The 22nd Annual Prostate Cancer Foundation Scientific Retreat Report

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The 22nd Annual Prostate Cancer Foundation (PCF) Scientific Retreat was convened in Washington, D.C. from October 8 to 10, 2015. This event is the foremost scientific conference in the world focusing on basic, translational, and clinical prostate cancer research with the highest potential for accelerating the understanding of prostate cancer biology and improving the lives and outcomes of prostate cancer patients. Topics highlighted during the 2015 Retreat included: (i) new strategies and treatments for localized high-risk, hormone-naive, oligometastatic, castrate-resistant, and treatment-refractory prostate cancer settings; (ii) the biology and genomics of tumor heterogeneity and tumor evolution; (iii) new understandings on the mechanisms and targeting of oncogenic drivers of prostate cancer; (iv) bioengineering of novel therapies and drug delivery methods; (v) innovative approaches to tumor immunotherapy; (vi) emerging molecular imaging technologies with improved sensitivity and specificity; and (vii) advancements in prognostic and predictive biomarkers and precision medicine strategies. Prostate © 2016 Wiley Periodicals, Inc.

KEY WORDS: androgen receptor; therapy; prognosis; diagnosis; tumorigenesis

INTRODUCTION

Every year since inception, the Prostate Cancer Foundation (PCF) has hosted an invitation-only Scientific Retreat. The 22nd Annual PCF Scientific Retreat was held from October 8 to 10, 2015 at the Omni Shoreham Hotel in Washington, D.C. The Retreat was originally a mechanism to bring together PCF-funded researchers to discuss progress on their projects in an intimate setting on the shores of Lake Tahoe. Over the years, this event has evolved to include global leaders from science, medicine, government, and business as attendees and speakers, and to enact a “first-in-field” agenda that champions emerging fields and important new concepts with the goal of accelerating this knowledge and application into the prostate cancer research community. The knowledge exchanges and interactions that have occurred at the Scientific Retreat over the last two decades have profoundly impacted the prostate cancer research field and the treatment landscape and outlook for prostate cancer patients and their families.

The 2015 Retreat was attended by 533 participants from 17 countries, representing 112 academic institutions, 10 medical research foundations, 36 biopharmaceutical companies, 13 other for-profit companies, and the NIH, NCI, and Department of Defense. There were 42 presentations in the plenary session and a poster session consisting of 133 poster presentations, all largely consisting of novel, unpublished data with high impact potential. A total of 75% of the speakers were presenting at the PCF Scientific Retreat for the first time. Session topics represented the convergence of many fields including: cellular and molecular biology, medical oncology, precision medicine, computational biology, genomics, tumor evolution, epigenetics, tumor immunology, immunotherapy, nuclear medicine, pathology, radiation oncology, surgery, urology.

Conflicts of interest: The authors have no conflicts of interest to disclose.

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Received 6 April 2016; Accepted 15 April 2016
DOI 10.1002/pros.23193
Published online in Wiley Online Library (wileyonlinelibrary.com).
molecular imaging, molecular pharmacology, drug discovery and development, bioengineering, drug delivery, and clinical trials.

This article seeks to disseminate the topics and findings discussed at the Retreat in order to broaden the impact of this knowledge and speed up research that will improve the lives of patients. A detailed summary of each presentation as well as the Retreat agenda can be downloaded at: http://www.pcf.org/2015retreatreport.

THE EVOLUTIONARY HISTORY OF PRIMARY AND METASTATIC PROSTATE CANCER

Analyses of prostatectomy specimens revealed that 70–80% of prostate cancer patients have multifocal primary disease, with multiple tumor clones and subclones coevolving, some of which possess metastatic potential. To study the biology and evolutionary history of multifocal primary prostate cancer, Colin Cooper (Institute of Cancer Research, UK) performed paired-end, massively parallel DNA sequencing on 3–5 tumor sites and one morphologically normal prostate site from fresh radical prostatectomy specimens for three patients. Two patients harbored high frequencies of genomic alterations in apparently benign prostatic tissue. While no mutations in known tumor driver genes were identified in the apparently normal prostate tissue, acquired alterations are likely drivers of clonal expansion. Comparisons with 21 genomic signatures of various mutational processes in cancer (Alexandrov et al. Nature, 2013 Aug 22;500(7463):415-21) identified one process associated with aging and two associated with unknown etiologies, in prostate tumor samples as well as in normal prostate tissues, indicating the same mutational processes are occurring in tumor and non-tumor tissues. Unique bioinformatic analysis identified both clonal and subclonal relationships between various tumor samples. Evolutionary trees were constructed to describe the relationships between the samples analyzed for each patient. In two patients, several distinct tumor lineages had evolved within a single tumor mass. Some but not all of the tumor sites shared mutations with normal tissues, possibly indicating that earlier clonal expansions had occurred. TMPRSS2-ERG translocations were not found in normal tissues while unique TMPRSS2-ERG translocations were acquired in 11 of 12 tumor sites from the three patients. This demonstrated that multiple TMPRSS2-ERG alterations occurred in a single prostate. These data suggest that the prostate persists in a pro-tumorigenic field state, within which morphologically normal prostate cells clonally expand and give rise to multifocal clonal and subclonal prostate cancer clones. How different primary tumor lineages contribute to metastasis remains to be determined.

