

STATE OF THE SCIENCE REPORT

Highlights from
the 22nd Annual PCF
Scientific Retreat
October 2015



Provided with the compliments of
the Prostate Cancer Foundation



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Introduction

The 22nd Annual Prostate Cancer Foundation (PCF) Scientific Retreat was convened in Washington D.C. on October 8-10, 2015. This event is the foremost scientific conference in the world on the biology and treatment of prostate cancer. The diversity, novelty, and extremely high impact of the topics presented as well as the diversity and excellence of the invited attendees make this a unique conference. The PCF Scientific Retreat reflects the unyielding commitment of PCF to ending death and suffering from prostate cancer. This investment by PCF fosters a collaborative culture unparalleled in any other cancer research area and has accelerated the understanding of prostate cancer biology and the treatment landscape. The science presented at the 22nd Annual Scientific Retreat was concept-energizing, and is reflective of the development of new therapies, biotechnologies and unprecedented progress made in 2015.

The 22nd Annual Scientific Retreat featured the following:

- 42 presentations in the Plenary Session
- 133 poster presentations
- 20 different scientific disciplines related to prostate cancer biology presented and discussed
- 75% of speakers presented first-in-field, unpublished data at the PCF Scientific Retreat for the first time
- 533 participants attended from 17 countries, including 233 PhDs, 178 MDs, 93 MD PhDs, 6 PharmDs, 1 JD, and 1 DMD
- 112 academic institutions, 36 biopharmaceutical companies, 10 medical research foundations, and 13 other for-profit companies were represented
- Leadership from NIH, NCI, and Dept. of Defense were present
- 131 PCF Young Investigators attended
- 43 members of the PCF Board of Directors, major donors, and special guests attended

The PCF Scientific Retreat stands on the shoulders of our “Global Research Enterprise,” currently extending to 19 countries. Since 1993, PCF has awarded over \$403 million in innovative prostate cancer research projects, led by an estimated 800 prostate cancer researchers. This includes over \$37 million awarded to 177 PCF Young Investigators since 2007 and nearly \$136 million to PCF Challenge Award teams since 2008.

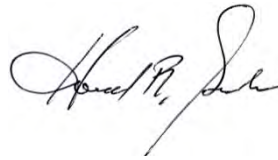
We thank the sponsors of the Retreat for their generous support: Sanofi-Aventis, Janssen, Millennium/Takeda, Astellas/Medivation, Bayer, Dendreon, and GenomeDx Biosciences.

The 2015 State of Science Report provides a detailed overview of the 22nd Annual PCF Scientific Retreat by summarizing each talk individually for the purpose of disseminating this knowledge throughout the global community and furthering the impact of the scientific findings and advancements presented. We hope that you find this report useful and that it stimulates further knowledge exchange, dialogue, and new research ideas and projects. If you have any specific questions, please contact Dr. Andrea Miyahira at amiyahira@pcf.org.

Yours sincerely,



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



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



Session 1: Androgen Axis Resistance in Patients

Prediction of AR Signaling Therapeutic Resistance through Single CTC Analysis of Phenotype, Heterogeneity and Genomics

Ryan Dittamore, BS, MBA
Epic Sciences

- Circulating tumor cells (CTCs) have disseminated from the primary or metastatic tumor sites and can be detected in patient blood. CTCs are emerging as a critical tool for monitoring disease progression and may be the basis for improved precision oncology.
- Mr. Ryan Dittamore presented detection of variable phenotypes and gene expression patterns in CTCs from patients who have developed resistance to androgen receptor (AR)-targeted therapies to identify biomarkers of therapeutic resistance and response. The Epic Sciences system was employed.
- The Epic Sciences CTC detection platform captures all nucleated cells from a 7.5 cc special blood draw which are deposited onto glass slides. Multiple slides from individual patients are archived in a biorepository until analysis. For analysis, the cells on the slide are stained with a cocktail of fluorescent antibodies targeting the epithelial cell marker CK that is expressed by traditional CTCs, the immune cell marker CD45 (to identify irrelevant white blood cells), DAPI to stain the nuclei and thereby mark all cells, and/or other markers of interest. The slides are then scanned by an imaging apparatus and analyzed using specialized software followed by validation by trained technicians and/or pathologists.
- In metastatic castrate resistant prostate cancer (mCRPC) patients, several distinct CTC phenotypes have been identified in addition to traditional CK-expressing epithelial CTCs. Non-traditional CTCs include small CTCs, apoptotic CTCs, CTCs lacking CK expression, CTCs with a speckled pattern of CK distribution, and CTCs with large nucleoli. Patients can exhibit clusters of CTCs and can have all of these CTC types simultaneously. Thus, a dynamic range of CTCs exists in mCRPC patients.
- AR is the primary driver of prostate cancer and is a critical therapeutic target. AR-V7 is a variant form of AR that lacks the androgen-binding domain and no longer requires androgens to maintain constant activity.
- Studies have indicated that AR-V7 expression may confer, in part, resistance to the AR-inhibitors Zytiga (abiraterone) and Xtandi (enzalutamide) but not to taxane chemotherapy.
- To validate the role for AR-V7 in Zytiga and Xtandi resistance, an assay was developed to evaluate AR-V7 expression in CTCs. A rabbit monoclonal antibody was generated that targets a cryptic region of AR that is only exposed in the AR-V7 splice variant. The specificity of this antibody for AR-V7 but not full-length AR was validated in prostate cancer cell lines.

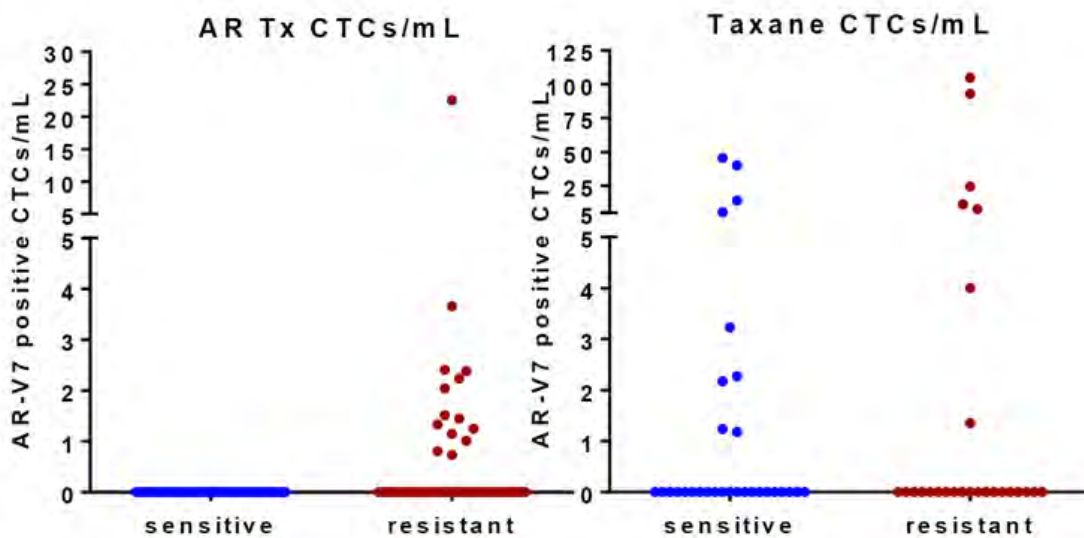
For further specificity, as AR-V7 is a constitutively active transcription factor and should therefore be present in the nucleus of the cell, the assay requires antibody detection of AR-V7 to be visualized in the nucleus to be considered an AR-V7 expressing cell.

- In collaboration with Memorial Sloan Kettering Cancer Center (MSKCC), CTCs were collected from a prospective cohort of 193 mCRPC patients. AR-V7 status was compared to outcome for treatment with Zytiga, Xtandi, or taxane chemotherapy.
- AR-V7 was expressed heterogeneously and was observed in a range of CTC phenotypes. In AR-V7-expressing patients, an average of 22% of total CTCs expressed AR-V7 (range of 0.3-100%). The fraction of AR-V7 expressing cells increased following therapy, from an average of 3% AR-V7+ CTCs with 1 line of treatment, to 31% after 3 lines of therapy.
- Patients were classified by PSA response as being *de novo* sensitive or resistant to therapy. All patients who had AR-V7 expression prior to therapy exhibited *de novo* resistance to Zytiga or Xtandi. In contrast, ~50% of AR-V7 expressing patients responded to taxanes, a response rate predicted for patients in the absence of knowledge of AR-V7 status (**figure**).
- For Zytiga and Xtandi-treated patients, AR-V7 expression was associated with a shorter progression-free survival, a shorter time on therapy and lower overall survival.
- Overall, there was 100% specificity of AR-V7 expression as a biomarker of *de novo* resistance to Zytiga or Xtandi by this CTC assay. Sensitivity however, was poor, indicating other mechanisms exist to confer Zytiga or Xtandi resistance. Other biomarkers are being studied.
- A platform was created to analyze the genomes of single CTCs, which was demonstrated to be as sensitive as analyzing 5 or 10 cells pooled together.
- In collaboration with MSKCC, whole genome sequencing was performed on 350 single CTCs from 17 patients to identify genome-wide copy number variations (CNVs).
- Numerous copy number losses in tumor suppressor genes were identified in CTCs. Inter-patient heterogeneity was common in which different CTCs harbored different tumor suppressor gene losses. This indicates that in studies that pooled CTCs before genomic analysis, mutations present in less frequent subclonal tumor populations may not have been identified.
- Commonly known genomic alterations that associate with drug-resistance were identified by comparing CNVs in CTCs from resistant versus responding patients who had been treated with AR-targeting therapy or taxane chemotherapy. Such alterations included amplification of the AR, MYC, and AURKA oncogenes and copy number losses of the PTEN, RB, BRCA2, and ATM tumor suppressor genes.
- Patients who exhibited *de novo* therapy resistance exhibited greater CNV heterogeneity in CTCs than responding patients, suggesting the existence of subclones that harbored different mutations in 3-75% of total CTCs. This indicates that patients with greater tumor

heterogeneity are at an increased risk of harboring tumor subclones that are able to resist a given therapy. The genomic alterations discovered in CTCs are proposed biomarkers to suggest appropriate treatment. This concept will require additional clinical validation.

- Overall, CTCs are an easily obtainable biospecimen that represents diverse clonal populations of tumor cells from an individual patient. The biology and genomic characteristics of CTCs may inform appropriate treatments for patients.

Patients with AR-V7+ CTCs by PSA status and treatment type



Scher, HI et al ESMO 2015

Figure: Epic AR-V7 status predicts resistance to AR-targeted therapy but not to taxane-based chemotherapy. CTCs were collected from a prospective cohort of 193 mCRPC patients. AR-V7 status was compared to outcome for treatment with Zytiga or Xtandi (AR Tx, left), or taxane chemotherapy (right). All patients who had AR-V7 expression prior to therapy exhibited *de novo* resistance to AR-targeting treatments. In contrast, ~50% of AR-V7 expressing patients had a response to taxanes.

Tumor Cell Plasticity and Immunity in Enzalutamide Resistant CRPC

Jennifer Bishop, PhD

Vancouver Prostate Centre, Canada

- Prostate cancer cells rely on the androgen receptor (AR) pathway for growth and survival, which has become a primary target for prostate cancer therapeutics.

- Xtandi (enzalutamide) is a second-generation AR-targeting therapy approved for the treatment of patients with castrate resistant prostate cancer (CRPC). Xtandi works by binding directly to AR to inhibit its activity.
- Prostate cancer cells eventually develop resistance to Xtandi. In some cases, resistant tumor cells can still express AR but lack classic AR activities such as PSA expression.
- Understanding AR-independent Xtandi-resistance mechanisms will enable new therapeutic strategies to be developed to treat patients with CRPC.
- Dr. Jennifer Bishop discussed mechanisms of disease progression in Xtandi-resistant tumors in which AR is expressed but is not performing classical functions.
- To generate a mouse model of Xtandi-resistant prostate cancer, immune-deficient mice are first injected with human prostate cancer cell lines. Once tumors have formed, mice are castrated in order to mimic the effect of androgen deprivation therapy (ADT) and force the development of ADT-resistant CRPC tumors. When tumors recur (now CRPC), mice are administered Xtandi, to which tumors initially respond but eventually become resistant.
- Importantly, in this mouse model, Xtandi-resistant CRPC tumors developed that mimic two types of disease observed in CRPC patients.
- The first type is "AR-driven", in which tumors express AR and AR is activated in a similar way to primary prostate cancer and Xtandi-naïve CRPC tumors. Patients with AR-driven Xtandi-resistant CRPC show elevated levels of PSA in the blood as their disease progresses (AR+PSA+).
- The second type of disease still expresses AR, but lacks hallmarks of classic AR activity, such as PSA expression (AR+PSA-), or other genes typically turned on by the AR. Xtandi resistant CRPC patients with this type of disease may be classified as "anaplastic" and they exhibit increasing tumor burden without a rise in serum PSA.
- Approximately 25% of Xtandi-resistant tumors that developed in mice were AR+PSA-, a fraction similar to the number of CRPC patients with Xtandi resistant tumors that show this "anaplastic" phenotype. These tumors provide a unique tool to study how the AR may regulate different mechanisms of resistance to Xtandi.
- To understand how the AR regulates mechanisms of Xtandi-resistance in CRPC, cell lines were derived from Xtandi-resistant AR+PSA- tumors, from Xtandi-resistant AR+PSA+ tumors, and from CRPC tumors not treated with Xtandi.
- Chip-SEQ is a method in which antibodies targeting a transcription factor of interest, such as AR, are used to isolate genes in the genome being targeted by that factor. Genomic sequencing then identifies the genes. AR Chip-SEQ was performed to determine which genes were activated by AR in AR+PSA- cells compared with AR+PSA+ cells and Xtandi-naïve CRPC cells.

- In Xtandi-resistant AR+PSA- cells, ~3,000 unique AR-binding sites not shared with Xtandi-naive CRPC cells or Xtandi-resistant AR+PSA+ cells were identified. Three major gene programs were found to be activated by AR in AR+PSA- cells: a cancer-stem cell-like program, a neuroendocrine cell-like program and an immune cell-like program. The expression of these genes was confirmed using RNA-SEQ, which sequences mRNA to identify expressed genes.
- Genes related to a neuroendocrine phenotype expressed by AR+PSA- cells included AURKA, a gene important for neuroendocrine differentiation in prostate cancer cells, and POU3F2, a transcription factor that is regulated by AR.
- Genes related to immune cells expressed by AR+PSA- cells included numerous immune cell transcription factors, surface receptors, cytokines and chemokines. Expression of these immune cell-like genes may allow tumor cells to resemble immune cells or differentially interact with immune cells to promote immune suppression.
- One immune gene highly expressed by AR+PSA- cells was PD-L1, a negative “checkpoint” molecule that functions to turn off the activity of T cells that express the PD-L1 binding-partner, PD-1. This blocks the ability of T cells to kill tumor cells.
- To determine whether PD-L1 expression by AR+PSA- tumors suppresses anti-tumor immune responses, the infiltration of tumors by innate immune cells was examined.
- Significantly fewer dendritic cells (DCs) and myeloid-derived suppressor cells including those that express PD-L1 or the related molecule PD-L2 were observed in AR+PSA- tumors compared with CRPC or AR+PSA+ tumors.
- Conversely, higher levels of DCs were observed in the blood of AR+PSA- tumor-bearing mice as compared to CRPC or AR+PSA+ tumor-bearing mice. These DCs exhibited lower expression levels of immune activating molecules CD80 and CD86, and higher levels of PD-L1 and PD-L2, indicating they have an immune-suppressive function.
- To determine if these observations are relevant in human prostate cancer patients, expression of PD-L1 and PD-L2 was examined in blood DCs from patients responding to versus progressing on Xtandi after 12 weeks of treatment.
- Patients progressing on Xtandi had significantly higher levels of PD-L1/2-expressing dendritic cells in their blood. Furthermore, the frequency of PD-L1/2-expressing dendritic cells continually increased throughout the duration of Xtandi treatment (figure).
- These studies indicate that DCs expressing PD-L1 and/or PD-L2 may be a biomarker of Xtandi resistance. It will be important to determine how early during therapy these cells expand in number or if they are involved in de novo resistance.
- Dr. Bishop also found that almost 95% of neuroendocrine-like AR+PSA- cells express PD-L1, indicating that AR concurrently turns on both gene programs.

- In summary, immune evasion, neuroendocrine features, and stem-cell-like features may be mechanisms of Xtandi resistance in CRPC. Ongoing studies will clarify the role of these gene programs in Xtandi resistance and prostate cancer progression.

PD-L1/2⁺ DC frequency increases with time on enzalutamide

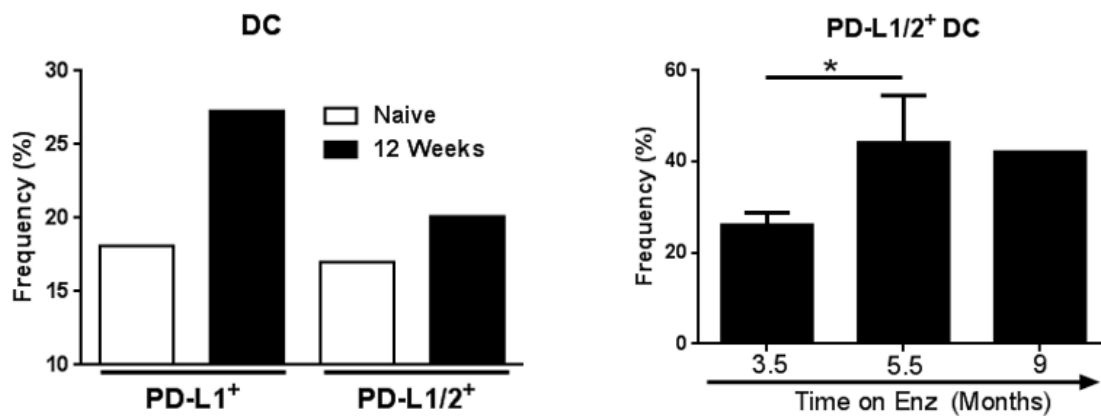


Figure: Left: Patients progressing on Xtandi had significantly higher levels of PD-L1/2-expressing dendritic cells in their blood. Right: The frequency of PD-L1/2-expressing dendritic cells in blood increased throughout the duration of Xtandi treatment.

Session 2: Ascorbic Acid in Prostate Cancer Treatment – Highlighting the Marcus Foundation

In 2015, in partnership with The Marcus Foundation, PCF issued a request for applications (RFA) for funding for projects studying the effect of ascorbic acid (vitamin C) on prostate cancer. Dr. Channing Paller and Dr. Jeffrey Karp were the recipients of these awards.

A Randomized Phase II Trial of Ascorbic Acid in Combination with Docetaxel in Men with Metastatic Prostate Cancer

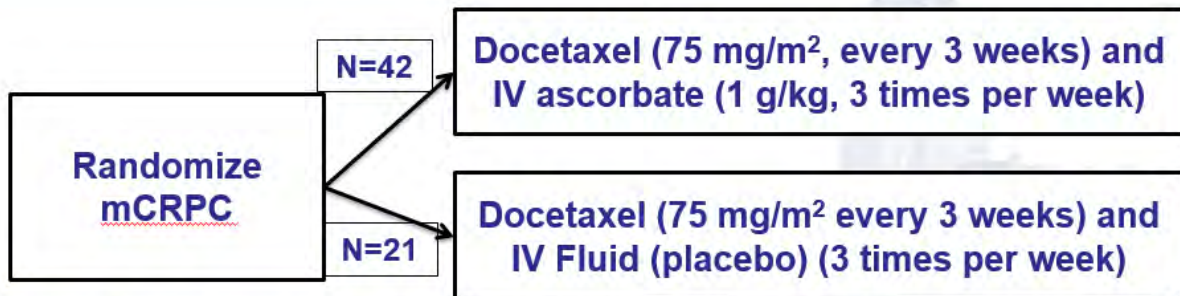
Channing Paller, MD

The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

- Ascorbic acid (vitamin C) is being used by complementary medicine practitioners to treat cancer, infections, and other conditions. Annual administration of more than 355,000 doses of intravenous (I.V.) ascorbic acid for more than 10,000 patients has been documented, although total industry sales are more than double that amount. The average prescribed dose is 28 grams every 4 days. Complications reported have been minimal, with fatigue reported in 1.2% of patients overall and rare reports of phlebitis and kidney stones.
- A long period of controversy over the efficacy of ascorbic acid in cancer patients began in 1976. Ewan Cameron with Linus Pauling published a retrospective study of untreatable cancer patients that demonstrated a survival benefit of 321 days with I.V. and oral ascorbic acid vs. 38 days for controls. In contrast, a 1979 Mayo Clinic study did not replicate this finding, although ascorbic acid was delivered only orally instead of IV. Phase I and II clinical trials in 2012, 2013, and 2014 demonstrated safety and anti-tumor activity for high dose I.V. ascorbic acid. These studies warranted testing the efficacy of I.V. ascorbic acid in randomized, placebo-controlled clinical trials.
- The effective therapeutic concentration of ascorbic acid is high, and can only be achieved with I.V. dosing but not oral use.
- At only these high concentrations, ascorbic acid will act as a pro-oxidant instead of an anti-oxidant. An electron lost from ascorbic acid is transferred to an iron-containing molecule, which results in formation of hydrogen peroxide (H₂O₂). In turn, H₂O₂ leads to formation of oxidants that cause DNA damage and metabolic stress in tumor cells, provoking cell death. Normal cells appear to be unaffected by these reactions. This creates the specificity by which high dose I.V. ascorbic acid is selectively toxic to tumor cells but not normal tissues, and underlies the safety of I.V. ascorbic acid.
- When ascorbic acid is administered by I.V. to patients at 0.9-1.5g/kg over the course of 90 minutes, effective therapeutic levels (10mM) can be maintained in blood for several hours.

- At 1mM, ascorbic acid inhibits 15% of growth for PC3 and DU145 prostate cancer cell lines. At 10mM, ascorbic acid completely inhibits the growth of PC3 cells and almost completely kills DU145 cells.
- Even at high doses, ascorbic acid is very poorly bioavailable when delivered orally. Maximal oral administration has been shown to result in concentrations of no more than 0.2 mM, far below effective therapeutic levels. Thus the findings of the 1979 Mayo Clinic study, which used oral administration only, do not conflict with Cameron and Pauling's findings that used I.V. plus oral administration.
- A recent study of high dose I.V. ascorbic acid in ovarian cancer patients indicated that ascorbic acid treatment combined with standard chemotherapy (paclitaxel) reduces certain toxicities associated with chemotherapy and might increase survival.
- Using the funds received from the Marcus Foundation, Dr. Channing Paller is conducting a randomized phase II clinical trial comparing docetaxel plus I.V. ascorbic acid (1g/kg, 3 times per week) versus docetaxel plus I.V. fluid (placebo) in mCRPC patients (figure). The primary outcomes will be PSA response and reduction of chemotherapy-related toxicities.
- Key secondary outcomes include radiographic progression free survival, safety, quality of life, and the need for dose reductions of docetaxel.
- Laboratory correlates including pharmacokinetics of ascorbic acid and docetaxel and oxidative stress levels will be studied. Biomarkers of resistance to docetaxel will also be investigated.
- This clinical trial activates in early 2016 and will be conducted at Johns Hopkins University and partnering sites including Thomas Jefferson University and Karmanos Cancer Center.

The Clinical Trial Schema



Co-primary endpoints:

- **Impact on cancer:** Percent change in PSA from baseline to 12 and 24 weeks and maximal change.
- **Impact on toxicity:** The proportions of toxicities (fatigue, nausea, bone pain, and anorexia) categorized by: no toxicities, grade 1-2, and grade 3-4.



Figure: Clinical trial schema for a randomized phase II clinical trial comparing docetaxel plus I.V. ascorbic acid (1g/kg, 3 times per week) versus docetaxel plus I.V. fluid (placebo) in mCRPC patients.

Targeting Prostate Cancer with Self-Assembled Vitamin C Nanofibers

Jeffrey Karp, PhD

Harvard: Brigham and Women's Hospital; Harvard Medical School

- Ascorbic acid (vitamin C) is being tested as a cancer therapeutic in various clinical trials. Ascorbic acid must reach high concentrations in blood which can only be reached by I.V. administration. The agent suffers from a short half-life and requires continuous and frequent infusion. New therapeutic administration methods may improve the efficacy of this treatment.
- Dr. Jeffrey Karp presented the development of a new technology to deliver ascorbic acid directly to tumor sites in vivo.
- Amphiphiles are molecules with hydrophobic (water-hating) and hydrophilic (water-loving) segments joined by an enzyme-cleavable bond. When placed into certain solvents

followed by heating and cooling, these molecules will dissolve and then self-assemble into vesicles with the hydrophobic ends facing inward, creating a nanofiber gel (figure).

- Drugs can be entrapped into the gel when associated with the hydrophilic (external) ends of the amphiphile. Enzymatic cleavage of the amphiphile releases the drug.
- Drug-carrying amphiphile nanofibers were created for the treatment of various diseases. In one example, amphiphiles loaded with immune-suppressive drugs could block immune-mediated graft rejection in rats.
- Tumor cells have been found to have greater sensitivity to cell death by ascorbic acid compared normal cells.
- An ideal amphiphile for prostate cancer therapy would be loaded with ascorbic acid and a chemotherapy drug and would enable site-specific release to minimize off-target toxicities.
- The FDA's generally recognized as safe (GRAS) list of molecules was scanned for amphiphiles with this potential. An amphiphile was identified that had ascorbic acid already attached to it and had chemical properties capable of entrapping docetaxel within the nanofiber gel.
- The ascorbic acid/docetaxel gels exhibited long-term stability.
- The gels were able to release the chemotherapy in the presence of tumor cells which is likely due to expression of the appropriate protein cleavage enzymes within the extracellular matrix of the tumor cells.
- Dye-loaded nanofibers administered systemically to mice bearing breast tumors accumulated preferentially in the breast tumor compared to the spleen. This indicates that tumor cells specifically express the cleavage enzymes necessary for drug-release from the amphiphile gel, and supports continued development of these drug-delivery platforms for cancer therapeutics.
- Studies to assess toxicity, pharmacokinetics, and efficacy in prostate cancer mouse models are ongoing.

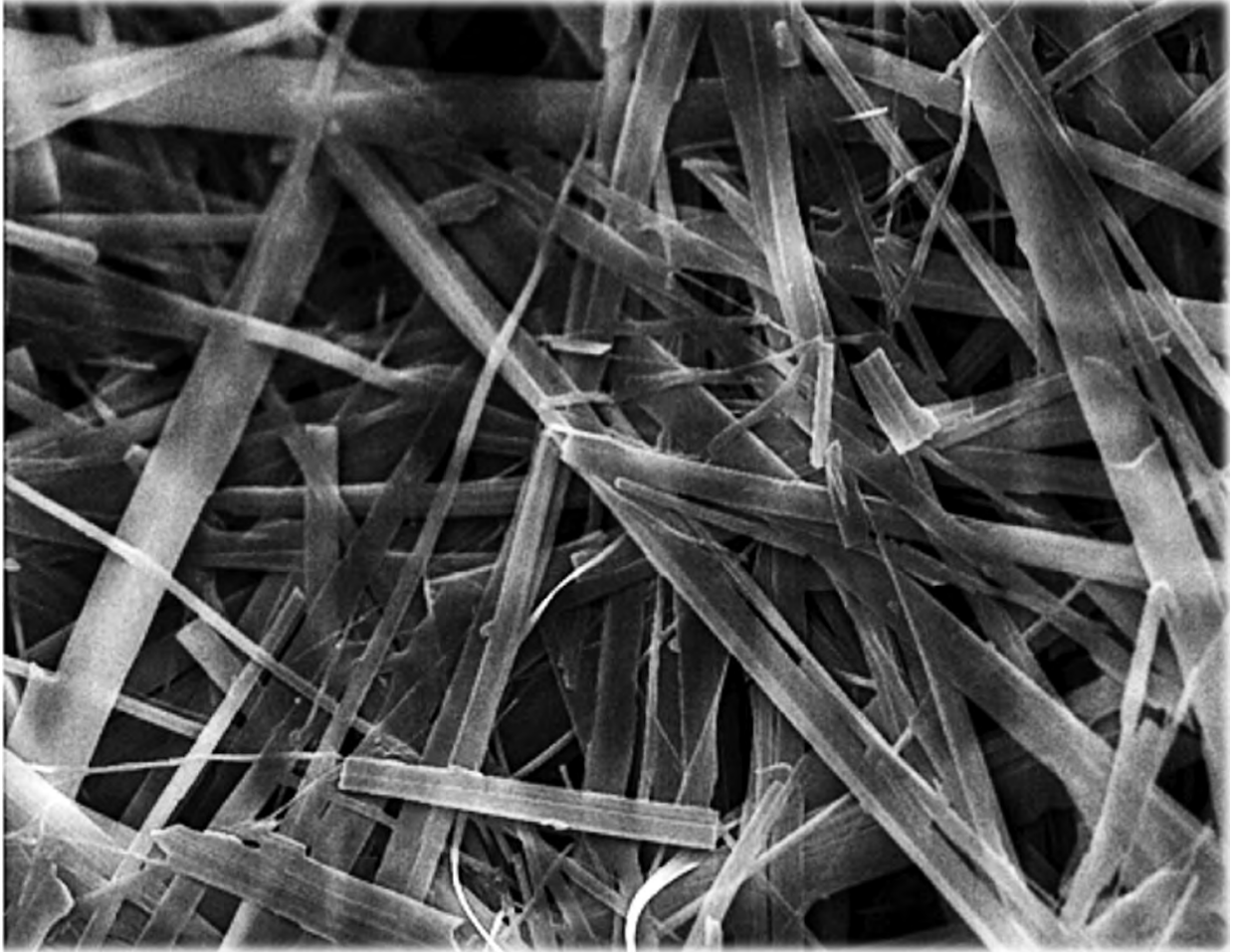


Figure: Electron microscope image of amphiphile nanofibers.

