset of breast or ovarian cancer in both the mother and father's families.
- Ubiquitination is a cellular mechanism in which proteins are tagged with ubiquitin molecules that direct their degradation. A critical step in the FA pathway during DNA repair is ubiquitination of FANCD2. The absence of FANCD2 ubiquitination was found to be a biomarker of FA pathway deficiency (Figure).
- Using FANCD2 ubiquitination as a biomarker, approximately 20% of high grade serous ovarian cancers were found to harbor FA pathway mutations. However, a study by The Cancer Genome Atlas (TCGA) found that up to 50% of high grade serous ovarian cancers have FA mutations.
- A study was performed to screen for DDR gene mutations in ovarian cancer and identify associations with sensitivity to PARP-inhibitors and cisplatin.
- Cisplatin sensitivity did not always overlap with PARP-inhibitor sensitivity.
- Mutations in BRCA1/2 did not always correspond with PARP-inhibitor sensitivity.
- In the absence of BRCA1/2 mutations, mutations in other DDR pathway genes may confer sensitivity to PARP-inhibitors.
- BRCA1/2-deficient cancer cells become resistant to PARP-inhibitors via various mechanisms. These include the acquisition of mutations that result in re-expression of BRCA1/2 and restoration of DDR activity, loss of the PARP gene, drug efflux, and mutations in the 53BP1/PTIP/RIF1/Artemis pathway.
- Ovarian tumors without DDR deficiencies did not respond to treatment with PARP-inhibitors.
- Novel drug combinations that can render DDR-proficient tumors sensitive to PARP-inhibitors are being tested. Combining PARP-inhibitors with inhibitors of CDK, PI3K, or ATR are showing promise against DDR-proficient ovarian cancer in early studies. Clinical trials are also testing the efficacy of combining PARP-inhibitors with immunotherapies such as pembrolizumab.
- Different PARP-inhibitors possess unique mechanisms of action. Understanding the mechanisms employed by various PARP-inhibitors is critical for identifying the settings in which they will be effective.
- One alternative function of PARP in BRCA1/2-deficient cells is to partner with the POL-theta enzyme to enact DNA repair. Targeting of the POL-theta pathway is being tested for treatment of BRCA1/2-deficient tumors. POL-theta-inhibitors may also be useful against tumors that have developed resistance to PARP-inhibitors.
- Not all DDR gene mutations have an impact on DNA repair. Tests that determine the functional impact of DDR gene mutations are needed to identify the patients most likely to benefit from treatment with PARP-inhibitors or cisplatin chemotherapy.
- Functional complementation is a method in which a mutated gene is inserted into cells deficient in the DNA repair gene and assessed for DNA repair activity. If the mutation

impairs gene function, DNA repair will not be restored in the cell line. If the mutation does not impact gene function, DNA repair will resume.

- Approximately 20-30% of castrate resistant prostate cancers have mutations in DDR genes. Like in ovarian cancer, DDR mutations render prostate tumors highly sensitive to treatment with PARP-inhibitors and cisplatin. Clinical trials are underway to further delineate which patients should receive these therapies.
- The studies presented provide numerous insights into the relationships between DDR mutations and sensitivity to various treatments in ovarian cancer. Translating these findings to prostate cancer will provide new precision therapies for prostate cancer patients.

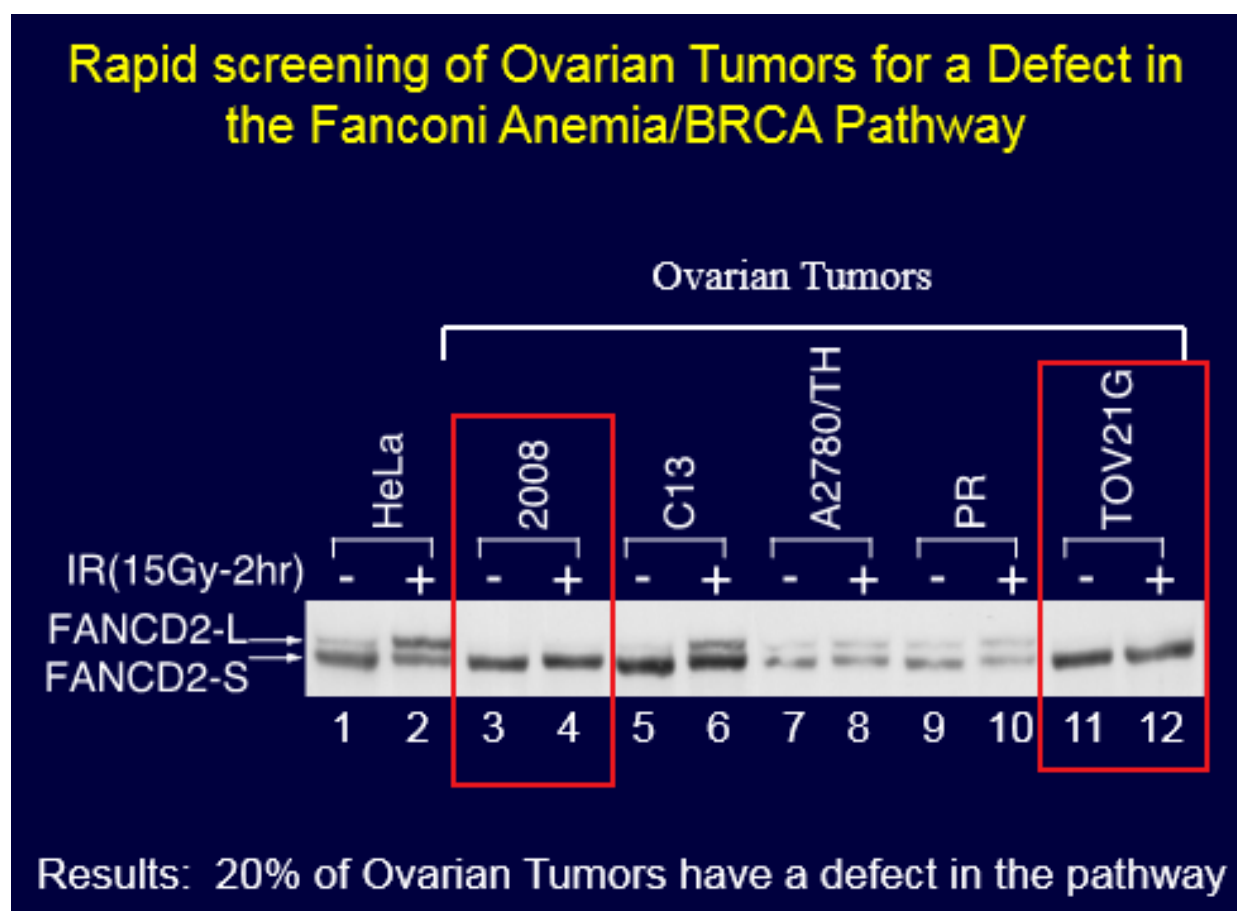


Figure: The absence of FANCD2 ubiquitination (top band) was found to be a biomarker of FA pathway deficiency. Using FANCD2 ubiquitination as a biomarker, approximately 20% of high grade serous ovarian cancers were found to harbor FA pathway mutations.

PARP Inhibitors: Opportunities and Challenges in Prostate Cancer

Joaquin Mateo, MD

The Institute of Cancer Research, UK

- Synthetic lethality is a concept in which tumor cells that have lost or mutated activity of one molecular pathway become highly dependent on a second pathway for survival. Therapeutically targeting the second pathway achieves selective killing of cancer cells while sparing normal cells that maintain function of the first pathway.
- Mutations in genes that function in DNA damage repair (DDR), such as BRCA1 and BRCA2, have been shown to render tumors sensitive to treatment with PARP-inhibitors due to synthetic lethality. (PARP is a “synthetic-lethal partner” of several DDR genes including BRCA1/2).
- This has led to FDA approvals for the PARP-inhibitors olaparib and rucaparib, for the treatment of BRCA1/2-mutated ovarian cancer.
- A recent series of studies found that 20-30% of metastatic castrate resistant prostate cancers (mCRPC) have DDR mutations and are sensitive to treatment with PARP-inhibitors. More work is needed in order to refine and streamline the use of PARP-inhibitors in prostate cancer and to achieve FDA approval for the use of these agents in prostate cancer.
- Dr. Joaquin Mateo discussed clinical trials testing the efficacy of PARP-inhibitors in the treatment of mCRPC and efforts needed to accelerate precision medicine for these patients.
- TOPARP is a multistage clinical trial being conducted in the UK. The recently completed TOPARP-A trial tested the efficacy of olaparib in a population of unselected mCRPC patients. Tumor biopsies from these patients were then evaluated for mutations that were associated with responses to olaparib.
- Of 49 patients tested, 16 responded to olaparib. Fourteen of the responders were found to have DDR mutations in their tumors (Figure). DDR mutations in mCRPC occurred primarily in the BRCA1, BRCA2, and ATM genes. There were only two cases in which DDR-mutated tumors did not respond to olaparib.
- In the ongoing TOPARP-B trial, tumor samples from patients are first evaluated for the presence of DDR mutations. Patients with DDR-mutated tumors receive treatment with olaparib.
- If efficacy is observed in olaparib-treated patients in TOPARP-B, then TOPARP-C will be initiated. In TOPARP-C, unselected mCRPC patients will be randomized to receive olaparib or placebo and will be subsequently assessed for DDR mutations.
- Dr. Mateo also discussed designing clinical trials that will optimize the clinical application of PARP-inhibitors for prostate cancer treatment.
- A critical question is whether PARP-inhibitors are only effective against prostate tumors with DDR mutations, or whether additional patients might benefit from these therapies. Randomized clinical trials testing PARP-inhibitors in all-comers are needed to answer this question.

- Clinical trials will also be needed to refine the “biomarker” -- the specific gene mutations and type of test -- used to select patients to receive PARP-inhibitors.
- The efficacy of PARP-inhibitors is dependent on inactivation of the synthetic-lethal gene partner. However, not all mutations impact a gene's function. Studies are needed to identify which DDR mutations do or do not inactivate DDR pathway activity.
- For a DDR pathway to be fully inactivated, loss of function of both gene copies is usually necessary. A recent study found that DDR mutations are inherited in ~12% of patients with metastatic prostate cancer. In most of these patients, the second copy of the gene was inactivated in tumor cells. However, in ~1/3 of these cases, the second copy of the DDR gene remained intact in tumor cells. Whether DDR mutations played a role in tumor development in these patients and whether these patients will benefit from treatment with PARP-inhibitors is uncertain.
- There are several strategies being pursued for combining PARP-inhibitors with other therapies. Therapeutic combinations that will cause a synthetic-lethal effect with PARP-inhibitors are being tested.
- PARP-inhibitors are also being tested in combination trials with androgen receptor (AR)-targeting agents (abiraterone or enzalutamide), and with platinum-based chemotherapy.
- Mechanisms by which some tumors develop resistance to PARP-inhibitors also need to be investigated. For example, secondary genomic mutations that restore DDR gene activity have been observed in tumors that initially responded to olaparib, but later progressed.
- Tumors can be heterogeneous. Mutations are acquired over time and thus mutations in one metastatic tumor site may not have been present in the primary tumor or in other coexisting metastases. Whether the primary tumor or single metastatic tumor biopsies are sufficient for identifying relevant genomic mutations is unclear and requires further study.
- Cell-free tumor DNA (ctDNA) is tumor DNA released into the circulation that can be harvested via patient blood draws and used to identify tumor mutations. ctDNA levels have also shown promise as a biomarker of tumor burden and treatment response. For instance, in the TOPARP trial, a $\geq 50\%$ decline in ctDNA levels predicted longer progression free survival and overall survival times. ctDNA is a promising tool for making precision medicine genomic studies easier to perform by avoiding the need for complicated invasive biopsies.
- Functional magnetic resonance imaging (MRI) is another potential treatment response biomarker that may be useful in measuring responses to PARP-inhibitors.
- These studies demonstrate huge promise for PARP-inhibitors as the first precision medicine therapy in prostate cancer and highlight steps needed to make this a reality for patients.

TOPARP-A

- 16/49 patients responded to Olaparib (PSA, RECIST, CTC)
- Association between responses and DNA repair mutations

ID	Maximum % PSA decline	Measurable disease	Best RECIST response	Confirmed CTC conversion	Baseline CTC count (/7.5ml blood)	Maximum % CTC decline	DNA repair genes defect
#1	84.7 %	No		No	87	100 %	ATM
#5	51.2 %	No		Yes	24	100 %	ATM
#6	29.3 %	Yes	SD	Yes	105	97.1 %	ATM, MLH3, MRE11, NBN
#8	47.3 %	No		Yes	38	94.7 %	HDAC2
#11	No decline	Yes	PD	Yes	6	83.3 %	Negative
#14	82.6 %	No		Yes	102	100 %	BRCA2 (g)
#15	79.9 %	Yes	PR	Yes	18	100 %	BRCA2 (g)
#16	86 %	Yes	PR	Yes	5	100 %	Neg (but copy loss BRCA2/PALB2)
#17	94.6 %	Yes	PR	Yes	8	100 %	BRCA2 (s)
#20	87.9 %	Yes	PR	N/E	<5	100 %	BRCA2 (s), FANCA, CHEK2
#26	No decline	No		Yes	12	100 %	BRCA1, FANCA
#30	69.9 %	No		Yes	100	100 %	BRCA2 (g)
#35	94.7 %	Yes	PR	Yes	513	100 %	BRCA2 (s)
#36	58.7 %	No		Yes	22	100 %	ATM
#39	68.2 %	Yes	PR	Yes	24	100 %	BRCA2 (s)
#48	No decline	Yes	SD	Yes	9	100 %	PALB2

Table: The TOPARP-A trial tested the efficacy of the PARP-inhibitor olaparib in a population of unselected mCRPC patients. Of 49 patients, 16 responded to olaparib, 14 of whom harbored DDR mutations in their tumors. Two responders did not have an identifiable DDR mutation (#11, #16).

Loss of DNA Repair in Prostate Cancer, Lessons from Breast and Ovarian Cancer

Richard Kennedy, D, PhD
Queen's University Belfast

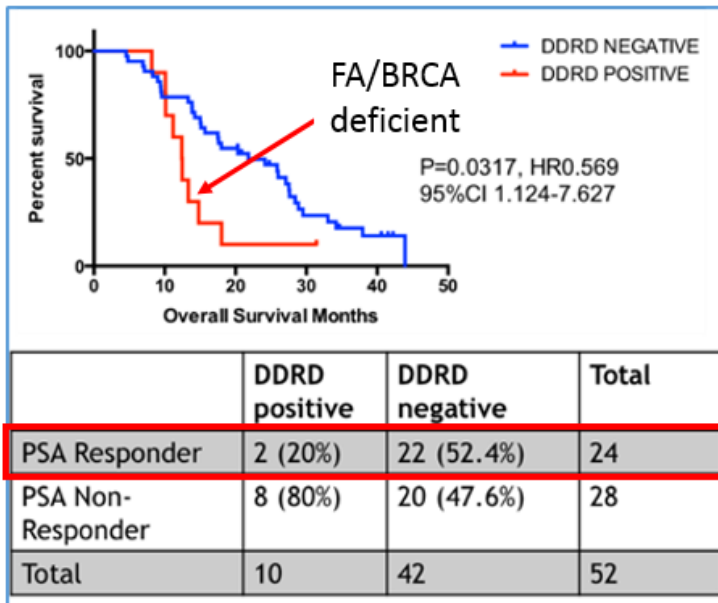
- BRCA1 and BRCA2 are genes that function to repair damaged DNA. Defects in BRCA1/2 and related Fanconi Anemia (FA) pathway genes results in loss of DNA repair, genomic instability, the accumulation of mutations, and a predilection for the development of cancer, namely breast, ovarian, and prostate cancer. This phenomenon is referred to as “BRCAness.”
- Dr. Richard Kennedy discussed how knowledge on BRCAness in breast and ovarian cancer can be applied to accelerate the development of treatment strategies for prostate cancer.