Christopher Hovens (Australian Prostate Cancer Research Centre; Epworth Hospital University of Melbourne Parkville, VIC, Australia) performed extensive whole genome sequencing of primary and metastatic tumor clones from seven prostate cancer patients in order to identify genomic aberrations that drive the evolution of metastasis. Blood samples were additionally assessed to identify relationships between circulating tumor cells (CTCs) and tumor sites. Targeted deep sequencing was performed to examine 3,500 genetic variants at ~2,500×. TP53 was further sequenced at up to 10,000×. In all seven patients, a minor primary tumor clone had evolved into metastatic disease while the major primary tumor clone was unrelated. This suggests that the primary tumor does not progress linearly until metastatic potential is reached. Rather, subclones can metastasize much earlier, while localized prostatic clones continue to evolve and expand in the prostate. Other phenomena observed included reseeding of the primary tumor by metastases, multiple waves of primary tumor clones seeding a metastatic site, and reseeding of metastatic sites by clones from other metastatic sites. In one patient, premetastatic primary tumor subclones were detectable in the patient’s blood 35 months post-prostatectomy, indicating that active micrometastatic sites can be maintained for long periods of time. These findings suggest that metastatic tumor clones have different potentials for driving further disease progression, continue to evolve, and can disseminate to already established tumor sites. Aberrations in TP53 and in multiple DNA-repair pathway genes including BRCA1/2, POLE, POLQ1, MSH2, MLH1, ATM, and RAD51D were identified in metastatic lineages from all patients but were less frequent in primary tumors. In a second cohort, 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 unique TP53 mutations were observed in different metastatic subclones. These studies suggest that mutations in DNA-repair genes and TP53 drive metastasis and may be useful as primary tumor biomarkers to stratify high risk patients. Ongoing studies are examining whether treatment resistance is driven by genomic heterogeneity of preexisting tumor subclones and identifying the associated genomic alterations.

Steven Bova (Tampere University Hospital, Finland) discussed studies in which the evolutionary histories of lethal metastatic castrate resistant prostate cancers (mCRPC) were mapped in order to
identify targetable tumor mutations. Whole genome sequencing was performed on multiple metastatic tumors from 10 mCRPC patients as well as the primary tumor and normal prostate tissues from five of these patients. Identified genomic alterations were used to create phylogenetic trees that reflect the evolutionary relationships between all tumor sites for each patient. In patients for whom the primary tumor was not available, primary tumor mutations were inferred based on sharing between metastatic sites. Despite heterogeneity in the primary tumor and subclonal diversity of metastases, all metastatic tumors for each patient were found to have a monoclonal origin. In five patients, multiple tumor subclones populated a single metastatic site, suggesting the occurrence of polyclonal seeding. In the other five cases, the metastatic sites examined were seeded by a single founder clone. Clonal, truncal mutations were unique to each patient and in all but one patient, comprised the vast majority of mutations identified. Copy number losses of tumor suppressor genes were the most frequent truncal alterations while copy number gains in oncogenes were also common. Almost all mutations in the androgen receptor (AR) were non-truncal and observed in metastases but not the primary tumor, suggesting that AR mutations do not contribute to the development of prostate cancer but instead are acquired during the course of therapies. Phylogenetic trees were constructed to understand the order of subclonal spread. Similar to data presented by Hovens, in four of five patients, all metastases arose from a minor primary tumor clone while other primary tumor clones persisted and continued to evolve. In some patients, metastatic spread occurred in distinct stages, with the primary site first seeding a few metastatic sites, followed by a wave of mass migration of multiple subclones between various metastatic sites as well as the primary. In one patient, a more in-depth mutational analysis identified a mutation in ASNA1 in all metastatic sites, but not the primary tumor, suggesting that either the founding subclone was not in the primary tumor region assessed or that an early metastatic lesion developed this mutation and then went on to seed all subsequent metastases. Potentially actionable targets identified in this patient included FGFR1, PIK3CG, ABCC4, ALDH9A1, and ASNA1, suggesting candidacy for experimental therapies targeting FGFR1 or PIK3. Distinguishing truncal from nontruncal mutations may help to identify optimal targeted therapies and avoid development of therapeutic resistance.