Session 3: New Prostate Cancer Targets and Treatments; What's a Patient to do when the Options Run Out? Part I

Overview on Mechanisms for Overcoming Castrate-Resistance and New Targets

Peter Nelson, MD

Fred Hutchinson Cancer Research Center

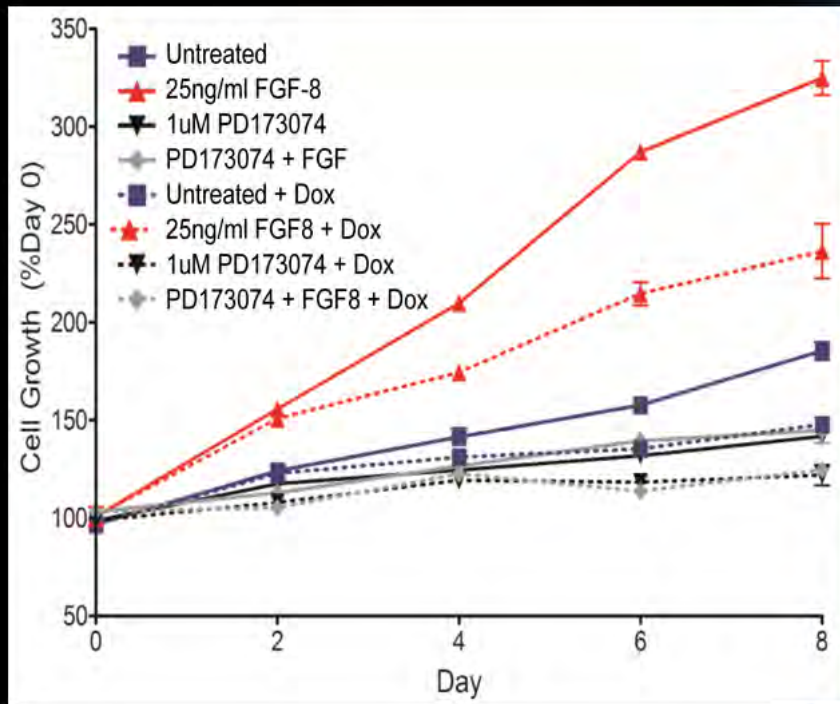
- The androgen receptor (AR) regulates the expression of growth and survival genes that drive prostate cancer pathogenesis. The AR pathway is a primary target for prostate cancer therapy. Tumors inevitably develop resistance to AR-targeted therapies and progress to castrate resistant prostate cancer (CRPC), a disease with lethal potential.
- When the AR pathway is therapeutically targeted, several outcomes may ensue: 1) the therapy may be unable to completely block the AR pathway, resulting in AR-driven remission followed by disease progression; 2) the therapy may achieve complete blockade of the AR pathway and result in a durable complete response; or 3) the therapy may achieve complete blockade of the AR pathway but aggressive AR-independent tumors may arise.
- Dr. Peter Nelson presented the mechanisms of AR-therapy resistance in CRPC and targeting strategies.
- In ~60% of CRPC patients, AR-therapy resistance is associated with activating alterations in the AR gene. These commonly include amplification of the AR gene and missense mutations in the AR ligand-binding domain (LBD) that cause AR to become independently active without standard androgen ligands.
- Other mechanisms of resistance include expression of AR-splice variants, de-repression of AR-repressed genes, and AR-bypass mechanisms.
- AR-splice variants (AR-Vs) are variations of the AR gene that can be expressed from combining different AR gene exons. AR-Vs that lack the LBD are rendered constitutively active and have been associated with resistance to Xtandi (enzalutamide) and Zytiga (abiraterone).
- Dr. Nelson's studies found that CRPC tumors commonly had reciprocal expression of AR-Vs and androgens. Tumors with high AR-V expression had low androgen levels and those with high androgen levels had low AR-V expression. This suggests existence of at least two independent mechanisms of adaptation to androgen deprivation therapy (ADT) that rescue AR activity.
- In addition to turning on expression of prostate cancer growth and survival genes, AR functions to repress the expression of numerous genes which number >800 in LNCaP cells. These genes become re-expressed in the absence of AR and may function to support

prostate cancer cell survival. Such genes include cMET, WNT10B, WEE1, NFκB1, CCL20, UGT2B15, and TNFRSF21. NFκB and cMET can function to turn on survival pathways in the absence of AR. Co-targeting of the AR and NFκB or cMET pathways may be a promising strategy for the treatment of CRPC.

- Resistance mechanisms can also include “AR-bypass,” in which tumor cells activate other survival and growth pathways and no longer rely on AR.
- AR-bypass mechanisms include subversion of the AR signaling program by related hormone receptors that can induce the expression of AR-regulated genes. In one study, some patients with high AR-activity but no AR protein expression were found to overexpress the highly related glucocorticoid receptor (GR) which binds to similar gene regulatory regions as AR.
- AR-independent mechanisms also include transdifferentiation of prostate cancer cells into those with neuroendocrine features (NEPC), which has been observed in patients that have developed resistance to treatment with Xtandi or Zytiga.
- To investigate other mechanisms of AR-independence, Dr. Nelson and colleagues created a model of androgen pathway-independent prostate cancer (APIPC). LNCaP cells were transduced with an AR-driven suicide gene construct that caused the death of cells expressing activated AR. This alteration encouraged the growth of cells that have devised strategies to grow in the absence of AR. An AR-null cell line was developed that is refractory to androgen-deprivation and Xtandi but did not express markers of neuroendocrine transdifferentiation.
- No activity of the AKT oncogene was observed in the APIPC cells. However, high MAPK activity was observed and was found to be driven by high levels of FGF8, a member of the fibroblast growth factor family which regulates the growth and differentiation of many cell types.
- FGF8 expression was unique to APIPC cells and was not observed in other prostate cancer cell lines including those with AR-independent neuroendocrine phenotypes. FGFR1-4 were highly expressed in APIPC cells.
- Patient tumors were identified with the APIPC phenotype that lacked expression of AR and neuroendocrine markers. This supports the clinical relevance for this AR-independent CRPC mechanism.
- Targeting of FGFR with an FGFR-inhibitor (PD173074) blocked in vitro growth of APIPC cells while treatment with FGF-8 enhanced APIPC cell growth (figure). Inhibiting both AR and FGFR had the greatest effect on blocking APIPC cell growth (figure). Treatment with an FGFR-inhibitor also blocked the growth of APIPC tumors in mice.
- Overall, these studies have revealed multiple mechanisms by which prostate cancer cells may gain resistance to AR-targeted therapies. AR remains a critical target for blocking the growth of prostate cancer. Combining AR-targeted treatment with therapies that prohibit AR-resistance mechanisms such as FGF/FGFR and neuroendocrine transdifferentiation, have promise for suppressing the development and progression of CRPC.

Exogenous FGF8 Sustains AR-Null Prostate Cancer Cell Growth

(medium depleted of androgens)



Dox – Induces AR knockdown
 PD173074 = FGFR antagonist

PNelson2015

Figure: Targeting of FGFR with an FGFR-inhibitor (PD173074) blocked *in vitro* growth of AIPIC cells while treatment with FGF-8 enhanced AIPIC cell growth. Inhibiting both AR (+ Dox) and FGFR (PD173074) had the greatest effect on blocking AIPIC cell growth.

In Vitro and In Vivo shRNA Screening for Candidate Prostate Cancer Drug Targets

Erik Danen, PhD

Universiteit Leiden, Netherlands

- Identifying molecules and pathways that regulate prostate cancer progression is critical for the development of new treatments for patients.
- Dr. Erik Danen discussed the discovery of two new regulators of prostate cancer metastasis.
- Two experimental systems were developed to identify and study regulators of the invasive properties of prostate cancer cells.

- A 3D in vitro cell culture system was developed in which an automated injector places clusters of tumor cells into nanowells filled with a gel composed of extracellular matrix (ECM) components. An imaging system is then used to visualize tumor cell activities such as invasion into the surrounding ECM, remodeling of ECM components and attraction of co-injected endothelial cells to the tumor cells.
- Tumor cells were found to reorganize the collagen fibers in the surrounding ECM gel as far away as five times their radius.
- Endothelial cells were injected into the gel at different distances from the tumor cluster. Those injected within the area of the reorganized collagen network could sense and move toward the tumor cells. This suggests that tumor cells remodel their surrounding environment to recruit supportive cells to the tumor.
- A zebrafish transplantation model was developed which expresses GFP in endothelial cells, resulting in a clear fish with green vasculature. This allows easy visualization of fish morphology using automated quantitative imaging methods.
- Zebrafish were injected with red fluorescent protein (RFP)-labeled tumor cells which were able to migrate through the zebrafish. More aggressive tumor cells disseminated further, indicating that tumor cell properties that enable metastasis could be studied in this model.
- A screening assay was used to identify regulators of prostate cancer cell migration. PC3 human prostate cancer cells were transformed with an adenovirus-based shRNA library that silences the expression of genes one at a time. The PC3 cells were then injected into zebrafish and monitored for any PC3 cells harboring shRNAs that disabled dissemination.
- MST1R is a molecule that has been implicated in a number of malignancies and is related to the MET oncogene.
- When MST1R expression was silenced by shRNA, PC3 cell dissemination through the zebrafish was inhibited. MST1R silencing also inhibited the ability of PC3 cells to invade ECM in the 3D nanogel system.
- MST1R has been shown to be overexpressed in primary and metastatic prostate tumors. In this study, MST1R was also found to be highly expressed in prostate cancer cell lines.
- MST1R expression was knocked down in a highly metastatic variant PC3 cell line in order to study the effects of this gene in animal tumor models. When immune-deficient mice were transplanted with PC3 cells, tumor cells without MST1R expression were less able to form bone metastases. This provides preclinical evidence that MST1R may be a target for preventing prostate tumor metastasis.
- SYK is a kinase in the B cell receptor (BCR) signaling pathway and is a therapeutic target in B cell leukemia. SYK signaling has also been implicated as a driver of epithelial cancers.

- SYK was identified in the zebrafish shRNA screen and validated in the 3D nanogel system as a regulator of prostate cancer cell migration. Treatment of PC3 cells with inhibitors of SYK also prevented dissemination in the 3D nanogel and zebrafish models.
- SYK was found to be expressed in a number of prostate cancer cell lines. Moreover, expression of SYK correlated with prostate cancer metastasis in publicly available prostate cancer gene expression datasets.
- SYK was more highly expressed in primary prostate cancer with higher Gleason grade and in metastases (figure). However, not all metastatic lesions expressed SYK (figure). This suggests that SYK plays a role in some but not all mechanisms of metastasis.
- The role of SYK was also studied in murine tumor models. When PC3 cells lacking SYK were transplanted into immune-deficient mice, they were less able to form bone metastases (figure).
- Future studies will clarify the role of the SYK signaling pathway in prostate cancer and will inform the discovery and development of improved SYK inhibitors.
- In summary, these studies have identified two new regulators of invasive properties of prostate cancer that can be targeted to inhibit metastasis.

Role for Syk in prostate cancer

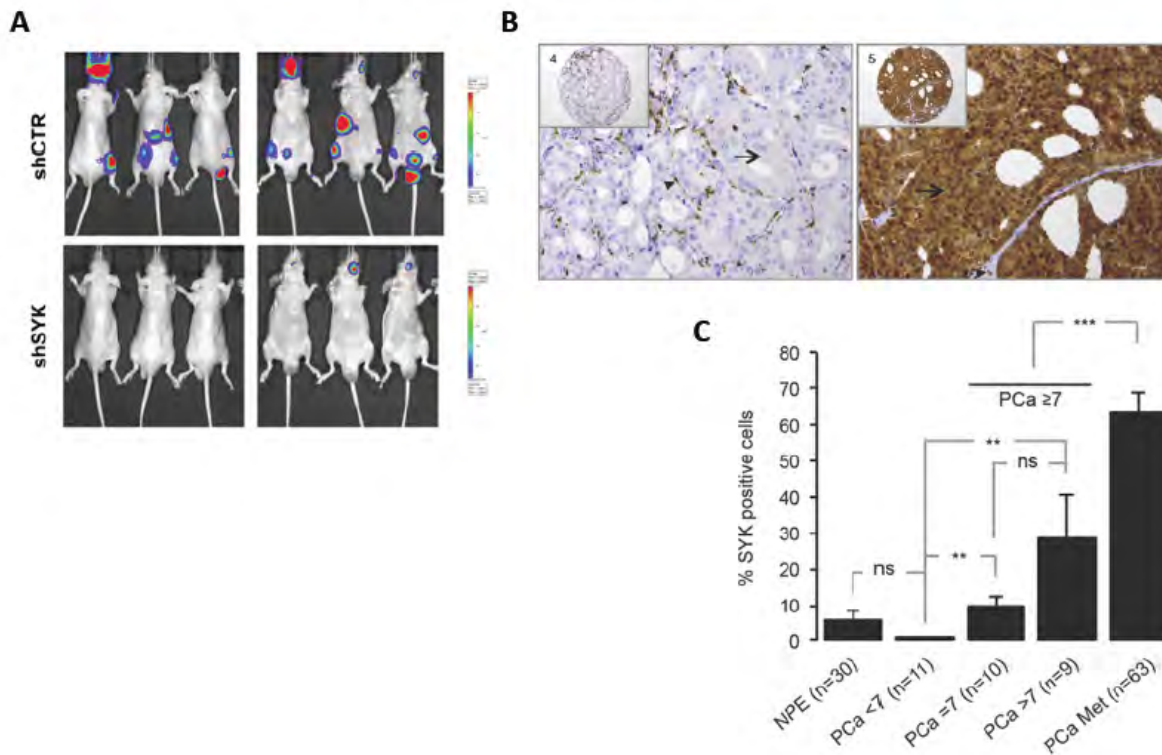


Figure: Role for SYK in prostate cancer. A) PC3 cells were injected in immune deficient mice and metastasis to the bones was examined. A shRNA knocking down expression of SYK prevented metastasis (shSYK) while control shRNA (shCTR) had no effect. B) In patients, SYK protein (brown staining) was highly expressed in some, but not all prostate cancer metastasis lesions (negative lesion and strongly positive lesion shown). C) Expression of SYK in prostate cancer is found in advanced disease stages.

LIM Kinase Inhibitors Impair Microtubule Dynamics and Prostate Cancer Proliferation

Michael F. Olson, PhD

Cancer Research UK Beatson Institute, UK

- LIM Kinase-1 (LIMK1) and LIMK2 are kinases that function to stabilize actin filaments and to regulate actin-myosin contraction and microtubule dynamics. LIMKs are important for tumor cell migration, invasion and metastasis.
- Dr. Michael Olson is studying the effects of targeting LIMK1/2 for prostate cancer therapy.

- LIMK1 and LIMK2 are members of the tyrosine kinase-like (TKL) family of kinases. TKL kinases are not affected by known kinase inhibitors that have broad activity across multiple other kinase families. The effects of targeting TKL kinases in cancer are relatively unknown.
- A LIMK-inhibitor with strong and selective activity has been developed by Bristol-Myers Squibb (BMS).
- Dr. Olson tested the BMS LIMK inhibitor for efficacy against prostate cancer cells.
- The LIMK inhibitor was found to block the ability of prostate cancer cell lines to migrate.
- Knockdown of LIMK1/2 expression in prostate cancer cell lines with siRNA induced prostate cancer cell death and inhibited proliferation, consistent with LIMKs being therapeutically relevant targets in prostate cancer.
- Prostate cancer cells that depend on androgens were found to be significantly more sensitive to the LIMK-inhibitor than androgen-independent prostate cancer cell lines. This suggests that LIMK inhibitors might be more effective in patients who have not yet progressed to castrate-resistance prostate cancer (CRPC).
- The androgen receptor (AR) uses microtubules to enter the nucleus when activated by androgen binding, where it then turns on the expression of genes required for prostate cancer growth and survival. The LIMK-inhibitor was found to block the interaction between AR and tubulin. AR stability was also reduced by the LIMK-inhibitor. Consequently, reduced levels of AR were observed in the nucleus of cells treated with the LIMK-inhibitor. These results indicate that LIMK-inhibitors block microtubule functions that then disable AR from entering the nucleus, resulting in the inability of cells to produce the factors needed for growth and survival.
- High levels of LIMK were observed by immunohistochemistry in human prostate tumor samples. High levels of LIMK1, LIMK2, and phosphorylated versions of the LIMK-target cofilin, were associated with significantly worse survival times in a cohort of 164 non-metastatic prostate cancer patients (figure). These factors were not predictive of survival in metastatic prostate cancer patients (figure).
- Two novel small molecule inhibitors of LIMK1 and LIMK2 have been identified by Dr. Olson and will be studied for activity against prostate cancer.
- These results suggest that LIMK-inhibitors are promising therapeutics, and could be beneficial for patients by both targeting microtubules to affect the stability and activity of AR, and by inhibiting prostate cancer cell motility, which will reduce the growth and spread of tumors.

Targeting LIMK kinases for prostate cancer therapy

Prostate cancer tissue microarray (n = 164 primary PC samples and 23 benign hyperplasia)

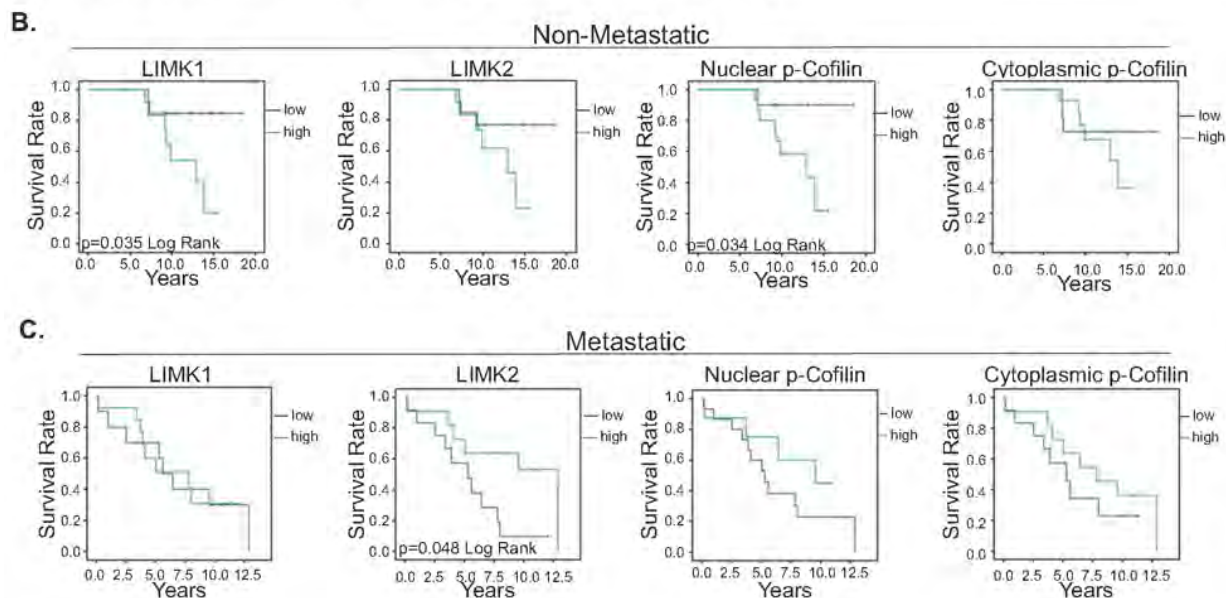


Figure: High levels of LIMK1, LIMK2, and nuclear activated cofilin, the target of LIMKs, were associated with significantly worse survival in a cohort of 164 non-metastatic prostate cancer patients. These factors were not predictive of survival in metastatic prostate cancer patients.

ODM-201 for Castration-Resistant and Castration-Sensitive Prostate Cancer

Martin Kornacker, MD
Bayer HealthCare

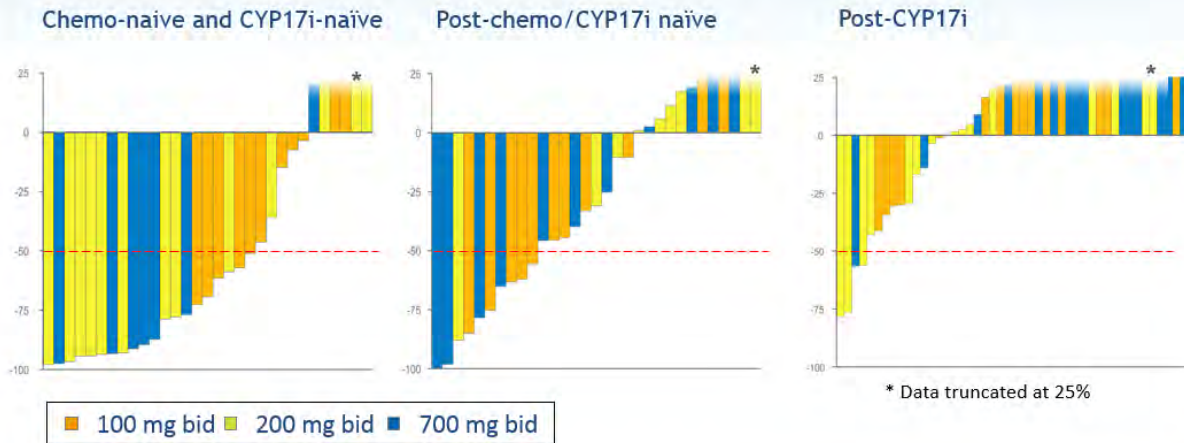
- The androgen receptor (AR), the major driver of prostate cancer, is also the main therapeutic target for prohibiting prostate cancer progression. Developing better AR-targeting medicines continues to be an important goal of researchers.
- Dr. Martin Kornacker discussed a novel AR-targeting non-steroidal small molecule inhibitor, ODM-201, which is being tested in phase III randomized clinical trials by Bayer and Orion.
- ODM-201 acts by binding to AR and blocking the interaction between AR and androgen ligands, thereby disabling AR from becoming activated by androgens. The same mechanism is employed by second generation AR-targeting therapies Xtandi (enzalutamide) and ARN-509. Studies were done to compare ODM-201 with Xtandi and ARN-509.

- ODM-201 and its main metabolite ORM-15341 were found to bind to AR with higher affinity than Xtandi or ARN-509 and were more effective at antagonizing AR at a lower drug concentration. Inhibition of prostate cancer cell proliferation occurred at drug levels similar to those of Xtandi and ARN-509.
- Neural toxicity seen at low frequency for this class of compound might be reduced for ODM-201, which does not cross the blood-brain barrier.
- Therapeutic doses of ODM-201 did not inhibit or induce CYP enzymes, which are involved in the production of androgens and other molecules by cells.
- Resistance of prostate cancer cells to Xtandi and ARN-509 can occur when tumor cells acquire mutations in AR that cause these drugs to become AR-agonists instead of antagonists. ODM-201 retained antagonistic properties against clinically important AR mutants (AR-F876L mutation).
- ODM-201 also retained some efficacy against an AR mutant that Xtandi and ARN-509 are no longer effective against (AR-W741L mutation).
- ODM-201 has been tested in phase I & II clinical trials. In the phase I/II ARADES trial, ODM-201 was tested in three metastatic castration-resistant prostate cancer (mCRPC) patient populations: patients who had not received either CYP17-inhibitors (abiraterone acetate (Zytiga), ketoconazole, VN/124-1 (TOK-001) and TAK-700) or chemotherapy; patients who had not received CYP17-inhibitors but had received chemotherapy; and patients who had received CYP17-inhibitors.
- Responses were seen in all three populations, but were most evident in the patients who had not received CYP17-inhibitors or chemotherapy (figure).
- ODM-201 was well tolerated with a favorable safety profile. The most frequent adverse events were grade 1-2 fatigue, back pain, and arthralgia.
- ODM-201 is being tested for efficacy in a randomized, double-blind, placebo-controlled phase III trial (ARAMIS) in non-metastatic CRPC patients. 1500 patients are planned to be enrolled in this trial.
- Additional indications for ODM 201 will be explored.



ARADES PSA Response at 12 Weeks

Expanded dose levels



The greatest PSA response in patients naïve to chemotherapy and CYP17i
Greatest response rates at 700 mg bid (86% of pt)

Fizazi et al 2014 Lancet Oncol

Figure: In the phase I/II ARADES trial, ODM-201 was tested in three metastatic castration-resistant prostate cancer (mCRPC) patient populations: patients who had not received either CYP17-inhibitors (CYP17i) or chemotherapy; patients who had not received CYP17-inhibitors but had received chemotherapy; and patients who had received CYP17-inhibitors. Responses were seen in all three populations, but were most evident in the patients who had not received CYP17-inhibitors or chemotherapy.

Session 4: Novel Macromolecular Drug Delivery

Cyclic Cell-Penetrating Peptides for Efficient Cytosolic Cargo Delivery

Dehua Pei, PhD

The Ohio State University

- The discovery of therapeutics that inhibit interactions between proteins and block their oncogenic activities is critical for advancing the treatment of men with prostate cancer.
- Dr. Dehua Pei presented data for a therapeutic class of molecules termed cyclic cell-penetrating peptides (CCPPs) that can enter cells and block specific protein-protein interactions.
- Peptides are promising therapies for blocking protein-protein interactions as they can be readily synthesized and modified to increase specificity and potency. However, linear peptides are rapidly degraded in blood and might lack biophysical properties consistent with crossing the cell membrane and accessing intracellular protein targets.
- Cyclic peptides are covalently bonded peptide rings that can occur in nature and are highly stable.
- To develop cyclic peptides as therapy candidates, Dr. Pei generated and screened libraries of millions of cyclic peptides for their ability to inhibit target proteins important in cancer and other diseases.
- The cyclic peptides discovered by the screen were typically too large and hydrophilic to penetrate the cell membrane and access targets in the cell cytoplasm. Methods to enhance intra-cellular delivery were developed.
- As an example, a cyclic peptide targeting Pin1, an enzyme overexpressed in many cancers, was discovered but was not able to penetrate cell membranes. To alter the Pin1-targeting peptide to enable cell permeability, the amino acids that specifically interact with Pin1 were identified. When three of the non-interacting amino acids were exchanged for arginine, cell permeability was significantly enhanced. The addition of two more arginine residues to the cyclic peptide further increased cell membrane permeability, although the inhibitor's potency was decreased. Thus, exchanging non-essential amino acids for arginine residues is one method to generally enhance the ability of cyclic peptides to permeate cell membranes.
- A series of cyclic cell-penetrating peptides (CCPPs) that may be harnessed for intracellular drug delivery was identified. cΦR4, a CCPP comprised of 4 arginine residues and 2 hydrophobic residues, had a cytosolic delivery efficiency of 14%, compared to <5% for other gold-standard cell-penetrating peptides. cΦR4 also exhibited excellent proteolytic stability.

- cF Φ R4 entered cells via endocytosis followed by escape from the early endosome into the cell cytosol.
- Additional CCPPs with even better delivery efficiency (up to 84%) were identified.
- These CCPPs were demonstrated to be orally bioavailable in mice.
- When these CCPPs were fused with impermeable cyclic peptides that target a specific protein, “bicyclic” peptides were generated that retained both membrane-permeable and protein-targeting properties. Bicyclic peptides were found to be significantly more stable than monocyclic peptides in serum.
- K-Ras is an oncogene that is mutated in ~30% of cancers and causes uncontrolled cell growth. The development of K-Ras inhibitors is critical for improving the treatment of cancer patients.
- By integrating CCPP sequences and K-Ras-targeting peptide sequences, Dr. Pei and colleagues developed an 11-amino acid cyclic peptide with high membrane permeability and high binding affinity for K-Ras (figure).
- Treatment of tumor cells with the K-Ras-targeting cyclic peptide inhibited the ability of K-Ras to activate downstream proteins AKT, MEK and ERK, and caused cell death. Cells expressing a constitutively active AKT mutant (myristolated AKT) were more resistant to the K-Ras inhibitor, supporting that the inhibitor acts upstream of AKT activation, directly on K-Ras.
- In summary, CCPPs are cell permeable, orally bioavailable, and can be engineered to target specific protein-protein interactions as well as carry otherwise non-permeable drugs into cells. These properties make them promising scaffolds for the development of cancer therapeutics that target intracellular cancer-causing protein-protein interactions.

Endocyclic Delivery: Development of a Direct K-Ras Inhibitor

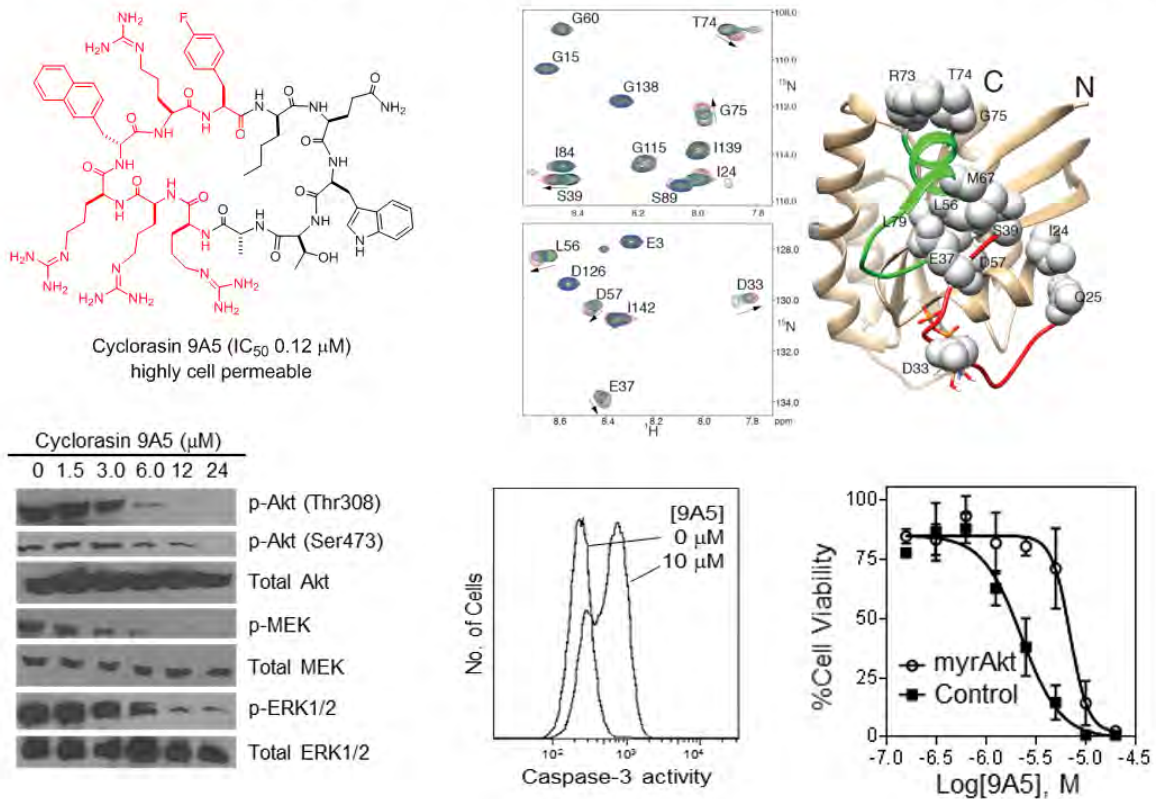


Figure: Top left: chemical structure of K-Ras-targeting CCPP, Cyclorasin 9A5. Top right: Binding site of Cyclorasin 9A5 on K-Ras (residues involved in/perturbed by 9A5 binding are shown as spheres). Bottom Left: Treatment of tumor cells with Cyclorasin 9A5 inhibited the activation of AKT, MEK and ERK. Bottom center: Cyclorasin 9A5 induced apoptosis, as measured by increased caspase-3 activity. Bottom right: Cells expressing a constitutively active AKT mutant (myrAKT) were resistant to Cyclorasin 9A5.