- In breast cancer, loss of BRCA1/2 is associated with more aggressive disease with lymph node and visceral metastases and with triple-negative (hormone therapy-insensitive) disease.
- In ovarian cancer, loss of BRCA1/2 is associated with a more aggressive disease with immune infiltration and lymph node, visceral and brain metastases.
- In prostate cancer, BRCA1/2 mutations have been associated with more advanced disease, higher Gleason grade, lymph node invasion, the presence of metastatic disease at diagnosis, shorter times to recurrence, and reduced overall survival.
- Tumors harboring mutations in BRCA1/2 are unable to repair DNA properly and are often sensitive to chemotherapy that damages DNA including cisplatin, cyclophosphamide, etoposide, and bleomycin.
- Treatment of BRCA1-deficient breast cancer patients with FEC chemotherapy (5-fluorouracil + epirubicin + cyclophosphamide) or cisplatin improved outcomes.
- Patients with BRCA1/2-mutated ovarian cancer also had better outcomes when given platinum chemotherapy.
- Several clinical trials have examined the efficacy of platinum chemotherapy (carboplatin, cisplatin, satraplatin, oxaliplatin, others) in prostate cancer. Responses have been observed in 10-70% of unselected patients across different studies.
- Although the genomic mutations associated with responsiveness were not studied in most of these cohorts, exceptional responses to carboplatin were observed in three patients with BRCA2-mutated prostate cancer. DDR pathway mutations are likely present in other patients who responded well to platinum chemotherapy.
- Taxane chemotherapy is a mainstay of treatment for advanced prostate cancer. Taxane chemotherapy is not a DNA damaging chemotherapy, but rather works through interfering with microtubule function.
- BRCA-deficient prostate cancers were less responsive to taxane chemotherapy compared with BRCA-normal tumors (23% vs 38% response rate; progression free survival of 2.2 vs 4.9 months).
- Likewise, BRCA-deficient breast cancer patients treated with taxane chemotherapy (paclitaxel) had poorer outcomes (reduced response rate, shorter progression free survival) compared with BRCA-normal patients.
- In ovarian cancer, the addition of taxane chemotherapy to platinum chemotherapy was only beneficial in patients with BRCA-normal tumors.
- In a study in 52 prostate cancer patients, DDR pathway deficiency, as measured by a gene expression assay, the "DNA Damage Response Deficient" (DDR-D assay) was associated with poorer overall survival following treatment with docetaxel monotherapy, suggesting taxanes are also not as effective in these patients (Figure). However, in a separate study, 7 prostate cancer patients with BRCA1/2 mutations responded to taxane chemotherapy. More studies are needed to address these discrepancies.

- Taking the data together from breast, ovarian and prostate cancer studies, it would appear that BRCA-mutant tumors are more aggressive, but respond well to platinum chemotherapy. Patients with BRCA-mutant prostate tumors are likely to have better outcomes if treated with platinum chemotherapy rather than taxane monotherapy.
- PARP-inhibitors are also more effective against BRCA-deficient tumors. However, PARP-inhibitors may increase risk for secondary malignancies if used in early disease for curative intent. More studies are needed to discern the long-term effects of PARP-inhibitors and compare efficacy with platinum chemotherapy if they are to be used in the curative, adjuvant setting.
- Unless the cell is in the final stage of division, DNA should be contained in the cell's nucleus. DNA in the cytosol is indicative of viral infection. Defects in DNA repair pathways can also result in the abnormal accumulation of DNA in the cytosol. All human cells are equipped with an innate immune pathway that detects cytosolic DNA and activates the immune system via the cGAS/STING-interferon signaling pathway.
- A study in breast cancer found that BRCA-deficient tumors tended to have higher levels of immune T-cell infiltration, higher interferon pathway activity, and expression of the immune checkpoint molecule PD-L1. This indicates that BRCAness may promote sensitivity to immunotherapy with novel agents such as PD-L1 inhibitors. This hypothesis is now being tested in clinical trials.
- In summary, data from breast, ovarian and prostate cancer studies indicate that BRCAness, while rendering tumors more aggressive, also sensitizes them to platinum chemotherapy, PARP-inhibitors, and potentially immunotherapy. Precision medicine clinical trials to define the best treatment strategies for these patients are ongoing.

Loss of FA/BRCA pathway (as defined by DDRD gene expression assay) is Prognostic in Advanced Prostate Cancer Following Docetaxel

OS from completion docetaxel



Median OS
12.4 months vs 24.8 months

Credit: Catherine Davidson
ESMO 2016

Figure: In a study in 52 prostate cancer patients, DDRD assay positive (FA/BRCA pathway deficient) patients were associated with poorer overall survival (OS) following treatment with docetaxel, suggesting taxanes are not effective in these patients.

Platinum Agents in Homologous Recombination Deficiency

Mark Pomerantz, MD

Dana-Farber Cancer Institute

Bruce Montgomery, MD

University of Washington

- Mutations in genes that function in DNA damage repair (DDR), such as BRCA1 and BRCA2, are associated with more aggressive prostate cancer.
- BRCA1/2 mutations are also associated with more aggressive breast and ovarian cancers. Studies have found that platinum chemotherapy is highly effective in these patients, prompting the testing of platinum chemotherapy in BRCA-mutant prostate cancer.

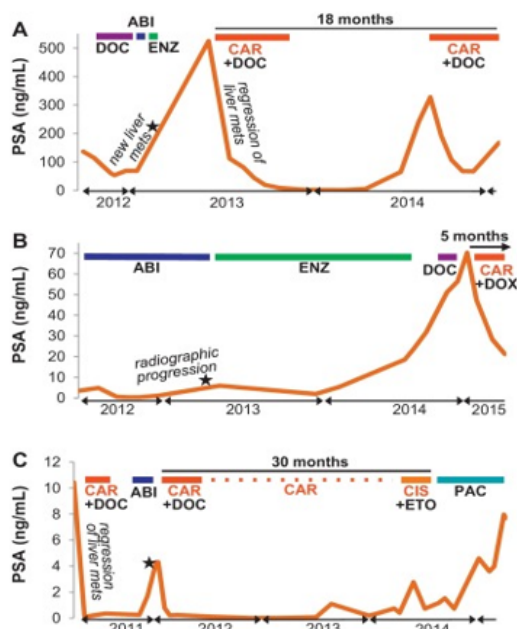
- Dr. Mark Pomerantz and Dr. Bruce Montgomery discussed clinical investigations into the efficacy of platinum chemotherapy in BRCA1/2-deficient prostate cancer.
- Several platinum chemotherapy agents have been tested in prostate cancer clinical trials, including satraplatin and carboplatin.
- A phase III trial testing satraplatin in castrate resistant prostate cancer (CRPC) was negative for overall survival, although approximately 20% of patients did have significant responses.
- A subsequent phase II trial testing carboplatin plus docetaxel in CRPC also found that a similar subset of patients benefitted.
- More recently, a study looking at potential markers for platinum response found that three CRPC patients with BRCA2-mutant tumors exhibited profound responses to carboplatin (Figure), suggesting that inactivation of BRCA2 is a biomarker for platinum sensitivity in prostate cancer.
- These studies indicate that a subset of prostate cancers, particularly those with BRCA1/2 mutations, are sensitive to platinum chemotherapy. Further investigation into identifying the patients who will benefit from treatment with platinum chemotherapy is warranted.
- An inherited mutation in a DDR gene is one mechanism by which these genes are inactivated in cancer. Germline (inherited) mutations in DDR genes, particularly BRCA1/2, are associated with an increased risk for the development of ovarian and breast cancer.
- Recently, studies supported by the PCF have found that ~12% of metastatic prostate cancer patients are carriers of germline DDR mutations, almost half of which are in the BRCA2 gene. Germline DDR mutations were identified in ~5% of men with localized prostate cancer and in 2.7% of individuals without a known cancer diagnosis. This suggests that inherited DDR mutations predispose men to developing more aggressive prostate cancer. Overall, germline mutations account for about half of the CRPC patients whose tumors harbor DDR-mutations (in the other half of patients, mutations or loss of the gene occurs only in the tumor cells).
- In a study at Dana Farber Cancer Institute, 5.6% of CRPC patients (8 of 141) with taxane-resistant disease were found to carry germline mutations in BRCA2. Seven of the eight germline BRCA2-mutation carriers responded to docetaxel plus carboplatin, six of whom experienced a PSA decline of >50%. This data suggests that the presence of a defective germline variant of BRCA2 predicts for responsiveness to DNA damaging agents.
- At the University of Washington, a patient with a germline mutation in the PALB2 DDR gene and a mutation on the second copy of the PALB2 gene in the tumor, exhibited an exceptional response to carboplatin + docetaxel after having failed all other therapies. Additional patients in this PCF sponsored study who carry BRCA2 mutations have also had exceptional responses.
- These studies indicate that prostate cancer patients with germline DDR mutations are likely to benefit from platinum chemotherapy. Further trials are needed to optimize the use of platinum chemotherapy in these patients.
- PARP-inhibitors, which are drugs which target a separate pathway in DNA repair, have demonstrated remarkable efficacy in prostate cancer patients with DDR-mutated tumors.

- The issue of relative efficacy of platinum chemotherapy or PARP inhibitors has not been addressed in prostate cancer but a study in DDR-mutated ovarian cancer found no differences in progression free survival (PFS) between patients treated with PARP-inhibitors and the DNA damaging agent pegylated liposomal doxorubicin (PLD). This suggests that DNA-damaging cytotoxic agents such as PLD have efficacy in some patients that is similar to PARP-inhibitors
- Phase II/III clinical trials led and coordinated by Dr. Montgomery and Dr. Matthew Rettig (UCLA) are being initiated in collaboration with the Prostate Cancer Foundation and the U.S. Department of Veteran Affairs (VA).
- This trial, POPCAP1 (Precision Oncology Program in Cancer of the Prostate), will screen U.S. veterans with metastatic prostate cancer for germline DDR mutations. Mutation carriers will be offered enrollment into either a carboplatin/docetaxel study or phase II and phase III PARP-inhibitor trials (Figure). Non-carriers will receive standard of care therapy, and may have tumors sequenced to identify any whose tumors harbor DDR mutations.
- In summary, both platinum chemotherapy and PARP-inhibitors have demonstrated promising activity against DDR-mutant prostate cancer. Clinical trials need to be conducted to determine the relative efficacy and appropriate sequencing of these agents in DDR-deficient prostate cancer in order to select the treatment that gives the most benefit with the lowest morbidity and cost.

Biallelic Inactivation of BRCA2 in Platinum-sensitive Metastatic Castration-resistant Prostate Cancer

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Eur Urol. 2016 Jun;69(6):992-5

Figure: Clinical course of three CRPC patients with BRCA2-mutant tumors who exhibited profound responses to carboplatin.

POPCAP1 – Germline screening and treatment of HR deficient prostate cancer

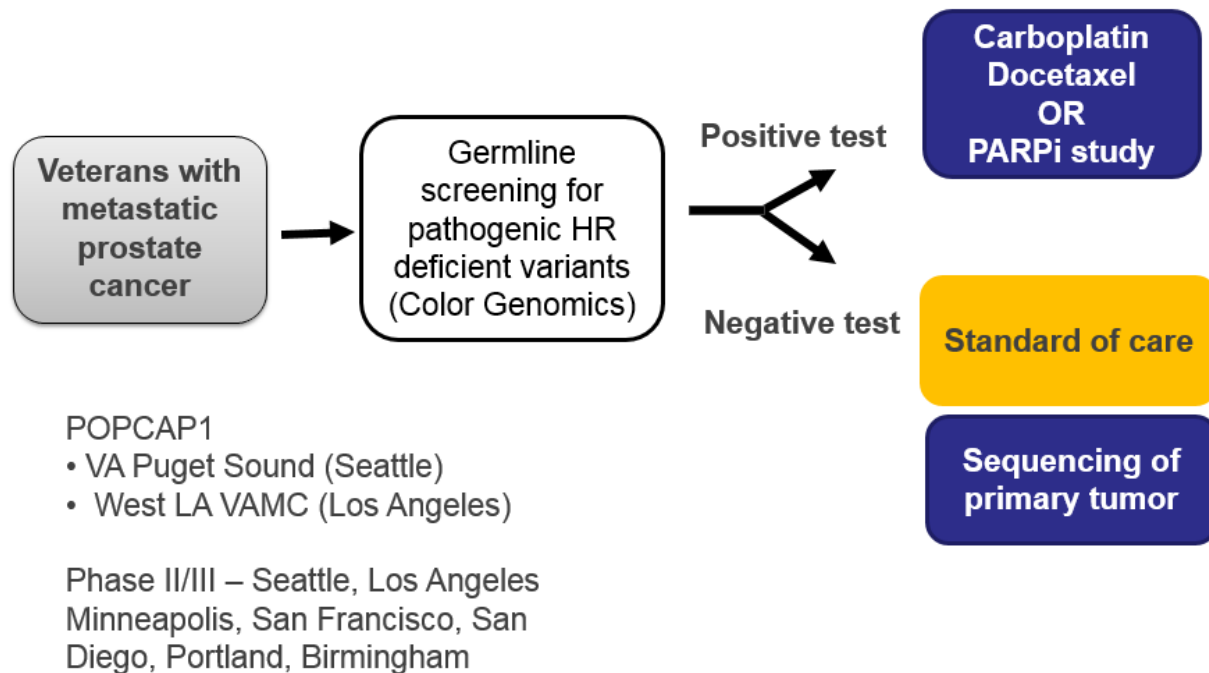


Figure: Outline for the POPCAP1 (Precision Oncology Program in Cancer of the Prostate) trial, which will screen U.S. veterans with metastatic prostate cancer for the presence of inherited DDR mutations. Carriers will be offered enrollment into either a carboplatin with docetaxel trial or PARP-inhibitor trials (Figure). Non-carriers will receive standard of care therapy, and will have tumors sequenced to determine the presence of DDR mutations.

Aggressive Variant Prostate Cancers: Clinical and Molecular Characterization

Ana Aparicio, MD

The University of Texas MD Anderson Cancer Center

- Prostate cancer is not a homogeneous disease, but behaves very differently in different people. Many different prostate cancer phenotypes can arise that have different morphological and molecular features and different treatment responses and clinical outcomes. Morphological features refer to how the tumor cells look under a microscope.
- The typical prostate cancer phenotype is adenocarcinoma. Prostate adenocarcinomas spread mostly to the bones and are driven by the androgen receptor (AR), which is fueled by the male hormone testosterone. These tumors typically shrink when AR is blocked or testosterone is withdrawn.