Gerhardt Attard (The Institute of Cancer Research; The Royal Marsden NHS Foundation Trust, UK) discussed genomic analyses of circulating tumor DNA (ctDNA) to identify mechanisms of treatment resistance in CRPC and to study temporal tumor clone evolutionary dynamics. Targeted next-generation sequencing was performed on sequential plasma DNA samples from 16 CRPC patients collected prior to and following progression on sequentially administered treatments for CRPC. The fraction of plasma DNA arising from tumor cells was estimated using 6–8 tumor-specific genomic lesions hypothesized to be early events in carcinogenesis and dominant in metastatic disease (present in all tumor cells). These aberrations included mono-allelic deletions of 21q22 and NKX3.1, point mutations in FOXA1, TP53, and SPOP and PTEN-deletion. Dominant pre-CRPC tumor clones were observed to lose dominance after progression to CRPC as indicated by variations in the relative abundance of these lesions, suggesting multiple distinct tumor clones give rise to metastatic disease. For example, ~20% of CRPC patients exhibited concurrent ERG-positive and ERG-negative metastatic tumor clones. In 15–20% of CRPC patients, somatic point mutations involving AR arose on treatment with abiraterone and were associated with abiraterone resistance and disease progression. The two most common mutations, resulting in amino acid changes at L702H and T878A, result in the AR becoming activated by prednisone or progesterone, respectively, supporting their role in driving resistance to treatment. AR copy number gain was detected in plasma in 50% of patients prior to abiraterone and was associated with a significantly lower response rate and shorter time to disease progression (all patients with AR copy number gain progressed on abiraterone by 21 weeks). The plasma tumor DNA fraction also associated with worse outcome but did not associate with PSA decline rate. These data support the prospective evaluation of plasma DNA to select patients for treatment and offer patients with plasma AR aberrations, who appear unlikely to benefit from AR-targeted therapies, alternative treatments as early as possible.

**UNDERSTANDING AND OVERCOMING ANDROGEN AXIS RESISTANCE IN PATIENTS**

Therapeutic targeting of AR in prostate cancer can result in several outcomes including: (i) incomplete AR pathway blockade resulting in AR-driven remission; (ii) complete blockade of the AR pathway resulting in a durable complete response; or (iii) complete blockade of the AR pathway but recurrence with aggressive AR-independent tumors. AR amplification and missense mutations in the ligand-binding domain (LBD) are associated with therapy resistance in ~60% of CRPC patients. Other mechanisms of
resistance include expression of ligand-independent AR-splice variants such as AR-V7, de-repression of AR-repressed genes, and AR-bypass mechanisms in which tumor cells activate other survival and growth pathways and no longer rely on AR. Identifying other resistance mechanisms to AR-targeted therapy and devising therapeutic strategies to overcome resistance is critical.

Peter Nelson (Fred Hutchinson Cancer Research Center) discussed additional AR-therapy resistance mechanisms in CRPC and strategies for targeting these. CRPC tumors commonly had reciprocal expression of AR-V7 and androgens, suggesting the existence of at least two independent mechanisms of adaptation to ADT that involve rescuing AR activity. Overexpression of the glucocorticoid receptor (GR) was found to be an AR-bypass mechanism responsible for rescuing the expression of AR-dependent genes in some AR-negative prostate tumors. AR was found to repress >800 genes in LNCaP cells including cMET, WNT10B, WEE1, NFkB1, CCL20, UGT2B15, and TNFRSF21. De-repression of genes such as NFkB and cMET may contribute to AR-therapy resistance by promoting survival in the absence of AR. Co-targeting of the AR and NFkB pathways in prostate cancer cells resulted in greater apoptosis and suppression of proliferation than targeting either pathway alone, suggesting this as a treatment strategy for CRPC. To investigate other mechanisms of AR therapy-resistance, a model of androgen pathway-independent prostate cancer (APIPC) was created. LNCaP cells were transduced with an AR-driven suicide gene construct to generate an AR cell line refractory to androgen deprivation or enzalutamide and negative for neuroendocrine marker expression. High MAPK activity occurred in APIPC cells in the absence of AKT activation. MAPK activity was found to be driven by FGF8, which was highly expressed exclusively in APIPC cells and absent in other prostate cancer cell lines including AR-negative neuroendocrine lines. FGFR1-4 were also highly expressed in APIPC cells. AR-negative FGF8-positive tumors lacking neuroendocrine marker expression were identified among patient samples, suggesting that this may be an important clinical phenotype. Treatment with exogenous FGF8 enhanced the growth of APIPC cell lines while the FGFR-inhibitor PD173074 blocked growth of APIPC cells in vitro and of APIPC tumor xenografts in mice. Overall, numerous mechanisms can confer resistance to AR-targeted therapies in prostate cancer cells. Combining AR-targeted treatment with therapies that block AR-resistance mechanisms such as FGF/FGFR or neuroendocrine transdifferentiation warrant further exploration.