Targeting Oncogenic Intracellular Protein-Protein Interactions with Ultrastable Cell Penetrating Micro Proteins

Julio Camarero, PhD
University of Southern California

- Oncogenic protein-protein interactions that differ between tumor and normal cells represent promising potential targets for cancer therapy.
- Polypeptides are short strands of amino acids that can be synthesized in a lab and can be designed to interact with various proteins or other molecules. As therapeutic agents, polypeptides have high specificity, high selectivity, and low off-target effects. However,

- polypeptides have low oral bioavailability, poor biological stability, and difficulty in crossing cell membranes to target intracellular protein-protein interactions.
- Dr. Julio Camarero discussed the development of a unique class of polypeptide inhibitors that can target and block the interactions between two proteins.
- Cyclotides are cyclic microproteins that naturally occur in plants. They are roughly 30 amino acids in length, are highly stable, orally bioavailable, can cross cell membranes, and exhibit diverse biological functions. Furthermore, they can be chemically synthesized and modified and therefore represent ideal scaffolds for developing specific protein-binding agents.
- The structure of cyclotides includes 5 hypervariable loops that can easily be modified. To create cyclotides with novel biological activities, bioactive polypeptides can be “grafted” to the cyclotide in place of these loops. Loop 6 is the most permitting for such modifications.
- P53 is a tumor suppressor gene that controls growth arrest, DNA repair, and cell death in response to cell stress signals including irradiation. Many cancers mutate p53 in order to avoid cell death. The amount of p53 in the cells is controlled by Hdm2 and HdmX, which maintain low levels of p53 in normal cells. Binding of Hdm2 to p53 leads to the ubiquitination and degradation of p53, while antagonization of this interaction stabilizes p53 and increases p53 levels.
- The site of p53 that interacts with Hdm2 was identified. A p53/Hdm2-inhibitory peptide was generated using a version of the p53 site that has been modified to enhance binding affinity to Hdm2. The p53/Hdm2-inhibitory peptide thus outcompetes normal p53 for binding to Hdm2. However, the peptide suffers from instability and poor cellular bioavailability.
- To stabilize and improve the bioactivity of the p53/Hdm2-inhibitory peptide, the peptide sequence was grafted onto loop 6 of the cyclotide MCoTI-I.
- The modified cyclotide, termed MCoTI-PMI, was recombinantly produced via expression in *E. coli* in the context of a thiol chemistry that promotes cyclization of the micro-protein. Nuclear magnetic resonance (NMR) was used to validate protein cyclization and binding of the cyclotide to Hdm2.
- The 3D structure of the MCoTI-PMI cyclotide in complex with Hdm2 was determined using x-ray crystallography. Analysis of the structure revealed other loops in MCoTI-PMI which come into contact with Hdm2 and may enhance affinity if modified.
- The IC₅₀ (the half maximal inhibitory concentration) of the cyclotide in competition with p53 for Hdm2 was 30nM, while the IC₅₀ of normal p53 for Hdm2-p53 was 2300nM.
- When MCoTI-PMI was used to treat a panel of cancer cell lines, those expressing p53 were highly sensitive, demonstrating that the cyclotide acts via reactivation of p53 to induce cell death.

- In LNCaP cells that express p53, the cyclotide was demonstrated to downregulate HdmX levels and upregulate p21, a marker of p53 pathway activation.
- MCoTI-PMI exhibited a half-life of 30 hours in human serum. In contrast, the half-life of a linearized version of the cyclotide was 0.7 hours.
- In vivo, MCoTI-PMI had a half-life of 0.8 hours.
- Treatment of colorectal tumor-bearing mice with MCoTI-PMI activated the p53 pathway and reduced the growth rate of tumors (figure).
- Overall, cyclotides offer a novel peptide-based scaffold with exceptional features, making these ideal for generating inhibitors that target protein-protein interactions.
- Ongoing studies are creating cyclotides that target other oncoproteins including Ras and HdmX.

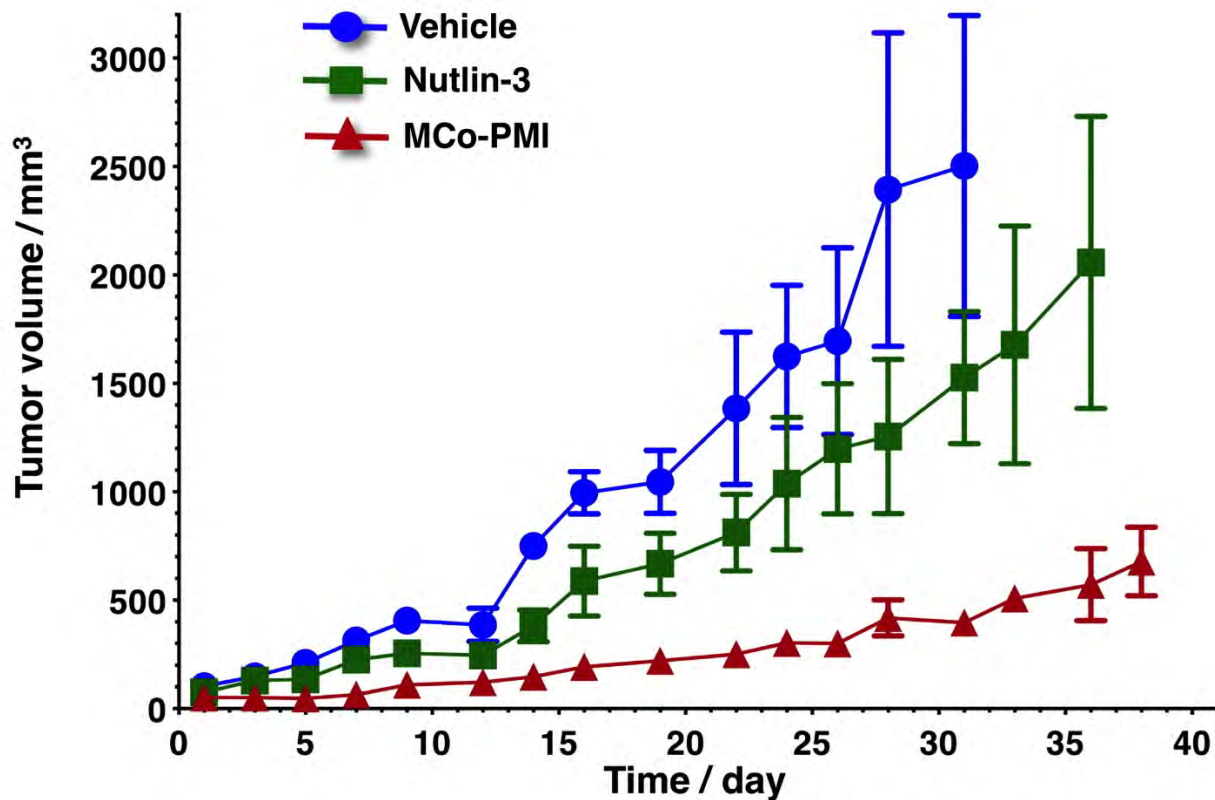


Figure: Treatment of p53-expressing colorectal tumor (HCT116) bearing mice with MCoTI-PMI (40 mg/kg by intravenous injection) reduced the growth rate of tumors.

Session 5: Prostate Cancer Heterogeneity

Circulating Tumor Clone Dynamics in Metastatic Prostate Cancer

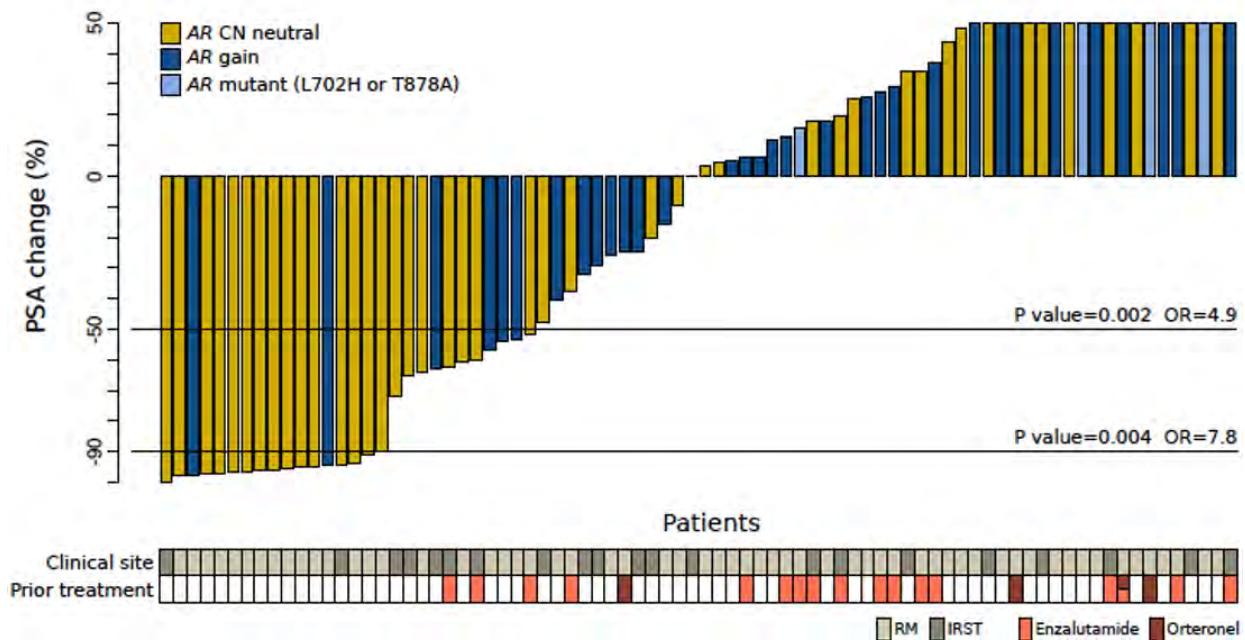
Gerhardt Attard, MD, PhD

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

- Circulating cell-free tumor DNA (ctDNA) is DNA that has been shed from tumors into the bloodstream and can be obtained from patient plasma. CtDNA is being harnessed to study primary and metastatic tumor genomics without the need for invasive biopsies and may be a useful tool for temporal monitoring of patient disease status.
- Dr. Gerhardt Attard discussed studies in which genomic aberrations in ctDNA were analyzed to identify mechanisms of treatment response and resistance in castrate resistant prostate cancer (CRPC) patients and to study the dynamics of tumor clones throughout the disease course.
- To study ctDNA, the fraction of DNA in plasma that is attributable to tumor cells must first be estimated. For this quantitation, Attard's group used 6-8 tumor-specific genomic aberrations that they hypothesized are clonal and dominant (i.e. very early events in prostate carcinogenesis that would be present in every tumor cell).
- To determine if drug-resistance and disease progression involve the outgrowth of preexisting tumor subclones carrying progression-enabling genomic aberrations, genomic analyses were performed on sequential plasma DNA samples from CRPC patients that were collected prior to the development of CRPC and at multiple time points over the course of various treatments after progression to CRPC.
- Genomic deletions that were identified in pre-CRPC samples did not maintain dominance once patients progressed to CRPC. Deletions in chromosome regions such as 21q and 8p varied in their relative abundance. This indicates that different tumor subclones changed in dominance over time and differentially contributed to drug resistance and disease progression.
- Multiple distinct tumor clones were found to contribute to metastases. For instance, metastatic tumor clones with and without rearrangements in the ERG oncogene occurred concurrently in ~20% of CRPC patients.
- In 15-20% of CRPC patients, emergence of clinically relevant subclonal aberrations in the androgen receptor (AR) gene were observed in response to treatment with the AR-pathway inhibitor Zytiga (abiraterone). AR copy number-gains and L702H and T878A point mutations in AR were consistently associated with resistance to Zytiga and disease progression (figure). All patients with an AR copy number-gain had progressed on Zytiga by 21 weeks of therapy. Other AR point mutations (H875Y) arose prior to Zytiga treatment and may have contributed to development of CRPC.

- Patients without detectable AR aberrations in ctDNA had significantly longer overall survival and progression free survival and remained on Zytiga longer than patients with AR aberrations.
- This demonstrates that ctDNA can be used to identify genomic aberrations in tumor clones that drive disease progression in CRPC.
- Studies were also done to determine if the fraction of tumor DNA in plasma can be used as a measure of response to therapies and to indicate clinical outcome. Overall survival and progression free survival following treatment with Zytiga was significantly increased in patients with lower ctDNA content. This indicates that ctDNA levels can be used as a biomarker of therapeutic response.
- Ongoing studies are exploring whether ctDNA levels and genomic aberrations can identify patients unlikely to benefit from AR-targeted therapies and should be offered alternative treatments. A 2014 PCF Challenge Award to Drs. Himisha Beltran, Francesca Demichelis, and Gerhardt Attard supports these studies.

Plasma AR associates with resistance to abiraterone



N=80

Romanel, Gasi Tandefelt et al. In Press

Figure: AR copy-number gain (dark blue bars) and L702H and T878A point mutations in AR (light blue bars) were consistently associated with resistance to abiraterone (Zytiga) and disease progression as measured by the % change in PSA levels.

Evolutionary History of Lethal Prostate Cancer

G. Steven Bova, MD

Tampere University Hospital, Finland

- As prostate tumors progress, new genomic alterations that confer resistance to therapies and enable metastasis and disease progression occur. Understanding when different mutations arise and the role they play is critical for identifying optimal therapeutic targets.
- Dr. Steve Bova presented studies to map the evolutionary history of lethal prostate cancer and identify targetable mutations that are present in tumor cells from a patient.
- In order to study the mechanisms leading to lethal metastatic castrate resistant prostate cancer (mCRPC), a rapid autopsy program was established to obtain metastatic and primary tumors from patients who succumbed to their disease.
- Whole genome sequencing was performed on the primary tumor, multiple metastatic tumors and normal prostate tissue from 10 mCRPC patients. (The primary tumor was only available in 5 of these patients.)
- Genomic alterations investigated included copy number changes, point mutations, substitutions, and insertions/deletions. Identified genomic alterations were used to create a phylogenetic evolutionary tree to show the relationships between all lesions in each patient.
- Bioinformatic methods were used to integrate copy number and point mutation data to derive estimations of the fraction of cells bearing each mutation and identify subclonal populations from each tumor site.
- Tumors from each patient harbored ~3,000-14,000 mutations.
- All of the tumors were found to have a monoclonal origin, (having developed from a single primary tumor), from which diverse subclonal metastatic disease later developed. This is surprising due to the known heterogeneity in the primary environment. These “clonal, truncal” mutations were unique to each patient. In patients for whom the primary tumor was not available, truncal mutations were inferred based on sharing between all metastatic sites.
- In all but one of these patients, the vast majority of mutations were truncal, while fewer mutations occurred following metastatic spread.
- Interestingly, in 5 of the patients, multiple tumor subclones were found populating a single metastatic site, indicating that tumor cells can migrate between and occupy different metastatic sites (“polyclonal seeding”). In the other 5 cases, a single founder cell seeded metastatic sites.

- Truncal mutations were compared with non-truncal mutations. The most frequent truncal alterations were copy number losses of tumor suppressor genes. Copy number gains in oncogenes were also frequent among truncal alterations.
- Almost all of the mutations found in the androgen receptor (AR) were non-truncal, being found in metastases but not the primary tumor. This is consistent with the hypothesis that AR mutations do not contribute to the development of prostate cancer but are treatment-induced due to AR-targeting therapies.
- By organizing the data into phylogenetic trees, the order of subclonal spread could be inferred. In some patients, distinct stages of metastatic spread could be seen, with the primary site first seeding a few metastatic sites in several waves, followed by mass migration of multiple subclones between various metastatic sites as well as the primary (figure).
- In 4 of the 5 cases with available primary tumors, all metastases were found to arise from a minor clone within the primary tumor. Other tumor clones in the primary site persisted and continued to evolve throughout the course of disease.
- In one patient, a more in-depth mutational analysis was performed. In this patient, a mutation in the ASNA1 gene was identified in all of the metastatic sites, but not the primary tumor. This suggests that either a small region of the primary that was not assessed was the seed for all metastases or that an early metastatic lesion developed this mutation and then went on to seed all subsequent metastases.
- The data from this patient was further assessed to identify any druggable truncal mutations. Potentially druggable targets identified included FGFR1, PIK3CG, ABCC4, ALDH9A1, and ASNA1. Inhibitors of FGFR1 are being tested in clinical trials. Mutations in other PIK3-family genes are known to confer responsiveness to PI3K-targeting drugs, though it is unknown if tumors with PIK3CG mutations would be responsive.
- In summary, genomic analysis of multiple prostate cancer metastases from men who consented to undergo autopsy revealed the evolutionary histories of their cancers. Distinguishing truncal from nontruncal mutations may help overcome therapy resistance and requires further study. The study highlights the need to study individual patients deeply and across the full lifecycle of their tumors, including autopsy studies.

Polyclonal seeding

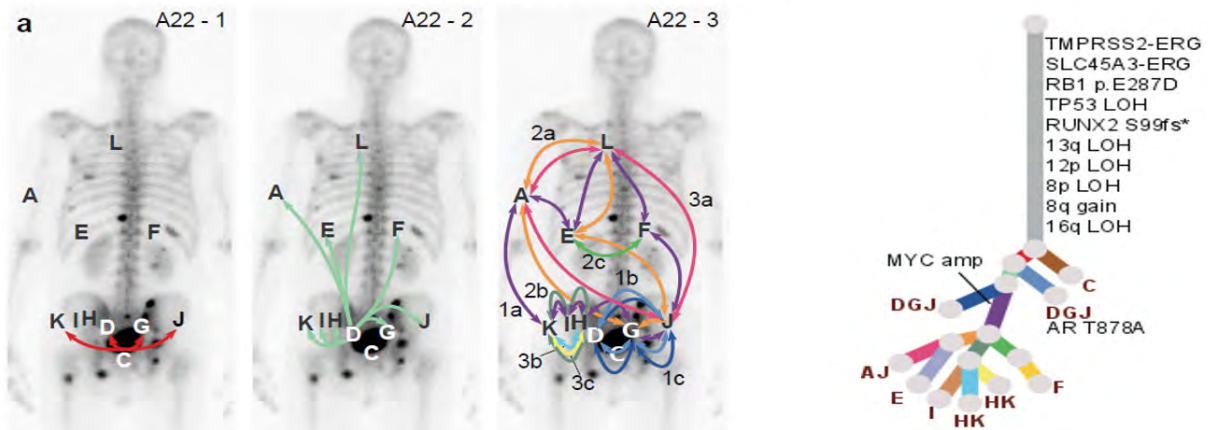


Figure: Right: Identified genomic alterations were used to create a phylogenetic evolutionary tree to show the relationships between all lesions in each patient. Left: Body map depicting the route of seeding of all tumor sites from one patient.

The Natural History of Primary Multifocal Prostate Cancer

Colin Cooper, PhD

University of East Anglia, UK

- Upon prostatectomy, 70-80% of men are found to have multifocal primary disease, in which multiple primary tumor sites can be found. Understanding how these tumor sites have evolved and their relative contribution to disease progression is critical for developing new therapeutic strategies for the treatment of prostate cancer.
- Dr. Colin Cooper discussed a study exploring the evolutionary relationships between multifocal primary prostate tumor sites.
- The presence of multiple co-developing primary tumors suggests that a “field effect” occurs in the prostate. In this hypothesis, a yet unknown process causes the prostate organ to persist in a pro-tumorigenic state that enables the development of many primary tumors.
- To study multifocal prostate tumors, 3D maps of prostate tumor regions were created. First, fresh radical prostatectomy specimens were sliced into 5mm sections and frozen. Frozen slices were then subjected to multiple punch biopsies which were further sectioned and analyzed by histology to identify regions with tumorigenic and normal morphology.
- Genomic sequencing was performed on different areas within individual and separate tumor masses as well as from adjacent normal prostate tissue, to identify genomic aberrations that occurred in each site. A total of 12 tumor sites and 3 normal sites were sequenced from 3 patients (3-5 tumor sites and 1 normal site per patient).

- In all 3 patients, prostate tissue that appeared morphologically normal was found to harbor numerous genomic mutations. In two of these patients, hundreds of mutations had been acquired, while in the third patient, 8 mutations were identified. None of the mutated genes was an obvious driver of tumorigenesis. However, some alterations are likely drivers of clonal expansion in “normal” prostate cells.
- TMPRSS2-ERG translocations occur in ~40% of prostate cancer patients and are thought to be one of the earliest prostate cancer-driving alterations to develop. Unique TMPRSS2-ERG translocations were identified in 11 of the 12 tumor sites examined.
- Researchers at the Sanger Institute have generated a set of 21 genomic signatures of various mutational processes in cancer (Alexandrov et al. Nature. 2013 Aug 22;500(7463):415-21).
- Three of these signatures were identified in the prostate cancer samples – one process caused by aging, and two of unknown causes. The normal tissues from the two patients with high mutational burden also harbored the same mutational signatures observed in their tumor regions. This indicates that the same mutational processes are contributing to the mutations found in the “normal” tissue as in the tumors.
- A Bayesian Dirichlet analysis was performed to identify clonal vs. subclonal relationships between the tumor regions. Both types of relationships were identified between various tumor samples.
- All of these data were used to construct evolutionary trees that describe the relationships between the samples analyzed for each patient.
- In the first patient, four samples were analyzed from a single tumor mass. Within these four tumor samples, three distinct tumor lineages had evolved.
- In a second case (figure), five different tumor lineages and five distinct TMPRSS2-ERG alterations were observed in three separate tumor masses. Some mutations were shared between two of these lineages and the “normal” tissue, indicating earlier or separate clonal cell expansions.
- In the third case, “normal” tissue shared mutations with two tumor lineages that had evolved within a single tumor mass. In a second tumor mass, another two tumor lineages were found: one shared ancestry with the normal tissue and the lineages within the first tumor mass, while the second lineage was completely genomically distinct. In this patient, shared mutations between the two cancers implied that clonal expansion had occurred prior to acquisition of a TMPRSS2-ERG alteration. This refutes the dogma that TMPRSS2-ERG alterations are the earliest genomic event to occur that can drive clonal expansion in all patients with TMPRSS2-ERG alterations.
- Overall, these data indicate that the “normal” prostate is in fact a pro-tumorigenic organ. Acquisition of mutations converts a normal prostate to a “field prostate,” within which morphologically normal prostate cells can clonally expand and multifocal clonal and subclonal prostate cancers can develop.

- How different primary tumor lineages contribute to metastases remains to be determined. Understanding which lineages contribute to progressive disease is also critical for developing biomarkers using tissues in which multiple primary tumor lineages have evolved.
- This project was performed as part of a larger prostate cancer genomics initiative by the International Cancer Genome Consortium (ICGC).

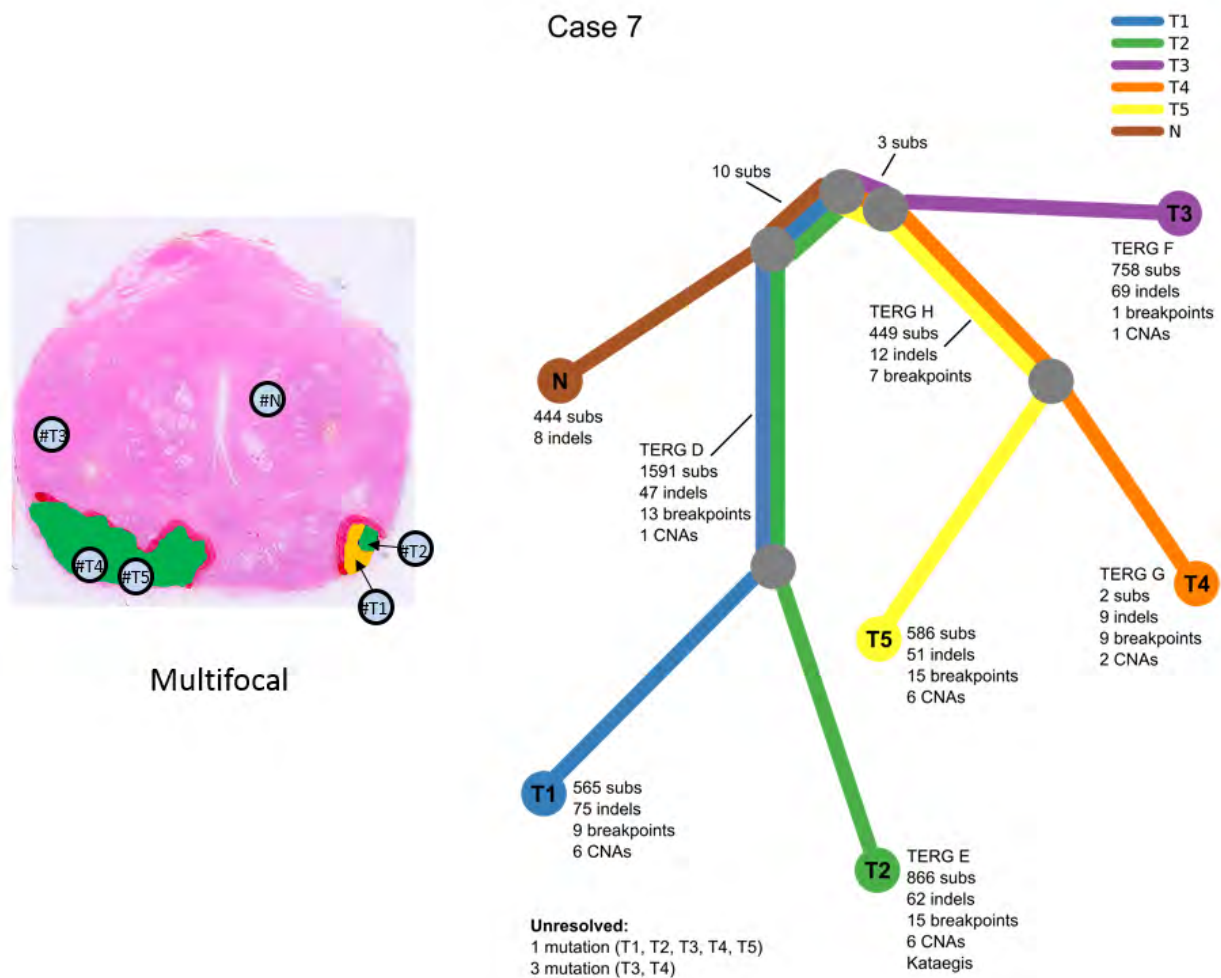


Figure: In one prostate cancer patient, at least five different tumor lineages (T1-T5) and five distinct ERG alterations (TERG D-H) were observed in three tumor separate masses. Mutations were shared between two of these lineages (T1, T2) and “normal” tissue (N). Left: map of prostate and regions where samples were taken from. Right: lineage tree depicting evolutionary relationships between normal and tumor samples.

Tracking the Origins and Drivers of Metastasis in Prostate Cancer

Christopher Hovens, PhD

Australian Prostate Cancer Research Centre; Epworth Hospital; University of Melbourne Parkville, VIC, Australia

- Primary prostate tumors are heterogeneous and multifocal, with multiple clones and subclones co-evolving within the prostate. Understanding the genomic drivers and biology of the clones that contribute to metastasis is critical for developing new therapies.
- Dr. Christopher Hovens discussed studies analyzing the genetic relationships between primary and metastatic tumor clones to identify genomic aberrations that drive the evolution of metastatic tumors.
- Seven metastatic prostate cancer patients with archived fresh primary tumor tissue and long term clinical follow-up data were identified for this study. Metastases were sampled from each patient using CT or ultrasound-guided biopsies.
- Extensive whole genome analyses were performed on metastatic and primary tumor samples to identify genomic alterations, gene expression patterns, and epigenetic modulation.
- In some patients, metastatic sites were sampled both before and after treatments to identify any genomic aberrations related to treatment response or resistance.
- Blood samples were also genomically analyzed to identify any circulating disseminated tumor clones and their relationships to the other tumor sites.
- Deep sequencing depth (number-x) refers to the number of times a nucleotide in the genome is read during genomic sequencing. More genomic reads are required for accurate reconstruction of individual genomes from a mixture of genetically heterogeneous cells.
- Because standard deep sequencing methods (20-50x) were not sensitive enough to reconstruct genomes when multiple genetically variant tumor subclones were present within a single sample, a targeted deep sequencing technology was applied which examined 3,500 genetic variants at a sequencing depth of ~2,500x. Targeted mutations in the tumor suppressor molecule TP53 (aka p53) were further sequenced at up to 10,000x.
- In one patient, metastatic sites had been seeded by tumor subclones, which then reseeded the prostate prior to clinical presentation and surgical removal of the primary tumor.
- In a second patient, a single metastatic site had been seeded by two waves of cells from the primary tumor.
- In a third patient, two metastatic sites were analyzed. The tumor clone that occupied one site was confined to that site, while the clone occupying a second metastatic site was shed

into the blood and had reseeded the first metastasis. This indicates that tumor clones able to form metastases can have different potentials for driving further disease progression.