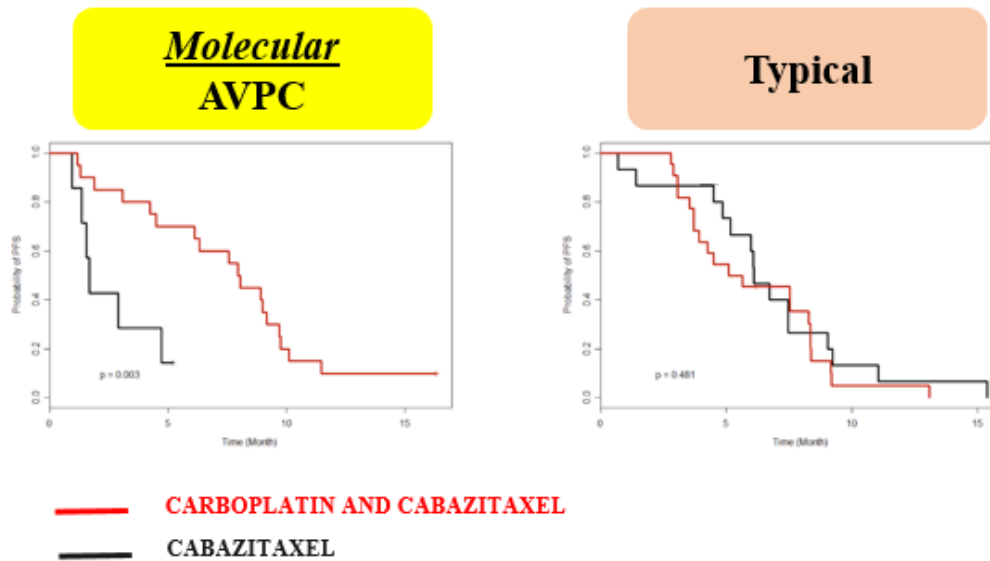
- The morphology of adenocarcinomas resemble that of normal prostate tissue, with glands stacked next to each other, but with imperfections such as different sized nuclei or growth into the surrounding tissues.
- There is a rare morphological prostate cancer variant called “small cell prostate carcinoma” (SCPC), which is composed of sheets of small cells without any glandular formation.
- SCPC does not express AR and is different from adenocarcinoma in that it has “atypical” clinical features (e.g. it will often spread to places other than the bone early on) and a very aggressive course.
- The best treatment for SCPC is combination chemotherapy that includes platinum drugs (carboplatin or cisplatin). The response rate is high but short-lived because small cell carcinomas are often identified late, when there is a large tumor burden.
- Dr. Aparicio presented results from clinical trials that investigated the molecular and clinical features of SCPC and related aggressive prostate cancer variants and their relationship to therapeutic responsiveness.
- In an effort to identify SCPC early, to prescribe chemotherapy before tumors have a chance to grow extensively, Dr. Aparicio and team biopsied lesions of men with prostate cancer that had atypical clinical features and examined their morphology.
- Many of the clinically atypical tumors exhibited adenocarcinoma morphology, but behaved clinically like SCPC.
- To understand whether the clinical features of SCPC, even if the morphological features of SCPC are not present, also predict for a high response to platinum-based chemotherapy, men whose prostate cancers exhibited clinicopathological features associated with SCPC were selected for treatment with platinum-based chemotherapy in a phase II clinical trial.
- The 7 SCPC-associated clinical features used for patient selection included: SCPC morphology; visceral metastases only; lytic bone metastases; bulky lymph node or primary tumors; low PSA levels relative to tumor volume; neuroendocrine markers and increased serum carcinoembryonic antigen (CEA) or lactate dehydrogenase (LDH) levels; or primary castration-resistance.
- This subset of prostate cancers were collectively referred to as clinically-defined “aggressive variant prostate cancers” (AVPC).
- Over 80% of patients with clinically-defined AVPC responded to carboplatin + docetaxel, which was sustained for at least 4 cycles of therapy. This indicates that clinical AVPC features predict for chemotherapy responsiveness, as would be expected if they had displayed SCPC morphology under the microscope.
- Of the 7 clinicopathological AVPC features evaluated, having a bulky primary tumor carried the worst prognosis. This suggests that definitive treatment of the primary tumor may improve the outcome of men with metastatic prostate cancer (including men with AVPC). A number of retrospective and population based studies support this hypothesis and several prospective randomized clinical trials are ongoing to test it.
- However, a wide-held view is that definitive treatment of the primary tumor will benefit predominantly men with a small number of metastases (the so-called 'oligometastatic')

prostate cancers). The data presented by Dr Aparicio do not support this view but rather, that the biological underpinnings of the disease, not limited to its anatomical distribution, will dictate the benefit from definitive treatment of the primary tumor. For this reason, all men with de novo metastatic prostate cancer, regardless of number of metastatic sites, are included in an ongoing prospective randomized phase II study of best systemic therapy, plus or minus definitive treatment of the primary tumor (NCT01751438), led by Dr. Brian Chapin.

- To understand the biology linked to the benefit from these interventions, the molecular features of AVPC tumor samples from participants in the phase II platinum-based chemotherapy study were examined. Over half of the clinically defined AVPC patients were found to harbor ≥ 2 co-occurring genomic alterations in the Tp53, RB1 and/or PTEN tumor suppressor genes. Mutations in these genes have previously been associated with SCPC.
- This genomic signature was used as the molecular definition of AVPC.
- To gain confidence in this signature, samples were examined from a phase I/II clinical trial led by Dr. Paul Corn in which men with metastatic castration resistant prostate cancer (mCRPC) were prospectively stratified into those with typical adenocarcinoma and those with clinical AVPC, and then randomized to receive either cabazitaxel alone or cabazitaxel + carboplatin.
- In this trial, the addition of carboplatin to cabazitaxel prolonged progression free survival (PFS) in the overall population of men with mCRPC. The clinical-AVPC group benefitted slightly more from the addition of carboplatin, indicating that the clinical-AVPC criteria enriched for a platinum-sensitive subset.
- However, when patients were stratified by the molecular-AVPC signature, the difference in benefit derived from the addition of carboplatin was far greater.
- Molecularly defined AVPC patients had a median PFS of 1.69 months with cabazitaxel alone and 7.99 months with cabazitaxel + carboplatin (Figure).
- "Typical" CRPC (molecularly defined non-AVPC) patients had a median PFS of 6.11 months with cabazitaxel alone and of 5.37 months with cabazitaxel + carboplatin (Figure).
- This indicates that patients with the molecular AVPC signature benefitted significantly from the addition of carboplatin, while those without ("typical" CRPC) did not. Moreover, men with the molecular AVPC signature who did not receive carboplatin did significantly worse.
- Analysis of cell free tumor DNA (ctDNA) and circulating tumor cells is ongoing to further expand on these observations. ctDNA is tumor DNA that has been released into the circulation and can be collected from patients via a simple blood draw, eliminating the need for invasive biopsies.
- Recent studies have shown that alterations in DNA damage repair (DDR) pathways are a therapeutically relevant biological feature in prostate cancer.
- Several observations suggest a link between AVPC and DDR pathway alterations, one of which is their unique platinum-sensitivity.
- DDR mutations are also known to sensitize tumor cells to treatment with PARP-inhibitors such as olaparib.

- Preliminary analyses showed that tumors displaying the molecular AVPC signature were enriched for DDR gene mutations.
- This observation led to the initiation of a clinical trial in which molecularly defined AVPC patients will receive six cycles of cabazitaxel + carboplatin. Patients will then be randomized to receive either olaparib maintenance therapy or undergo observation.
- Whether AVPC tumors are more sensitive to immunotherapy is also worthy of consideration.
- In order to integrate these observations with those related to the immune microenvironment of prostate cancer as well as the AR pathway, Dr. Aparicio and team are conducting the 'DynAMo' clinical trial. In this trial, men with mCRPC are allocated to early chemotherapy or randomized to immunotherapy depending on their early response to maximal AR ablation with abiraterone, prednisone and apalutamide.
- This trial will accelerate the development of biologically informed combination therapies in a rational and efficient manner, and validate biomarker signatures such that specific, biologically substantiated therapies can be given earlier in the disease process with curative intent.
- These studies demonstrate that molecular AVPC tumors are a clinically distinct CRPC subset that are highly sensitive to treatment with platinum chemotherapy. The dissemination of this knowledge along with precision medicine strategies to identify molecular AVPC patients is of utmost critical need, so that these patients can receive treatment with a life-prolonging therapy.

Combined Defects in Tp53, RB1 and/or PTEN Predict for Carboplatin Benefit



Credit: Lianchun Xiao

Figure: Patients with molecularly defined AVPC benefited significantly from the addition of carboplatin to cabazitaxel treatment while those with "typical" CRPC did not. Molecular AVPC patients had a median PFS of 1.69 months with cabazitaxel alone and of 7.99 months with cabazitaxel + carboplatin (left). "Typical" CRPC (molecularly defined non-AVPC) patients had a median PFS of 6.11 months with cabazitaxel alone and of 5.37 months with cabazitaxel + carboplatin (right).

Session 7: Targeting Developmental Pathways in Prostate Cancer

Fundamentals of Canonical and Non-Canonical WNT Signaling in Cancer

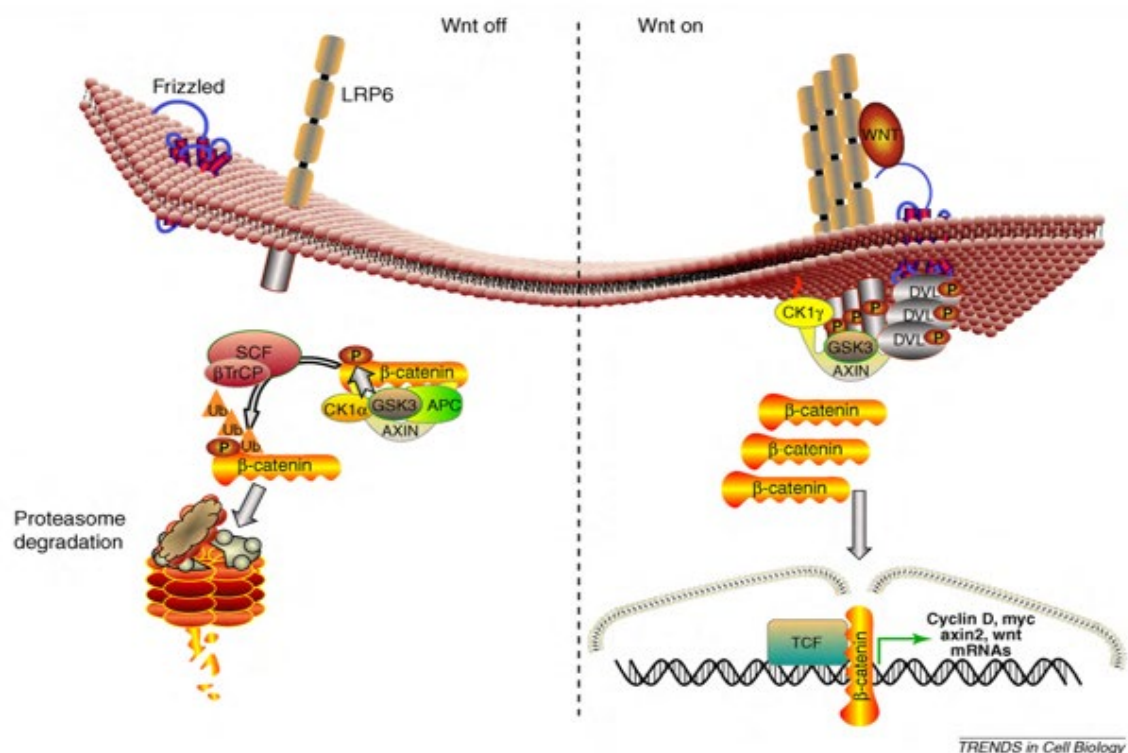
Stuart Aaronson, MD

Icahn School of Medicine at Mount Sinai

- WNT signaling is an evolutionarily conserved molecular pathway that plays a major role during fetal development and in the ongoing maintenance of tissue stem cells.
- The classical WNT pathway is commonly mutated and involved in the development of a large variety of cancer types, especially colorectal cancer.
- Recent genomic studies have found that a subset of prostate tumors possess mutations in components of the WNT pathway.
- Targeting the WNT pathway may be appropriate for some patients with prostate cancer.
- Dr. Stuart Aaronson gave an overview on the role of classical and alternative WNT pathways in driving cancer development and progression.
- In the classic (“canonical”) WNT signaling pathway, cells triggered by WNT molecules activate the β -catenin transcription factor which turns on the expression of genes involved in cell differentiation and cell proliferation (Figure).
- The alternative (non-canonical) WNT signaling pathway shares some components with the canonical pathway but is independent of β -catenin.
- Some tumor types produce their own WNT proteins to drive activation of the WNT pathway.
- WNT pathway mutations have been observed in 2% of primary prostate cancer. These mutations occur primarily in β -catenin.
- In a metastatic prostate cancer cohort, ~18% of tumors harbored WNT pathway mutations in genes including β -catenin, APC, RNF43, ZNRF3, and R-spondin.
- Other studies have implicated a role for the non-canonical WNT pathway in metastatic castrate resistant prostate cancer.
- These studies suggest that aberrations in WNT pathways play an important role in the development of many cancer types including prostate cancer.
- Understanding the role that canonical and non-canonical WNT signaling pathways play in prostate cancer deserves further study.
- A variety of therapeutic approaches have been taken to target the WNT pathway. Inhibitors of the porcupine and tankyrase proteins have exhibited promising activity in preclinical models.

- Due to the critical role of WNTs in regulation of tissue stem cells, caution needs to be taken when targeting the WNT pathway, as side effects including gastrointestinal track toxicities may occur.

Wnt canonical signaling



Davidson and Niehrs (2010)

Figure: In the classic ("canonical") WNT signaling pathway, WNT molecules send signals that activate the β -catenin transcription factor, which turns on the expression of genes involved in cell differentiation and cell proliferation.

Notch Signaling Promotes Prostate Cancer Metastasis

Li Xin, PhD

Baylor College of Medicine

- The prostate is a male organ that functions to secrete sperm-protective factors. The prostate consists of many tiny glands, each composed of a layer of luminal cells which line the lumen (the cell-free space within the gland that collects secretions), backed by a layer of basal cells. There are also interspersed rare neuroendocrine cells, which function to transmit communications between the prostate and neurons.

- Studies have indicated that both prostate basal and luminal cells can be the source of prostate cancer under different conditions.
- Notch is a signaling pathway that regulates cell lineage commitment.
- Activation of the Notch pathway causes cleavage of Notch receptors at the inner cell membrane and release of a protein fragment called the Notch-intracellular domain (ICD). The Notch-ICD moves into the cell nucleus and activates downstream target genes. This process can control the identities of individual cell lineages in a variety of organs.
- Activation of the Notch pathway and upregulation of Notch pathway molecules have been observed in prostate cancer cells, indicating that Notch may play a role in driving prostate cancer.
- Other studies have suggested tumor-suppressive roles for Notch.
- Dr. Li Xin discussed studies conducted to clarify the role of the Notch pathway in normal prostate and prostate cancer.
- To study the role of Notch in prostate cancer, genetically engineered mice were developed that either lacked active Notch signaling or overexpressed an activated version of the Notch protein (Notch-ICD) in prostate cells.
- The Notch pathway was found to play different roles in prostate luminal and basal cells.
- In basal stem cells, Notch cooperated with the TGF β pathway and suppressed cell proliferation.
- In luminal cells, Notch partnered with the NF- κ B pathway and drove cell proliferation and resistance to anoikis. This resulted in the development of precancerous lesions from prostate luminal cells that overexpressed the activated form of Notch.
- Anoikis is a cell death program that is activated when cells detach from their surrounding matrix. Anoikis-resistance is a hallmark of metastatic cancer, allowing cells to detach from tissue sites without dying, and become metastatic.
- These data indicate that Notch may drive the development of prostate cancer from prostate luminal cells.
- Higher expression of Notch-regulated genes was associated with worse clinical outcomes in two independent human prostate cancer datasets.
- In the human prostate cancer dataset generated at the Memorial Sloan Kettering Cancer Center, Notch-regulated gene expression was inversely correlated with expression of the tumor suppressor gene PTEN. The PTEN gene is commonly lost in prostate cancer, which activates the tumor-driving AKT pathway. Enhanced Notch activity may act in synergy with PTEN-loss/ AKT-activation to promote prostate cancer.
- To study the relationship between Notch activity and PTEN-loss, genetically engineered mice were developed that overexpress the activated form of Notch and/or lacked the PTEN gene in prostate cells.
- PTEN-loss alone promoted the development of prostate cancer in mice.