Ryan Dittamore (Epic Sciences) presented analysis of prostate cancer CTC phenotypes and gene expression patterns in order to identify biomarkers of resistance and response to AR-targeted therapies in CRPC. A dynamic range of CTC phenotypes were observed in CRPC patients that included traditional cytokeratin (CK)-positive CTCs, CK-negative CTCs, small CTCs, apoptotic CTCs, CTCs with a speckled pattern of CK distribution, and CTCs with large nucleoli. Patients could exhibit clusters of CTCs or have many CTC phenotypes simultaneously. An assay was developed to evaluate AR-V7 status in CTCs using an antibody targeting a cryptic region of AR that is only exposed in AR-V7. The relationship between AR-V7 status and outcome was prospectively evaluated in 193 mCRPC patients treated with abiraterone, enzalutamide, or taxane chemotherapy. AR-V7 was heterogeneously expressed in CTCs (range 0.3–100%) and observed in all of the CTC phenotypes. AR-V7 expression increased with additional lines of therapy, from an average of 3% AR-V7-positive CTCs in patients with one line of treatment, to 31% AR-V7-positive CTCs after three lines of therapy. All patients with AR-V7-positive CTCs exhibited de novo resistance to abiraterone or enzalutamide and AR-V7 expression was associated with shorter progression-free survival, shorter time on therapy, and lower overall survival. AR-V7 status was not associated with response to taxanes. These results confirm previous findings that AR-V7 expression in CTCs from CRPC patients is associated with resistance to abiraterone and enzalutamide but not taxanes. While AR-V7 status exhibited a 100% specificity for resistance to abiraterone or enzalutamide, sensitivity was poor, indicating the existence of other resistance mechanisms. The EPIC Sciences CTC analysis platform was adapted to allow isolation of single CTCs for whole genome sequencing. Analysis of 350 single CTCs from 17 patients revealed significant inter-patient heterogeneity as different CTCs harbored different copy number variations (CNVs) in tumor suppressor genes and oncogenes. This suggests that genomic analysis of circulating tumor DNA, tissue, or pooled CTCs may not identify all mutations present in less frequent subclonal populations. AR, MYC, and AURKA amplifications, and PTEN, RB, BRCA2, and ATM losses were commonly associated with resistance to AR-targeting therapy or taxanes. Patients who exhibited de novo therapy resistance had greater CNV heterogeneity in CTCs than responding patients, indicating that tumor heterogeneity increases the risk of having de novo therapy-resistant tumor subclones. Overall, these studies highlight the subclonal diversity of tumor populations and emphasize the need for single cell or highly
sensitive assays to fully elucidate tumor biology and discover biomarkers that best inform appropriate clinical treatment.