- In a fourth patient (figure), the pre-metastatic primary tumor subclones remained detectable in the patient's blood 35 months post-prostatectomy. This indicates that there are additional body sites that can harbor prostate tumor cells long term where they remain at subclinical levels.
- In all patients, a smaller tumor subclone present in the prostate evolved into metastatic disease while the major clone occupying the prostate was unrelated to the metastasis. This indicates that the primary tumor does not progress in a linear fashion until metastatic potential is reached. Rather, subclones can metastasize much earlier, while primary tumor clones continue to evolve in the prostate.
- Metastatic lineages from all patients harbored aberrations in DNA mismatch-repair or DNA break-repair pathway genes including BRCA1/2, POLE, POLD1, MSH2, MLH1, ATM, and RAD51D. Mutations in these genes were less prevalent in primary tumors.
- TP53 is the most commonly mutated gene in cancer, although it is not generally recognized as important in primary prostate cancer. TP53 mutations were present in metastatic lesions from all patients. In another patient cohort composed of metastatic and non-metastatic prostate cancer patients, TP53 mutations were present in the primary tumor of 10/19 patients with metastatic disease but only 1/19 patients with localized disease. In some patients, multiple separate TP53 mutations occurred in different metastatic subclones.
- These data indicate that TP53 is important in prostate cancer metastasis and the presence of TP53 mutations in the primary tumor may be used as a biomarker to identify prostatectomy patients at risk for metastasis.
- In summary, these studies have identified clinically relevant mutations in DNA-repair genes and TP53 that occur in metastatic prostate tumors and in the primary tumors of patients at risk for metastasis. These mutations may be drivers of metastasis and may be useful as primary tumor biomarkers to stratify high risk patients. Moreover, metastatic tumor clones can continue to evolve and disseminate to already established tumor sites.
- Ongoing studies will determine whether treatment resistance is driven by preexisting genetically heterogeneous tumor subclones and identify the associated genomic alterations.

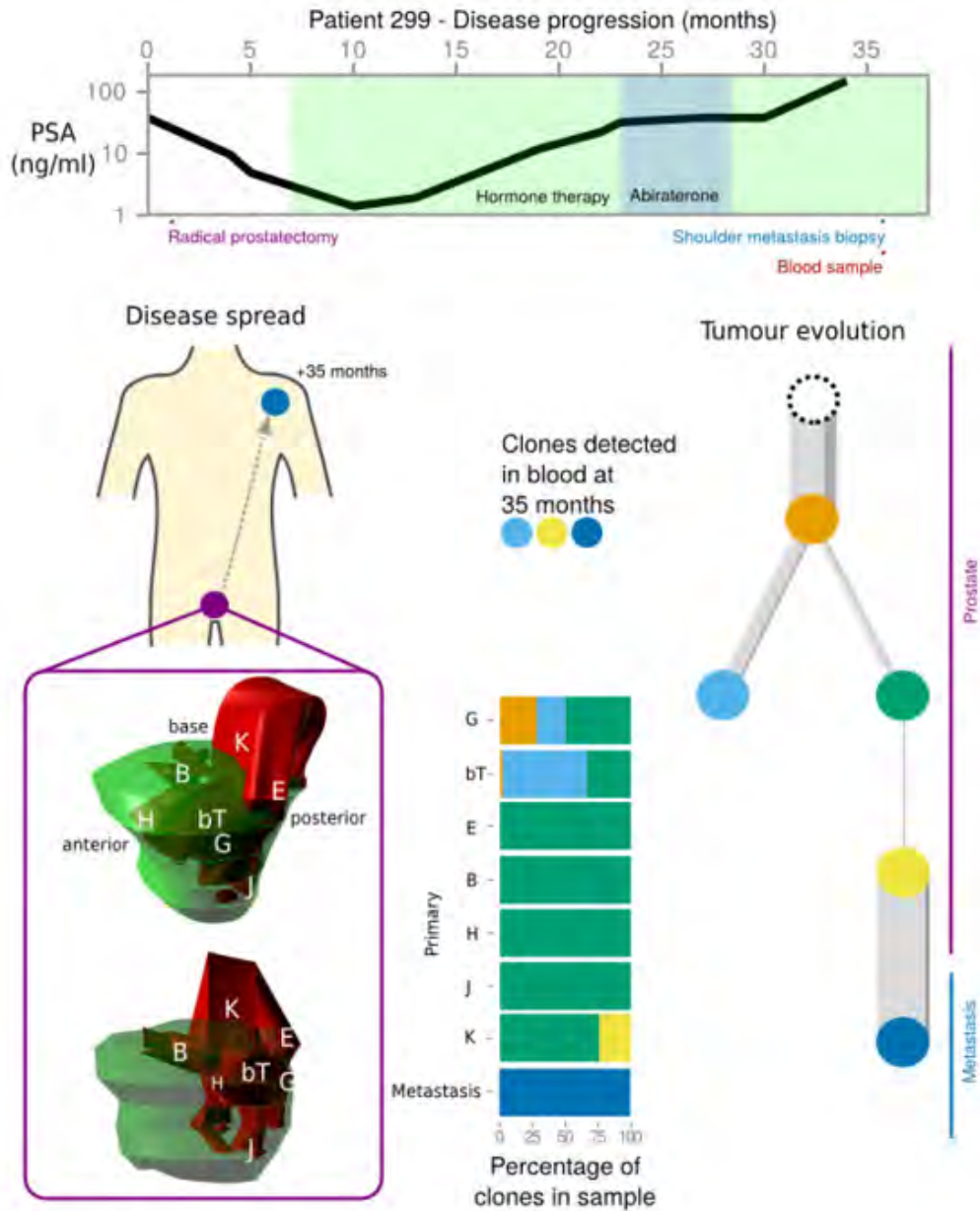


Figure: In one patient, a smaller tumor subclone present in the prostate (green/yellow) evolved into metastatic disease (dark blue) while the major clone occupying the prostate (light blue) was unrelated to the metastasis. The pre-metastatic primary tumor subclones (light blue, yellow) remained detectable in the patient's blood 35 months post-prostatectomy. Top: patient's clinical history. Bottom left: map of primary tumor samples taken from prostatectomy. Bottom right: evolutionary history of tumor clones present in primary and metastatic sites and in blood.

Session 6: Reports from Innovative Conferences

Emerging Concepts in High Risk Prostate Cancer: Update from the Coffey-Holden Prostate Cancer Academy

Joshua Lang, MD

University of Wisconsin Carbone Comprehensive Cancer Center

- The Coffey-Holden Prostate Cancer Academy (CHPCA) Meeting is an annual conference hosted by PCF that brings together ~75 investigators in a think-tank setting to address a critical unmet medical need in prostate cancer.
- The 2015 CHPCA Meeting, held from June 25-28 in La Jolla, California, was themed “Multidisciplinary Intervention of Early, Lethal Metastatic Prostate Cancer” and focused on how to best identify and treat patients with localized high risk or oligometastatic (≤ 5 metastatic lesions) prostate cancer.
- Dr. Joshua Lang, who chaired the 2015 CHPCA Meeting, presented an overview.
- 71 investigators, including 31 PCF-funded young investigators, attended the meeting. 10 minute talks were followed by 20 minutes of discussion. This highly interactive format resulted in over 400 questions from attendees over the 3-day meeting.
- The topics of the major sessions included: optimizing treatment strategies, the biology of high-risk disease, targeting the tumor microenvironment, new molecular imaging and molecular biomarkers, and rationale for multi-modal interventions.
- Surgical removal of the primary tumor (radical prostatectomy) in high-risk patients was previously deemed an option in patients for whom chances of cure were high. However, improved surgical techniques, earlier diagnosis from PSA screening, and anecdotal evidence that elimination of the primary lesion can prolong survival even without being curative, has recast a wider net for patients who could be candidates for radical prostatectomy.
- Treatment of the primary tumor with radiation combined with androgen deprivation therapy (ADT) has also been shown to extend survival compared with ADT alone.
- Overall, these studies indicate that treatment of the primary tumor can potentially extend survival in patients with advanced prostate cancer.
- Pathologists measure the aggressive potential of prostate tumors by assigning a “Gleason Score” which sums the “Gleason patterns” (GP) of the two most prominent tumor phenotypes observed in primary tumor samples. GP3 tumors are usually considered indolent while GP4 suggests more aggressive disease. Analysis of mutations present in adjacent GP3 and GP4 tumors from Gleason Score 7 patients found that these tumors shared many mutations, suggesting a clonal origin. Shared mutations included oncogenic

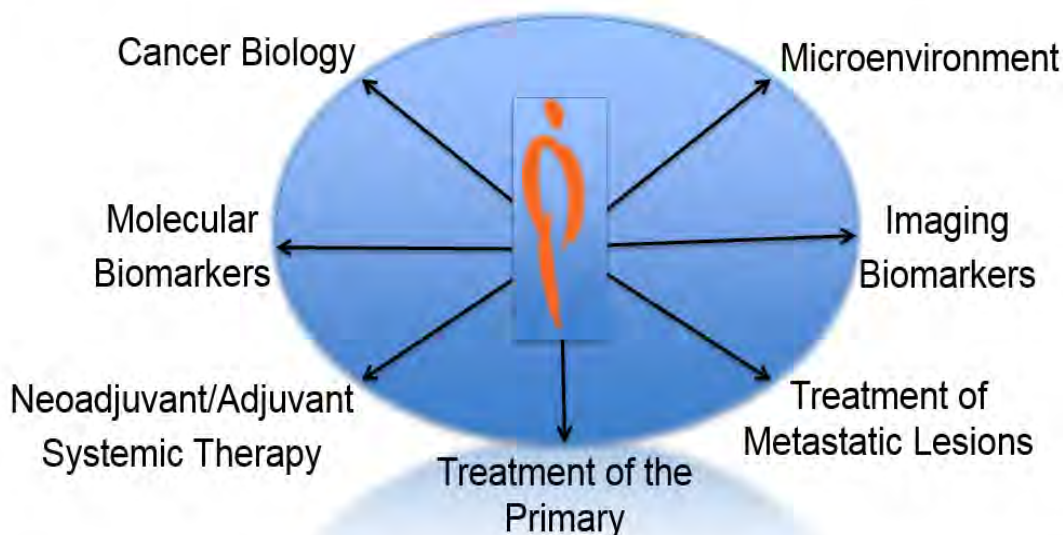
H-Ras mutations. However, GP4 tumors had gained additional mutations in tumor suppressor genes. A higher rate of mutations was observed in tumors with mutations or loss of DNA repair genes. These studies provide insight into the processes associated with the biology of aggressive prostate cancer.

- The tumor microenvironment includes cell types such as fibroblasts, smooth muscle cells, neuroendocrine cells, endothelial cells, nerve cells, and immune cells in addition to prostate tumor cells. Other properties of the tumor microenvironment that play a role in tumor growth include matrix components and secreted growth factors, nutrients, hormones, and cytokine signaling proteins.
- Understanding the biology of the tumor microenvironment will provide rationale for therapeutic targeting of components that promote tumor growth.
- Examples of tumor-promoting microenvironment factors include androgen production by stromal cells which drives the growth of prostate tumors and may buffer tumors from changes in hormone levels elicited by treatments such as ADT. Also found to be important are WNT proteins secreted by tumor and stromal cells, which drive tumor cell growth and inhibit anti-tumor immune responses by reducing the proliferation and functional capacity of T cells.
- Infiltration of tumors by immune cells is important for native and therapy-induced anti-tumor immune responses. However, the tumor microenvironment is highly immune suppressive via various mechanisms. T cells activated by the Sipuleucel-T cancer vaccine were found at tumor peripheries but rarely within tumor centers. While T cell diversity was found to increase with treatment, additional measures are needed to bring T cells into tumors and maintain their activity once there for maximal tumor-killing effects.
- Newly emerging molecular biomarkers and molecular imaging technologies will allow earlier detection of prostate cancer and improved assessment of treatment responses. Specialized imaging agents (theranostics) can be used for both imaging and targeted therapy.
- One of the most promising new PET (positron emission tomography) imaging technologies uses radiolabeled imaging agents that target PSMA, a prostate cancer-associated prostate cell surface molecule. Multiple PSMA-PET agents are in development and have shown superior specificity and sensitivity compared with traditional bone scans.
- Whole body magnetic resonance imaging (WB-MRI) is another emerging technology that can detect multiple structural and functional aspects of tissues, has demonstrated similar diagnostic accuracy as sodium-fluoride-PET/CT and was superior to bone scans.
- Novel quantitative imaging methods are being used to study heterogeneous responses to therapy in different metastatic lesions in a patient. Genomic analysis of the most responsive versus most resistant lesions from the same patient will lend significantly to understanding the biology of treatment resistance.

- The cost of these new imaging technologies is significantly higher than current standard bone scans. Clinical efficacy in improving patient outcomes must be demonstrated in clinical trials before widespread adoption of these technologies can occur.
- Recently completed and ongoing clinical trials in patients with high risk or oligometastatic disease will aid in defining the most optimal treatment strategies for these patients.
- Several recently completed neoadjuvant clinical trials have demonstrated efficacy in upfront aggressive androgen axis blockade with Xtandi or Zytiga followed by radical prostatectomy in high risk prostate cancer patients. However, treatments were not curative. Genomic characterization of residual tumor foci in one of these trials found significant heterogeneity in different tumor foci in individuals as well as de novo resistant disease. An ongoing clinical trial is testing even more complete neoadjuvant androgen axis blockade with Zytiga plus ARN-509 in intermediate and high-risk prostate cancer patients.
- A novel strategy of using an agent to drive bone metastatic tumor cells from the protective bone marrow microenvironment followed by chemotherapy to kill mobilized tumor cells is being tested in an upcoming clinical trial at Johns Hopkins University. Bone marrow biopsies and CTCs will be studied in these patients to analyze tumor cell phenotypes and validate mechanisms of therapeutic activity.
- Clinical trials are also testing the efficacy of radical prostatectomy combined with standard treatment versus standard treatment alone in patients with oligometastatic disease.
- Another clinical trial in oligometastatic prostate cancer patients is testing the efficacy of neoadjuvant chemotherapy followed by radical prostatectomy and stereotactic body radiation therapy, which is radiotherapy targeted to individual metastatic tumors.
- Patients with low-burden high-risk disease may be an optimal population to treat with immunotherapy. A clinical trial is investigating the curative potential of the combination of the T cell activating therapy ipilimumab with degarelix hormone therapy followed by radical prostatectomy in patients with metastatic hormone-sensitive prostate cancer.
- In summary, the integration of improved imaging methods, molecular biomarkers and multi-modal therapeutic strategies may enable curative treatment of men with localized high risk or oligometastatic prostate cancer.
- A detailed summary of the 2015 CHPCA Meeting written by the organizing committee was published in the scientific journal The Prostate:
<http://onlinelibrary.wiley.com/doi/10.1002/pros.23107/abstract>

Can We Treat for Cure?

Intersection of Biology, Biomarkers and Multi-modal Therapeutics



It is time for multimodal therapy to become the standard of care for many of our patients.

-Dr. Patrick Walsh



Highlights from the Advanced Prostate Cancer Consensus Conference

Silke Gillessen, MD

Kantonsspital, Switzerland

- In March 2015, the first St. Gallen Advanced Prostate Cancer Consensus Conference (APCCC) was held in St. Gallen, Switzerland, to generate recommendations for clinical management of prostate cancer patients in areas where little or no evidence exists.
- Dr. Silke Gillessen provided an overview of the recommendations generated at the conference.
- 41 panel members who are experts in prostate cancer participated in creating the consensus recommendations. Panelists consisted of urologists, medical oncologists, clinical oncologists, radiologists, pathologists, statisticians, geneticists, biologists, and nuclear medicine experts from around the world.

- Topics of critical unmet need that were discussed included: management of castration-naive metastatic prostate cancer; definition of castration-resistance; management of non-metastatic castration-resistant prostate cancer (CRPC); the value of hormonal therapy without proven survival-benefit; treatment sequencing for metastatic CRPC; disease staging and treatment monitoring; use of bone-targeted agents for prevention of skeletal related events; value and use of predictive biomarkers; oligometastatic prostate cancer; and general management of patients.
- The modified Delphi process was used to generate the consensus recommendations. Prior to the conference, consensus questions were drafted based on the most important areas of controversy in prostate cancer and sent to 41 selected panel members. All comments and revised questions based on comments were circulated for a total of three rounds.
- At the conference, the consensus questions were discussed and voted on by the expert panel.
- Following the conference, a manuscript detailing the consensus recommendations was circulated among the panel before peer-reviewed publication in the scientific journal *Annals of Oncology*.

Consensus recommendations included:

- Zytiga (abiraterone) or Xtandi (enzalutamide) treatment in addition to androgen deprivation therapy (ADT) was recommended as first-line therapy for the majority of asymptomatic or minimally symptomatic CRPC patients. No general consensus preference was obtained for Zytiga versus Xtandi in this treatment strategy.
- Chemotherapy was generally not recommended or only recommended for a minority of asymptomatic or minimally symptomatic CRPC patients. However, more panelists recommended chemotherapy for healthy but symptomatic CRPC patients as first-line therapy.
- The bone-targeted radionuclide radium-223 was generally not recommended, or only recommended for a minority of symptomatic CRPC patients with bone but no visceral metastases as first-line therapy.
- The skeletal fracture prevention agent zoledronic acid was generally not recommended in every 3-4 week doses for castration-naive patients with bone metastases. The bone-loss treatment denosunab was also not generally recommended every 4 weeks for these patients. For castration-resistant patients with bone metastases the agents were generally recommended. However, no consensus was reached for the optimal dosing and timing of these agents in this setting with roughly a third of panelists favoring each of the following regimens: every 3-4 weeks; less frequently than every 3-4 weeks from the start of therapy; or every 3-4 weeks until ~2 years followed by less frequent administration.
- Other recommendations include: measuring of testosterone levels to determine castration resistance, performing baseline staging examinations that include imaging before starting a

treatment for CRPC, using imaging and other methods beyond PSA to monitor treatment of CRPC patients, and encouraging patients to enter clinical trials.

- Practitioners are recommended to NOT: treat castration-naive metastatic prostate cancer patients with bisphosphonates (zoledronic acid) or denosumab in the dose for reduction of skeletal related events; treat non-metastatic CRPC patients with survival prolonging agents outside of a clinical trial; treat men progressing on ADT with bicalutamide if Zytiga or Xtandi are available, or to stop treatment of CRPC patients based only on a rise in PSA.
- Topics that consensus were not reached on included diagnosis and therapy of oligometastatic disease and how to use novel imaging methods to guide treatment decisions.
- Another consensus conference to address additional topics of critical unmet need in prostate cancer is planned for 2017.
- The consensus recommendations published in Annals of Oncology can be accessed free of charge at: <http://annonc.oxfordjournals.org/content/26/8/1589.long>

Session 7: The Collision of Technology and Immunotherapy for Patients

Biomaterials as Therapeutic Cancer Vaccines

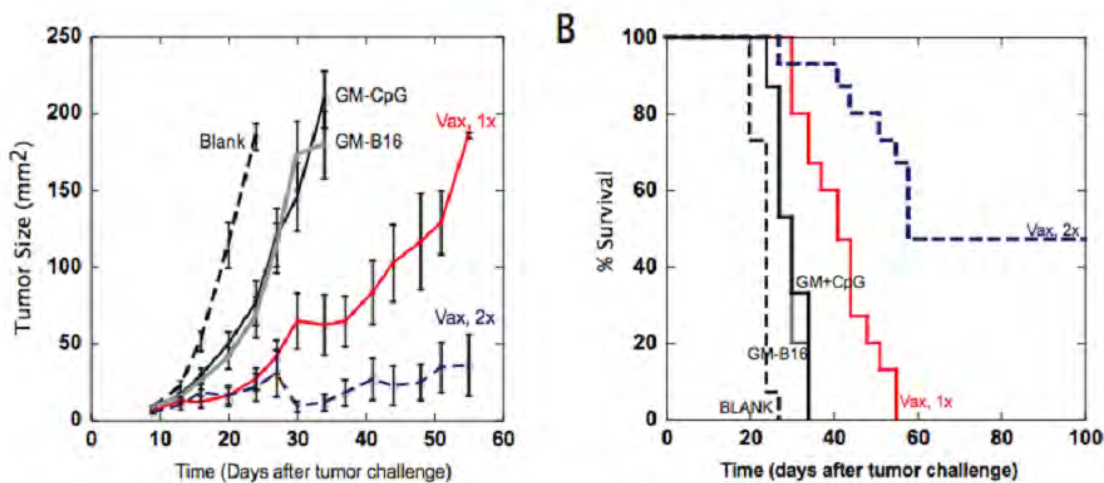
David Mooney, PhD
Harvard University

- The Sipuleucel-T prostate tumor vaccine is the only immunotherapy that has received FDA-approval for the treatment of prostate cancer patients. Sipuleucel-T harnesses the immune-activating power of dendritic cells (DCs), which function to detect dangers such as pathogens or tumors, and activate antigen-specific T cell responses. In this vaccine strategy, DCs are removed from patients and loaded with a prostate tumor antigen linked to the DC growth factor, GM-CSF. DCs are then reinfused into patients where they activate T cells to target and kill tumor cells. This vaccine extends lives for only ~4months, but costs ~\$100,000 and is a complex and cumbersome procedure done for individual patients. More effective and less expensive cancer vaccine strategies are needed.
- Dr. David Mooney has created a biomaterial-based vaccine which functions as an in vivo alternative to Sipuleucel-T but is much simpler and has the potential to be a more affordable procedure. In this strategy, a highly porous biomaterial tablet the size of an aspirin pill is loaded with dead tumor cell particles and GM-CSF and injected under a patient's skin.
- The biomaterial releases GM-CSF to recruit immature DCs into the material. Inside the biomaterial, DCs pick up tumor cell particles and become activated. They then traffic to patient lymph nodes where they activate anti-tumor T cell responses.
- The biomaterial dissolves in the body over time, leaving nothing permanent behind.
- This highly porous biomaterial can house millions of cells at a time. When injected under the skin of mice, the biomaterial was highly infiltrated by DCs. Infiltration required loading of the biomaterial with GM-CSF. Increasing the dose of GM-CSF in the biomaterial increased the number of recruited DCs.
- Various adjuvants and immune activation molecules can be added to the biomaterial to boost the activation of arriving DCs. CpG is a form of DNA associated with bacteria and is a strong vaccine adjuvant. CpG nanoparticles were generated and used to decorate the surface of the biomaterial. The CpG nanoparticles were picked up by entering DCs and enhanced their activation.
- To confirm that DCs can pick up antigens from the biomaterial and carry them to lymph nodes, the biomaterial was loaded with the fluorescent protein FITC and injected under the skin of mice. When the biomaterial was also loaded with GM-CSF and CpG, DCs carrying FITC could be found entering lymph nodes.

- In mice with very aggressive melanoma tumors that do not typically trigger anti-tumor immune responses, a single vaccination with the biomaterial placed under the skin prohibited tumor growth better than GVax, a cancer vaccine composed of irradiated tumor cells loaded with GM-CSF, that is currently in phase III clinical trials. Mice that were given a second vaccination with the biomaterial experienced complete regression of their melanoma (figure).
- Very high numbers of melanoma-specific T cells were generated and could be found in tumors in these mice.
- The efficacy of the biomaterial vaccine was hypothesized to be due to the ability of the biomaterial to recruit and activate diverse types of DCs. CD8+ DCs and plasmacytoid DCs were both seen to increase in frequency at the vaccine site and correlated with efficacy.
- Anti-CTLA4 (ipilimumab) and anti-PD1 (nivolumab) antibodies are highly effective immunotherapeutic agents that have been approved for the treatment of multiple cancers. These agents act to block inhibitory “checkpoint” signals on T cells, allowing T cells to better kill tumor cells.
- Because the biomaterial vaccine activates DCs which then elicit anti-tumor T cells, synergy with anti-CTLA4 or anti-PD1 is likely. Combination of the vaccine with anti-CTLA4 or anti-PD1 antibodies significantly increased survival of tumor-bearing mice and led to a significant number of tumor regressions.
- WDVax is a form of the biomaterial vaccine that is being tested in a melanoma patient clinical trial underway at the Dana Farber Cancer Institute (DFCI). To obtain the patient-specific dead tumor particles needed to generate the vaccine, each patient’s tumor is biopsied and freeze-dried to create a powder that is loaded into the biomaterial.
- Thus far, 12 patients have been treated and no safety concerns have been observed. The majority of patients are showing stable disease or no disease at this time. Two patients have experienced disease progression and are being studied for mechanisms of vaccine failure.
- Another biomaterial vaccine technology being developed by Dr. Mooney and team are injectable cryogels. These “gels” are highly elastic and have a structure that can be compacted with pressure and regained within 200 milliseconds after removal of the pressure. This allows loading of cryogels into a syringe and injection under the skin, where they regain their original shape.
- A third biotechnology under development employs mesoporous silica microparticles that form a 3D structure following injection under the skin. These microparticles have nanopores that can be loaded with immune activating molecules, bioactive drugs of interest, or tumor cell particles.
- When injected into mice, recruitment of immune cells into the microparticle structures was demonstrated.

- Mesoporous silica particle-based vaccines were able to generate humoral (antibody) responses in mice. High serum levels of IgG1 and IgG2 antibodies were found following a single injection.
- In summary, several novel biomaterial-based vaccination strategies have been developed and have demonstrated efficacy in numerous mouse tumor models. These vaccines will hopefully soon be tested in prostate cancer.

Therapeutic Effect



(Ali et al., *Science Translational Medicine*, 2009)

Figure: In mice with B16 melanoma, a single vaccination (Vax, 1x) with the biomaterial placed under the skin prohibited tumor growth better than GVax, another cancer vaccine strategy currently in phase III clinical trials. Mice that were given a second vaccination (Vax, 2x) experienced complete regression of their melanoma.

Lymph Node Targeting Nanoparticle Cancer Vaccines

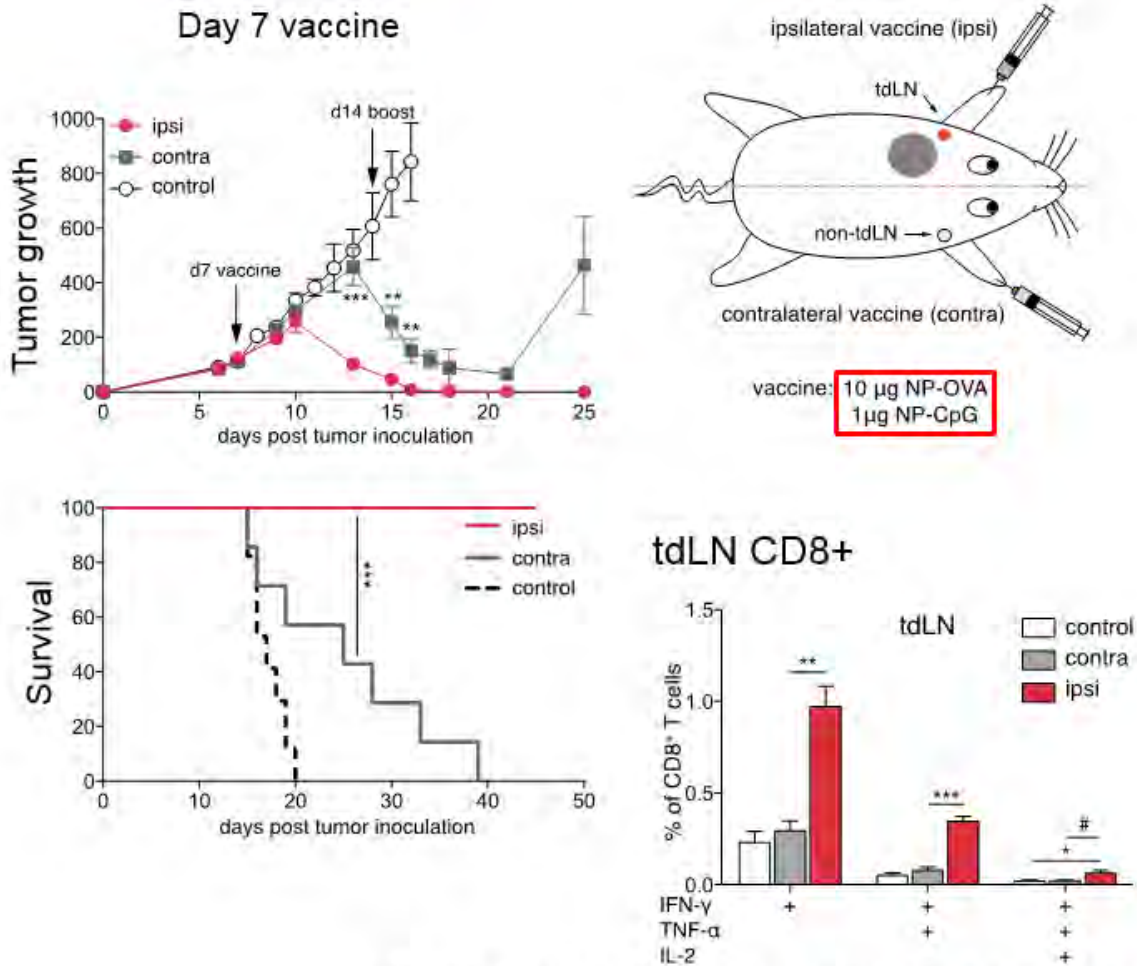
Jeffrey Hubbell, PhD
University of Chicago

- Lymph nodes are specialized immune organs where the initial activation of antigen-specific T cell and B cell responses occur. Targeting tumor vaccine materials to lymph nodes may enhance the generation of anti-tumor immune responses and improve vaccine efficacy.
- Dr. Jeffrey Hubbell discussed the development of a novel tumor vaccine technology using ultrasmall nanoparticles that can traffic to lymph nodes and carry tumor vaccine components.
- Particles smaller than 100nm are able to efficiently pass through the interstitial space between capillaries and reach draining lymph nodes within 10-20 minutes following intravenous or intramuscular injection. Larger particles tend to remain stuck in tissues at the site of injection.
- To create nanoparticles (NP) that can elicit an immune response and be used for tumor vaccinations, tumor antigens or adjuvant molecules were conjugated to the surface of ultrasmall (30nm) nanoparticles using a disulfide bond-mediated cross-linking chemistry. This chemistry allows release of the molecules after nanoparticles are taken up into the endosomes of cells and the disulfide bonds are reversed by the reducing environment.
- The efficacy of a tumor vaccine comprised of nanoparticles carrying the CpG adjuvant (NP-CpG) plus nanoparticles carrying the model antigen OVA (NP-OVA) were tested in mice with OVA-expressing lymphoma tumors.
- Vaccination of mice with NP-CpG + NP-OVA delayed tumor growth and induced significantly more antigen-specific cytotoxic T cells than vaccination with free OVA and CpG or NP-CpG alone. This indicates that NPs carrying adjuvants and tumor antigens can target and activate more lymph node immune cells and are thus a superior vaccine delivery method compared with freely injected adjuvants and tumor antigens.
- To test if there is a difference between targeting tumor-draining lymph nodes versus non-tumor-draining lymph nodes with the vaccine, mice with a tumor growing on one flank were vaccinated either on the same flank (ipsilateral) or on the opposite flank (contralateral), and tumor growth and anti-tumor immune responses were monitored.
- Ipsilateral vaccination suppressed tumor growth, extended survival of mice, and activated cytotoxic anti-tumor T cells significantly better than contralateral vaccination. This indicates that tumor-draining lymph nodes may be more antigen-experienced and already harbor a population of anti-tumor T cells that are boosted by the vaccine.
- Dendritic cell (DC) vaccines have been developed for metastatic prostate cancer. The FDA-approved Sipuleucel-T vaccine requires isolating DCs from patients, loading them with

tumor antigens and activating them with the DC growth factor GM-CSF, before reinfusing them into patients.