- In mice with both PTEN-loss and Notch-hyperactivity, a morbid increase in abdominal girth developed due to enlargement of organs including the prostate seminal vessels, vas deferens, and epididymis, though not the prostate itself.
- Notch-hyperactivity was found to promote two opposing activities: cell proliferation and cell death, the latter due to increased DNA damage and activation of the tumor suppressor gene p53.
- Interestingly, PTEN-loss/Notch-hyperactive mice had smaller primary prostate tumors than mice with PTEN-loss and normal Notch activity, but increased numbers of metastases.
- Both prostate and seminal vesicles developed primary tumors in this model. Seminal vesicles are glands adjacent to the prostate that produce ~60% of the components of semen. Tumors in seminal vesicles are very rare.
- To determine the origin of metastases in these mice, gene expression patterns from metastases were compared to patterns from normal prostate and seminal vesicle tissues. Results indicated that both prostate and seminal vesicles could have served as the primary tumor source of different metastatic lesions.
- Notch-hyperactive/PTEN-loss mice in which seminal vesicles were removed at a young age (prior to tumor onset), still developed metastases. This confirmed that Notch activation is indeed capable of driving the development of metastatic prostate cancer.
- Notch-hyperactivity promoted the expression of genes involved in epithelial-mesenchymal transition (EMT), including FoxC2. EMT is a change in cell phenotype from epithelial cells which require attachments to other cells and tissue structures, to mesenchymal cells which lack rigid structural interactions with surrounding tissue. EMT is a primary mechanism by which cancer cells acquire the ability to metastasize. FoxC2 was found to be required for metastatic activity in tumor cells from Notch-hyperactive/PTEN-loss mice.
- Overall, these studies demonstrate that activation of the Notch pathway is capable of driving prostate cancer metastasis. Studies to delineate how best to manipulate the Notch pathway for the treatment of prostate cancer are ongoing.

Increased Notch Activity Drives Distal Metastases

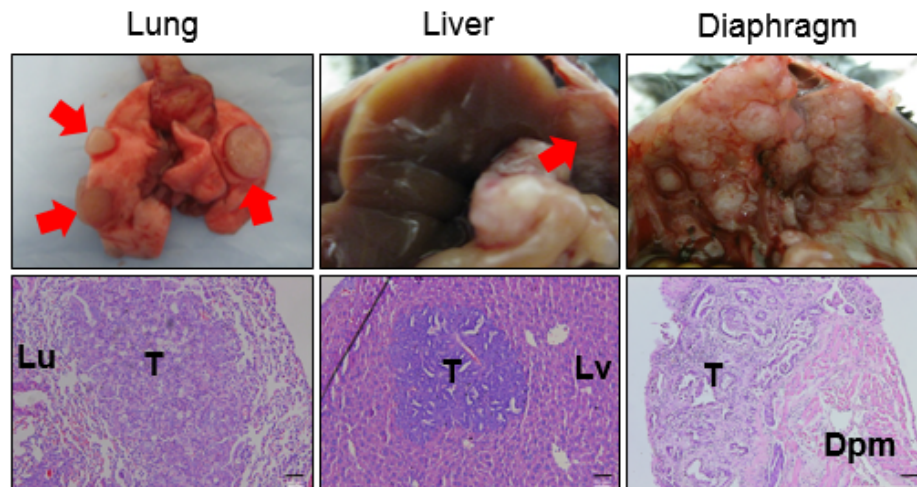


Figure: Notch-hyperactivity increased the development of metastases in PTEN-loss mice. Metastases found in the lung, liver, and diaphragm are noted.

SOX4 in PI3K/AKT and WNT Signaling in Prostate Cancer

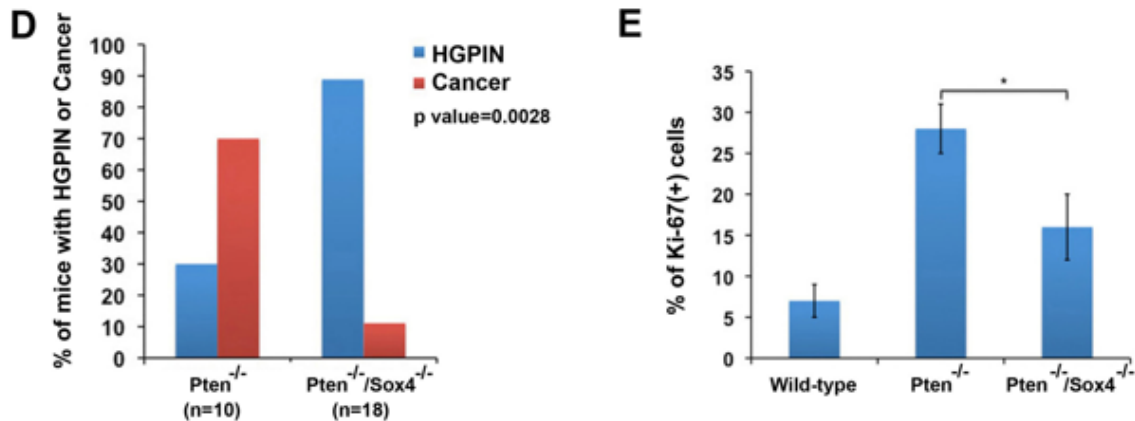
Carlos Moreno, PhD

Winship Cancer Institute at Emory University

- Molecular pathways that regulate development are often misappropriated in cancer cells to support abnormal growth activities.
- SOX4 is a transcription factor with a role in normal development and is expressed in stem cells, progenitor cells, and transit-amplifying cells. These cell types function to maintain tissues by birthing new cells.
- SOX4 is also highly expressed in many cancers including prostate, breast, colon, lung, leukemia, lymphoma, medulloblastoma, and glioblastoma.
- SOX4 has been shown to promote self-renewal of liver tumor-initiating cells.
- In breast cancer, SOX4 is essential for metastasis by turning on epithelial-mesenchymal transition (EMT), a process that allows cells to lose requirements for being attached to other cells and gain the ability to metastasize.
- The role of SOX4 in prostate cancer progression is not yet clear.
- Dr. Carlos Moreno discussed studies examining the role of SOX4 in prostate cancer.
- In human prostate cancer samples, SOX4 expression was found to increase with tumor progression and was highest in metastatic disease.

- Overexpression of SOX4 in prostate cancer cells increased metastatic activities such as cell migration.
- To examine the role of SOX4 in prostate cancer, genetically engineered mice were created that lack the SOX4 gene in prostate cells. These mice were also engineered to lack the PTEN tumor suppressor gene, which acts to suppress the tumor-promoting AKT pathway. In PTEN-null cells, the AKT pathway becomes hyperactivated and drives the development and progression of prostate cancer.
- PTEN-null mice developed invasive prostate tumors. However, mice that additionally lacked SOX4 only developed precancerous lesions (prostatic intraepithelial neoplasia) that did not progress to invasive cancer (Figure). This suggests that SOX4 is required for tumor development driven by PTEN-loss/AKT-activation.
- Examination of tumors from these mice confirmed that the AKT pathway was turned on by PTEN-loss, but turned off when SOX4 was additionally deleted.
- In PTEN-null prostate cancer cells from mice, SOX4 expression was highly enhanced.
- SOX4 was found to be highly expressed in PTEN/AKT-mutant human prostate cancer samples compared with prostate tumors lacking those mutations.
- Treatment of prostate cancer cells with AKT pathway-inhibitors reduced the expression of SOX4. Inhibition of other prostate cancer-driving pathways (androgen receptor, MEK) did not affect SOX4 expression.
- These data suggest that AKT activity resulting from PTEN-loss drives the expression of SOX4, which then feeds back to promote further AKT activity.
- Genes regulated by SOX4 include other developmental pathway genes, such as Notch and WNT. These developmental pathways are commonly hijacked by cancer cells to promote tumor growth and progression.
- SOX4 was found to directly interact with the WNT pathway facilitator, β -catenin and promoted the ability of β -catenin to induce expression of WNT pathway-regulated genes.
- β -catenin activity was increased in PTEN-null prostate cancer cells. However, when SOX4 was deleted in PTEN-null cells, β -catenin activity was turned off.
- These studies indicate that β -catenin activity requires the activation of the AKT pathway, which requires SOX4.
- Collectively, these studies demonstrate that SOX4 is required for activation of the AKT pathway following PTEN-loss, and drives the development of prostate cancer. SOX4 can also promote the activity of other pro-cancer pathways, such as WNT/ β -catenin.
- Targeting SOX4 may have promise for the treatment of PTEN-null tumors, which comprise the majority of advanced prostate cancer cases.
- Future studies will further clarify the role for SOX4 in prostate cancer and explore the potential for targeting SOX4 for the treatment of prostate cancer.

Sox4 is essential for Pten-mediated Prostate Carcinogenesis



Bilir et al, *Cancer Res.* (2016) **76**(5):1112-21.

Figure: Mice with PTEN-loss developed invasive prostate cancer, while mice that additionally lack SOX4 only developed precancerous lesions (high grade prostatic intraepithelial neoplasia, HG PIN) (left). PTEN-loss promoted prostate cancer cell proliferation (%Ki-67+ cells), which was reduced in cells that additionally lack SOX4 (right).

Pharmacologically Targeting WNT Signals in Cancer Stem Cells

Michael Kahn, PhD

University of Southern California

- The WNT signaling pathway is a critical regulator of embryonic development and maintenance of tissue stem cells.
- The WNT pathway is commonly hijacked in cancer and is particularly important for the development of colorectal cancer.
- Activation of the WNT pathway culminates in activation of β -catenin, a protein that regulates the expression of genes involved in development, cell proliferation, and cell migration. β -catenin partners with other gene expression-regulating proteins such as CBP and p300 to control the expression of WNT pathway-regulated genes.
- Dr. Michael Kahn discussed strategies to target the β -catenin/CBP interaction to eliminate cancer stem cells, a population of specialized cancer cells with stem cell properties that are thought to continuously regenerate the entire tumor cell population.
- Normal stem cells sustain both stem cell and differentiated cell populations by undergoing asymmetric cell division. This produces one cell that retains stem cell identity and a second

cell that becomes a transit-amplifying cell. Transit-amplifying cells undergo many symmetric cell divisions and eventually differentiate into normal tissue cells.

- Like normal tissues, a cancer stem cell population sustains tumors. However, cancer stem cells preferentially undergo symmetric division, which generates two cancer stem cells, increasing the size of the cancer stem cell population and "crowding out" normal stem cells. Manipulating cancer stem cell division can drive cancer stem cells to differentiate, thereby reducing the stem population and increasing sensitivity to cytotoxic therapies.
- CBP and p300 are two related genes that function as major regulators of gene expression.
- The WNT pathway regulates two apparently divergent outcomes of stem cell activity: stem cell proliferation and differentiation. The choice between these two outcomes depends on whether β -catenin partners with CBP or p300 to regulate gene expression. Partnering with CBP induces maintenance of potency and proliferation, which expands the stem cell population. Partnering with p300 initiates the expression of cell differentiation genes, leading to more differentiated cells. Many cellular factors likely affect the balance between binding of β -catenin to CBP or p300.
- A screen of small molecule inhibitors in colorectal cells identified ICG-001 as a modulator of β -catenin activity. ICG-001 was found to strongly bind to CBP, thereby disrupting the CBP/ β -catenin interaction and block expression of cell survival genes that are critical in cancer cells. This tool compound can be used as a selective inhibitor of the β -catenin/CBP interaction which regulates stem cell proliferation and maintenance.
- Stem cells from mice lacking the stem cell regulator p73 exhibit a reduced amount of asymmetric divisions (which generate one stem cell and one transit-amplifying cell). Treatment of p73-null mice with ICG-001 reactivated asymmetric stem cell division in neural stem cells indicating that ICG-001 regulates stem cell maintenance.
- Cancer stem cells are required for the establishment of new "metastatic" disease, drug resistance and disease relapse.
- Leukemia stem cells treated with ICG-001 and then implanted under the skin of mice still grow. However, when these tumor cells were extracted and engrafted into the bone marrow of new mice, they no longer possessed the ability to form leukemia, whereas originally untreated leukemia stem cells put through this process retained the ability to generate leukemia. This suggests that ICG-001 eliminated the leukemia stem cells from the tumor population.
- Treatment of mice bearing leukemia tumors with ICG-001 in combination with nilotinib (a Bcr-Abl kinase inhibitor used to treat leukemia) resulted in complete leukemia elimination, which did not recur for the life of the mouse (Figure). Treatment with nilotinib alone initially reduced the leukemia burden, but the leukemia eventually recurred as lethal disease due to the leukemia stem cells. Similar results were observed when ICG-001 was combined with VDL (vincristine, dexamethasone, and L-asparaginase).
- A second generation CBP/ β -catenin antagonist, PRI-724, was developed by Prism Pharma and exhibited low toxicity in preclinical studies and phase Ia clinical trials.
- Phase Ib clinical trials for PRI-724 in patients with various tumor types have been initiated.

- These studies suggest that targeting the β -catenin/CBP interaction can tip the balance in the cancer stem cell population from proliferation to differentiation, effectively reducing the cancer stem cell population, without deleterious effects on normal stem cell populations. This may be a promising therapeutic strategy to eliminate the cancer stem cell population and prevent tumor recurrence, drug resistance and metastasis.

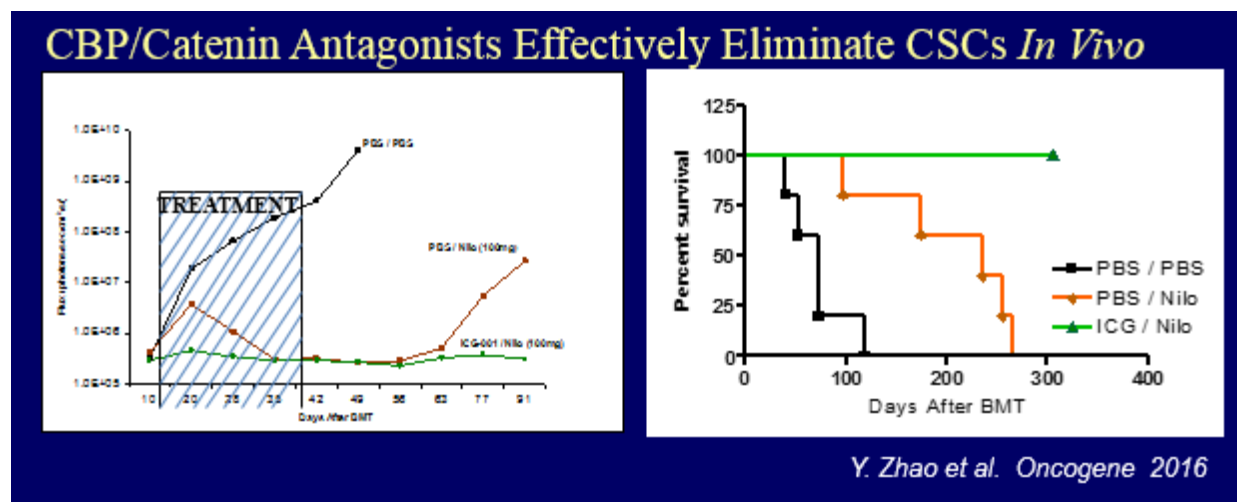


Figure: Treatment of mice bearing leukemia with ICG-001 in combination with nilotinib resulted in complete leukemia elimination which did not recur for the life of the mice (green). (Actually the mice live for 2 years as long as their control litter mates, but this is a separate figure). Treatment with nilotinib alone resulted in initial leukemia regression which eventually recurred as lethal disease (red). Left: Leukemic load over time following different treatments. Right: Percent survival of leukemia bearing mice treated with different regimens. PBS = saline control (black).