Jennifer Bishop (Vancouver Prostate Centre, Canada) discussed mechanisms of disease progression in enzalutamide-resistant tumors in which AR is expressed but is not performing classical functions. A xenograft mouse model of enzalutamide-resistant CRPC was developed by establishing prostate-specific antigen (PSA)-positive tumors in mice, which are then castrated and treated with enzalutamide. This causes tumors to initially regress followed by the emergence of enzalutamide-resistant tumors. Recurrent tumors mimic two types of disease observed in CRPC patients: AR-driven tumors that express PSA (AR-positive/PSA-positive) and anaplastic AR-positive tumors that lack PSA expression and other hallmarks of classic AR activity (AR-positive/PSA-negative). Cell lines were derived from enzalutamide-resistant AR-positive/PSA-negative and AR-positive/PSA-positive tumors, and from enzalutamide-naïve CRPC tumors. AR-Chip SEQ performed on these cell lines identified ~3,000 unique AR-binding sites in AR-positive/PSA-negative cells. Three major gene programs were found to be activated by AR in AR-positive/PSA-negative cells. The programs included those similar to cancer stem cells, neuroendocrine cells, and immune cells. Neuroendocrine-related genes expressed by AR-positive/PSA-negative cells included AURKA and the POU3F2 transcription factor. Immune-related genes expressed by AR-positive/PSA-negative cells included immune cell transcription factors, surface receptors, cytokines, and chemokines as well as the negative checkpoint molecule PD-L1. Expression of immune-related genes may allow tumor cells to resemble immune cells or differentially interact with immune cells to promote immune suppression. Almost 95% of neuroendocrine-like AR-positive/PSA-negative cells expressed PD-L1, indicating that AR concurrently turns on both gene programs. To determine whether PD-L1 expression by AR-positive/PSA-negative tumors suppresses anti-tumor immune responses, infiltration of xenograft tumors by innate immune cells was examined. Significantly fewer dendritic cells (DCs) and myeloid-derived suppressor cells including those that express PD-L1 and/or PD-L2 were observed in AR-positive/PSA-negative tumors compared with CRPC or AR-positive/PSA-positive tumors. Conversely, higher levels of DCs were observed in the blood of AR-positive/PSA-negative tumor-bearing mice as compared to CRPC or AR-positive/PSA-positive tumor-bearing mice. Peripheral blood DCs exhibited lower expression levels of CD80 and CD86 and higher levels of PD-L1 and PD-L2, indicating an immunosuppressive function. Patients progressing on enzalutamide after 12 weeks of treatment exhibited significantly higher levels of PD-L1/2-positive peripheral blood DCs compared with responding patients. The frequency of PD-L1/2-positive DCs continually increased throughout the duration of enzalutamide treatment. Thus, PD-L1/2-positive DCs may be a biomarker of enzalutamide resistance. Overall, these studies indicate that neuroendocrine features, stem cell-like features, and immune suppression may be mechanisms of enzalutamide resistance and deserve further study.

Martin Kornacker (Bayer Healthcare) discussed ODM-201, a novel non-steroidal small molecule inhibitor that binds selectively and with high affinity to AR and blocks interaction with testosterone or dihydrotestosterone (DHT). ODM-201 and its primary metabolite ODM-15341 exhibited higher affinity and lower IC₅₀ for AR compared with enzalutamide and the newer second generation anti-androgen ARN-509. ODM-201 inhibited VCaP cell proliferation at a concentration similar to enzalutamide and ARN-509. No alteration of CYP enzyme activity is expected with therapeutic doses of ODM-201. ODM-201 retained antagonistic properties against the AR-F876L mutant which converts enzalutamide and ARN-509 into AR agonists. ODM-201 also retained some efficacy against the enzalutamide and ARN-509-refractory AR-W741L mutant. In the phase I/II ARADES trial, ODM-201 demonstrated efficacy in three mCRPC populations: patients who were naïve for both CYP17-inhibitors and chemotherapy, CYP17-inhibitor-naïve patients who had received chemotherapy, and patients who had received CYP17-inhibitors. PSA responses at 12 weeks were best in chemotherapy-naïve, CYP17-inhibitor-naïve patients. ODM-201 was well tolerated up to the highest pre-specified dose of 1,800 mg/day with a favorable safety profile. The most frequent adverse events were grade 1–2 fatigue, back pain, and arthralgia. Negligible brain entry was observed for ODM-201, suggesting a reduced likelihood of neural toxicity which occurs at low frequency for this class of compound. ODM-201 is being tested by Bayer and Orion in the randomized, double-blind, placebo-controlled ARAMIS phase III trial in non-metastatic CRPC patients.

Samuel Denmeade (Johns Hopkins University School of Medicine) discussed results from clinical trials testing bipolar androgen therapy (BAT) in CRPC patients. The BAT strategy consists of inducing rapid cycling between polar extremes of supra-physiologic and castrate levels of testosterone by administering monthly injections of an FDA-approved supra-physiologic level of testosterone (400 mg) in combination with constitutive ADT. BAT takes advantage of the