- Exosomes are small vesicles that are secreted from cells and carry proteins and nucleic acids from that cell. Exosomes are sufficiently small (~100nm) to efficiently drain through the interstitial fluid and reach lymph nodes.
- Dr. Hubbell explored whether dendritic cell exosomes (“dexosomes”) could perform similarly to DC tumor vaccines. Dexosomes can carry molecules that DCs use to activate immune responses, including the MHC surface molecules that present antigens to T cells in order to activate antigen-specific T cell responses.
- Dexosomes were collected via ultracentrifugation of supernatant from ovalbumin (OVA)-loaded DCs that had been activated in the presence of various adjuvants, and used to vaccinate mice bearing lymphoma tumors.
- Dexosomes from DCs that had been activated with an adjuvant that mimics viral RNA (polyI:C) carried significantly more MHC molecules and elicited superior anti-tumor T cell responses compared with dexosomes from DCs activated with a bacterial membrane molecule (LPS) adjuvant.
- The OVA antigen induces robust immune responses that may not be physiologically relevant in the context of cancer immunity. To test the vaccination efficacy of dexosomes in a setting more typical to patients, dexosomes were collected from DCs that had been pulsed with dead melanoma cell debris (no model antigen added) and activated with polyI:C
- Mice bearing aggressive melanoma tumors were given priming and booster vaccinations with the melanoma debris-dexosomes. Some delay in tumor growth was elicited by dexosomes from DCs activated with either melanoma debris or polyI:C, while dexosomes from DCs activated with both factors induced significant cytotoxic melanoma-specific T cell activity and resulted in long term survival of mice.
- This strategy also reduced the expression of the T cell inhibitory molecule PD1, compared with other vaccination strategies.
- In summary, novel nanoparticle-based vaccination strategies that are able to carry vaccine components to lymph nodes and induce robust anti-tumor immune responses were developed. These vaccines may be promising future therapies for prostate cancer patients.

Targeting Vaccine to the Tumor-draining LN is Superior to a Non-draining LN



Laura Jeanbart et al., *Cancer Immunol Res* 2:436-47 (2014)

Figure: To test if there is a difference between targeting tumor-draining LNs (tdLN) vs non-tumor-draining LNs with the vaccine, mice with a tumor growing on one flank were vaccinated either on the same flank (ipsilateral) or on the opposite flank (contralateral), and tumor growth and anti-tumor immune responses were monitored. Ipsilateral vaccination suppressed tumor growth (top left), extended survival of mice (bottom left), and activated cytotoxic anti-tumor T cells (bottom right) significantly better than contralateral vaccination. Top right: vaccination strategy; gray circle indicates tumor location.

Enhancing Cell Therapy of Cancer with Nanotechnology

Darrell Irvine, PhD

Massachusetts Institute of Technology

- Adoptive T cell therapy has produced long-term regressions and apparent cures in some leukemia patients. However, responses are not as significant in the treatment of solid tumors, as the solid tumor microenvironment includes large numbers of immune-suppressive cells that restrain the activity of the T cells.
- Treatments that have been administered to patients to enhance the function of adoptively transferred T cells, such as interleukin-2 (IL-2), interferon-alpha, anti-CD40, and anti-CD137, are accompanied by severe side effects including cardiopulmonary toxicity, respiratory distress, neutropenia, opportunistic infections and liver damage. Better ways of delivering supportive signals to T cells are needed to enhance the efficacy of adoptive T cell therapies.
- Dr. Darrell Irvine developed a technology to attach drug-carrying nanoparticle “backpacks” to T cells to either stimulate the T cell itself, or to allow the T cell to deliver a drug to other cells in the tumor microenvironment.
- A cell surface cross-linking chemistry was designed to link a drug-carrying nanoparticle to the surface of a cell. Nanoparticles were coated with reactive chemical groups that form bonds with molecules on the cell surface, linking the nanoparticle to the cell. When particles were mixed with phagocytic cells such as macrophages, they ended up being engulfed, whereas they remained attached to the surface of non-phagocytic cells such as T cells and hematopoietic stem cells for extended periods of time. Nanoparticles also were kept on the surface of non-phagocytic cells after cell division, getting divided between daughter cells.
- Nanoparticles did not affect the ability of T cells to traffic to tumors in mouse tumor models. Nanoparticles were also efficiently delivered to tumors by T cells.
- In a B16 metastatic melanoma mouse model, when melanoma-specific T cells were given nanoparticle backpacks loaded with T-cell proliferation inducers IL-21 and IL-15, massive expansion of T cells was observed. The T cells were maintained as long term memory T cells and 100% of mice experienced tumor regressions compared with no regressions in untreated mice or in mice given T cells along with separate injections of IL-21 and IL-15. This indicates that nanoparticles can efficiently deliver supportive autocrine signals to T cells in vivo.
- T cells were tested for their ability to carry potent SN-38 chemotherapy-containing backpacks to the tumor site (figure). In this assay, T cells were expanded in the presence of rapamycin and IL-2 which maintains their ability to home to tumors but causes the cells to stop dividing, which allows the T cell to be resistant to the chemotherapy. In a lymphoma model, when T cells delivered the chemotherapy, 10-fold less drug was able to kill tumor cells at a significantly higher efficacy compared with injection of chemotherapy-nanoparticles or chemotherapy alone.

- In summary, novel nanoparticle therapies that arm tumor-homing T cells with supportive factors or anti-tumor drugs were developed and will be tested in prostate cancer models.

Paracrine “pharmacy” strategy allows active chemotherapy targeting to tumors

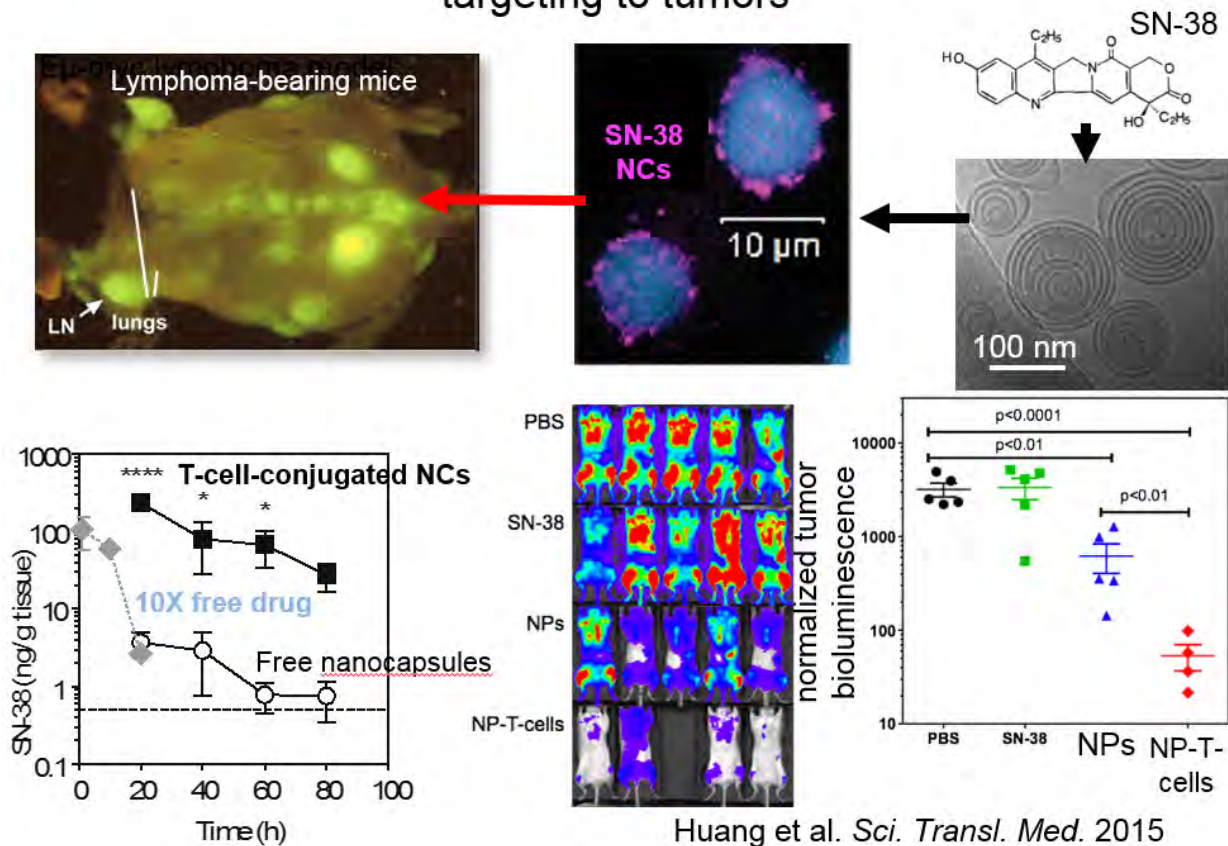


Figure: T cells were tested for their ability to carry potent SN-38 chemotherapy-containing backpacks (top center) to the tumor site (top left). Bottom: in a lymphoma model, when T cells delivered the chemotherapy, 10-fold less drug was able to kill tumor cells at a significantly higher efficacy compared with injection of chemotherapy-nanoparticles or chemotherapy alone (SN-38).

Bispecific Antibody Armed Activated T-Cells (BATs) in Prostate Cancer

Ulka Vaishampayan, MD

Wayne State University; Karmanos Cancer Institute

- There are several therapeutic strategies to induce T cells to target and kill tumor cells.
- Chimeric antigen receptor (CAR) T cells are genetically modified to express a CAR molecule that consists of a cell surface tumor-targeting antibody fragment connected to an intracellular T cell activating molecule fragment. CAR T cells exhibit brisk clinical responses

against tumor cells and can persist for years in a patient. However, CARs exhibit significant autoimmune toxicities as they may kill any normal cell that also expresses the tumor-associated target gene. For instance, anti-CD20 CAR T cells have demonstrated robust clinical responses against B cell lymphomas. However, CD20 is expressed on all B cells, and patients given anti-CD20 CAR T cells lose normal B cells as well. CARs are also extremely expensive, with an estimated cost of \$500,000 per patient.

- Bispecific antibodies (BiTEs) are composed of fragments of two different antibodies conjugated together. One of the antibodies targets a tumor cell antigen and the other targets the T cell activation molecule, CD3. BiTEs function by bridging a contact between T cells and tumor cells, prompting the T cell to kill the tumor cell. BiTEs must be continually infused into patients and are associated with significant toxicities including cytokine release syndrome and neurotoxicity. Blinatumomab is an anti-CD20/anti-CD3 BiTE that has been FDA approved for the treatment of B cell leukemia.
- Bispecific antibody armed activated T-Cells (BATs) are generated by coating patient T cells with anti-CD3/anti-tumor bispecific antibodies. This technique avoids the toxicities associated with continual infusion of BiTEs, while being able to deliver T cells with CAR-like activity but at a far lower cost than CARs.
- Dr. Ulka Vaishampayan discussed the development and clinical trial results of a BAT therapy in prostate cancer.
- To produce BATs, peripheral blood immune cells are first isolated from a patient by apheresis. T cells are expanded for several days in an incubator by culturing with OKT3, another anti-CD3 antibody that activates T cells, and IL-2, a cytokine that induces T cell proliferation. Expanded T cells are coated with the bispecific antibody and reinfused into patients.
- A BAT therapy was created that targets HER2, a human epidermal growth factor receptor commonly expressed in breast cancer.
- In a phase I trial in heavily pre-treated metastatic breast cancer patients, the HER2-BAT was well tolerated. The only noted toxicities were some rigors and infusion reactions. The BATs induced cytokine release, which depleted immunosuppressive cells and promoted anti-tumor cytotoxicity.
- Clinical responses were observed in some patients, with a median overall survival of 57.4 months in patients with HER2+ tumors and 27.4 months for patients with HER2-negative/low tumors.
- A number of prostate cancer cell lines were found to be sensitive to HER2-BATs in cell culture assays, prompting the testing of this agent in prostate cancer patients.
- A phase I study in eight symptomatic metastatic castrate resistant prostate cancer (mCRPC) patients was conducted. Men received 2 infusions per week for 4 weeks in the presence of IL-2 and the immune activating molecule GM-CSF. Doses ranged from 2.5 -10 billion BAT cells per infusion.

- No dose-limiting toxicities were observed. Of 7 evaluable patients, one partial response was observed with a PSA reduction of >50% for 4 months (figure). Two additional patients had significant PSA decreases and reductions in pain scores (figure).
- Patient immune cells were analyzed before versus after BAT treatment. Increased T cell activity was observed following BAT administration.
- BATs exhibited enhanced activity when cultured with ipilimumab (Yervoy), an immunotherapy that blocks inhibitory signals on T cells. This indicates that therapeutic synergy might be achieved by combining BATs with ipilimumab.
- A phase II clinical trial testing the combination of HER2-BATs with an inhibitor of PD-1/PDL-1, another set of regulatory T cell molecules, is being planned in mCRPC.

Change in PSA levels in three CRPC patients (60202 and 91760) who had minor or partial responses (FG60163; PSA levels declining by >50% post therapy).

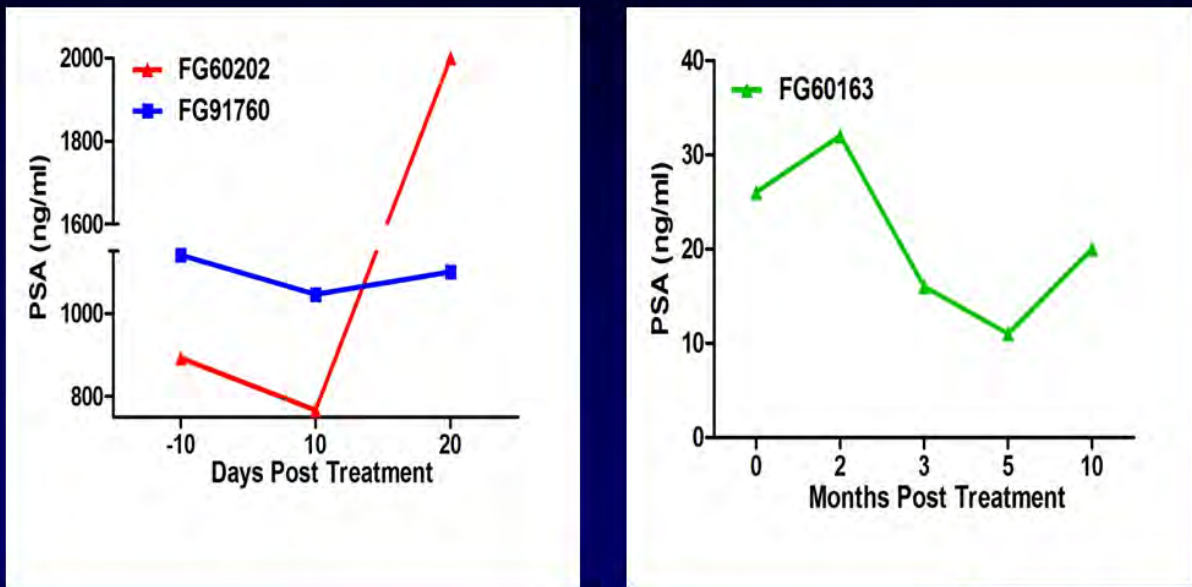


Figure: A phase I study in eight symptomatic metastatic castrate resistant prostate cancer (mCRPC) patients was conducted. Of 7 evaluable patients, two minor responses were observed (left) and one partial response was observed with a PSA reduction of >50% for 4 months (right).

Session 8: Global Research in the UK

Exploring and Exploiting the Crosstalk between Androgen and MicroRNA Signaling in Prostate Cancer

Claire Fletcher, PhD

Imperial College London; London Movember Centre of Excellence, UK

- The androgen receptor (AR) is a crucial regulator of prostate cancer growth and survival. Multiple therapies have been developed to target AR, but drug-resistance is common. New strategies to block this pathway are urgently needed.
- MicroRNAs (miRs) are small (18-22 nucleotides) non-coding RNA molecules that turn off the expression of genes with related RNA sequences. MicroRNAs regulate diverse cellular functions and can function as tumor suppressors or oncogenes.
- Dr. Claire Fletcher discussed the discovery of oncogenic microRNAs that regulate the AR pathway and represent new potential targets for prostate cancer therapy.
- The prohibitin (PHB) tumor suppressor protein suppresses the expression of AR and blocks the growth and progression of prostate tumors.
- The expression of PHB was found to be repressed by the oncogenic microRNA, miR-27a. This required binding of miR-27a to the 3' untranslated region of the PHB mRNA.
- Treatment with an inhibitor targeting miR-27a suppressed the growth of prostate cancer cells in culture and prostate tumors in mice, demonstrating that miR-27a is a prostate cancer oncogene that may be targeted for prostate cancer treatment (figure).
- miR-27a is coded on the genome beside two other miR genes: miR-23a and miR-24-2.
- The expression of all three miRs was activated by androgens and prohibited by treatment with the AR-targeting drug bicalutamide.
- AR was found to be recruited to the promoter of the miR gene cluster. AR was also found to promote processing of the miR-27a RNA to its active suppressive form. These data demonstrate that miR-27a and AR co-regulate one another and suggest that miR-27a inhibition may be effective in combination with inhibitors of the AR pathway.
- A screening assay was performed to identify other miRs that positively or negatively regulate AR activity. Of 950 miRs tested, 80 were identified that could regulate AR in one of two prostate cancer cell lines. Eight miRs regulated AR activity in both cell lines and were selected for further study.

- An example of a miR identified in the screen as a positive regulator of AR was discussed. Overexpression of this miR in prostate cancer cells enhanced AR activity while an inhibitor of this miR reduced AR activity, confirming the AR-promoting activity of this miR.
- Inhibition of the miR blocked prostate cancer cell growth and caused cell death.
- Overexpression of the miR promoted prostate cancer cell migration and invasive properties including epithelial-to-mesenchymal transition (EMT), a phenotypic change that cancer cells undergo in order to gain metastatic potential.
- These data suggest that, like miR-27a, inhibition of this miR and other miRs identified in the screen may be used in combination with AR-inhibitors in the treatment of prostate cancer.

miR-27a is an OncomiR in PCa – Inhibition Represses Tumour Growth *in Vitro* and *in Vivo*

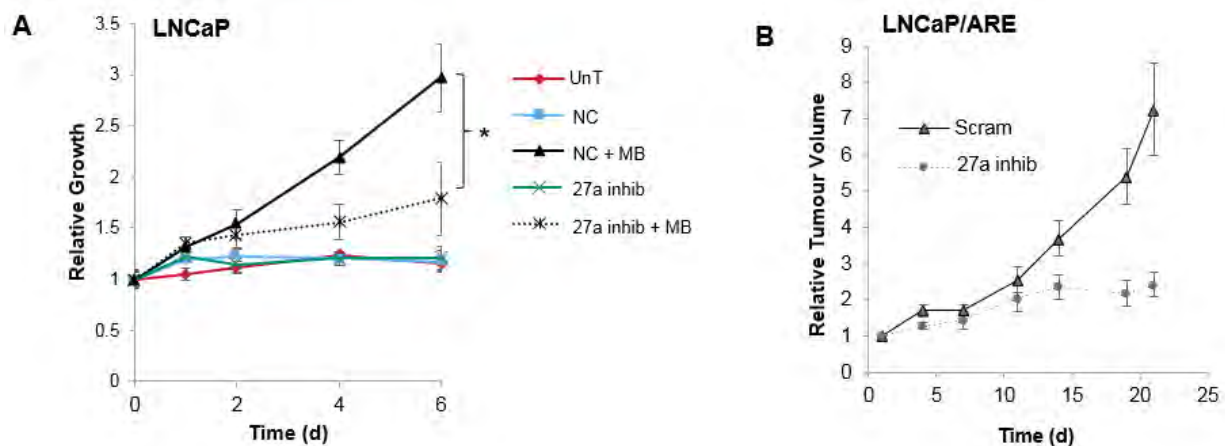


Figure: Left: treatment with an inhibitor targeting miR-27a (27a inhib) suppressed the growth of prostate cancer cells in culture, while a miR-27a mimic (MB) promoted cell growth. Right: treatment with an inhibitor targeting miR-27a (27a inhib) suppressed the growth of prostate tumors in mice compared to control (Scram).

TRoMbone: Testing Radical Prostatectomy in Men with Prostate Cancer and OligoMetastases to the Bone

Prasanna Sooriakumaran, MD, PhD
University of Oxford, UK

- Prostate cancer patients almost always die from their disease due to the adverse effects of metastases as opposed to direct effects of the primary tumor. Because treatment of the primary tumor subjects patients to surgery or radiation-associated morbidities, the current standard treatment for men who present with metastatic disease is systemic androgen deprivation therapy (ADT).
- Whether or not treatment of the primary tumor extends the lives of patients who already have metastatic disease is unclear.
- Dr. Prasanna Sooriakumaran discussed the rationale behind a new clinical trial that will test whether men who present with a low burden of metastases will benefit from surgical removal of the primary tumor.
- Patients with circulating tumor cells (CTCs) and an intact prostate are five-times more likely to develop distant metastases than patients who have had their prostates removed. This indicates that the presence of primary tumors promotes the ability of CTCs to successfully colonize metastatic sites. Possible mechanisms include providing a persistent source of potentially metastatic cells, secreting systemic tumor-supportive factors, or modulating the immune system. Additionally, recent studies have demonstrated that metastatic tumor cells are able to re-seed the primary tumor site, which can then seed new metastatic sites.
- In renal and ovarian cancer patients with metastatic disease, removal of the primary tumor has been shown to extend survival. Surgical removal of the primary tumor is standard of care in the treatment of metastatic renal cancer.
- Epidemiologic studies using the SEER Database and Munich Cancer Registry have indicated that radical prostatectomy confers a survival benefit in prostate cancer patients who present with metastatic disease. However, these are not true population-based registries as not all patients in the population are captured, and co-morbidity and clinical outcome data are limited.
- To validate these findings, a study was performed using the comprehensive PCBaSe which tracks the entire Swedish population throughout life and records over 98% of all relevant medical and life event information. Outcomes for >1000 men who presented with metastatic prostate cancer were significantly better if men received treatment with localized therapy (radiation therapy or radical prostatectomy) compared with ADT.
- In another SEER Database study, cancer-specific survival for men presenting with metastatic disease was improved following treatment with radical prostatectomy compared to radiation therapy. Radical prostatectomy was hypothesized to reduce metastases by taking

away a source of new metastatic tumor clones or removing a haven for metastatic clone reoccupation and site of evolution of further metastatic and aggressive potential.

- Finally, in a multi-institutional analysis, radical prostatectomy with extended pelvic lymphadenectomy was found to be safe and tolerable in patients presenting with metastatic disease.
- Several clinical trials are underway to investigate whether metastatic prostate cancer patients benefit from radical prostatectomy and/or various other adjuvant treatments beyond ADT.
- STAMPEDE is a multi-armed clinical trial being run in the UK, which is testing the efficacy of ADT alone or combined with various other treatments (zoledronic acid; docetaxel; zoledronic acid + docetaxel, abiraterone (Zytiga); radiation therapy) in newly diagnosed metastatic prostate cancer patients as well as all other patients in separate arms.
- The HORRAD trial is testing ADT with or without local external radiotherapy in newly diagnosed metastatic prostate cancer patients.
- A clinical trial at MD Anderson Cancer Center is testing the efficacy of best systemic therapy alone versus best systemic therapy combined with radical prostatectomy or primary tumor radiation therapy in metastatic prostate cancer patients.
- These trials are all studying men presenting with metastatic prostate cancer without differentiating between men with high and low burdens of metastatic disease.
- Oligometastatic prostate cancer is a setting of early metastasis in which men have 5 or fewer metastatic lesions.
- Oligometastatic patients have superior outcomes compared with patients with more overt metastases. In bronchial, liver, and renal cell carcinomas, 20-30% of oligometastatic patients can be cured with surgery or radiotherapy targeted to metastatic lesions. Oligometastatic patients also have a different response and better prognosis with chemotherapy treatment compared with polymetastatic prostate cancer patients.
- Altogether, this indicates that oligometastatic disease may have a distinct biology and may benefit from different treatments, particularly radical prostatectomy.
- Dr. Sooriakumaran is planning the TRoMbone clinical trial, which will test the efficacy of treatment as usual versus treatment as usual plus radical prostatectomy in previously untreated oligometastatic patients.
- Outcomes that will be assessed include survival, the development of castrate resistance, treatment feasibility, cost-effectiveness and quality of life.
- In addition to radical prostatectomy tissue, bone marrow, urine or blood samples may be collected for biobanking or further study.

- Funding applications for the clinical trial and correlative science will be submitted in 2016 to various US and international funding agencies including PCF.

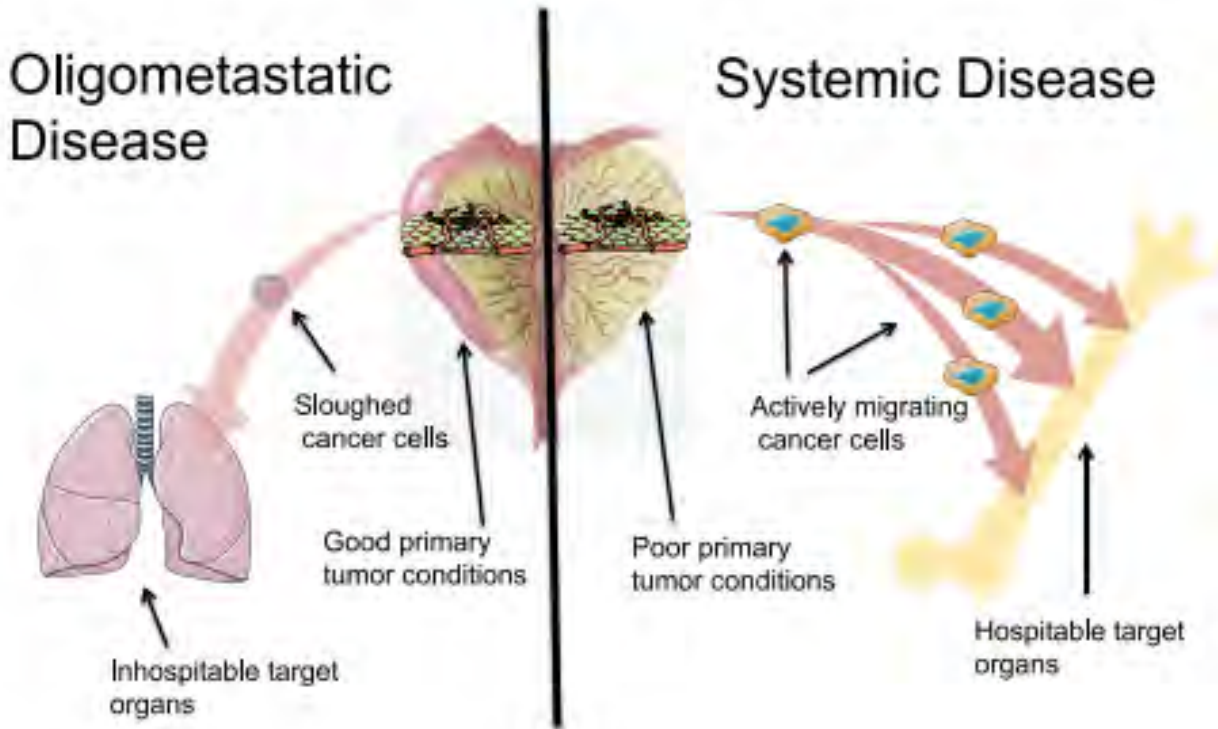


Figure: Potential mechanisms contributing to the unique disease biology and superior outcomes of oligometastatic prostate cancer patients compared with prostate cancer patients with overt systemic disease.

Exploring the Utility of Detecting HER Receptor Rewiring in Circulating Exosomes for Overcoming Treatment Resistance in Castration-Resistant Prostate Cancer Patients

Tony Ng, MD, PhD

Kings College London; University College London Comprehensive Cancer Imaging Centre, UK

- Tumor cells are constantly evolving and adapting in order to evade the immune system, colonize new metastatic sites, and resist tumor-targeting therapies. The development of technologies that can monitor the biology of evolving tumor cells is critical for optimal treatment of prostate cancer patients.

- Dr. Tony Ng discussed applying novel imaging methods to monitor dynamic oncogenic signaling pathways and the role these pathways play in therapeutic resistance and response.
- Fluorescence Lifetime Imaging (FLIM) is an imaging technology that produces an image based on the decay rate of fluorescent molecules instead of fluorescence intensity. This technology uses specialized antibodies to visualize when two molecules of interest are interacting with one another in cells and tissues. An antibody labeled with a donor fluorophore targets one molecule and an antibody labeled with a receptor fluorescent dye targets the other molecule. When the targeted molecules of interest are within 10 nanometers of one another, energy transfer occurs from the donor fluorophore to the receptor dye and quenching of fluorescence is observed and depicted.
- HER2 is a critical oncogene and therapeutic target in HER2+ breast cancer. HER2 is one of four HER (aka ErbB) family proteins that can exist in at least 8 homodimers or heterodimers. Targeting of HER2 is difficult because HER proteins will reconfigure into alternate dimer confirmations to rewire and reactivate the signaling pathway if any HER is blocked. This leads to pre-existing as well as drug-induced resistance to HER-targeting therapies.
- HER2:HER3 heterodimers have been found to be crucial in prostate cancer by activating a signaling pathway that enhances the stability of the androgen receptor (AR). The mechanisms underlying the cross-talk between HER and AR signaling pathways are being studied by Dr. Ng's research group.
- The FLIM technique was used to analyze the levels of HER2:HER3 heterodimers in tumor tissues from breast cancer patients. High levels of the dimer were found to be associated with a 5.5-fold increased risk of aggressive disease/distant metastasis.
- The levels of HER2:HER3 heterodimers were completely independent of HER2 expression levels. HER2-positive tumors were associated with a 2-fold increased risk of aggressive disease. Thus, the HER2:HER3 heterodimer status was superior to HER2 status in predicting disease progression.
- In collaboration with MSKCC, the EGFR (ErbB1/HER1):HER3 dimer was monitored in biopsy tissues from triple negative breast cancer patients who had been enrolled in a clinical trial testing the efficacy of neoadjuvant treatment with cetuximab (Erbix), which targets the EGFR (figure).
- Prior to treatment with cetuximab, EGFR and HER3 did not interact in triple negative breast cancer cells. However, following treatment with 6 cycles of cetuximab, high levels of EGFR:HER3 heterodimers appeared, causing rewiring of the HER signaling pathway.
- EGFR:HER3 rewiring was found to be a cetuximab-resistance mechanism and could be prohibited by combining cetuximab with a HER3-inhibitor.
- Exosomes are small vesicles secreted from cells that carry proteins and nucleic acids from that cell. In cancer patients, tumor cell-derived exosomes are observed in high levels in the circulation and can be isolated from blood as proxies for studying tumor cell biology.