Special Announcement: PyL Molecular Imaging Clinical Investigation

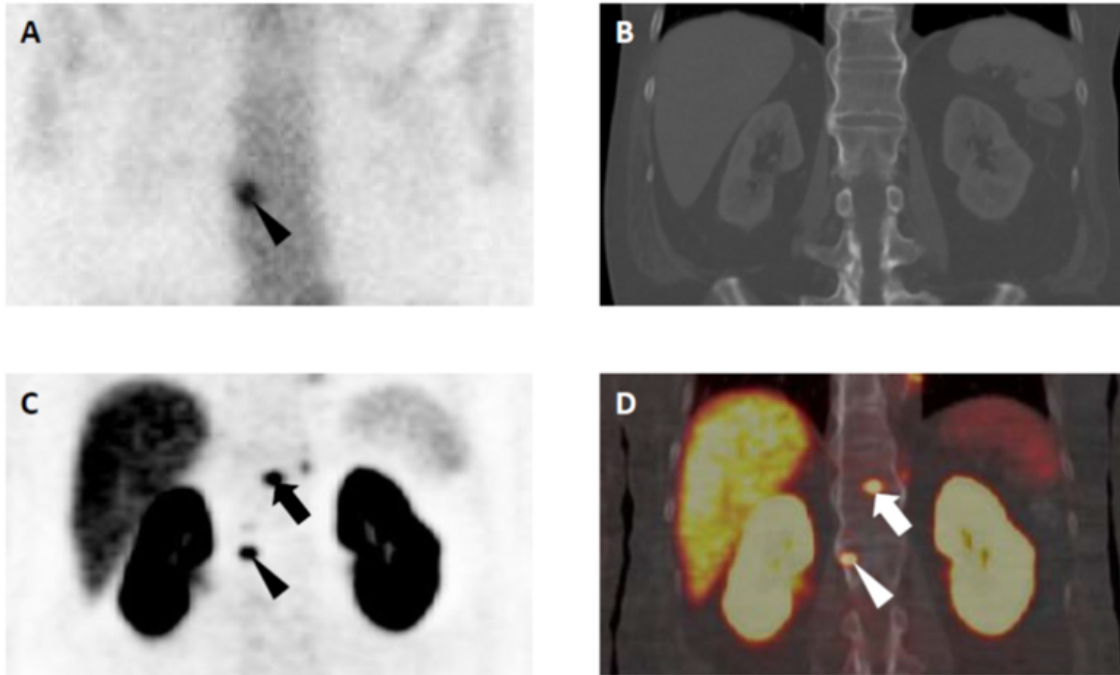
Mark Baker, JD

Progenics Pharmaceuticals

- Designing the most optimal treatment strategies for patients relies on accurate patient staging, including detection and accurate identification of the location and number of metastatic lesions. Advancing these efforts requires the development of new molecular imaging technologies with improved sensitivity and specificity for detecting prostate tumors.
- 18F-DCFPyL (PyLTM) is a radiolabeled small molecule that binds with high affinity to the extracellular domain of prostate specific membrane antigen (PSMA), a protein expressed by virtually all prostate cancers.
- Preliminary data has shown that PyL positron emission tomography (PET) is more sensitive and specific for the detection of metastatic prostate cancer than standard imaging modalities (bone scans and CT) (Figure).

- Definitive positive clinical evidence for PyL is needed before this imaging modality can receive FDA approval and become a standard of care practice.
- A phase II/III clinical trial (OSPREY) is being conducted to assess the diagnostic performance of PyL PET/CT imaging to detect prostate cancer in the prostate gland and sites of metastasis or local recurrence.
- PyL was developed at Johns Hopkins University and was licensed to Progenics Pharmaceuticals, Inc. who has undertaken further clinical development.
- Mark Baker, CEO of Progenics, announced a special initiative by his Company in which PyL will be made freely available to the scientific community for investigational research studies. It is hoped that the PyL imaging data generated from this initiative could be useful in further understanding prostate cancer biology and may lead to better disease management.
- Beginning in January 2017, 18F-radiolabeled PyL may be available for investigational studies at no cost; or, researchers may obtain PyL precursor and perform radiolabeling at their own qualified facilities.
- To obtain PyL, researchers must be part of an academic institution, a non-profit organization, or a for-profit company, who are able to hold an IND and conduct sponsored clinical trials.
- Researchers must agree to use PyL as specified by Progenics. Researchers must also agree to share their data including images and minimal clinical information, as part of an open source, publically available, cloud-based database for prostate cancer research.
- This offer was initially open only to PCF researchers; however, in 2017, PCF researchers will be able to recommend other researchers to whom this resource can be made available.
- In January 2017, Progenics will also make a web-based version of the Bone Scan Index freely available to PCF researchers. This FDA-approved technology quantifies the burden of disease in metastatic prostate cancer patients using technetium bone scans.
- The information necessary to access the PyL Research Access Program™ online was provided to the attendees of the 2016 Annual PCF Retreat.

Imaging metastatic prostate cancer with DCFPyL



A) ^{99m}Tc -MDP bone scan; B) CT scan; C) DCFPyL PET scan; D) DCFPyL PET/CT scan

Figure: ^{18}F -DCFPyL has a higher sensitivity for metastatic prostate cancer compared with current standard imaging modalities (bone scans and CT).

Session 8: New Platforms to Attack the Undruggable Targets

Targeting Refractory Cancer Drivers using Cell-Penetrating Miniproteins

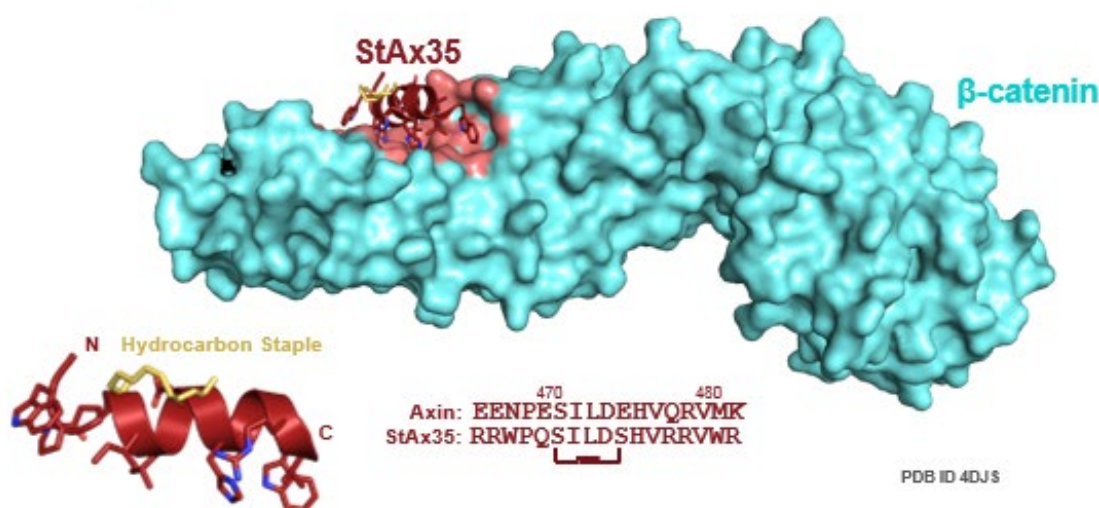
Gregory Verdine, PhD

Fog Pharmaceuticals, Inc.

- More progress has been made identifying new targets for cancer therapy than successfully developing new therapeutics. Major drivers of cancer including Ras, Myc, β -catenin, and Notch represent complex targets that have not been successfully drugged to date.
- Larger biological therapeutics such as antibodies have proven to be versatile therapies with great specificity and potency but to date only target extracellular molecules and do not enter cells.
- Small molecule inhibitors are able to penetrate cells and target intracellular molecules. However, ~90% of proteins lack a “greasy pocket” motif required for binding by small molecule inhibitors.
- Finding new methods to target currently undruggable intracellular cancer drivers is critical for the advancement of new therapies for patients with cancer.
- Dr. Gregory Verdine discussed the development of a novel class of cell-penetrating miniproteins (CPMPs) that are able to target intracellular proteins.
- CPMPs are synthetically produced, hyper-stabilized peptides consisting of short protein sequences that are “stapled” with a brace that maintains their 3D structure. The specialized structure of CPMPs confers resistance to protein-degrading enzymes and enables cell penetration. CPMPs possess desirable pharmacological properties including a long half-life in the body, and lack of immune-activating features.
- The development of CPMPs typically involves identification of a helical (coil-structured) peptide that blocks the activity of the intended target. The peptide is then chemically “stapled” to generate a CPMP.
- A CPMP that targets the tumor-driving genes HDM2 and HDMX has been developed and is currently in phase I clinical trials.
- A CPMP that targets β -catenin has also been developed and optimized and is being tested in preclinical cancer models (Figure).
- A mini-protein (SAHM1) that targets Notch was identified and used to derive a Notch-targeting CPMP. Treatment of leukemia cells with SAHM1 blocked the expression of Notch-regulated genes. SAHM1 treatment also blocked the growth of acute T-cell leukemia in mice.
- Developing CPMPs for targets that do not interact with known helical peptides, such as the Ras tumor-driver, is more complicated.

- Specialized techniques are being developed to discover and create novel helical molecules that can act as CPMPs. Using this technology, a potential CPMP for Ras has been discovered and is being tested and refined in preclinical models.
- These studies demonstrate that stapled cell-penetrating mini-proteins are promising new drug modalities that can target currently undruggable intracellular cancer drivers.
- FogPharma has set aggressive goals to develop CPMPs suitable for clinical trials in 2017 and to enter clinical trials shortly thereafter.

Discovery of direct-acting β -catenin antagonists



Grossman, TN; Yeh, JT; Bowman, BR, Chu, Q; Moellering, RE; Verdine, GL
PNAS 109, 17942 (2012).

Figure: StAx35, a CPMP that targets β -catenin, has been discovered and is being optimized and tested in preclinical cancer models.

Drugging the Spliceosome: Therapeutic Modulation of Splicing in Human Malignancies

Markus Warmuth, MD
H3 Biomedicine, Inc.

- Genes are often not encoded in a contiguous sequence within the genome. Instead, genes can be encoded in several pieces called “exons,” that are interrupted by non-coding sequences called “introns.”

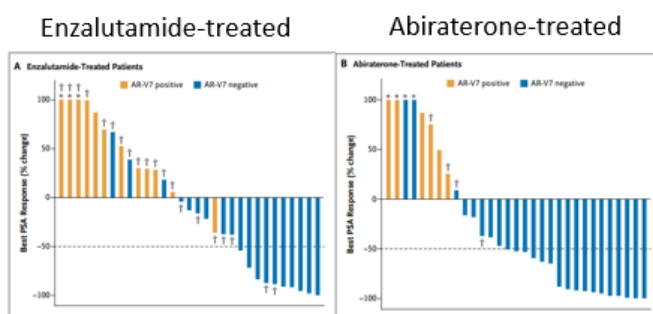
- In the process of translating genes into proteins, a premature RNA is first transcribed that includes both exons and introns. A process called “splicing” removes the introns to generate a mature RNA, which is then translated into proteins.
- RNA splicing is performed by the spliceosome, a large complex of many proteins.
- Spliceosome dysfunction can cause abnormal retention of introns or removal (skipping) of exons and result in the production of proteins with altered or no function.
- A number of spliceosome genes are mutated in many cancer types. Recurrent mutations have been observed in the SF3B1, U2AF1, SRSF2 and ZRSR genes, which are core components of the spliceosome. These mutations result in aberrant splicing of many genes.
- Dr. Markus Warmuth discussed how aberrant splicing can be targeted as a novel cancer therapeutic approach.
- Cancer-associated mutations in splicing genes were always observed on only one copy of the gene, while the other copy remained normal. This indicates that slightly altered splicing is beneficial for cancer cells, while any further splicing alterations could not be tolerated and would result in cell death. Thus, splicing mutations may be an Achilles’ heel of the cancer cell that can be therapeutically exploited to kill cancer cells while leaving normal cells unharmed.
- A number of naturally occurring small molecules that inhibit splicing have been identified.
- Compounds were identified that selectively kill cells harboring a SF3B1-mutation but not SF3B1-normal cells.
- A promising candidate was identified and optimized to derive an orally-available lead compound, H3B-8800.
- H3B-8800 exhibited highly selective killing of SF3B1-mutant cancer cell lines and prevented the growth of SF3B1-mutant tumor cells in mice.
- To study potential mechanisms of resistance to H3B-8800, a cancer cell line sensitive to splice modulation was treated with H3B-8800 until resistant cells developed. These cells were found to have acquired drug resistance mutations in the SF3B1 and PHF5A genes.
- Examination of the 3D structure of the core spliceosome revealed that all of the H3B-8800-resistance mutations occurred in the regions of SF3B1 and PHF5A that interact with the RNA being spliced.
- H3B-8800 was found to impair spliceosome activity by causing the retention of introns rich with GC-nucleotides, a feature common in splicing genes. H3B-8800 specifically affected the splicing of RNA that encode for splicing components, setting off a cascade of events resulting in cell death.
- Different splicing modulators can differentially affect splicing by interacting with different regions of RNA or the spliceosome. Splicing modulators that can selectively target certain types of splicing events are being developed.
- Altered splicing plays a role in the development of some forms of castrate resistant prostate cancer (CRPC), including neuroendocrine prostate cancer and AR-V7-positive prostate cancer.

- The androgen receptor (AR) is the primary driver of prostate cancer and remains a critical therapeutic target. Resistance to the AR-targeting therapies enzalutamide and abiraterone is associated with expression of AR-V7, an alternatively spliced AR protein that lacks the region targeted by these therapies and is constantly activated.
- Treatment of AR-V7-expressing prostate cancer cell lines with a splicing modulator blocked the expression and activity of AR-V7 (Figure). This demonstrates promise for splicing modulators for the treatment of prostate cancer.
- A precision medicine strategy is being developed that will generate RNA splicing profiles from tumors and match them to the splicing modulator most likely to be effective.
- In summary, using precision medicine to target aberrant RNA splicing in tumor cells is a promising approach for the treatment of prostate and other cancers. These compounds are undergoing further preclinical optimization in preparation for clinical trials.

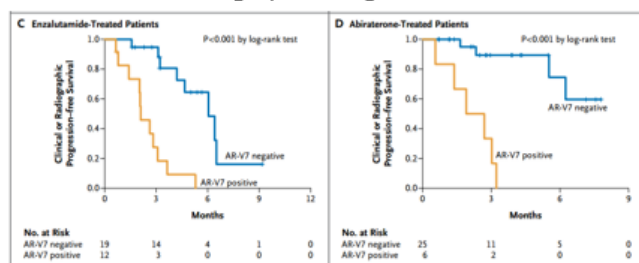
Splicing modulation of AR pathway might overcome resistance to anti-AR treatment



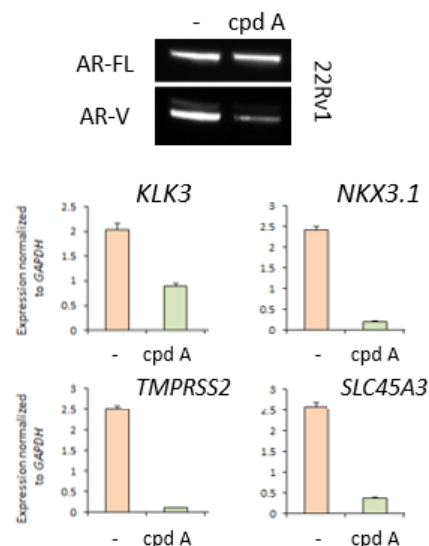
Prostate-Specific Antigen (PSA) Responses



Clinical or Radiographic Progression-free Survival



H3 splicing modulators downregulate AR-V7 and AR target genes



H3 Biomedicine – PCF Retreat

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Figure: Resistance to enzalutamide and abiraterone is associated with expression of AR-V7 (left). Treatment of AR-V7 expressing prostate cancer cell lines with a splicing modulator (cpd A) blocked the expression of AR-V7 and AR-regulated genes KLK3, NKX3.1, TMPRSS2, and SLC45A3 (right).