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observation that AR acts as a DNA licensing factor and must be released from DNA and degraded following each round of cell division in order for DNA to be relicensed for subsequent rounds of cell division. Thus, at supra-physiologic androgen levels, cells die due to DNA licensing failure while at castrate androgen levels, cells die due to an insufficiency of AR-regulated growth and survival genes. Cells that adapt to either polar extreme by upregulating or down-regulating the androgen signaling axis will become sensitive to death during the next cycling between polar extremes. In a pilot trial, 14 CRPC patients received three cycles of BAT combined with etoposide. Responders were then treated with continuous testosterone. Eight patients experienced a PSA decline below baseline and 30% had a >50% PSA decline. The median response was 248 days with a 50% objective response by RECIST criteria. Four patients received >12 cycles of testosterone and one patient exhibited a complete response. All 10 patients who went on to receive treatment with abiraterone, enzalutamide, or other anti-androgens following BAT exhibited a response regardless of whether or not they had responded to BAT. A >50% PSA decline was observed in seven of eight patients treated with abiraterone or enzalutamide following BAT. All three patients who had previously developed resistance to abiraterone or enzalutamide and were re-challenged with the same anti-androgen following BAT, exhibited a response. These data suggest that BAT can resensitize CRPC to androgen ablative therapy. In the ongoing phase II RESTORE trial, patients failing enzalutamide or abiraterone are given three cycles of BAT. Responders remain on BAT until progression while non-responders were treated with the anti-androgen they had been failing prior to BAT. Of 34 patients treated thus far, 17 exhibited some PSA decline and 10 exhibited a >50% PSA decline. One complete response occurred, lasting for 505 days when subsequently treated with enzalutamide. Similarly, AR-V7-positive cells lost AR-V7 expression when rechallenged with abiraterone or enzalutamide. Similarly, AR-V7-positive cells lost AR-V7 expression within 4 hr following testosterone treatment in vitro but regained AR-V7 expression within 5 days when subsequently treated with enzalutamide. Thus down-regulation of AR-V7 may be one means by which BAT resensitizes prostate cancer cells to enzalutamide or abiraterone. The randomized phase II TRANSFORMER trial was recently initiated to compare the efficacy of BAT versus enzalutamide with cross-over in asymptomatic CRPC patients. This study will enroll 180 patients at over 17 U.S. sites, and is designed to detect a 50% improvement in radiographic progression-free survival. Overall, these findings suggest that BAT has promise for the treatment of CRPC and may resensitize patients to AR-targeted therapies.

### NEW PROSTATE CANCER TARGETS AND TREATMENTS

Erik Danen (Universiteit Leiden, Netherlands) presented the discovery of two regulators of prostate cancer cell migration using novel in vitro and in vivo experimental systems. An in vivo prostate cancer cell migration assay was developed in which RFP-labeled tumor cells are visualized disseminating through zebrafish engineered to express GFP in endothelial cells. To identify genes necessary for prostate cancer cell dissemination, PC3 cells were transformed with an adenovirus-based genome-wide shRNA library, labeled with RFP, and injected into zebrafish. Zebrafish were then monitored for PC3 cells with disabled dissemination. SYC, a kinase in the B cell receptor (BCR) signaling pathway, and MST1R, a relative of MET that cross-talks with the MET pathway, were identified as candidate regulators of PC3 cell migration. Silencing of SYC or MST1R in PC3 cells also disabled migration in an in vitro culture system in which tumor cells were loaded into nanowell plates filled with a gel composed of extracellular matrix (ECM) components, followed by imaging of tumor cell migration. Knockdown of MST1 or SYC in a highly metastatic PC3 variant line reduced the formation of bone metastases in murine xenograft models. Both MST1R and SYK were found to be highly expressed in prostate cancer cell lines. Expression of SYK was correlated with metastasis in publicly available prostate cancer gene expression datasets. SYK expression was associated with metastasis in publicly available prostate cancer gene expression datasets. SYK expression was correlated with Gleason grade and was higher in metastases than primary tumors. However, not all metastatic lesions expressed SYK, suggesting that SYK plays a role in some but not all mechanisms of metastasis. These studies suggest that SYC and MST1R regulate the invasive properties of prostate cancer cells and may be targets for preventing prostate tumor metastasis.