- In order to non-invasively monitor HER pathway rewiring without requiring tissue from biopsies or surgery, a method was developed to perform FLIM in tumor cell-derived exosomes.
- Exosomes expressed the same oncogenic proteins and activated protein states observed in parental cancer cells.
- Heterogeneity of HER-family member expression was observed in tumor-derived exosomes from several different patients with lung cancer. When treated with cisplatin chemotherapy, 40% of patients exhibited a decrease in the EGFR:HER3 heterodimer.
- Expression of EGFR was found to be associated with cisplatin resistance. However, it is unclear whether the addition of HER3-targeting therapy will benefit these patients.
- Studies are underway to determine whether EGFR:HER3 heterodimer levels in tumor-derived exosomes inform resistance to treatment with various therapies that cross-talk with HER signaling pathways, including chemotherapy.
- In addition, whether EGFR:HER3 heterodimers play a role in the development of castration-resistance in prostate cancer is being studied.
- Overall, HER pathway rewiring has been found to contribute to aggressive prostate and other cancers. Future studies will more clearly delineate these mechanisms and identify combination therapy regimens to target HER pathway molecules to prevent therapeutic resistance.

HER1 (EGFR) rewired to HER3 under anti-EGFR therapy pressure in TNBC patients

Weitsman, Ng, et al. in collaboration with José Baselga and Maurizio Scaltriti, *Science Signaling*, 2014

>70% of patients with residual cancer burden post therapy
Had evidence of EGFR-HER3 rewiring

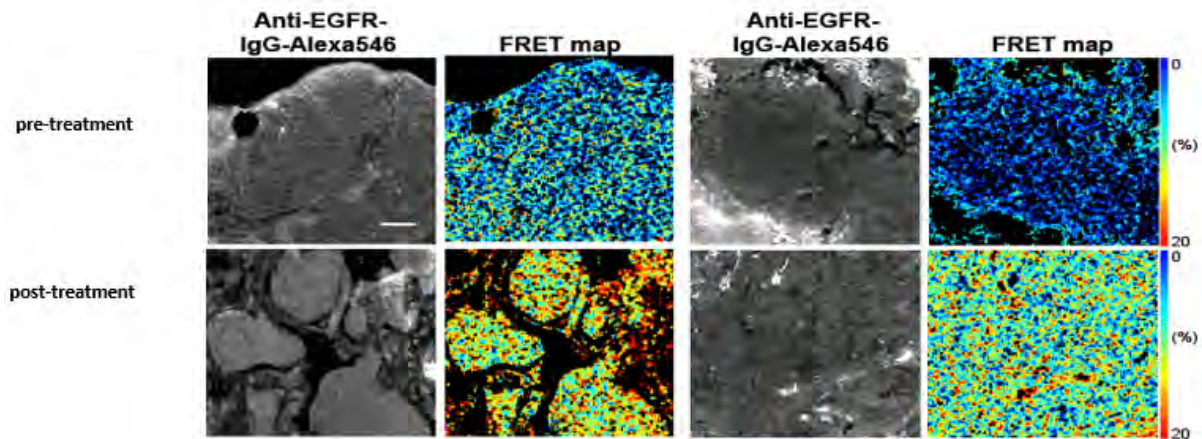


Figure: The EGFR(ErbB1/HER1):HER3 dimer was monitored in biopsy tissues from triple negative breast cancer patients who had been enrolled in a clinical trial testing the efficacy of neoadjuvant treatment with cetuximab (Erbix), which targets the EGFR. Prior to treatment with cetuximab (top row), EGFR and HER3 did not interact in triple negative breast cancer cells (blue color in FRET map). However, following treatment with 6 cycles of cetuximab (bottom row), high levels of EGFR:HER3 heterodimers were observed, causing rewiring of the HER signaling pathway (red color in FRET map). Blue indicates a low level of FLIM activity (EGFR:HER3 interaction) while red indicates high FLIM activity; FLIM level color bar indicator is to right side of figure.

SPECIAL LECTURE

New Direction in Prostate Cancer Research: Resolvin' Tumor Growth with Resolvins

Dipak Panigrahy, MD

Harvard Medical School; Harvard: Beth Israel Deaconess Medical Center

- Side effects of cytotoxic therapy for cancer can sometimes be severe. Tumor cells not killed can become more threatening to the patient by converting to a stem-like state. Moreover, tumor debris produced by dying tumor cells can activate inflammatory responses that support the growth of remaining tumor cells. New treatments that block tumor debris-induced inflammation may strengthen the effects of cytotoxic therapy.
- Dr. Dipak Panigrahy discussed a class of molecules termed resolvins, which function to modify the intensity of inflammation.
- Resolvins are lipid signaling molecules derived from EPA and DHA omega-3 fatty acids.
- Resolvins were discovered by Dr. Charles Serhan during investigations of the role of lipids in the resolution of inflammation.
- Resolvins were found to possess anti-inflammatory activity by blocking recruitment of neutrophils, which are inflammatory immune cells, and by inhibiting production of inflammatory cytokine molecules such as IL-1, IL-6 and TNF α .
- Resolvins also modify the inflammatory response by enhancing phagocytosis by macrophages, which clean up the inflammatory microenvironment by engulfing dying neutrophils and other cells.
- Resolvins can be synthesized in a laboratory and have been tested for their effects in treating a variety of inflammatory diseases.
- Resolvins have shown efficacy in multiple experimental disease models including inflammatory bowel disease, sepsis, obesity, asthma, infection, stroke, atherosclerosis, Alzheimer disease, periodontitis, and pain.
- Resolvins are currently in clinical trials for eczema, dry eye disease and periodontal disease.
- In addition to anti-inflammatory properties, resolvins are anti-fibrotic, anti-angiogenic, anti-infective, and anti-hyperalgesic.
- Notably, resolvins are not immunosuppressive.

- Autopsy studies have found that >90% of people have dormant tumors. Over 90% of individuals have dormant thyroid tumors, 40% of men have dormant prostate tumors, and 35% of women have dormant breast tumors.
- A mouse model of tumor dormancy was developed in which 10,000 Lewis lung carcinoma (LLC) cells are injected into mice. With this number of LLC cells, half of the mice will develop tumors while tumor development remains dormant in the other half.
- To determine if dead tumor cell debris can stimulate dormant tumor cells to grow again, mice that had dormant LLC tumors were further inoculated with dead tumor cell debris.
- Mice that received more tumor cell debris exhibited greater LLC tumor growth. The same phenomenon was observed in immune-deficient mice bearing dormant human head and neck tumors that were subsequently administered dead tumor cell debris. These studies demonstrate that tumor cell debris stimulates tumor dormancy escape.
- Cells can die by various mechanisms. Apoptosis is programmed cell death, in which cells condense their parts and fragment into smaller bodies which are picked up by phagocytic cells for disposal. Necrosis is an unprogrammed form of cell death, in which cells end up lysing and spilling their contents into the surrounding space. Apoptosis does not activate immune responses while necrosis incites a significant inflammatory response.
- Debris from apoptotic tumor cells stimulated growth of LLC tumors while debris from tumor cells that had died via necrosis did not promote LLC tumor growth.
- Apoptosis can be induced by cytotoxic therapies including docetaxel and cisplatin chemotherapy.
- When mice with dormant tumors were given a low dose of cisplatin chemotherapy, tumors escaped from dormancy and grew rapidly.
- When mice with dormant pancreatic tumors were administered resolvins along with dead tumor cell debris, tumor growth was inhibited significantly better than in mice treated with 5-FU or gemcitabine chemotherapy or with Celebrex or dexamethasone anti-inflammatory therapies.
- The efficacy of resolvin treatment was tested in a prostate cancer model in which immune-deficient mice were administered human prostate tumors (figure). Without the addition of dead tumor cell debris to stimulate tumor growth, resolvins had an anti-tumor efficacy similar to that of cisplatin chemotherapy. However, when tumor growth was enhanced by the administration of dead tumor cell debris, cisplatin had almost no effect while resolvins significantly inhibited tumor growth.
- In the genetically engineered TRAMP mouse prostate cancer model, daily resolvin treatment delayed the growth of prostate tumors.

- Resolvins were found to increase pro-tumor M2 macrophages and could polarize these cells into phagocytic debris-clearing cells.
- The resolvin D1 receptor was found to be expressed by tumor macrophages and tumor endothelial cells within the tumor microenvironment.
- Resolvins reduced pro-inflammatory cytokine production (IL-6, IL-8, TNF α , CCL4, CCL5) by macrophages stimulated with dead prostate cancer cell debris.
- Resolvin also stimulated phagocytosis of dead cell debris by macrophages.
- In summary, resolvins are pro-resolving molecules that inhibit primary tumor growth by activating macrophages to phagocytize tumor cell debris and by prohibiting the production of pro-inflammatory cytokines.
- Treatment of prostate cancer patients with resolvins may be a promising strategy to prevent the tumor promoting side effects of dead tumor cell debris created by treatment with chemotherapies and other cytotoxic therapeutics.

Resolvins Inhibits Human Prostate (PC3M-LN4) Tumor Growth

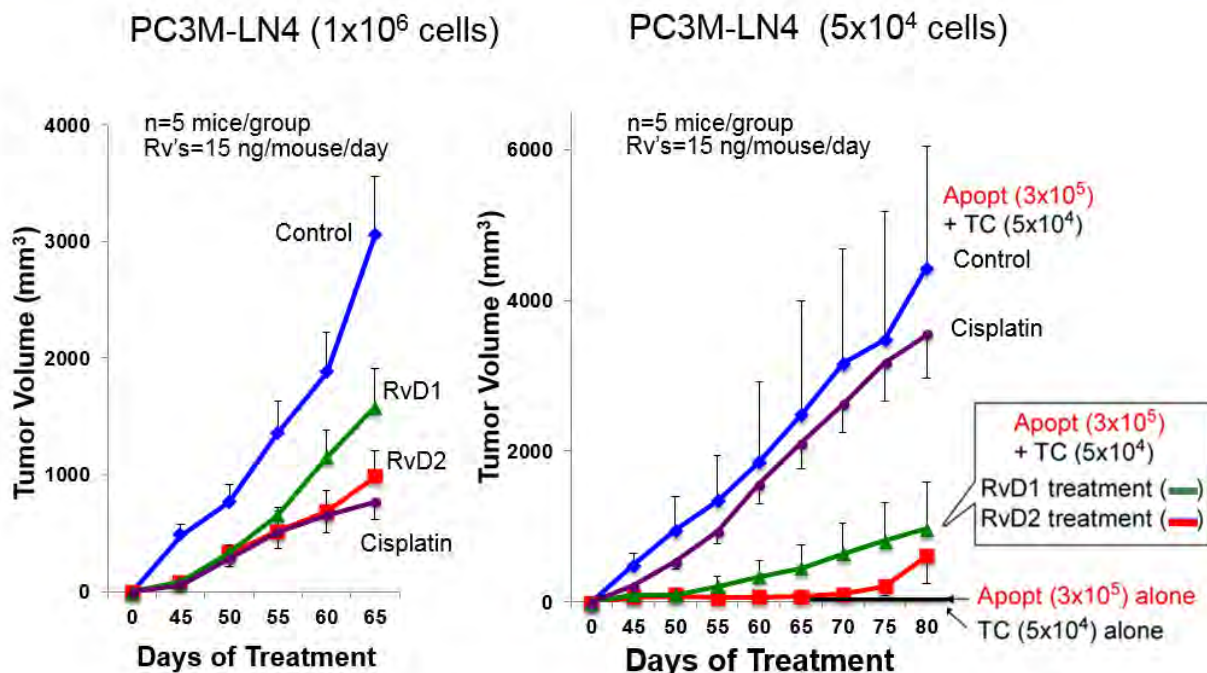


Figure: The effects of resolvin treatment were tested in a prostate cancer model in which immune-deficient mice were given human prostate tumors. Without the addition of dead tumor cell debris to stimulate tumor growth, resolvins (Rv) had an anti-tumor efficacy similar to that of cisplatin. However, when tumor growth was enhanced by the administration of dead tumor cell debris, cisplatin had almost no effect while resolvins significantly delayed tumor growth.

Session 9: New Prostate Cancer Targets and Treatments; What's a Patient to do when the Options Run Out? Part II

High-Testosterone Therapy in Post-Abiraterone and Post-Enzalutamide Era

Samuel Denmeade, MD

Johns Hopkins University School of Medicine

- Prostate cancer cells rely on testosterone for growth and survival. Androgen deprivation therapy (ADT) acts as a form of “shock-therapy” by rapidly removing testosterone from the system which leads to extensive prostate cancer cell death and a clinical response to therapy. This is followed by a time period where the prostate cancer cells that have survived adapt to low testosterone levels and may eventually develop resistance mechanisms to overcome ADT.
- High levels of testosterone in the supra-physiologic range can also induce prostate cancer cell death. There is a homeostatic window of androgen levels that prostate cancer cells prefer.
- Dr. Samuel Denmeade discussed how low and high testosterone levels can be used in the treatment of prostate cancer patients.
- ADT resistance mechanisms include adapting to the low levels of androgens by titrating androgen receptor (AR) activity back into the homeostatic window. Mechanisms include AR overexpression, AR gene amplification, AR mutations, expression of ligand-independent AR variants, and androgen synthesis by tumor cells.
- Adaptation of AR levels by the tumor cells in response to low androgen levels creates a therapeutic vulnerability that can be targeted for therapy.
- AR was found to not only be a transcription factor that drives expression of growth and survival genes, but also functions as a “DNA licensing factor”. When there are normal androgen levels, AR binds to sites on DNA in a complex with other DNA licensing factors, which allows the genome to undergo one round of replication. In order for the cell to proceed through division and set up for a second round of replication, the AR bound in DNA licensing complexes must detach and be degraded.
- Supra-physiologic androgen levels have been found to drive prostate cancer cell death, and the role of AR as a DNA licensing factor is one mechanism by which this occurs. When there are high levels of AR due to adaptation to ADT, if there are also supra-physiologic levels of androgens, AR becomes stabilized and will not detach from the licensing complex and become degraded. This leads to an inability of the cell to relicense DNA for a subsequent round of replication and the cells instead die. Physiologic levels of androgens however, do not promote cell death.

- This suggests that men with castrate resistant prostate cancer (CRPC) may respond to a therapeutic strategy consisting of rapid cycling between polar extremes of supra-physiologic and castrate testosterone levels, which is termed Bipolar Androgen Therapy (BAT).
- At supra-physiologic androgen levels, cells with high AR levels will become sensitive to death due to problems with DNA licensing. At castrate levels of androgens, cells with low AR levels will die due to an insufficiency of the growth and survival genes regulated by AR. Cells that adapt to either polar extreme by upregulating or downregulating AR levels will become sensitive to death during the next cycling between polar extremes.
- The BAT strategy consists of monthly injections of an FDA-approved supra-physiologic level of testosterone while co-administering continuous ADT. The ADT will cause testosterone levels to drop back to near-castrate levels.
- In a pilot trial, 3 cycles of BAT were given with etoposide. Patients who responded were then treated with continuous testosterone. Fourteen CRPC patients were treated with BAT. Eight patients experienced a PSA decline below baseline and 30% of the patients had a >50% PSA decline. The median response was 248 days with a 50% objective response by RECIST criteria. One patient exhibited a complete response.
- Ten patients received subsequent treatment with Zytiga (abiraterone), Xtandi (enzalutamide) or other anti-androgens. In all ten patients, a PSA response was observed. This occurred whether or not the patients had responded to BAT and despite being heavily pre-treated and having developed resistance to other AR-targeting therapies. A >50% PSA decline was observed in 7/8 patients treated with Zytiga or Xtandi following BAT. All 3 patients who had previously developed resistance to Zytiga or Xtandi, exhibited a response when they were rechallenged following BAT. This indicates that BAT can resensitize prostate tumor cells to AR-targeting therapies.
- A second, larger study was designed to validate these findings. In this trial, termed RESTORE (RE-sensitizing with Supraphysiologic Testosterone to Overcome Resistance), patients failing Zytiga or Xtandi would be given 3 cycles of BAT. Patients exhibiting a response would remain on BAT while patients who did not respond would resume treatment with the anti-androgen therapy they had been failing prior to BAT. A total of 60 patients will be enrolled in this study (30 on Zytiga and 30 on Xtandi). Thus far, 34 patients have been treated. Some PSA decline was observed in 17/34 patients while >50% PSA decline was observed in 10/34 patients. One patient exhibited a complete response which has lasted for over 6 cycles of BAT. Of 19 men who progressed on BAT, 13 exhibited a PSA decline when rechallenged with Zytiga or Xtandi, 7 of whom had a >50% PSA decline (figure).
- AR-V7 is an androgen-independent variant of AR that can be expressed by prostate cancer cells and contributes to Zytiga and Xtandi resistance. All six patients who expressed AR-V7 in circulating tumor cells (CTCs) prior to BAT, lost expression of AR-V7 while on BAT. However, 4 of these patients regained AR-V7 expression after BAT when rechallenged with Zytiga or Xtandi.

- Studies in cell lines validated these findings. In culture, prostate cancer cells which express AR-V7 lost expression within 48 hours of testosterone treatment. If Xtandi was then added to the cell culture, the cells regained AR-V7 expression within 5 days.
- This indicates that BAT may resensitize prostate cells to Zytiga and Xtandi by downregulating AR-V7. Methods to maintain low levels of AR-V7 once a patient is back on Zytiga or Xtandi may improve treatment responses.
- The TRANSFORMER Trial (Testosterone Revival Abolishes Negative Symptoms, Fosters Objective Response and Modulates Enzalutamide Resistance) is a randomized phase II trial that was recently initiated to compare the efficacy of BAT vs. Xtandi in asymptomatic CRPC patients. The study will enroll 180 patients at over 17 U.S. sites, and is designed to detect a 50% improvement in radiographic progression-free survival. Patients who progress can cross-over to the other arm and will be monitored for progression by PSA response.
- Overall, these studies suggest that BAT is a promising therapeutic strategy for CRPC patients and may resensitize patients to AR-targeted therapies.

RESTORing Sensitivity to Androgen Ablative Therapy

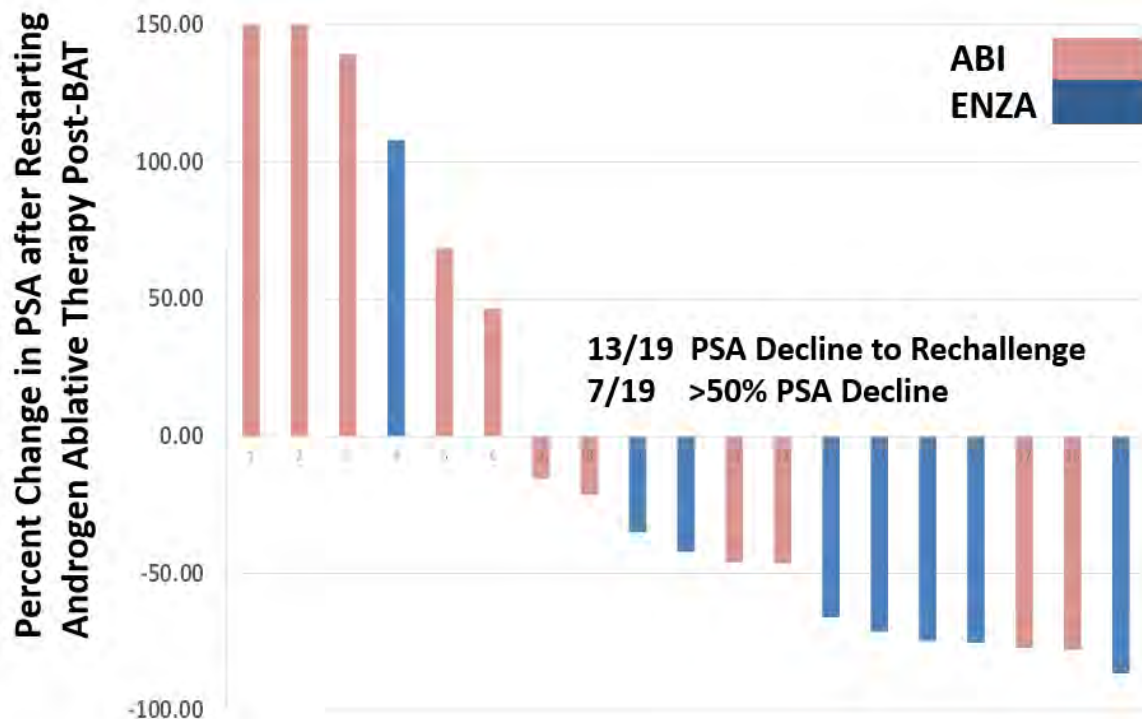


Figure: Of 19 men who progressed on BAT in the RESTORE trial, 13 exhibited a PSA decline when rechallenged with Zytiga (Abi) or Xtandi (enza), 7 of whom had a >50% PSA decline.

Targeting Estrogen Receptors

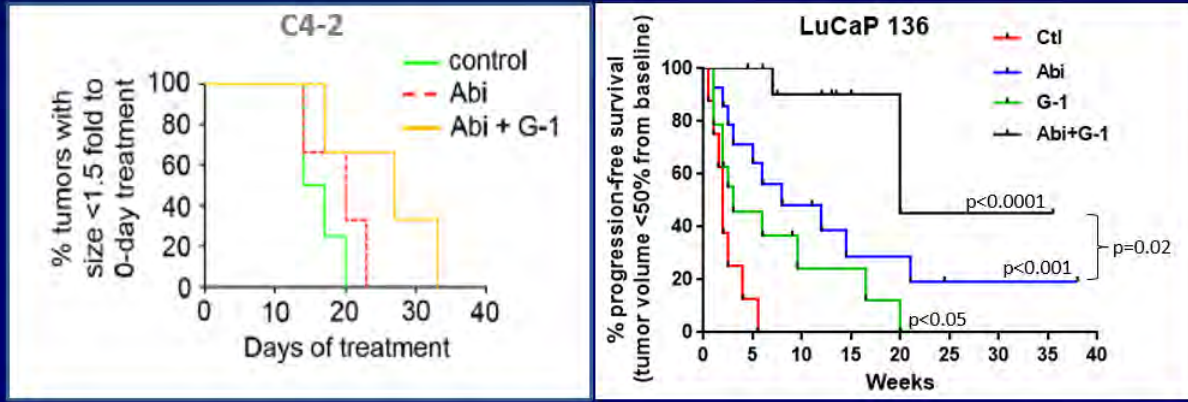
Hung-Ming Lam, PhD

University of Washington

- Estrogens were once the primary therapy used to treat metastatic prostate cancer. However, the side effects caused in men by estrogen treatment, including blood clots, heart attack, loss of libido, weight gain, and breast enlargement, led clinicians to no longer use this treatment.
- Dr. Hung-Ming Lam presented preclinical work on new methods to target estrogen receptors in prostate cancer.
- Estrogens exert their effects on cells by binding and sending signals through one of three estrogen receptors: ER α , ER β , and a newly discovered structurally unique receptor GPR30.
- Estrogen receptors are expressed on normal and cancerous prostate cells. Treatment of prostate cancer cells with estrogens inhibits growth. ER α is expressed primarily by stromal cells while prostate epithelial cells express ER β . GPR30 is expressed by both stromal and epithelial cells and expression levels increase as prostate cancer progresses.
- GPR30 promotes breast, endometrial and ovarian cancer, but inhibits prostate cancer. GPR30 has diverse roles in many other cell types, such as promoting vasodilation and alleviating depression.
- Different estrogen family compounds differentially activate the three estrogen receptors. Beta-estradiol strongly activates ER α and ER β but only poorly activates GPR30. G-1 is an estrogen-like steroidal compound that was recently discovered and found to specifically activate GPR30 but not ER α or ER β .
- Administration of G-1 to mice with human PC3 castrate resistant prostate cancer (CRPC) tumors inhibited tumor growth. Tumor growth blockade was also observed with LNCaP tumors in castrated but not androgen-intact mice. This indicates that anti-tumor GPR30 activity is not effective in the presence of androgens.
- Examination of tumors from mice treated with G-1 revealed that G-1 induced necrotic cell death in ~70% of the tumor area. Necrotic regions were infiltrated by large numbers of neutrophils, which are phagocytic immune cells that engulf dead or dying cells and activate inflammatory immune responses. In tumors not treated with G-1, necrosis was seen in ~20% of the tumor area, however no neutrophil infiltration was observed. Neutrophil infiltration was also observed in non-necrotic tumor regions of castrated mice treated with G1. This indicates that G-1 treatment induces both tumor cell necrotic death and neutrophil recruitment to tumor areas.

- Gene expression was examined in prostate tumors from mice treated with G-1. In non-castrated mice, no gene expression changes were observed with G-1 treatment, confirming the lack of activity of G-1 in the presence of androgens.
- In castrated mice, G-1 treatment altered the expression of numerous genes by prostate tumors including numerous chemokines (molecules that recruit immune cells), cytokine signaling molecules and inflammatory response genes.
- GPR30 expression was suppressed by androgens and promoted by anti-androgen therapy in prostate cancer cells in culture. GPR30 expression was low in LNCaP prostate tumors in non-castrated mice but was increased in prostate tumors from castrated mice. This indicates that an AR-driven pathway suppresses the expression of GPR30.
- GPR30 was highly expressed in ~50% of primary prostate tumors and in 80% of CRPC tumors from autopsied prostate cancer patients. Heterogeneity was observed for GPR30 expression in metastases, ranging from high expression in some lesions to none in others. High GPR30 expression levels were observed in metastatic tumors from patients resistant to Zytiga (abiraterone), an inhibitor of androgen synthesis.
- These data further indicate that G-1 may have enhanced therapeutic activity when AR is suppressed.
- The combination of G-1 and Zytiga was found to more significantly delay progression of CRPC in mice compared with either treatment alone (figure). Moreover, while 43% of mice exhibited de novo resistance to Zytiga and 50% of mice exhibited de novo resistance to G-1, only 8% of mice exhibited de novo resistance to the combination of G-1 plus Zytiga.
- The combination of G-1 plus Zytiga suppressed expression of AR, while either therapy alone actually increased the levels of AR.
- G-1 did not induce any notable toxicities in mice, indicating this may be a safe therapy for patients.
- In summary, G-1 is an inducer of the estrogen-receptor GPR30, and may be effective in CRPC patients, particularly in combination with AR-pathway inhibitors such as Zytiga.
- Ongoing studies will clarify the molecular mechanisms underlying the effect of G-1 on prostate tumors and define patient populations for whom G-1 therapy may be especially effective.

Abi and G-1 combination delayed CRPC progression



Lam and Ho, unpublished

Figure: The combination of G-1 and Zytiga was found to more significantly delay progression of CRPC in two different mouse tumor models compared with either treatment alone.

Co-Targeting Stress-Activated Chaperones, CLU and Hsp27

Martin Gleave, MD

Vancouver Prostate Centre, Canada

- The current therapeutic landscape for prostate cancer patients is complex and includes therapies targeting the androgen receptor (AR), chemotherapy, immunotherapy, and new experimental targeted therapies. The sequencing of therapies likely contributes to disease phenotypes and insensitivity to subsequent therapeutics, as each successive treatment is being used against more resistant and aggressive tumor cells.
- Understanding when and how to combine treatments to avoid or target resistance mechanisms will enhance therapeutic efficacy.
- Dr. Martin Gleave discussed preclinical and clinical studies on targeting stress response mediators alone and in combination with other therapies for the treatment of prostate cancer.
- Stress responses are highly conserved mechanisms that enable cells to adapt to changes in the environment. Stress responses can be activated by a variety of stresses including nutrient deprivation, low oxygen, and cancer therapeutics, and proceed through a number of signaling pathways to converge upon the regulation of protein translation. This allows selective translation of proteins that are important for adapting to stress and cell survival.

- Clusterin and HSP27 are two stress-activated “molecular chaperone” proteins that are turned on in prostate cancer cells that have gained resistance to androgen deprivation therapy (ADT) and have become castrate resistant prostate cancer (CRPC). Studies have indicated that these molecular chaperones, which function to assist the assembly or disassembly of proteins and cellular structures, have a functional role in resistance to AR-targeted therapy.
- Both Clusterin and HSP27 are difficult to target using small molecule inhibitors. Antisense inhibitors, which target the RNA molecules encoding these proteins and inhibit the number of Clusterin or HSP27 proteins that get translated, were developed and are being tested in preclinical models and clinical trials.
- Under stress conditions, Clusterin is upregulated to inhibit cell death pathways and promote autophagy, which is the degradation and recycling of cellular components.
- Treatments targeting AR such as Xtandi (enzalutamide) can activate autophagy. Clusterin inhibition was found to synergize with Xtandi in inhibiting the growth of prostate tumors in mice by abrogating Xtandi-induced autophagy and by blocking the AKT oncogene pathway.
- Inhibition of Clusterin was also found to synergize with taxane chemotherapy in promoting the death of prostate cancer cells.
- A phase II trial testing the Clusterin antisense inhibitor OGX-11 (aka custirsen) in combination with docetaxel demonstrated a 7 month survival advantage compared with docetaxel alone in 82 CRPC patients. However, the subsequent phase III SYNERGY trial in metastatic CRPC patients failed to demonstrate any benefit for the addition of OGX-11 to docetaxel.
- The failure of the SYNERGY trial despite significant preclinical indications for a benefit from OGX-11 may stem from imprecise preclinical models as well as clinical trial design issues. The changing landscape of therapies that trial participants received after study, for example with Zytiga or Xtandi, may also affect overall survival in the phase III trial.
- In addition, being able to enrich for patients that will respond well to Clusterin inhibition is critical. Clusterin is adjacent to the NKX3.1 gene which is deleted in 20% of patients. An assay has been developed to determine whether Clusterin has also been deleted.
- In depth analysis of the SYNERGY trial results indicated that Clusterin inhibition had no effect in patients with a good prognosis, but extended median overall survival from 13.7 to 16 months in patients with a poor prognosis. Patients that demonstrated a reduction in serum levels of Clusterin while on therapy also fared better than patients who had no reduction of Clusterin levels. This information may help to better identify patients who will respond to Clusterin inhibition in future trials.
- The mechanisms of synergy between Clusterin inhibition and taxane chemotherapy are being studied.