Expression Studies Implicate Neuroendocrine Prostate Cancer as a Novel Indication for Rovalpituzumab Tesirine – A Delta-Like Protein 3 (DLL3)-Targeted Antibody-Drug Conjugate (ADC)

Laura Saunders, PhD
AbbVie-Stemcentrx LLC

- The expression of neuroendocrine genes is a hallmark of neuroendocrine prostate cancer (NEPC), a highly aggressive form of castrate resistant prostate cancer (CRPC).
- Other cancers can also exhibit neuroendocrine features, including small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC), two subtypes of highly aggressive lung cancer.
- Identifying therapies for NEPC is a crucial need and may be accelerated by progress made in other neuroendocrine cancer types due to overlapping mechanisms.
- Dr. Laura Saunders discussed the therapeutic targeting of Delta-Like Protein 3 (DLL3), a protein expressed in SCLC and LCNEC, which is also expressed in NEPC.
- DLL3 is normally expressed in the developing brain and lung, residing in the Golgi apparatus.
- DLL3 inhibits the Notch signaling pathway, a major embryonic regulator of development. DLL3 acts by retaining Notch receptors within the cell, preventing them from reaching the cell surface where they can interact with their ligands to become activated.
- In normal lung development, upregulation of ASCL1 and downregulation of Notch promotes differentiation of lung progenitor cells into cells of neuroendocrine lineage instead of an epithelial lineage.
- In SCLC, almost universal loss of the tumor suppressor genes p53 and RB1 can skew this pathway by increasing ASCL1 activity, prompting lung cancer cells to differentiate into neuroendocrine cells. ASCL1 drives DLL3 expression, which may alone mediate oncogenic suppression of Notch signaling.
- DLL3 was found at high levels on the surface of SCLC cells, suggesting that DLL3 may be a good antibody therapeutic target in neuroendocrine lung cancer.
- Antibodies that can specifically target DLL3 were developed.
- An antibody-drug conjugate, Rovalpituzumab Tesirine (Rova-T), was developed by linking the DLL3-specific antibody to a pyrrolobenzodiazepine (PBD) dimer toxin.
- Binding of Rova-T to DLL3 on the cell surface triggers internalization of Rova-T, followed by release of the PBD toxin that kills cells by damaging DNA.
- Treatment of mice bearing SCLC tumors with Rova-T resulted in sustained tumor regression. Rova-T was significantly more effective than standard-of-care cisplatin/etoposide chemotherapy in shrinking tumors and preventing recurrence of established SCLC and LCNEC tumors in mice.

- The efficacy of Rova-T directly correlated with the expression level of DLL3 in tumor cells.
- When tumors were removed from mice following the initiation of treatment but prior to complete regression, those from mice treated with Rova-T were unable to form tumors when transplanted into new mice. This suggests that Rova-T eliminated “tumor-initiating cells,” a population of stem-like cancer cells that serve as the source of new tumor cells. Treatment with cisplatin/etoposide did not eliminate tumor-initiating cells.
- Rova-T was tested in a phase I dose-escalating trial in SCLC patients. At active doses of Rova-T (0.2-0.4 mg/kg), responses were observed particularly in patients whose tumors expressed higher levels of DLL3 (Figure).
- At least two DLL3-high, third-line SCLC patients who received 3 doses of Rova-T remain alive and progression-free for over 2 years without any further treatment.
- Based on these studies, Rova-T represents a promising new therapy for SCLC cancer patients whose tumors express DLL3. This is the first biomarker-directed therapy in SCLC.
- Comparing phase I Rova-T data in patients in whom DLL3 was expressed in $\geq 50\%$ of tumor cells, Rova-T outperformed confirmed response rate and overall survival benchmarks for topotecan as a second line therapy and conventional chemotherapy as a third line therapy. The response rates for Rova-T were similar when used as second or third line therapy.
- DLL3 expression is also common in melanoma, small cell bladder cancer, neuroendocrine pancreas cancer, neuroendocrine colorectal cancer, medullary thyroid cancer and glioblastoma.
- Interrogation of RNA expression databases showed that expression of DLL3 in prostate cancer is associated with a neuroendocrine phenotype. DLL3 was not expressed in benign prostate tissues or prostate adenocarcinoma, while expression was observed in some NEPC. These results suggest that targeting DLL3 with Rova-T may be a promising treatment option for select prostate cancer patients.
- Ongoing studies are evaluating DLL3 expression at different stages of prostate cancer progression. Whether the expression of DLL3 associates with factors including neuroendocrine marker expression, Notch pathway activity, androgen receptor (AR) pathway activity, NEPC-associated genomic alterations, PSA levels, and clinical outcomes is being studied.
- Preclinical and early clinical studies evaluating the efficacy of Rova-T in prostate cancer are planned and underway (NCT02709889).
- These studies nominate Rova-T as a promising treatment for DLL3-expressing tumors including NEPC.

Rova-T: Best Response Data in Evaluable DLL3^{Hi} Patients

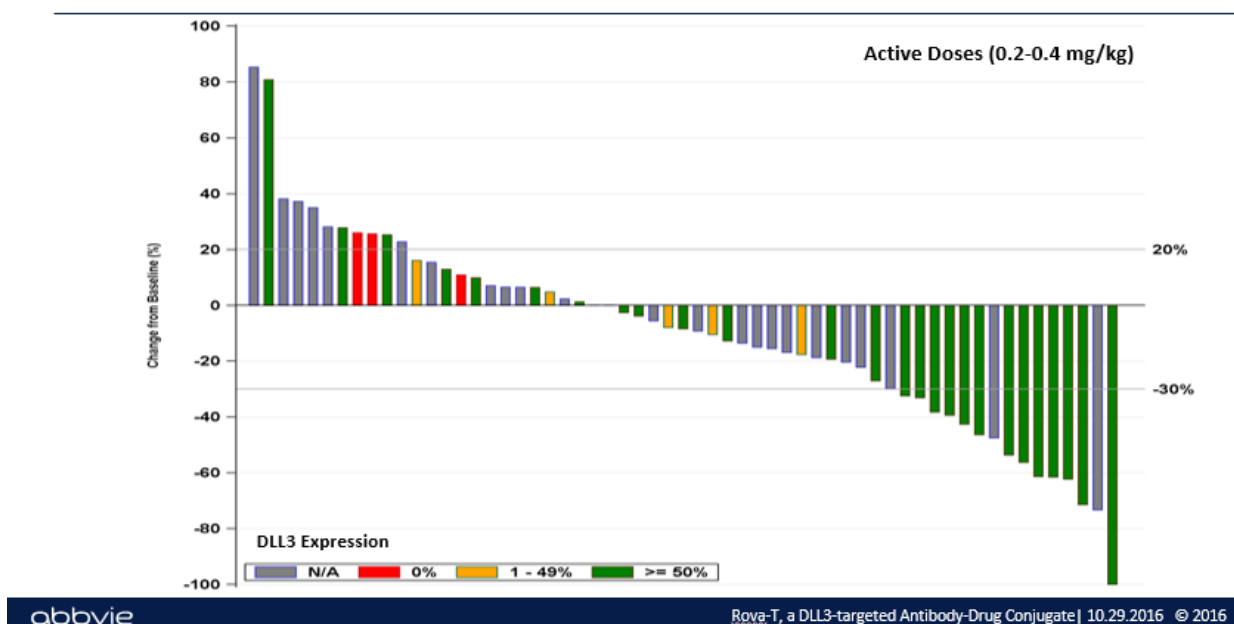


Figure: Rova-T was tested in a phase I dose-escalating trial in SCLC patients. At active doses of Rova-T (0.2-0.4 mg/kg), responses were observed particularly in patients whose tumors expressed higher levels of DLL3. Depicted is the change in tumor size from baseline for each patient in the cohort. Colors of bars indicate degrees of DLL3 expression in tumors.

Session 9: Precision Survivorship

Precision Survivorship: A Personalized Medicine Approach to Prostate Cancer Survivorship

Alicia Morgans, MD, MPH

Vanderbilt University Medical Center

- Precision survivorship is the idea that an individual's unique characteristics, including genetics, lifestyle, and environment, can affect the risk of developing various complications from cancer treatments.
- In precision medicine, a patient's unique characteristics such as clinical factors, biomarkers, and genetics or molecular markers are used to select treatments predicted to be of greatest benefit. These same factors may be predictive of treatment-related complications and are a subject of precision survivorship studies.
- Dr. Alicia Morgans discussed a precision survivorship initiative that will address topics related to complications associated with prostate cancer treatments.
- In addition to bone and metabolic complications, effects on cognitive and cardiovascular function have recently been identified mostly in observational studies. Further studies are needed to validate and better understand these associations.
- The goals of precision survivorship studies include developing methodology to prospectively assess treatment-related complications, validating associations between treatments and complications previously made by observational studies, developing biomarkers to identify vulnerable populations, and improving our knowledge of the biology of complications to enable the development of prevention and treatment strategies.
- Past prostate cancer precision survivorship efforts, with significant PCF funding, include identification of the association between hormonal therapy and bone complications, which led to the development of bisphosphonates and RANK ligand inhibitors to limit bone damage.
- Observational studies have reported associations between androgen receptor (AR)-targeted therapy and cognitive complications in prostate cancer patients. Prospective clinical trials are needed to validate these findings.
- To address the potential relationship between AR-targeted therapy and cognitive complications, Dr. Morgans is initiating the PCF-supported COGCaP clinical trial (Figure).
- Men with metastatic castrate resistant prostate cancer (mCRPC) without dementia or prior to chemotherapy exposure who are initiating AR-targeted therapy will be randomized to receive either abiraterone or enzalutamide.
- Cognitive testing will be performed at baseline, and at 3 months, 6 months, and 12 months after treatment initiation to identify any associations between AR-targeted therapy and the development of cognitive impairment over time. Patients will undergo cognitive tests

performed by neuropsychologists as well as take a computer-based cognitive assessment test. A secondary objective of the study is to validate the ability of the computer-based test to assess cognitive function and identify cognitive impairments in this population.

- Advanced imaging techniques will be used to examine functional and structural changes in the brain of these patients to support studies examining the biology of any cognitive changes found.
- Patient genetics will also be assessed to identify any genetic factors that predispose individuals to developing treatment-related complications.
- This collaborative study will be conducted at multiple centers including Vanderbilt University, University of San Francisco (Dr. Charles Ryan) and University of Southern California (Dr. Tanya Dorff).

COGCaP Schema

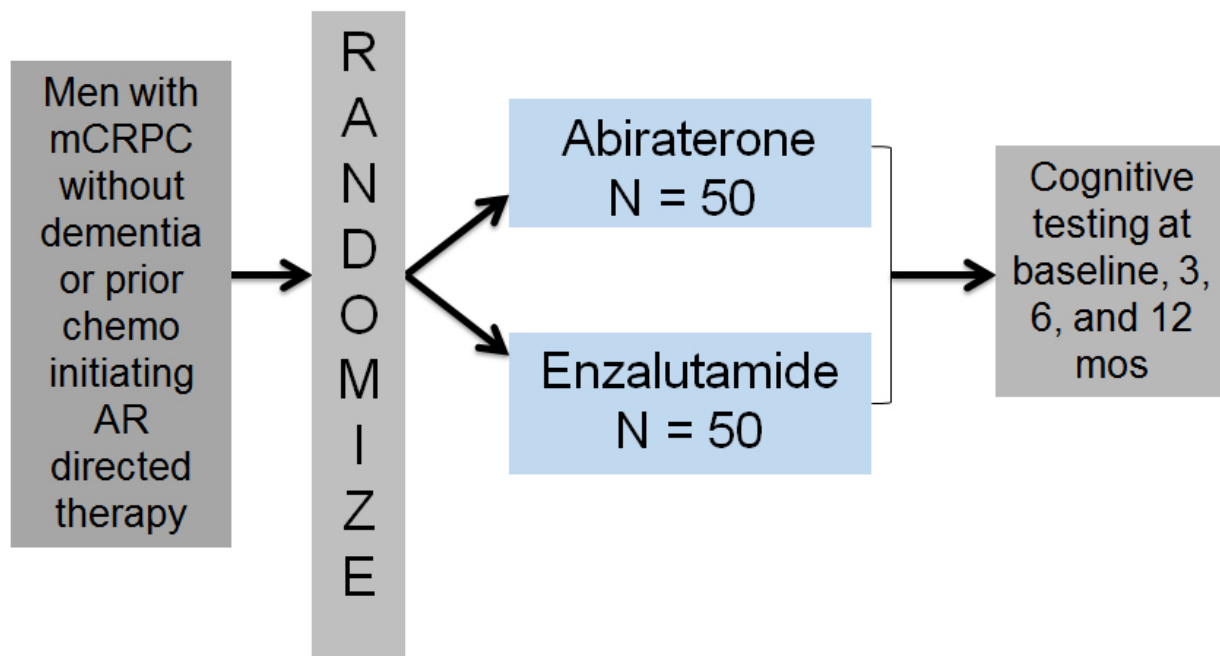


Figure: In the PCF-supported COGCaP clinical trial, men with metastatic castrate resistant prostate cancer (mCRPC) without dementia or prior to chemotherapy exposure who are initiating AR-targeted therapy will be randomized to receive either abiraterone or enzalutamide. Cognitive testing will be performed at baseline, and at 3 months, 6 months, and 12 months to identify any associations between AR-targeted therapy and the development of cognitive impairment over time.

ADT and the Brain: The Unwanted Target

Charles Ryan, MD

University of California, San Francisco

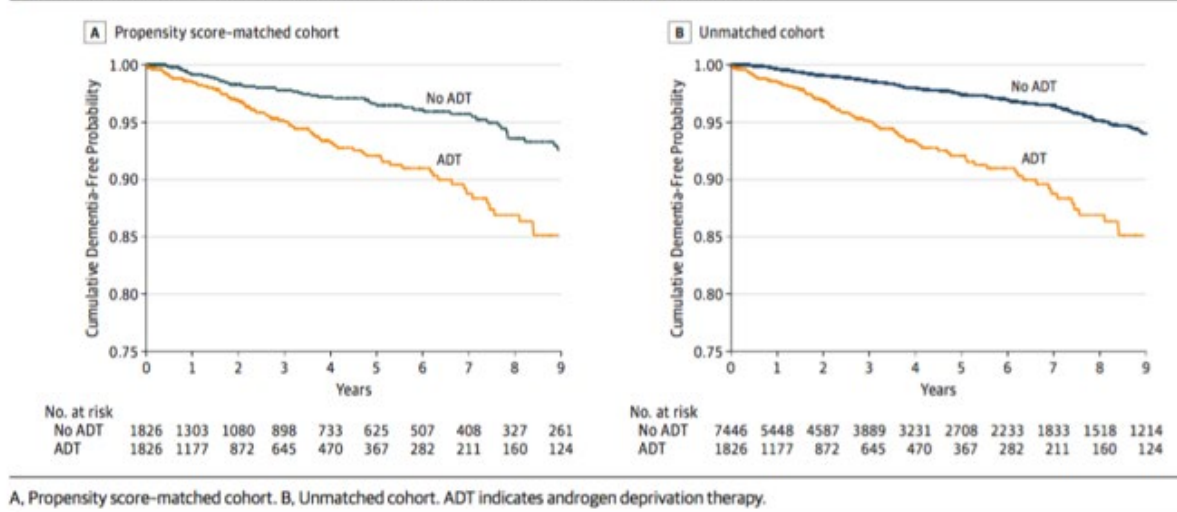
- The androgen receptor (AR) is the primary driver of prostate cancer and is the target of mainstay therapy including androgen deprivation therapy (ADT) and the second generation androgen axis-inhibitors, abiraterone and enzalutamide.
- However, AR can be expressed on tissues outside of the prostate and contribute to their functions.
- This has led to concerns that ADT, which is prescribed for years to decades, may have negative effects on other AR-expressing tissues that affect patient quality of life.
- Dr. Charles Ryan discussed biological evidence and critical clinical questions regarding potential associations between treatment with ADT and the onset of cognitive disorders.
- AR is widely expressed in the brain and is postulated to play a neuro-protective role by prolonging neuron lifespan, preventing neuron deterioration, and decreasing levels of β -amyloid, a causative factor of Alzheimer's disease.
- The activity of AR is regulated by androgens, which are male hormones including testosterone.
- The level of androgens in the brain declines with age. Estrogens do not undergo an age-related decline. This suggests that decreased levels of androgens may cause age-related cognitive decline.
- Low levels of testosterone in the brain have been observed in individuals with mild cognitive impairment.
- ADT has been observed to shrink the brain following 6 months of treatment.
- These studies indicate that ADT may have a negative impact on cognitive function by blocking the neuro-protective activity of AR. Randomized, prospective clinical trials are required to confirm these observations.
- These observations prompted studies examining the effects of ADT on cognitive function in prostate cancer patients.
- A study was performed to examine a variety of brain functions in prostate cancer patients undergoing ADT. Of 14 cognitive domains measured, 3 were found to be moderately impaired following 12 months of ADT: immediate span of attention, visual-spatial ability, and executive function.
- Spatial cognition was one of the earliest and most affected cognitive functions impacted by treatment with ADT. Spatial cognition is the ability to perceive and assess objects in a 3D space, and is tested by tasks such as evaluating images of spatially rotated geometric objects.