Michael Olson (Cancer Research UK Beatson Institute, UK) discussed targeting LIM Kinase-1 (LIMK1) and LIMK2, which are convergent signaling nodes downstream of RhoA-C, Rac1-3, and CDC42 GTPase pathways. LIMK1/2 function in stabilization of actin filaments, regulation of actin-myosin contraction and microtubule dynamics, and have roles in tumor cell...
migration, invasion, and metastasis. LIMK1/2 are tyrosine kinase-like kinases, which have not yet been extensively examined as therapeutic targets in cancer. In vitro treatment of DU145 and PC3 cells with a small molecule LIMK-inhibitor (developed by BMS) reduced cell migration. Additionally, LIMK-inhibitor treatment or siRNA knockdown of LIMK1/2 expression induced apoptosis and inhibited proliferation more substantially in androgen-dependent prostate cancer cell lines compared with androgen-independent lines, suggesting that LIMK-inhibitors might have greater efficacy in hormone-sensitive prostate cancer patients. LIMK-inhibition also blocked the interaction between AR and tubulin and induced AR degradation, leading to reduced levels of nuclear AR. LIMKs were highly expressed in human prostate tumor samples and correlated with reduced survival along with phosphorylated-cofilin, a LIMK target, in 164 non-metastatic prostate cancer patients. Conversely, these factors were not predictive of survival in metastatic prostate cancer patients. Overall, targeting LIMKs may be a strategy to prevent prostate tumor metastasis by affecting microtubule stability, AR activity, and prostate cancer cell motility.

Hung-Ming Lam (University of Washington) discussed activation of GPR30, a recently discovered structurally unique member of the estrogen receptor (ER) family, for the treatment of prostate cancer. GPR30 was found to be expressed on both stromal and prostate cancer epithelial cells, and levels of expression correlated with disease progression. GPR30 activation promotes the growth of breast and ovarian cancers but is inhibitory in prostate cancer. G-1 is a recently developed GPR30-specific agonist that lacks activity against ERα and ERβ. G-1 treatment inhibited the growth of PC3 xenografts and of LNCaP tumors in castrated but not androgen-intact mice, indicating that GPR30 activation is only effective in the absence of androgen signaling. G-1 treatment induced tumor necrosis and led to mass infiltration of necrotic regions with neutrophils. Numerous cytokines, chemokines, and inflammatory response genes were induced in tumors from castrated but not uncastrated mice treated with G-1. In LNCaP cells, expression of GPR30 was inhibited by treatment with androgens and promoted by bicalutamide treatment or AR-silencing by siRNA. Castration of mice bearing LNCaP tumors led to upregulation of GPR30 expression. Together, these data suggest that an AR-driven pathway suppresses GPR30 expression. GPR30 was found to be highly expressed in ~50% of primary prostate tumors and in 80% of CRPC tumors from autopsy patients. GPR30 was heterogeneously expressed in prostate cancer metastases, ranging from no expression in some lesions to high expression in others. High expression of GPR30 was observed in metastatic tumors from abiraterone-refractory CRPC patients. In mouse models in which hormone-sensitive C4-2 or LuCaP xenograft tumors are established, followed by castration to promote development of CRPC, G-1 treatment reduced tumor growth and was synergistic with abiraterone. While 43% of mice exhibited de novo resistance (defined by tumor progression >50% from baseline at 6 weeks of treatment) to abiraterone and 50% of mice exhibited de novo resistance to G-1, only 8% of mice exhibited de novo resistance to the combination. Expression of AR was found to be inhibited by the combination of G-1 plus abiraterone, while either alone enhanced AR expression. No notable toxicities were observed following G-1 treatment of mice. Overall, these studies indicate that activation of GPR30 with G-1 may have efficacy in prostate cancer, in particular in combination with AR-axis inhibitors.

Martin Gleave (Vancouver Prostate Centre, Canada) discussed targeting of Clusterin and HSP27, two stress-activated molecular chaperones that act to block apoptosis and promote protein stabilization. Both were found to be upregulated in prostate cancer and promote adaptation to hormone withdrawal and subsequent development of CRPC. OGX-011, a second generation antisense drug targeting Clusterin, synergized with enzalutamide and promoted prostate cancer cell apoptosis by blocking enzalutamide-induced autophagy and cross-talk activation of AKT. Treatment with OGX-011 also synergized with enzalutamide, taxane chemotherapy, and with AKT-inhibition to induce prostate cancer cell death and reduced the growth of LNCaP xenografts in mice. In a phase II trial in 82 CRPC patients, OGX-011 in combination with docetaxel resulted in a 7 month survival advantage compared with docetaxel alone. However, in the subsequent phase III SYNERGY trial in metastatic CRPC, the addition of OGX-011 to docetaxel failed to confer a survival benefit. Post hoc hypothesis-generating analysis revealed that patients with a good prognosis derived no benefit from OGX-011, while in patients with a poor prognosis, the addition of OGX-011 extended overall survival from 13.7 to 16 months. In poor prognosis patients, the survival benefit from OGX-011 correlated with decreases in serum levels of Clusterin. Reasons for trial failure are being explored. The Clusterin gene is adjacent to the NKX3.1 gene that is deleted in 20% of patients. An assay to determine whether Clusterin has also been deleted has been developed. The ongoing phase III AFFINITY trial is testing OGX-011 in
combination with cabazitaxel in patients previously treated with docetaxel.