- A phase III trial, AFFINITY, is ongoing, in which OGX-11 is being tested in combination with cabazitaxel in patients who have been previously treated with docetaxel.
- HSP27 is also a molecular chaperone that functions to inhibit cell death following stress.
- HSP27 expression correlates with higher grade tumors, metastases, and poorer outcome in patients with various types of solid cancers and is associated with broad-spectrum resistance to cancer therapeutics.
- OGX-427, an HSP27 antisense inhibitor, was found to prohibit prostate tumor growth and inhibit AR activity in mouse models of CRPC.
- A phase I trial of OGX-427 indicated some efficacy in prostate and ovarian cancer patients, with a >15% decrease in measurable disease in 27% of patients.
- In a phase II study in metastatic CRPC patients, a PSA decline of >50% was observed in 47% of patients treated with OGX-427 plus prednisone compared with 24% of patients treated with prednisone alone. Patients failing on the prednisone alone arm could be crossed over and receive OGX-427. Over 50% of cross-over patients experienced some PSA decline, with 20% of patients experiencing a PSA decline of >50%. Objective responses were observed in 31% of patients treated with OGX-427 plus prednisone compared with 18% of patients treated with prednisone alone (figure). However, no delay in time to progression was observed, with ~50% of patients in both treatment arms showing disease progression at 12 weeks.
- OGX-427 is currently being test in nine phase I and II studies in prostate, bladder, and lung cancer patients.
- In a PCF-funded study, the molecular structure of HSP27 was determined. The interactions between HSP27 and other proteins are being modeled in order to identify regions of the protein which may be effectively targeted by small molecule therapies.
- In summary, these studies have identified two proteins, Clusterin and HSP27, which contribute to CRPC and may represent promising therapeutic targets. Results from ongoing clinical trials testing inhibitors of these proteins in prostate cancer patients are awaited.

Phase II Study of Prednisone +/- OGX-427 in mCRPC - RR and PFS



Objective Responses: 5/16 (31%) OGX-427 + Prednisone vs. 4/22 (18%) Prednisone



	OGX-427 + Prednisone (N=36)	Prednisone (N=38)
No Disease Progression at 12 weeks	18 (50%) (95%CI: 32.9%, 67.1%)	14 (48%) (95% CI: 26.3%, 59.2%)

Figure: In a phase II study in metastatic CRPC patients, objective responses were observed in 31% of patients treated with OGX-427 plus prednisone compared with 18% of patients treated with prednisone alone. No delay in time to progression was observed, with ~50% of patients in both treatment arms showing disease progression at 12 weeks.

From West Coast Dream Team Research to the Discovery of New Targeted Therapies

Matthew Rettig, MD
University of California, Los Angeles

- MAPK pathways are oncogenic pathways that play a major role in driving progression of numerous cancer types including prostate cancer. MAPK pathways are activated by many signals including growth factors, and cross talk with many other pathways. These complex pathways involve a hierarchy of signaling proteins that successively activate one another and culminate in activation of the MEK kinase which activates several transcription factors that regulate genes that control cell growth and survival.

- Dr. Matthew Rettig discussed the role and effect of targeting MEK in prostate cancer patients.
- Previous studies sequencing the genomes of prostate tumors have identified mutations in MAPK pathway proteins, including PTPN11, KRAS, NRAS, BRAF and RAF1, that may confer constitutive activation of the MAPK pathway in 43% of primary tumors and 90% of metastases.
- Increasing MAPK activity correlated with disease progression in prostate cancer clinical specimens, with activated MAPK seen in ~80% of CRPC patients. This study was performed prior to Xtandi (enzalutamide) and Zytiga (abiraterone) treatments becoming available and thus the prevalence of MAPK activity in the current CRPC treatment landscape is unknown.
- In a mouse model of prostate cancer, activation of K-Ras in combination with deletion of the tumor suppressor gene PTEN generated aggressive tumors that readily formed liver and lung metastases. Pharmacologic inhibition of MEK in these mice prohibited the formation of metastases.
- Epithelial-mesenchymal transition (EMT) is a phenomenon in which epithelial cells (such as prostate cells) lose their adherent phenotype and transition into migratory mesenchymal cells. EMT is thought to be a critical step in the development of metastatic potential by prostate adenocarcinoma cells. MAPK activity was found to promote EMT in the K-Ras/PTEN mouse model of prostate cancer.
- In another study, treatment with MEK inhibitors suppressed the growth of human CRPC tumors that had been implanted in mice. This provides further preclinical evidence that MEK-inhibition may benefit prostate cancer patients.
- The West Coast Prostate Cancer Dream Team (WCDDT), funded by PCF, has analyzed the expression of genes in 83 metastatic tumors from castrate resistant prostate cancer (CRPC) patients who have been treated with Zytiga or Xtandi. The expression of genes in this cohort was compared with those in primary prostate tumors from The Cancer Genome Atlas (TCGA) database. ERK was found to be the most hyper-active kinase in metastatic CRPC tumors versus primary tumors (figure).
- Development of resistance to AR-targeting therapies such as Xtandi and Zytiga by CRPC tumors has sometimes been associated with a transition from the typical adenocarcinoma phenotype into a small cell phenotype or an androgen-independent phenotype with intermediate morphology.
- MEK activation was found in all of these CRPC subtypes, suggesting that ERK activation occurs in the general metastatic setting and may be further promoted by the acquisition of resistance to Xtandi or Zytiga.
- PCF has supported a neoadjuvant study of Trametinib, an inhibitor of MEK, in localized high risk prostate cancer patients, followed by radical prostatectomy. MEK activity was higher in

treatment-naive needle biopsies compared with post-treatment radical prostatectomy samples, demonstrating that Trametinib inhibits MEK activity in patients.

- On an off-label usage, one patient who had progressed on androgen deprivation therapy, Zytiga, Sipuleucel-T, Xtandi, and Xofigo exhibited a ~90% reduction in PSA following Trametinib treatment (figure).
- Based on these data, a phase II study testing Trametinib in progressive metastatic CRPC patients is being planned. The primary endpoint will be PSA response at 12 weeks.
- Biopsies will be taken pretreatment and at disease progression to perform correlative studies to identify mechanisms of treatment response and resistance.

MEK activation in WCDT samples vs TCGA samples

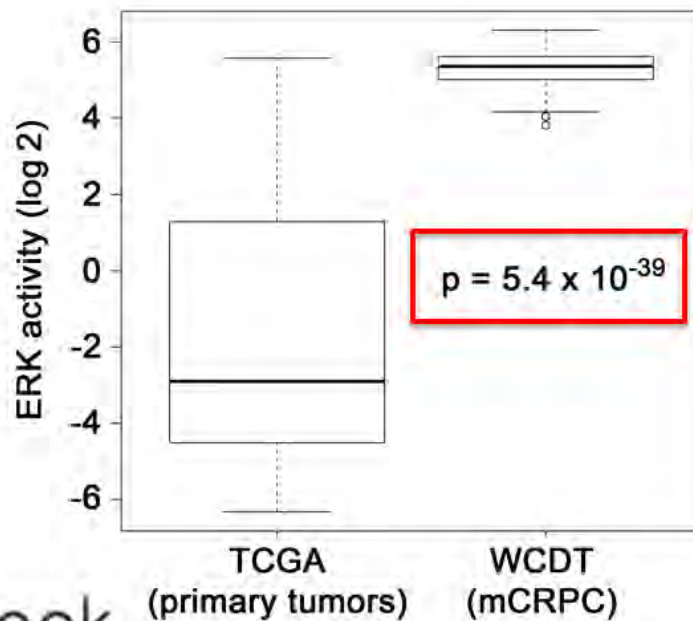


Figure: Comparison of ERK activity between WCDT metastatic CRPC tumors and primary prostate tumors from TCGA.

MEK inhibition reduces PSA in a patient with mCRPC

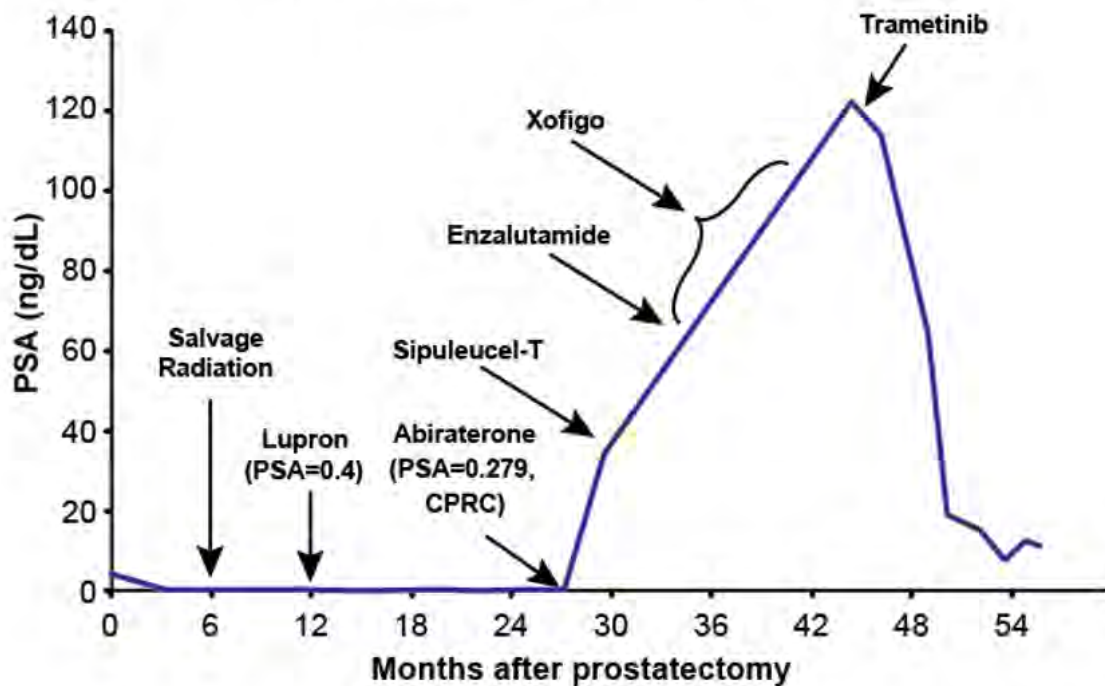


Figure: A patient who had progressed on androgen deprivation therapy (Lupron), Zytiga (abiraterone), Sipuleucel-T, Xtandi (enzalutamide), and Xofigo exhibited a ~90% reduction in PSA following Trametinib treatment.

When the Guardian Becomes the Enemy: Targeting ATM in PTEN-Deficient Cancers

Nuala McCabe, PhD

Queen's University Belfast, UK

- Synthetic lethality is a concept in which loss of two genes induces cell death, while cells that have lost only one of these genes can survive. This concept is important in identifying new therapeutic targets in cancer cells, which commonly lose or mutate genes important for cell survival. Targeting a synthetic lethal partner of a gene that has been lost by the cancer cells enables selective killing of cancer cells while sparing normal cells that have not lost either gene.
- The DNA damage repair gene PARP1 has been found to be a synthetic lethal partner of other DNA damage repair genes including BRCA1 and BRCA2. Targeting PARP1 in tumors with

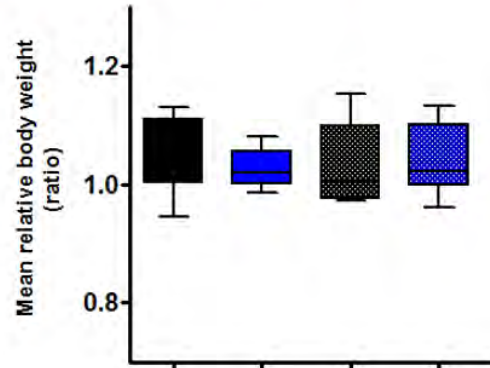
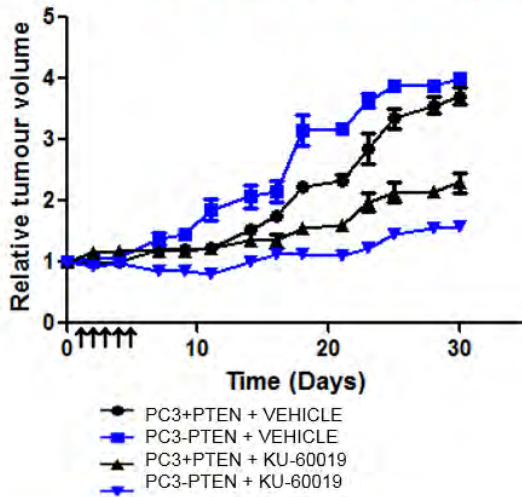
mutations in BRCA1/2 or other DNA damage repair molecules has demonstrated clinical efficacy and is one successful example of applying the concept of synthetic lethality to the treatment of cancer patients.

- The ATM kinase is a tumor suppressor gene that senses double strand breaks in DNA and activates cell cycle arrest, senescence, and apoptosis (cell death). This protects the host by preventing the survival and growth of cells with broken DNA.
- Dr. Nuala McCabe performed a study to identify any genes that when targeted, can induce death in cancer cells that harbor ATM-loss or mutations (i.e. synthetic-lethal partners of ATM).
- A custom siRNA library was used to knock down the expression of each of 178 tumor suppressor genes in patient-derived fibroblast cells carrying a mutation in ATM (AT221JE-T cells) compared with AT221JE-T cells in which normal ATM had been reexpressed. After 10 days, cells were assayed for viability to identify any of the tumor suppressor genes whose loss caused death of ATM-mutant but not ATM-normal cells.
- Nine genes that may function as synthetic-lethal partners of ATM were identified: BRIP1/FANCL, CDKN2C, PTEN, STEAP4, NKTR, CASP8, TP53, FANCG, and CAV1.
- Of these genes, loss of PTEN, a tumor suppressor gene that acts to inhibit the PI3K oncogene, is commonly associated with advanced prostate and other cancers.
- To determine if targeting of ATM in tumor cells with PTEN-loss has therapeutic efficacy, ATM knockdown with siRNA or ATM-inhibitors (KU-55933) were used to treat colorectal and prostate cancer cells with versus without normal PTEN. In both cell types, cells that had PTEN-loss were more sensitive to death following ATM-knockdown or ATM-inhibition than cells with normal PTEN. PTEN-null cells treated with the ATM-inhibitor exhibited enhanced cell cycle arrest, apoptosis, and chromosome instability.
- The mechanisms underlying ATM-PTEN synthetic lethality were further studied.
- PTEN-null cells are known to have elevated levels of reactive oxygen species (ROS), which are chemically reactive oxygen-containing molecules that damage DNA and other molecules in the cell.
- PTEN-null colorectal and prostate cancer cells were confirmed to have elevated ROS levels compared with PTEN-normal cells. Treatment with the ATM-inhibitor increased the levels of ROS, suggesting that oxidative stress is a mechanism of lethality in these cells.
- Phosphorylation of ATM and of gamma-H2AX, a marker of DNA double stranded breaks and a substrate of ATM, was increased in PTEN-null cells. This indicates that ATM becomes constitutively active in PTEN-null cells in order for cells to survive.
- Treatment of PTEN-null cells with the antioxidants β -carotene or N-Acetyl Cysteine (NAC) reduced ATM activity and gamma-H2AX activation and inhibited the death of PTEN-null

cells that had been treated with ATM-inhibitors. This further confirms that the mechanism of synthetic lethality between PTEN and ATM is due to oxidative stress.

- To explore ATM-inhibition as a therapeutic strategy for PTEN-null prostate cancer, immune-deficient mice harboring PTEN-normal vs. PTEN-null prostate tumors were treated with the ATM-inhibitor KU-60019 (figure). KU-60019 is a derivative of KU-55933 which has been optimized for in vivo stability. PTEN-null prostate tumors grew more rapidly than PTEN-normal tumors. ATM-inhibition reduced the growth of both PTEN-normal and PTEN-null tumors, but had a more profound effect on PTEN-null tumor growth. No differences were noted in mouse weight for any of the treatment groups indicating low/negligible off-target toxicity.
- Altogether, these studies indicate that loss of PTEN results in increased ROS. ROS then causes double stranded DNA breaks, activating ATM to induce cell cycle arrest and DNA repair for cell survival. Targeting ATM in PTEN-null cells leads to loss of cell cycle arrest and DNA repair and results in cell death. PTEN-null cells are thus sensitized to ATM inhibition, as a novel therapeutic strategy for cancer.
- Dr. McCabe also discussed a novel biomarker discovery program being instituted at Queen's University Belfast in collaboration with the Karolinska Institute and the University of Cambridge.
- 134 primary prostate cancers, 40 lymph node metastases, and normal prostate and lymph node tissues have been collected and profiled for gene expression using DNA microarrays.
- A bioinformatics method called “unsupervised hierarchal clustering” was used to identify primary prostate cancers that have gene expression profiles similar to metastatic disease. A 70-gene signature was generated that predicts this subgroup of patients.
- The 70-gene signature was tested in another publicly available prostate cancer dataset and found to be highly statistically significant in predicting time to metastatic recurrence and biochemical recurrence.
- These findings are being further validated in additional datasets.

Single-agent treatment of KU-60019 in PC3 PTEN Dox-inducible xenografts



COMPARISON	T-Test
PTEN WT VS DEFICIENT (VEHICLE)	0.3218
PTEN WT VEHICLE VS PTEN WT KU60019	0.1213
PTEN DEFICIENT VEHICLE VS KU60019	0.0004
PTEN WT VS PTEN DEFICIENT (KU60019)	0.0060

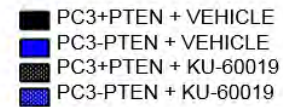


Figure: Immune-deficient mice harboring PTEN-normal (PC3+PTEN) vs. PTEN-null (PC3-PTEN) prostate tumors were treated with the ATM-inhibitor KU-60019 versus control (vehicle). Left: PTEN-null prostate tumors grew more rapidly than PTEN-normal tumors. ATM-inhibition reduced the growth of both PTEN-normal and PTEN-null tumors, but had a more profound effect on PTEN-null tumor growth. Right: No differences were noted in mouse weight for any of the treatment groups.

Session 10: Molecular Imaging for Improved Detection of Prostate Cancer Recurrence; Focus on Heterogeneity of Response

MRI of Advanced Prostate Cancer

Anwar Padhani, MD

Mount Vernon Cancer Centre; Institute of Cancer Research, UK

- Current standard molecular imaging technologies (bone scans) used by clinicians to visualize tumors in prostate cancer patients have poor sensitivity and specificity. This leads to late detection of metastases and the inability to identify all metastases that are present, resulting in improper staging of patients and the inability to accurately follow treatment responses. New imaging technologies with improved sensitivity and specificity can meet this unmet medical need for the optimal management of patients.
- Dr. Anwar Padhani discussed the application of whole body magnetic resonance imaging (WB-MRI) for more accurate detection of metastatic lesions in prostate cancer patients.
- WB-MRI was found to detect significantly more malignant lesions in metastatic castrate resistant prostate cancer (CRPC) patients compared with standard bone scans (figure).
- An analysis of various imaging technologies found that bone scans have a sensitivity of 76-86% and a specificity of 80-84%. In contrast, MRI has a sensitivity of 95% and a specificity of 96% and Choline-PET/CT has a sensitivity of 87% and a specificity of 97%.
- The poor sensitivity of bone scans results in an inability to accurately determine when treatments begin to fail.
- Moreover, different metastatic lesions in a patient can differentially respond to therapy. While some lesions may respond, others may progress and new lesions may form.
- This heterogeneity of response is not taken into account in the current clinical paradigm in which clinicians use bone scans and PSA levels to determine if patients are responding and make decisions on when to discontinue treatment.
- This leads to unnecessary therapy-associated morbidities and financial costs.
- Additionally, patients may decline in health and therefore cannot move onto other potentially more effective approved and experimental therapies.
- MRI is unique among imaging technologies as different pulse sequences and imaging parameters that examine different properties of tissues can be combined into a single 30-minute scan. For instance, the constituents that can be analyzed to assess the biology of

metastatic bone disease include: cellular, water, and fat content of tissues, vascularization, trabecular bone structure and density, and tumor density and size.

- Composite biomarkers that include modern imaging technologies such as WB-MRI, PSA levels, and other biomarkers of response should be used to determine disease progression and therapeutic responses and guide the care of patients.

WB-MRI detects more malignant lesions/ patient than bone scans

74 mCRPC on Enzalutamide. PSA 0.4 ng/ml
3 lesions on planar bone scan; 6 lesions on WB-MRI

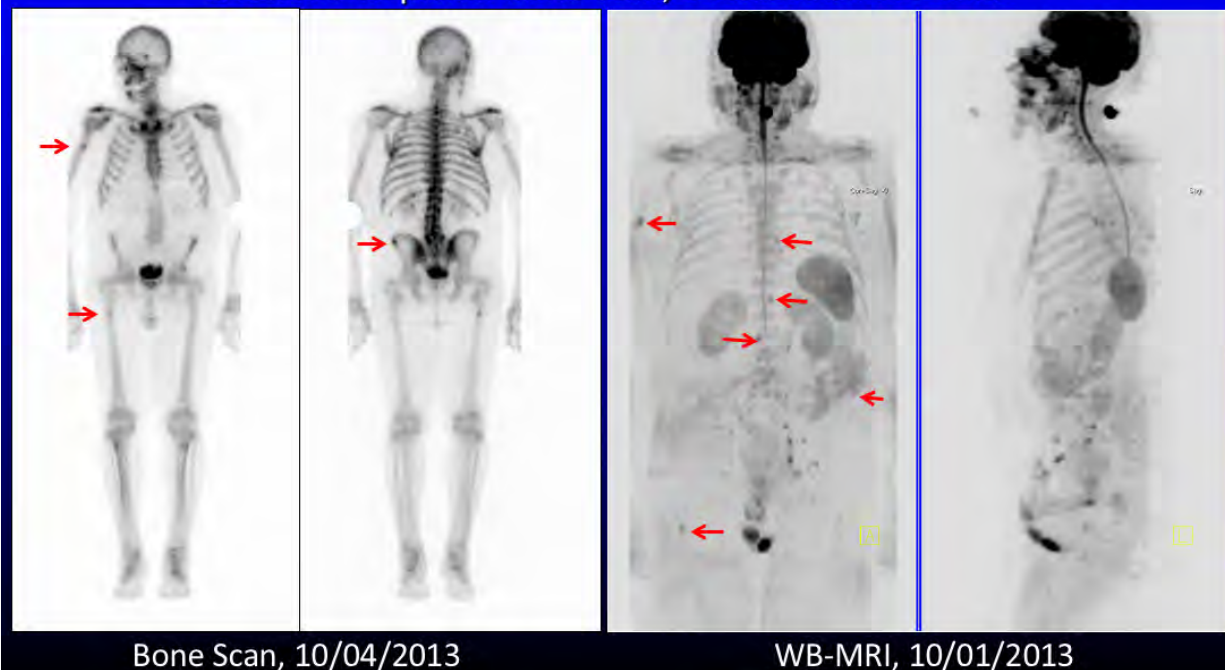


Figure: WB-MRI (right) is able to detect more malignant lesions (red arrows) in metastatic CRPC patients compared to standard bone scans (left).

Understanding Treatment Response in mCRPC Using Quantitative Total Bone Imaging

Robert Jeraj, PhD
University of Wisconsin Carbone Cancer Center

- Significant heterogeneity has been observed in treatment responses of different metastatic lesions in a patient. While some lesions may respond, others may progress and new lesions

may form concurrently. The ability to measure these responses and understand what they mean for patient outcome is critical for the optimal treatment of patients.

- Dr. Robert Jeraj discussed the use of a Quantitative Total Bone Imaging (QTBI) method for studying treatment responses in prostate cancer patients.
- QTBI combines anatomic images, typically from CT (computerized tomography) scans, with functional imaging, typically from PET (positron emission tomography) or MRI (magnetic resonance imaging) scans. By imaging patients over the course of treatment, lesions that are responding versus those that are progressing can be identified and quantified.
- Fluorine-18 sodium fluoride (^{18}F -NaF) PET/CT scans can be used to identify metastatic lesions in bone.
- While it is normally difficult to accurately count and follow the growth of ^{18}F -NaF-PET/CT-detected lesions that are close to one another, the QTBI algorithm is able to deconvolute individual lesions and their properties in order to follow the unique response of each.
- PCF sponsored a study at the University of Wisconsin Carbone Cancer Center, Memorial Sloan Kettering Cancer Center, and the National Cancer Institute to examine the reproducibility of ^{18}F -NaF-PET/CT scans in prostate cancer patients undergoing treatment with docetaxel chemotherapy or androgen receptor (AR)-targeted therapy and to identify imaging biomarkers of response and progression.
- With harmonized protocols and instruments, reproducibility across the three institutions was found to be ~95%, suggesting that with harmonization, ^{18}F -NaF-PET/CT can be reproducibly used in the clinic to image bone metastatic prostate cancer.
- The univariate parameter most predictive for progression-free survival was found to be total disease burden at the end of treatment as determined by the QTBI. Total disease burden at the beginning of treatment was also highly predictive of outcome.
- A 2014 PCF-Challenge Award is supporting a study led by Dr. Jeraj to apply QTBI to understanding the biology of response heterogeneity in metastatic prostate tumors. Patients are imaged by ^{18}F -NaF-PET/CT over the course of treatment. The most responsive and nonresponsive lesions will be biopsied and genomically profiled to identify mechanisms of response as well as imaging biomarkers that predict biology.
- All patients assessed thus far exhibited concurrent responding, progressing, and newly developing metastatic lesions (figure).
- A clinical trial sponsored by Medivation (MDV3100-18) will use ^{18}F -NaF-PET/CT to assess the treatment responses of individual metastatic lesions to Xtandi (enzalutamide) in order to guide time on treatment and potentially treat patients beyond clinical progression.
- One point of controversy concerning ^{18}F -NaF-PET/CT, is that it is a measure of bone metabolism and suffers from the same specificity limitations as technetium bone scans.

- More specific molecular imaging agents, such as PSMA-PET/CT, which targets the prostate cancer associated PSMA protein, could measure tumor more directly. Multi-modality imaging, such as DWI-MR (diffusion weighted imaging (DWI) form of magnetic resonance (MR) imaging) could provide additional information about lesion characteristics. Although QTBI is an improvement for 18F-NaF-PET/CT as a biomarker for disease progression, biological validation will be required before routine clinical use, a goal of the 2014 PCF-Challenge Award.
- In summary, different metastatic lesions in patients respond differently to a given therapy. Technologies such as QTBI will enable identification of the mechanisms that are driving treatment resistance and disease progression. This knowledge will impact clinical decision making and drug development.

Complexity of response heterogeneity

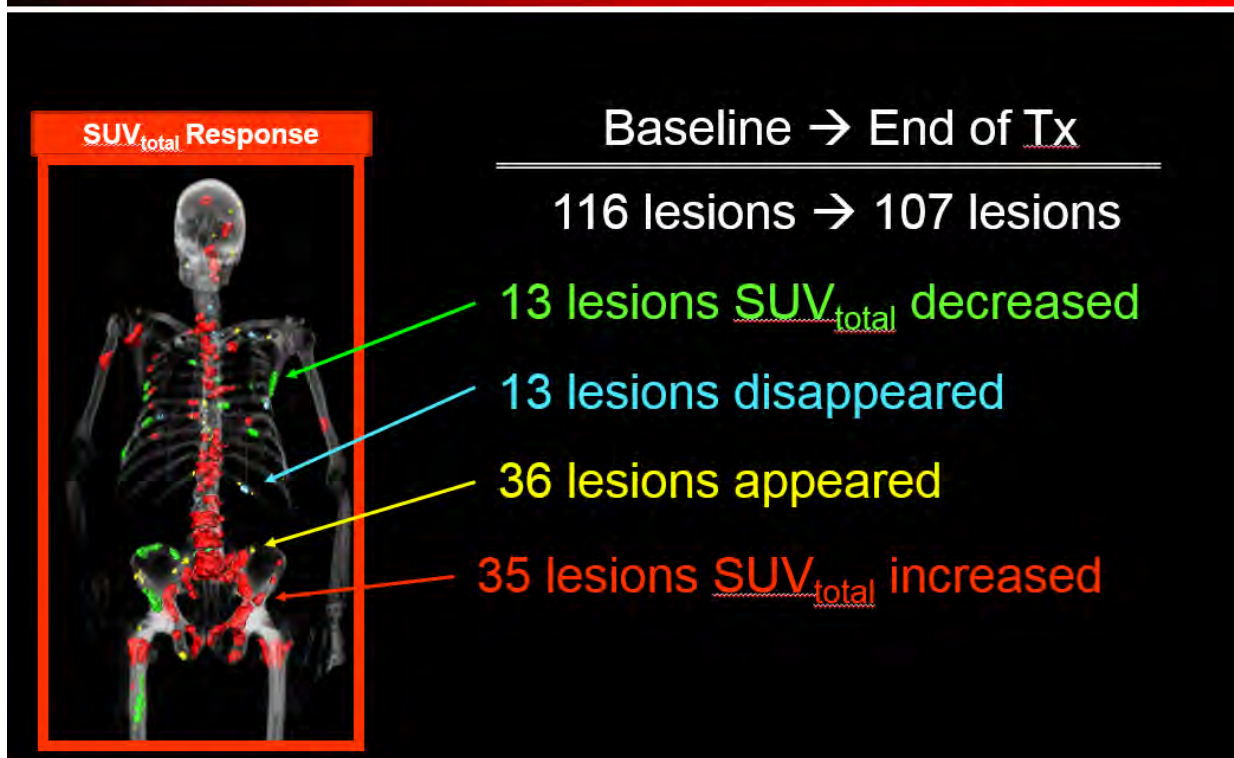


Figure: QTBI was used to observe response heterogeneity in metastatic lesions by imaging prostate cancer patients over the course of treatment. Lesions which are responding versus those that are progressing as well as new lesions that develop can be identified and quantified.