- Some patients have cited cognitive impairment due to ADT as their reason for needing to retire from their jobs.
- Patients can be treated with ADT for years to decades. A more recent study examined cognitive decline in prostate cancer patients undergoing ADT for a longer period of time.
- At baseline, about 40% of patients exhibited some level of cognitive impairment, which may be a reflection of the advanced age of patients being treated for prostate cancer.
- After 12 months of ADT, 55-60% of patients exhibited cognitive impairment. Whether this reflects a worsening of baseline impairment due to aging of the cohort or is a result of ADT deserves further study.
- This study also identified genetic polymorphisms that were associated with increased risk for impaired cognitive function.
- The promoter of the AR gene contains a number of repeats of a CAG nucleotide sequence that are highly variant in the population. The number of CAG repeats appears to regulate the activity level of the AR. Fewer CAG repeats associate with increased AR transcriptional activity, while more CAG repeats associate with lower AR activity.
- The relationship between the number of CAG repeats and cognition was examined in another study. A longer chain of CAG repeats, correlating to less AR activity, were associated with poorer cognitive function. Patients with reduced baseline AR levels may be more susceptible to cognitive impairment when treated with ADT. This hypothesis will be explored in future studies.
- In a different study however, fewer CAG repeats were associated with an increased risk for Alzheimer's disease. Understanding the paradox between these two studies will likely require a better understanding of the role that androgens play in the brain.
- A study published in 2015 examined the relationship between ADT and the diagnosis of Alzheimer's disease in prostate cancer patients. This was not a prospectively conducted study, but was performed by retrospectively evaluating diagnosis codes in patient medical records. In this study, an increased risk for the diagnosis of Alzheimer's disease was observed in patients treated with ADT for 7 years or longer.
- A 2016 study by the same group examined the relationship between ADT and the diagnosis of all types of dementia (again by retrospectively evaluating diagnosis codes in patient medical records) (Figure). An earlier diagnosis of dementia was associated with treatment with ADT for 2 years or longer.
- These studies suggest a link between low androgens (as a result of age and/or ADT) and the onset of cognitive disorders including Alzheimer's disease.
- Androgen supplementation has been tested as a treatment for Alzheimer's disease, but was not successful. Androgens may be able to protect from brain injury but unable to repair already damaged brain tissues.
- Individuals at higher risk for cognitive impairment may be more likely to become impaired when treated with ADT. Cognitive impairment risk factors include a positive β -amyloid status and positive Apolipoprotein E baseline status. In a typical ADT population, 40% of men have one of these risk factors and 5% have both risk factors.

- These studies indicate that ADT treatment strategies should be modified in patients who are at risk for cognitive impairment. However, modifying the ADT treatment course must be done carefully, to avoid increasing risk for prostate cancer progression in these patients.
- Studies to identify factors that increase risk for Alzheimer's disease and other cognitive impairments in prostate cancer patients undergoing treatment with ADT are warranted. Such studies would allow the generation of alternate treatment and protection strategies for those at risk.
- Prolonged use of ADT may increase risk for development of Alzheimer's disease and dementia, although these observations must be validated in randomized, prospective clinical trials. The identification of factors that increase risk for Alzheimer's disease would allow at-risk patients to be identified and offered alternate treatment strategies.

ADT And Dementia (of all types)

Figure 1. Kaplan-Meier Curves Examining the Cumulative Probability of Remaining Dementia Free



Nead et al

JAMA Oncology Published online October 13, 2016

Figure: A study examined the relationship between ADT and the diagnosis of dementia by retrospectively evaluating diagnosis codes in patient medical records. An earlier diagnosis of dementia was associated with treatment with ADT for 2 years or longer.

Session 10: Circulating Tumor DNA: A Model for Public-Private Partnership

Circulating Tumor DNA: A Model for Public-Private Partnership

Gerhardt Attard, MD, PhD

The Institute of Cancer Research and the Royal Marsden
NHS Foundation Trust, UK

- Precision medicine is an emerging discipline in medicine where unique tumor genomic alterations are matched to treatments most likely to be of benefit.
- Determining these genomic alterations requires the acquisition of tumor tissue by biopsies of the primary or metastatic lesions.
- Biopsies are invasive procedures that can be painful, expensive, and technically difficult to perform. Simplifying the procedures needed to determine clinically actionable tumor biology is critical for precision medicine to become standard of care for cancer patients.
- Recent studies have found that tumor mutations can be assessed from circulating tumor DNA (ctDNA). ctDNA is tumor DNA that has been shed into the circulation and can be collected via blood draws.
- Dr. Gerhardt Attard discussed a recently funded PCF Challenge Award project that will develop a best in class plasma DNA test to be called “PCF-SELECT” for metastatic prostate cancer that will identify tumor mutations and enable clinicians to make precision medicine decisions.
- The PCF-SELECT test will use targeted next generation genomic sequencing to identify mutations in a preselected set of genes with clinical relevance in prostate cancer.
- Different methodologies and gene sets will be tested by each of the team members and compared in order to identify the best method for performing the test.
- The PCF-SELECT test will then be developed and optimized in a clinically certified lab in order to provide a FDA-acceptable test.
- Once developed, the PCF-SELECT test will be implemented at consortium sites to obtain stage 1 clinical qualification in a range of scenarios and to interrogate biological and biomarker questions.
- Finally, an academic-industry partnership will be established in order to commercialize the test and initiate prospective clinical trials for clinical qualification. The goal for a commercially available test is 2019.

Circulating Tumor DNA and Clinical Outcomes in mCPRC Patients Commencing AR-Targeted Therapy

Alexander Wyatt, PhD

Vancouver Prostate Centre

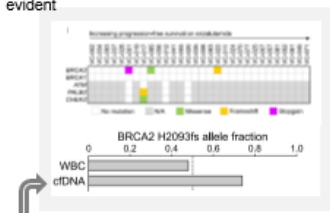
- Precision medicine requires the acquisition of tumor DNA that can be assessed for genomic alterations. These alterations are used to guide the selection of treatments most likely to target the tumor's unique weaknesses.
- Typically, tumor sampling has been through biopsies of tumor lesions. However, biopsies can be invasive and difficult to perform, limiting their use in monitoring changes in tumor biology over time.
- Circulating tumor DNA (ctDNA) is DNA released from dead or dying tumor cells that can be obtained from patient plasma and genomically sequenced to identify tumor mutations.
- Studies have found that ctDNA can comprise <1 to >90% of total circulating DNA found in plasma. The fraction of ctDNA has been shown to be a biomarker of tumor burden.
- Dr. Alexander Wyatt presented studies using ctDNA to identify tumor mutations and as a biomarker of tumor burden.
- Mutations and amplifications in the androgen receptor (AR) gene are commonly observed in castrate resistant prostate cancer (CRPC) and have been associated with poor responses to AR-targeted therapy.
- AR mutations and amplifications are also observed in ctDNA from CRPC patients and associate with resistance to AR-targeted therapy.
- Clinically informative tumor mutations were detected in ctDNA from CRPC patients progressing on enzalutamide, and were concordant with mutations observed in tumor biopsy samples. Tumor suppressor gene loss, hotspot mutations, and other gene losses could be observed (Figure).
- ctDNA analyses have identified tumor mutations not observed in biopsies of single metastatic sites. This suggests that ctDNA contains DNA from multiple metastatic lesions which can have unique mutations and may be more representative of total disease than a single biopsy.
- Thus, ctDNA appears to be a strong surrogate for tissue biopsy in the majority of patients with CRPC.
- A randomized phase II cross-over trial of enzalutamide vs. abiraterone was recently completed in treatment-naïve mCRPC, in which patients were randomized to receive either enzalutamide or abiraterone and at progression were crossed over to the other therapy. ctDNA was collected from patients at baseline and at times of progression on the first and second therapy, and subjected to deep, targeted genomic sequencing.
- In these patients, the fraction of tumor DNA in total plasma DNA was highly prognostic and allowed for the identification of clinically informative tumor mutations.

- ctDNA analyses also allowed temporal monitoring of changes in tumor mutations.
- A liquid biopsy program to study tumor mutations using ctDNA is now being developed at the Vancouver Prostate Centre. As part of this program, ctDNA is being profiled from patients at all clinical stages who are undergoing treatment with various therapies. An umbrella trial is being conducted in which patients are prospectively enrolled and stratified to receive different targeted therapies based on mutations identified in ctDNA.
- The use of ctDNA will allow clinicians to easily study tumor mutations over time in patients without the need for painful and invasive biopsies. Ongoing studies are creating standardized methods and validating the use of ctDNA for monitoring tumor mutations and tumor burden.
- Dr. Wyatt is a member of the “PCF-SELECT” PCF Challenge Award team, who are developing a clinically validated ctDNA test for prostate cancer precision medicine.

CLINICALLY-INFORMATIVE ALTERATIONS ARE ROBUSTLY DETECTED IN CRPC

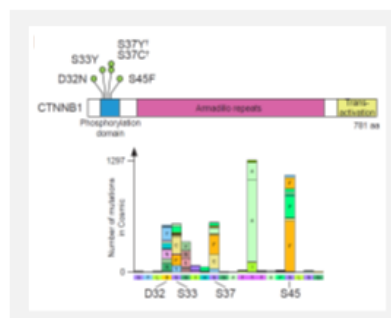


Tumor suppressor loss evident



Somatic LOH in a patient with germline BRCA2 mutation

- Deep targeted sequencing of 19 frequently mutated prostate cancer genes
- Somatic mutations and/or copy number changes were detected in all samples; median VAF of 19.7%.



Suggests that ctDNA may be a surrogate for tissue biopsy in the majority of patients with mCRPC: requires clinical validation

Wyatt et al., JAMA Onc 2016

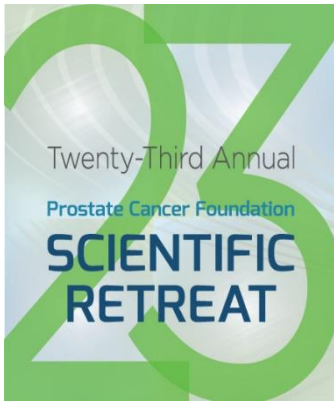
Figure: Clinically informative tumor mutations were detected in ctDNA from CRPC patients progressing on enzalutamide, and were concordant with mutations observed in tumor biopsy samples. Tumor suppressor gene loss, hotspot mutations, and other gene losses could be observed.

APPENDIX:

23rd ANNUAL PROSTATE CANCER FOUNDATION SCIENTIFIC RETREAT

OCTOBER 27-29, 2016

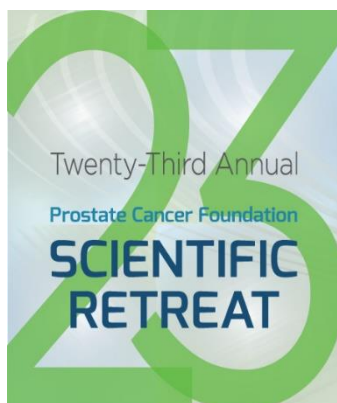
PROGRAM AGENDA



***23rd Annual Prostate Cancer Foundation
Scientific Retreat***

October 27 - 29, 2016

***La Costa Resort
Carlsbad, California***



AGENDA

Thursday, October 27, 2016

GENERAL SESSIONS

Location: Costa Del Sol Ballroom

8:00 am

Registration

Costa Del Sol Foyer

Welcome & Introduction

1:30 PM - 1:40 PM

Howard Soule, PhD
Prostate Cancer Foundation

Session 1: New Treatments in Prostate Cancer

1:40 PM - 3:00 PM

Moderator: Joshua Lang, MD
University of Wisconsin Carbone Cancer Center

1:40 PM - 1:55 PM

Biomarker-Driven Contexts of Use for a TROP2 Antibody-Drug Conjugate in Prostate Cancer

Joshua Lang, MD
University of Wisconsin Carbone Cancer Center

1:55 PM - 2:00 PM

Discussion

2:00 PM - 2:15 PM

Understanding PREX2 and PTEN in Cancer

Ramon Parsons, MD, PhD
Icahn School of Medicine at Mount Sinai

2:15 PM - 2:20 PM

Discussion

2:20 PM - 2:35 PM

IRE1 Signaling Drives Prostate Cancer

Fahri Saatcioglu, PhD
University of Oslo

2:35 PM - 2:40 PM

Discussion

Thursday, October 27, 2016

- 2:40 PM - 2:55 PM ***ROR- γ Nuclear Receptor as a New Therapeutic Target in Advanced CRPC***
Hongwu Chen, PhD
University of California, Davis
- 2:55 PM - 3:00 PM **Discussion**

Session 2: Prostate Cancer Research in the Netherlands

3:00 PM - 4:00 PM

Moderators:

Guido Jenster, PhD

Erasmus Medical Centre, The Netherlands

Jack Schalken, PhD

Radboud University Medical Centre, The Netherlands

- 3:00 PM - 3:15 PM ***Prostate Cancer Grading Beyond Gleason Score: 3D, Molecular and Clinical Perspective***

Arno van Leenders, MD, PhD

Erasmus Medical Centre, The Netherlands

- 3:15 PM - 3:20 PM **Discussion**

- 3:20 PM - 3:35 PM ***Epigenetics in Prostate Cancer: From Prognostication to Tailored Therapy***

Wilbert Zwart, PhD

Netherlands Cancer Institute, The Netherlands

- 3:35 PM - 3:40 PM **Discussion**

- 3:40 PM - 3:55 PM ***Identifying Factors Governing Androgen Therapy Resistance Through Transdifferentiation, an Organoid Approach***

Wouter Karthaus, PhD

Memorial Sloan Kettering Cancer Center

- 3:55 PM - 4:00 PM **Discussion**

Session 3: Radium for CRPC

4:00 PM - 5:20 PM

Moderator:

James Gulley, MD, PhD

National Cancer Institute

- 4:00 PM - 4:15 PM ***Radium-223: Is Bone Targeted Therapy Meeting its Full Potential?***