Detecting Prostate Cancer with DCFPyL

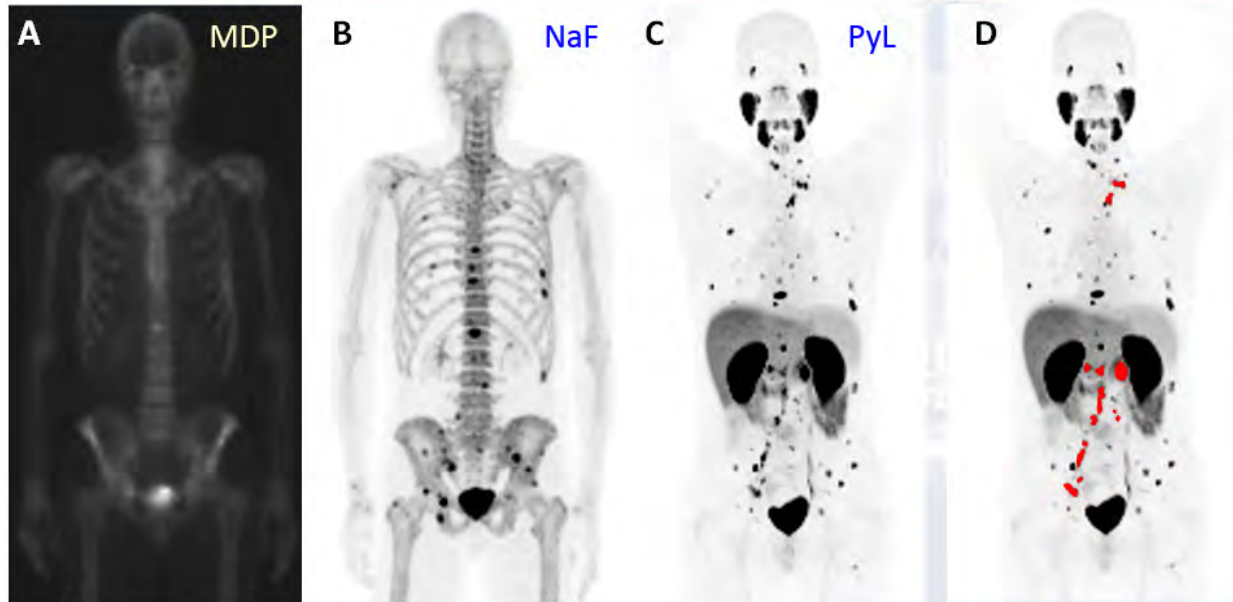
Martin Pomper, MD, PhD

Johns Hopkins University School of Medicine

- Technology for the imaging of prostate cancer needs improvement in sensitivity and specificity to more accurately determine site and extent of primary and metastatic disease.
- Dr. Pomper has developed and tested in patients, prostate-specific membrane antigen (PSMA)-targeting PET (positron emission tomography) imaging agents for prostate cancer.
- PSMA is a protein that is expressed on the surface of prostate cells and is an indicator of androgen signaling. PSMA expression is associated with advanced prostate cancer and is an excellent imaging biomarker for identification of very small tumor deposits not visualized by current imaging methods.
- PSMA-PET imaging can be used to manage prostate cancer patient care by informing candidates for active surveillance, presurgical staging of patients at risk for occult metastases, and for therapeutic monitoring.
- Dr. Pomper and colleagues have generated 18F-DCFBC and 18F-DCFpyL, a series of small molecule ligands of PSMA that have been radiolabeled with 18F for PET imaging. 18F-DCFBC is the first generation agent while 18F-DCFpyL is a newer derivative.
- 18F was selected as the PET imaging isotope due to good imaging properties, a high number of patients who could be studied per synthesis, and a known mechanism for distribution of the compound throughout the US (follows that of the commonly used PET agent 18F-FDG).
- 18F-DCFBC was able to distinguish prostate cancer from benign prostatic hypertrophy and correlated with pathologic Gleason grade, which may be useful for directing biopsies or focal therapies.
- Both 18F-DCFBC and 18F-DCFpyL are imaged in patients at 1 hour post-administration. Very good signal to noise ratios were observed.
- A high concentration of these PSMA-PET imaging agents distribute in tumors, enabling high sensitivity. A standardized uptake value (SUV) of ~100 is can be reached while SUV levels for other PET imaging agents are generally much lower.
- 18F-DCFpyL PET imaged significantly more lesions and had superior signal to noise ratios compared with bone scans and sodium-fluoride (18F-NaF) PET imaging (figure). 18F-DCFpyL PET was able to detect four times more lesions than the combination of bone scans and CT imaging.
- Because of the high number of target sites per cell, lesions as small as 2-3 mm could be detected by 18F-DCFpyL-PET.

- 18F-DCFPYL was also useful for imaging primary tumors.
- 18F-DCFPYL may also be combined with MRI imaging for additional anatomic and functional information.
- Compared with 68Ga-PSMA that is being used in Europe, 18F-DCFPYL provides crisper images in part due to a shorter path and a greater amount of imaging agent that can be administered. More radioactivity can also be administered for the 18F agent due to dosimetry considerations, further contributing to image clarity. 18F-DCFPYL is beginning to be used in Europe.
- A clinical trial in patients with PSA recurrence (≥ 0.2 ng/ml) but no visible metastases by bone or CT scans is being conducted at Johns Hopkins University by Dr. Ashley Ross. In current practice, these patients would either continue on active surveillance or receive salvage external beam radiation or androgen deprivation therapy (ADT). However, with more precise imaging and more thorough identification of metastatic lesions, salvage pelvic lymph node dissection or stereotactic body radiation therapy (SBRT) targeted to individual oligometastatic sites could be performed.
- An algorithm named Auto-PERCIST is being used to quantify the size of bone and soft tissue lesions obtained by PET scans. This algorithm may be used as a readout of disease progression in clinical trials.
- PSMA is expressed in tumor neovasculature and therefore may also be a useful imaging target for other tumor types. The ability of 18F-DCFPYL to detect lesions was demonstrated in clear cell renal cell carcinoma.
- A phase II trial testing 18F-DCFPYL-PET/CT in men with biochemical recurrence after prostatectomy or radiation but with negative bone and CT scans, is being designed. In patients with positive scans, sensitivity will be indicated if 18F-DCFPYL-detected lesions regress upon ADT administration.

DCFPyL in mCRPC: Comparison to bone scan



Rowe *Clin Genitourin Cancer*, in press



Figure: Comparison of imaging a prostate cancer patient with bone scan (MDP, A), Sodium-Fluoride (NaF, B), ^{18}F -DCFPYL (PyL, C). In (D), soft tissue lesions visualized by ^{18}F -DCFPYL are indicated in red.

PET and Optical Imaging of Prostate Cancer with Engineered Antibodies

Robert Reiter, MD

University of California, Los Angeles

- Sensitive and specific molecular imaging technologies that better inform the extent of disease are necessary for proper staging and treatment of prostate cancer patients.
- Antibodies are specialized proteins generated by immune cells that have high specificity and high affinity for their target. They are also extremely stable in vivo and have been successfully developed into therapeutics. These properties make antibodies promising imaging agents for prostate cancer.
- Dr. Robert Reiter discussed the application of radiolabeled and fluorescently-labeled antibodies and antibody-fragments for positron emission tomography (PET) and fluorescence imaging of prostate cancer.

- Antibodies are relatively large and may take a long time to reach tissues of interest and to clear from the body and are not optimal for molecular imaging.
- However, antibodies with any specificity can be reengineered into smaller fragments (minibodies and diabodies) that retain specificity and affinity and have pharmacological properties more optimal for molecular imaging. Both minibodies and diabodies retain the antigen-binding domains of the original antibody, while minibodies but not diabodies also retain another small portion of the antibody.
- While intact antibodies could take 1 week to image following administration, minibodies can be imaged the next day and diabodies can be imaged within a few hours.
- A minibody targeting prostate stem cell antigen (PSCA) labeled with iodine-124 (¹²⁴I) was developed as a PET imaging agent. PSCA is highly expressed by prostate cancer cells.
- This imaging agent has demonstrated high specificity in mouse tumor models and is being tested in phase I prostate cancer clinical trials.
- An antibody targeting the prostate cancer-specific protein PSMA (prostate-specific membrane antigen) was also reengineered into a minibody and radiolabeled with zirconium-89 (⁸⁹Zr) for PET imaging. The utility of the PSMA-minibody, termed IAB2M, was tested in a phase I clinical trial to identify metastatic prostate tumors and compared to bone scans and FDG-PET (figure).
- The specificity and sensitivity of these imaging modalities was investigated by performing biopsies of all lesions that were not unanimously detected. Biopsy specimens were then histologically analyzed to determine which lesions were truly malignant.
- A total of 393 metastatic tumors were identified in 28 patients by the 3 imaging modalities. ⁸⁹Zr-IAB2M PET identified 82% of all detected bone lesions and 86% of all detected soft-tissue lesions and detected 30% more lesions than the other scans. ⁸⁹Zr-IAB2M PET was found to have an overall specificity of 89.5%.
- An ongoing phase II clinical trial is comparing the specificity and sensitivity of ⁸⁹Zr-IAB2M PET/CT versus ¹¹¹In-capromab pendetide or conventional imaging in detecting lymph-node metastases in prostate cancer patients who had not yet undergone a prostatectomy. ¹¹¹In-capromab pendetide is a PET probe made with a different PSMA-targeting antibody. Lymph node tissues are surgically removed during a subsequent prostatectomy and examined by histology for presence of tumor. Thus far, 6 patients have been analyzed. ⁸⁹Zr-IAB2M PET detected 54.5% of tumor-positive lesions compared to 0% detected by the other modalities. The specificity of ⁸⁹Zr-IAB2M PET was 97%, with only one of 37 imaged lymph nodes being incorrectly called.
- Radiolabeled minibodies that target T cells are also being developed to visualize anti-tumor immune responses in cancer patients using PET imaging. T cells could be seen infiltrating implanted tumors in mice. Administration of a T cell activating immunotherapy significantly

enhanced T cell infiltration into tumors and led to tumor regression. This suggests that T cell PET imaging can be used to observe responses to immunotherapy in cancer patients.

- Positive surgical margins have been reported in ~20% of radical prostatectomy patients and are significantly more common in patients with higher grade tumors.
- New technologies utilizing fluorescent imaging agents and fluorescent probes attached to surgical tools are being developed to image tumors at the time of surgery. This would allow surgeons to determine the precise location of tumors and achieve full resection of primary prostate tumors with negative margins while preserving normal structures.
- A fluorescently labeled PSCA-targeted diabody was generated for fluorescent imaging during surgery. The diabody was injected into mice in order to make the tumor visible to the surgeon. Operations under white light during surgery resulted in positive margins while operations under white plus fluorescent light resulted in negative margins.
- Overall, these studies demonstrate that engineered antibodies have significant promise as sensitive and specific molecular imaging agents for detecting prostate tumors and other critical cell types in cancer patients.

^{89}Zr -Df-IAB2M imaging Comparison with Bone scan and FDG PET

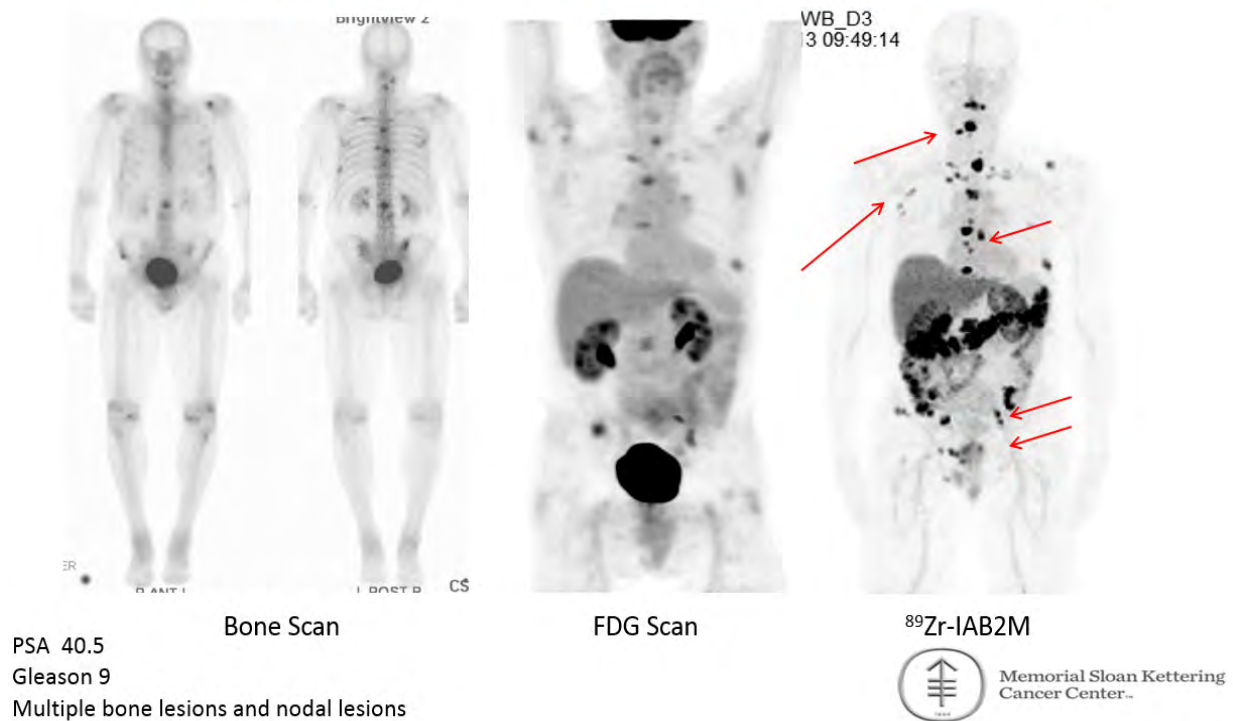
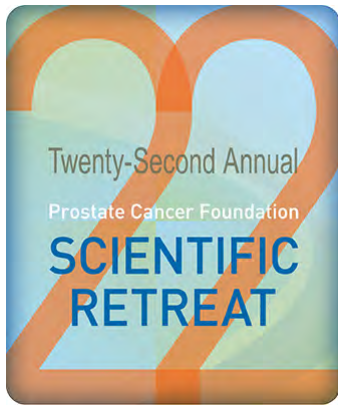


Figure: Comparison of imaging a prostate cancer patient with bone scan (left), FDG-PET (center), and ^{89}Zr -IAB2M PET (right). Significantly more metastatic lesions were detected by ^{89}Zr -IAB2M PET.

APPENDIX:

**22nd ANNUAL PROSTATE CANCER FOUNDATION
SCIENTIFIC RETREAT
OCTOBER 8-10, 2015**

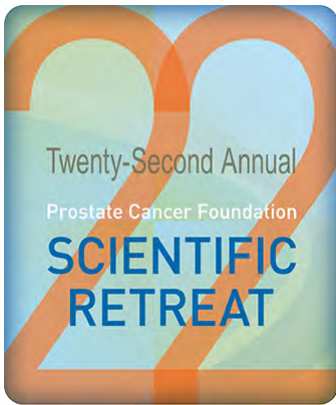
PROGRAM AGENDA



**22nd Annual Prostate Cancer Foundation
Scientific Retreat**

October 8 - 10, 2015

**Omni Shoreham Hotel
Washington D.C.**



AGENDA

Thursday, October 8, 2015

GENERAL SESSIONS

Location: Regency Ballroom

Welcome & Introduction

2:00 PM - 2:10 PM

Howard Soule, PhD
Executive Vice President
Chief Science Officer
Prostate Cancer Foundation

Session 1: Androgen Axis Resistance in Patients

2:10 PM - 3:10 PM

Moderator: Andrew Armstrong, MD
Duke Cancer Institute

2:10 PM - 2:25 PM *SNAIL Drives Enzalutamide Resistance through AR-V7 Regulation & PCF-Movember Global Treatment Sciences Challenge Grant Update*

Andrew Armstrong, MD
Duke Cancer Institute

2:25 PM - 2:30 PM **Discussion**

2:30 PM - 2:45 PM *Prediction of AR Signaling Therapeutic Resistance through Single CTC Analysis of Phenotype, Heterogeneity and Genomics*

Ryan Dittamore, BS, MBA
Epic Sciences

2:45 PM - 2:50 PM **Discussion**

2:50 PM - 3:05 PM *Tumor Cell Plasticity and Immunity in Enzalutamide Resistant CRPC*

Jennifer Bishop, PhD
Vancouver Prostate Centre, Canada

3:05 PM - 3:10 PM **Discussion**

Thursday, October 8, 2015

Session 2: Ascorbic Acid in Prostate Cancer Treatment – Highlighting the Marcus Foundation

3:10 PM - 3:50 PM

Moderator: Channing Paller, MD

The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

3:10 PM - 3:25 PM

A Randomized Phase 2 Trial of Ascorbic Acid in Combination with Docetaxel in Men with Metastatic Prostate Cancer

Channing Paller, MD

The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

3:25 PM - 3:30 PM

Discussion

3:30 PM - 3:45 PM

Targeting Prostate Cancer with Self-Assembled Vitamin C Nanofibers

Jeffrey Karp, PhD

Harvard: Brigham and Women's Hospital; Harvard Medical School

3:45 PM - 3:50 PM

Discussion

Session 3: New Prostate Cancer Targets and Treatments; What's a Patient to do when the Options Run Out? Part I

3:50 PM - 5:30 PM

Moderator: Howard Soule, PhD

Prostate Cancer Foundation

3:50 PM - 4:05 PM

Overview on Mechanisms Overcoming Castrate-Resistance and New Targets

Peter Nelson, MD

Fred Hutchinson Cancer Research Center

4:05 PM - 4:10 PM

Discussion

4:10 PM - 4:25 PM

In Vitro and In Vivo shRNA Screening for Candidate Prostate Cancer Drug Targets

Erik Danen, PhD

Universiteit Leiden, Netherlands

4:25 PM - 4:30 PM

Discussion

4:30 PM - 4:45 PM

LIM Kinase Inhibitors Impair Microtubule Dynamics and Prostate Cancer Proliferation

Mike Olson, PhD

Cancer Research UK Beatson Institute, UK

4:45 PM - 4:50 PM

Discussion

Thursday, October 8, 2015

- 4:50 PM - 5:05 PM *Targeting the DNA-Binding Domain of Nuclear Receptors – A Paradigm Shift in Rational Drug Discovery*
Artem Cherkasov, PhD
Vancouver Prostate Centre, Canada
- 5:05 PM - 5:10 PM **Discussion**
- 5:10 PM - 5:25 PM *ODM-201 for Castration-Resistant and Castration-Sensitive Prostate Cancer*
Martin Kornacker, MD
Bayer HealthCare
- 5:25 PM - 5:30 PM **Discussion**

Session 4: Novel Macromolecular Drug Delivery

5:30 PM - 6:30 PM

Moderator: Bradley Pentelute, PhD
Massachusetts Institute of Technology

- 5:30 PM - 5:45 PM *Precision Delivery of Proteins into Cancer Cells*
Bradley Pentelute, PhD
Massachusetts Institute of Technology
- 5:45 PM - 5:50 PM **Discussion**
- 5:50 PM - 6:05 PM *Cyclic Cell-Penetrating Peptides for Efficient Cytosolic Cargo Delivery*
Dehua Pei, PhD
The Ohio State University
- 6:05 PM - 6:10 PM **Discussion**
- 6:10 PM - 6:25 PM *Targeting Oncogenic Intracellular Protein-Protein Interactions with Ultrastable Cell Penetrating Micro Proteins*
Julio Camarero, PhD
University of Southern California
- 6:25 PM - 6:30 PM **Discussion**

Thursday, October 8, 2015

Dinner

7:15 PM - 8:45 PM

Dinner Location: Palladian Ballroom

Poster Session and Dessert

8:45 PM - 11:00 PM

Poster Session and Dessert Location: Ambassador Ballroom

Friday, October 9, 2015

6:45 AM - 7:45 AM ***Breakfast***

Location: Empire Ballroom

7:45 AM - 8:00 AM **Move to Session 5**

GENERAL SESSIONS
Location: Regency Ballroom

Session 5: Prostate Cancer Heterogeneity

8:00 AM - 9:20 AM

Moderator: Gerhardt Attard, MD, PhD

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

8:00 AM - 8:15 AM ***Circulating Tumor Clone Dynamics in Metastatic Prostate Cancer***
Gerhardt Attard, MD, PhD

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

8:15 AM - 8:20 AM **Discussion**

8:20 AM - 8:35 AM ***Evolutionary History of Lethal Prostate Cancer***
Steven Bova, MD

Tampere University Hospital, Finland

8:35 AM - 8:40 AM **Discussion**

8:40 AM - 8:55 AM ***The Natural History of Primary Multifocal Prostate Cancer***
Colin Cooper, PhD

University of East Anglia, UK

8:55 AM - 9:00 AM **Discussion**

9:00 AM - 9:15 AM ***Tracking the Origins and Drivers of Metastasis in Prostate Cancer***
Christopher Hovens, PhD

Australian Prostate Cancer Research Centre; Epworth Hospital University of
Melbourne Parkville, VIC, Australia

9:15 AM - 9:20 AM **Discussion**

Friday, October 9, 2015

Session 6: Reports from Innovative Conferences

9:20 AM - 10:00 AM

Moderator: Howard Soule, PhD
Prostate Cancer Foundation

9:20 AM - 9:35 AM

Emerging Concepts in High Risk Prostate Cancer: Update from the Coffey-Holden Prostate Cancer Academy

Joshua Lang, MD

University of Wisconsin Carbone Comprehensive Cancer Center

9:35 AM - 9:40 AM

Discussion

9:40 AM - 9:55 AM

Highlights from the Advanced Prostate Cancer Consensus Conference

Silke Gillessen, MD

Kantonsspital, Switzerland

9:55 AM - 10:00 AM

Discussion

SPECIAL AWARD

10:00 AM – 10:10 AM

Project Data Sphere - Prostate Cancer DREAM Challenge Winner

Award Presenters:

Carsten Goessl, MD

AstraZeneca

Liz Zhou, MD

Sanofi US

Award Recipient:

Tuomas Mirtti, MD, PhD

HUSLAB, Helsinki University Hospital, Finland

Introduced by Howard Soule, PhD

Prostate Cancer Foundation

Friday, October 9, 2015

SPECIAL LECTURE

10:10 AM – 10:40 AM

State of the Science 2015

Jonathan Simons, MD

President and CEO

Prostate Cancer Foundation

Introduced by Howard Soule, PhD

Prostate Cancer Foundation

10:40 AM - 10:45 AM

Discussion

KEYNOTE ADDRESS

10:45 AM - 11:45 AM

Mike Milken

Founder and Chairman

Prostate Cancer Foundation

Introduced by Stuart Holden, MD

PCF Medical Director

Professor of Urology and Associate Director, UCLA Institute of Urologic Oncology

University of California, Los Angeles

Group Photo

11:45 AM

Lunch

12:00 PM - 1:00 PM

Location: Empire Ballroom

Friday, October 9, 2015

1:00 PM - 1:15 PM **Move to Session 7**

Location: Regency Ballroom

Session 7: The Collision of Technology and Immunotherapy for Patients

1:15 PM - 2:35 PM

Moderator: David Mooney, PhD
Harvard University

1:15 PM - 1:30 PM ***Biomaterials as Therapeutic Cancer Vaccines***

David Mooney, PhD
Harvard University

1:30 PM - 1:35 PM **Discussion**

1:35 PM - 1:50 PM ***Lymph Node Targeting Nanoparticle Cancer Vaccines***

Jeffrey Hubbell, PhD
University of Chicago

1:50 PM - 1:55 PM **Discussion**

1:55 PM - 2:10 PM ***Enhancing Cell Therapy of Cancer with Nanotechnology***

Darrell Irvine, PhD
Massachusetts Institute of Technology

2:10 PM - 2:15 PM **Discussion**

2:15 PM - 2:30 PM ***Bispecific Antibody Armed Activated T-Cells (BATs) in Prostate Cancer***

Ulka Vaishampayan, MD
Wayne State University; Karmanos Cancer Institute

2:30 PM - 2:35 PM **Discussion**

Session 8: Global Research in the UK

2:35 PM - 3:55 PM

Moderator: Charlotte Bevan, PhD
Imperial College London, UK

2:35 PM - 2:50 PM ***Exploring and Exploiting the Crosstalk between Androgen and MicroRNA Signaling in Prostate Cancer***

Claire Fletcher, PhD
Imperial College London; London Movember Centre of Excellence, UK

2:50 PM - 2:55 PM **Discussion**

Friday, October 9, 2015

- 2:55 PM - 3:10 PM *TRoMbone: Testing Radical Prostatectomy in Men with Prostate Cancer and OligoMetastases to the Bone*
Prasanna Sooriakumaran, MD, PhD
University of Oxford, UK
- 3:10 PM - 3:15 PM **Discussion**
- 3:15 PM - 3:30 PM *Systemic Host-Tumour Interactions Drive Castration-Resistant Prostate Cancer*
Rachana Patel, PhD
Beatson Institute for Cancer Research, University of Glasgow, UK
- 3:30 PM - 3:35 PM **Discussion**
- 3:35 PM - 3:50 PM *Exploring the Utility of Detecting HER Receptor Rewiring in Circulating Exosomes for Overcoming Treatment Resistance in Castration-Resistant Prostate Cancer Patients*
Tony Ng, MD, PhD
Kings College London; University College London Comprehensive Cancer Imaging Centre, UK
- 3:50 PM - 3:55 PM **Discussion**

SPECIAL LECTURE

3:55 PM - 4:15PM

***New Direction in Prostate Cancer Research:
Resolvin' Tumor Growth with Resolvins***

Dipak Panigrahy, MD

Harvard Medical School; Harvard: Beth Israel Deaconess Medical Center

Introduced by Andrea Miyahira, PhD

Prostate Cancer Foundation

4:15 PM - 4:20 PM

Discussion

Friday, October 9, 2015

FIRESIDE CHAT

4:20 PM - 4:40 PM

An Unusual Career Path to Big Data Expertise for Cancer Genomics

Jeffrey Hammerbacher

Icahn School of Medicine at Mount Sinai; Cloudera Inc.

Introduced & Interviewed by William Oh, MD

Icahn School of Medicine at Mount Sinai

4:40 PM - 4:45 PM

Discussion

Dinner, Awards and Entertainment

7:15 PM - 10:00 PM

Location: Regency Ballroom

2015 PCF Young Investigator Awards

2015 The Movember Foundation-PCF Challenge Awards



2014 Fall PCF Challenge Awards

2015 PCF Challenge Awards

Saturday, October 10, 2015

6:45AM - 7:45 AM ***Breakfast***

Location: Ambassador Ballroom

7:45 AM - 8:00 AM **Move to Session 9**

GENERAL SESSIONS

Location: Regency Ballroom

Session 9: New Prostate Cancer Targets and Treatments; What's a Patient to do when the Options Run Out? Part II

8:00 AM - 10:00 AM

Moderator: Hung-Ming Lam, PhD
University of Washington

8:00 AM - 8:15 AM ***High-Testosterone Therapy in Post-Abiraterone and Post-Enzalutamide Era***

Samuel Denmeade, MD
Johns Hopkins University School of Medicine

8:15 AM - 8:20 AM **Discussion**

8:20 AM - 8:35 AM ***Targeting Estrogen Receptors***

Hung-Ming Lam, PhD
University of Washington

8:35 AM - 8:40 AM **Discussion**

8:40 AM - 8:55 AM ***Co-Targeting Stress-Activated Chaperones, CLU and Hsp27***

Martin Gleave, MD
Vancouver Prostate Centre, Canada

8:55 AM - 9:00 AM **Discussion**

9:00 AM - 9:15 AM ***From West Coast Dream Team Research to the Discovery of New Targeted Therapies***

Matthew Rettig, MD
University of California, Los Angeles

9:15 AM - 9:20 AM **Discussion**

9:20 AM - 9:35 AM ***Sigma 1 Targeted Treatment of Prostate Cancer***

Felix Kim, PhD
Drexel University

9:35 AM - 9:40 AM **Discussion**

Saturday, October 10, 2015

- 9:40 AM - 9:55 AM *When the Guardian Becomes the Enemy: Targeting ATM in PTEN Deficient Cancers*
Nuala McCabe, PhD
Queen's University Belfast, UK
- 9:55 AM - 10:00 AM **Discussion**

Session 10: Molecular Imaging for Improved Detection of Prostate Cancer Recurrence: Focus on Heterogeneity of Response

10:00 AM - 11:20 PM

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

- 10:00 AM - 10:15 AM *MRI of Advanced Prostate Cancer*
Anwar Padhani, MD
Mount Vernon Cancer Centre; Institute of Cancer Research, UK

10:15 AM - 10:20 AM **Discussion**

- 10:20 AM - 10:35 AM *Understanding Treatment Response in mCRPC Using Quantitative Total Bone Imaging*
Robert Jeraj, PhD
University of Wisconsin Carbone Cancer Center

10:35 AM - 10:40 AM **Discussion**

- 10:40 AM - 10:55 AM *Detecting Prostate Cancer with DCFPyL*
Martin Pomper, MD, PhD
Johns Hopkins University School of Medicine

10:55 AM - 11:00 AM **Discussion**

- 11:00 AM - 11:15 AM *PET and Optical Imaging of Prostate Cancer with Engineered Antibodies*
Robert Reiter, MD
University of California, Los Angeles

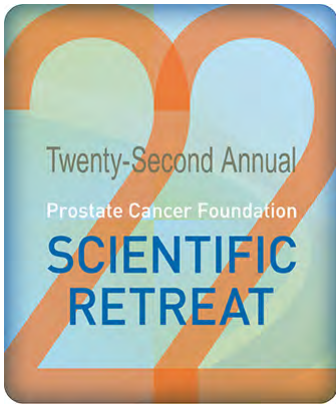
11:15 AM - 11:20 AM **Discussion**

Meeting Adjourned

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

If you are staying on Saturday evening, there will be an informal dinner at 6:00 pm in the Diplomat Ballroom.

Advanced RSVP required during online registration to attend this dinner.



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)

Andrew Armstrong, MD (Duke Cancer Institute)

Charlotte Bevan, PhD (Imperial College London, UK)

Hung-Ming Lam, PhD (University of Washington)

David Mooney, PhD (Harvard University)

Bradley Pentelute, PhD (Massachusetts Institute of Technology)

We thank our Retreat supporters for providing funding for this educational initiative.

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