Michael Morris, MD

Memorial Sloan Kettering Cancer Center

- 4:15 PM - 4:20 PM **Discussion**

Thursday, October 27, 2016

- 4:20 PM - 4:35 PM ***Radiation Induced Immunogenic Modulation: Insights for Combination Therapy***
James Gulley, MD, PhD
National Cancer Institute
- 4:35 PM - 4:40 PM **Discussion**
- 4:40 PM - 4:55 PM ***Targeting the Bone Compartment in Metastatic Prostate Cancer***
Nora Navone, MD, PhD
The University of Texas MD Anderson Cancer Center
- 4:55 PM - 5:00 PM **Discussion**
- 5:00 PM - 5:15 PM ***Radium-223: A Novel Approach to Prevention and Early Treatment of Bone Metastases from Prostate Cancer***
Robert Coleman, MD
University of Sheffield, UK
- 5:15 PM - 5:20 PM **Discussion**

Session 4: Cancer Immunotherapy: Overcoming T Cell Exhaustion in the Tumor Microenvironment
5:20 PM - 7:40 PM

Moderator: William Redmond, PhD
Providence Portland Medical Center

- 5:20 PM - 5:35 PM ***Immune Suppression in the Tumor Microenvironment of Pancreatic Cancer***
Sunil Hingorani, MD, PhD
Fred Hutchinson Cancer Research Center
- 5:35 PM - 5:40 PM **Discussion**
- 5:40 PM - 5:55 PM ***DNA Repair, the Immune Response and Therapeutic Strategies***
Johann de Bono, MD, PhD
Royal Marsden Hospital, UK
- 5:55 PM - 6:00 PM **Discussion**
- 6:00 PM - 6:15 PM ***Overcoming T-cell Exhaustion in Prostate Cancer***
Haydn Kissick, PhD
Emory University School of Medicine
- 6:15 PM - 6:20 PM **Discussion**
- 6:20 PM - 6:35 PM ***PD-1 Blockade in Prostate Cancer***
Julie Graff, MD
Oregon Health & Science University
- 6:35 PM - 6:40 PM **Discussion**

Thursday, October 27, 2016

- 6:40 PM - 6:55 PM ***MMR Mutations and Microsatellite Instability in Prostate Cancer***
Colin Pritchard, MD, PhD
University of Washington
- 6:55 PM - 7:00 PM **Discussion**
- 7:00 PM - 7:15 PM ***DNA Damage Histological Score & Checkpoint Immunotherapy***
Mark Linch, MD, PhD
University College London, UK
- 7:15 PM - 7:20 PM **Discussion**
- 7:20 PM - 7:35 PM ***Novel Immunotherapy Strategies in Prostate Cancer***
Sumit Subudhi, MD, PhD
The University of Texas MD Anderson Cancer Center
- 7:35 PM - 7:40 PM **Discussion**

Dinner

7:40 PM - 8:30 PM

Dinner Location: Costa Del Sol Patio

Poster Session and Dessert

8:30 PM - 11:00 PM

Poster Session and Dessert Location: Costa Del Sol ABC

Friday, October 28, 2016

6:30 AM - 7:30 AM Breakfast

Location: Costa Del Sol Patio

7:30 AM - 7:45 AM Move to Session

GENERAL SESSIONS

Location: Costa Del Sol Ballroom

Panel Discussion:

Precision Clinicopathologic Conference (CPC):

Hype vs. Hope vs. Hypothesis

7:45 AM - 8:45 AM

Moderator: Johann de Bono, MD, PhD

Royal Marsden Hospital, UK

Felix Feng, MD (University of California, San Francisco)

Maha Hussain, MD (Northwestern University)

Christopher Logothetis, MD (University of Texas MD Anderson Cancer Center)

Silke Gillesen, MD (Kantonsspital, Switzerland)

William Nelson, MD, PhD (Johns Hopkins School of Medicine)

Todd Morgan, MD (University of Michigan)

Scott Tomlins, MD, PhD (University of Michigan)

8:45 AM - 8:50 AM Break

Session 5: Immunotherapy for Prostate Cancer

8:50 AM - 10:10 AM

Moderator: Howard Soule, PhD

Prostate Cancer Foundation

Friday, October 28, 2016

- 8:50 AM - 9:05 AM ***Targeting Escape from the NKG2D Pathway with a Novel Cancer Vaccine***
Kai Wucherpfennig, MD, PhD
Dana-Farber Cancer Institute
- 9:05 AM - 9:10 AM **Discussion**
- 9:10 AM - 9:25 AM ***Improving PSMA Specific Eradication of Metastatic Prostate Cancer Using TGF β Resistant CAR T Cells***
Christopher Kloss, PhD
University of Pennsylvania
- 9:25 AM - 9:30 AM **Discussion**
- 9:30 AM - 9:45 AM ***CAR T Cells for Treatment of Solid Tumors***
Saul Priceman, PhD
City of Hope
- 9:45 AM - 9:50 AM **Discussion**
- 9:50 AM - 10:05 AM ***Marrow Infiltrating Lymphocytes: Their Biology and Clinical Implications***
Ivan Borrello, MD
Johns Hopkins Sidney Kimmel Comprehensive Cancer Center
- 10:05 AM - 10:10 AM **Discussion**

SPECIAL LECTURE

10:10 AM - 10:50 AM

State of the Science 2016

Jonathan Simons, MD

Prostate Cancer Foundation

Introduced by Howard Soule, PhD

Prostate Cancer Foundation

10:50 AM - 10:55 AM

Discussion

Friday, October 28, 2016

KEYNOTE ADDRESS

10:55 AM - 11:55 AM

Michael Milken

Founder and Chairman
Prostate Cancer Foundation

*Introduced by Stuart Holden, MD
Prostate Cancer Foundation*

Group Photo

11:55 AM - 12:10 PM

Location: TBD

Lunch

12:10 PM - 1:05 PM

Location: Costa Del Sol Patio

1:05 PM - 1:15 PM Move to Session 6

Location: Costa Del Sol Ballroom

Session 6: Targeting DNA Repair Deficiency in Prostate Cancer

1:15 PM - 3:10 PM

Moderator: Bruce Montgomery, MD
University of Washington

1:15 PM - 1:45 PM Special Lecture: Targeting DNA Repair in Cancer Therapy

Alan D'Andrea, MD
Harvard Medical School

1:45 PM - 1:50 PM Discussion

Friday, October 28, 2016

- 1:50 PM - 2:05 PM ***PARP Inhibitors: Opportunities and Challenges in Prostate Cancer***
Joaquin Mateo, MD
The Institute of Cancer Research, UK
- 2:05 PM - 2:10 PM **Discussion**
- 2:10 PM - 2:25 PM ***Loss of DNA Repair in Prostate Cancer, Lessons from Breast and Ovarian Cancer***
Richard Kennedy, MD, PhD
Queen's University Belfast
- 2:25 PM - 2:30 PM **Discussion**
- 2:30 PM - 2:45 PM ***Platinum Agents in Homologous Recombination Deficiency***
Bruce Montgomery, MD
University of Washington
- 2:45 PM - 2:50 PM **Discussion**
- 2:50 PM - 3:05 PM ***Aggressive Variant Prostate Cancers: Clinical and Molecular Characterization***
Ana Aparicio, MD
The University of Texas MD Anderson Cancer Center
- 3:05 PM - 3:10 PM **Discussion**

Session 7: Targeting Developmental Pathways in Prostate Cancer

3:10 PM - 4:50 PM

Moderator: Carlos Moreno, PhD
Winship Cancer Institute at Emory University

- 3:10 PM - 3:25 PM ***Fundamentals of Canonical and Non-Canonical WNT Signaling in Cancer***
Stuart Aaronson, MD
Mount Sinai Hospital
- 3:25 PM - 3:30 PM **Discussion**
- 3:30 PM - 3:45 PM ***Notch Signaling Promotes Prostate Cancer Metastasis***
Li Xin, PhD
Baylor College of Medicine
- 3:45 PM - 3:50 PM **Discussion**
- 3:50 PM - 4:05 PM ***Molecular Signatures in Circulating Prostate Cancer Cells***
Daniel Haber, MD, PhD
Massachusetts General Hospital Cancer Center
- 4:05 PM - 4:10 PM **Discussion**

Friday, October 28, 2016

- 4:10 PM - 4:25 PM ***SOX4 in PI3K/AKT and WNT Signaling in Prostate Cancer***
Carlos Moreno, PhD
Winship Cancer Institute at Emory University
- 4:25 PM - 4:30 PM **Discussion**
- 4:30 PM - 4:45 PM ***Pharmacologically Targeting WNT Signals in Cancer Stem Cells***
Michael Kahn, PhD
University of Southern California
- 4:45 PM - 4:50 PM **Discussion**

Special Announcement

4:50 PM - 5:00 PM

PyL Molecular Imaging Clinical Investigation

Mark Baker, JD

Progenics Pharmaceuticals

Friday, October 28, 2016

Dinner, Awards Ceremony, and Special Lecture

7:15 PM - 10:00 PM

Location: Costa Del Sol Ballroom

Special Lecture:

Genomic-Based Medicine and the Impact on Cancer

8:15 PM - 8:35 PM

J. Craig Venter, PhD

Human Longevity, Inc.

Introduced by:

Michael Milken

Prostate Cancer Foundation

PCF Awards Ceremony

8:45 PM - 9:30 PM

Featuring:

PCF Career Recognition Award

Gary Gallick, PhD

University of Texas MD Anderson Cancer Center

Presented by:

Nora Navone, MD

University of Texas MD Anderson Cancer Center

Christopher Logothetis, MD

University of Texas MD Anderson Cancer Center

Saturday, October 29, 2016

6:30 AM - 7:30 AM **Breakfast**

Location: Costa Del Sol Patio

7:30 AM - 7:45 AM **Move to Session 8**

GENERAL SESSIONS

Location: Costa Del Sol Ballroom

Session 8: New Platforms to Attack the Undruggable Targets

7:45 AM - 9:50 AM

Moderator: Marco Gottardis, PhD
Janssen Research & Development, LLC

7:45 AM - 7:50 AM

Introduction
Marco Gottardis, PhD
Janssen Research & Development, LLC

7:50 AM - 8:05 AM

NK cells and Prostate Cancer
David Raulet, PhD
University of California, Berkeley

8:05 AM - 8:10 AM

Discussion

8:10 AM - 8:25 AM

Targeting Recalcitrant Cancer Drivers using Cell-Penetrating Miniproteins
Gregory Verdine, PhD
Fog Pharmaceuticals, Inc.

8:25 AM - 8:30 AM

Discussion

8:30 AM - 8:45 AM

PROTACs: Making the Problem Go Away via Induced Protein Degradation
Craig Crews, PhD
Yale University

8:45 AM - 8:50 AM

Discussion

8:50 AM - 9:05 AM

Drugging the Spliceosome: Therapeutic Modulation of Splicing in Human Malignancies
Markus Warmuth, MD
H3 Biomedicine, Inc.

9:05 AM - 9:10 AM

Discussion

Saturday, October 29, 2016

- 9:10 AM - 9:25 AM ***Development of a Pyrrole-Imidazole Polyamide for Treatment of Enzalutamide Resistant Prostate Cancer***
Nickolas Nickols, PhD, MD
David Geffen School of Medicine at UCLA
- 9:25 AM - 9:30 AM **Discussion**
- 9:30 AM - 9:45 AM ***Expression Studies Implicate Neuroendocrine Prostate Cancer as a Novel Indication for Rovalpituzumab Tesirine – A Delta-Like Protein 3 (DLL3)-Targeted Antibody-Drug Conjugate (ADC)***
Laura Saunders, PhD
StemCentrx, Inc.
- 9:45 AM - 9:50 AM **Discussion**

Session 9: Precision Survivorship

9:50 AM - 11:05 AM

Moderators:

Alicia Morgans, MD, MPH

Vanderbilt University Medical Center

Charles Ryan, MD

University of California, San Francisco

- 9:50 AM - 9:55 AM ***Introduction***
Alicia Morgans, MD, MPH
Vanderbilt University Medical Center
- 9:55 AM - 10:10 AM ***Cardiovascular Perils of Prostate Cancer Survivorship: A Personalized Approach to Prevention***
Javid Moslehi, MD, PhD
Vanderbilt University Medical Center
- 10:10 AM - 10:15 AM **Discussion**
- 10:15 AM - 10:30 AM ***ADT and the Brain: The Unwanted Target***
Charles Ryan, MD
University of California, San Francisco
- 10:30 AM - 10:35 AM **Discussion**
- 10:35 AM - 11:00 AM **Special Lecture: On the Etiologies of Dementing Diseases Caused by Prions**
Stanley Prusiner, MD
University of California, San Francisco
- 11:00 AM - 11:05 AM **Discussion**

Saturday, October 29, 2016

Session 10: Circulating Tumor DNA: A Model for Public-Private Partnership

11:05 AM - 12:10 PM

Moderator: Gerhardt Attard, MD, PhD

The Institute of Cancer Research and the Royal Marsden NHS Foundation Trust,
UK

11:05 AM - 11:10 AM ***Introduction***

Gerhardt Attard, MD, PhD

The Institute of Cancer Research and the Royal Marsden NHS Foundation Trust,
UK

11:10 AM - 11:25 AM ***Quantification of Circulating Tumor DNA***

Francesca Demichelis, PhD

University of Trento, Italy

11:25 AM - 11:30 AM ***Discussion***

11:30 AM - 11:45 AM ***Circulating Tumor DNA and Clinical Outcomes in mCPRC Patients
Commencing AR-Targeted Therapy***

Alexander Wyatt, PhD

Vancouver Prostate Centre

11:45 AM - 11:50 AM ***Discussion***

11:50 AM - 12:05 PM ***Early Cancer Detection with Cell-Free Nucleic Acids: Challenges and
Opportunities for Prostate Cancer***

Mark Lee, MD, PhD

GRAIL, Inc.

12:05 PM - 12:10 PM ***Discussion***

Closing Remarks

12:10 PM - 12:15 PM

Howard Soule, PhD

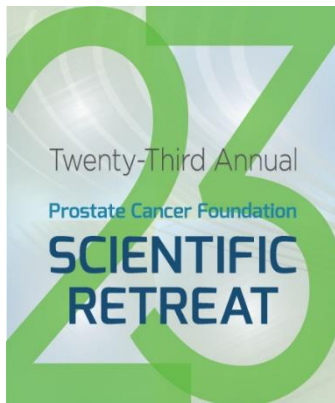
Prostate Cancer Foundation

Jonathan Simons, MD

Prostate Cancer Foundation

Meeting Adjourned

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



Program Committee:

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

Program Committee Co-Chair: Andrea Miyahira, PhD (Prostate Cancer Foundation)

Jonathan Simons, MD (Prostate Cancer Foundation)

Gerhardt Attard, MD, PhD (The Institute of Cancer Research and the Royal Marsden NHS Foundation Trust, UK)

Johann de Bono, MD, PhD (Royal Marsden Hospital, UK)

Marco Gottardis, PhD (Janssen Research & Development, LLC)

James Gulley, MD, PhD (National Cancer Institute)

Guido Jenster, PhD (Erasmus Medical Centre, The Netherlands)

Joshua Lang, MD (University of Wisconsin Carbone Cancer Center)

Bruce Montgomery, MD (University of Washington)

Carlos Moreno, PhD (Winship Cancer Institute at Emory University)

Alicia Morgans, MD, MPH (Vanderbilt University Medical Center)

William Redmond, PhD (Providence Portland Medical Center)

Charles Ryan, MD (University of California, San Francisco)

Jack Schalken, PhD (Radboud University Medical Centre, The Netherlands)

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Prostate Cancer
Foundation

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