OGX-427, a second generation antisense drug targeting HSP27, prohibited AR activity and prostate tumor growth in mouse models of CRPC. In a phase I trial, OGX-427 demonstrated some dose-dependent grade 1–2 infusion reactions but reduced tumor markers and CTCs in prostate and ovarian cancer patients. Overall, a >15% decrease in measurable disease was observed in 27% of patients. In a phase II study with cross-over in 65 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. Over 50% of patients failing on prednisone who were crossed over to OGX-427 experienced some PSA decline, and 20% of patients experienced 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. However, ~50% of patients in both treatment arms exhibited 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. The crystal structure of HSP-27 was recently solved and is being used to identify regions targetable by small molecule inhibitors. A candidate small molecule inhibitor was identified that could inhibit HSP27 nuclear translocation and phosphorylation following heat shock. Ongoing studies will clarify whether targeting Clusterin or molecules that may function as synthetic-lethal partners of the DNA damage checkpoint kinase ATM in prostate cancer cells. The patient-derived AT22IJE-T fibroblast cell line, which harbors an ATM mutation, was transduced with a siRNA library targeting 178 tumor suppressor genes and monitored for cell viability as compared to AT22IJE-T cells in which ATM was re-expressed. Nine tumor suppressors that may function as synthetic-lethal partners of ATM were identified: BRIPI/FANCJ, CDKN2C, PTEN, STEAP4, NKTR, CASP8, TP53, FANC, and CAV1. PTEN is commonly deleted in advanced prostate cancer and was selected for further study. Targeting of ATM with siRNA or an ATM-inhibitor (KU-55933) in colorectal and prostate cancer cells with PTEN-loss led to enhanced cell cycle arrest, caspase 3/7 activation, apoptosis, and chromosome instability compared with PTEN-WT cells. PTEN-null cells exhibited constitutive ATM activation and elevated γ-H2AX phosphorylation, a marker of DNA damage. Reactive oxygen species (ROS) were also elevated in PTEN-null cells and exacerbated by ATM-inhibition, suggesting that ATM becomes constitutively active in PTEN-null cells in order to survive oxidative stress. Treatment of PTEN-null cells with antioxidants β-carotene or N-Acetyl Cysteine (NAC) reduced ATM activity and γ-H2AX phosphorylation and inhibited apoptosis of PTEN-null cells treated with ATM-inhibitors, supporting the hypothesis 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, mice bearing PTEN-WT versus PTEN-null prostate tumors xenografts were treated with the ATM-inhibitor KU-60019. 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 but were more profoundly affected by ATM-inhibition. In summary, targeting ATM may be an effective therapeutic strategy against PTEN-null prostate and other cancers.

Tony Ng, MD, PhD (Kings College London; University College London Comprehensive Cancer Imaging Centre, UK) discussed the role of HER pathway rewiring in cancer. HER1 (EGFR), HER2, and HER3 are druggable targets in breast cancers and have been suggested to also play an important role in CRPC by enhancing the stability and DNA binding activity of AR. Targeting of the HER molecules can be problematic however, because the four HER/ErbB family members are able to reconfigure into various heterodimers and reactivate the HER signaling pathway, conferring resistance to HER-targeting therapy. Fluorescence Lifetime Imaging (FLIM) images energy transfer between donor and receptor fluorophore-labeled antibodies when under 10 nm apart. FLIM was used to examine the levels of EGFR:HER3 heterodimers in tumor tissues from triple negative breast cancer patients pre versus post neoadjuvant treatment with cetuximab. EGFR:HER3 heterodimers were not observed prior to treatment, but were highly induced after six cycles of cetuximab in >70% of patients with residual tumor burden. In order to non-invasively monitor HER pathway rewiring, a method was developed to perform FLIM in tumor-derived exosomes harvested from peripheral blood. EGFR: HER3 heterodimers were homogeneously expressed in tumor-derived exosomes from NSCLC patients and were down-regulated in 40% of patients following treatment with cisplatin. Studies are underway to determine whether EGFR:HER3 heterodimer levels in tumor-derived exosomes inform resistance to treatment with therapies that cross-talk with HER.

The Prostate
signaling pathways, including chemotherapy. Whether EGFR:HER3 heterodimers play a role in the development of CRPC is also being explored.

Claire Fletcher (Imperial College London; London Movember Centre of Excellence, UK) discussed the discovery of oncogenic microRNAs (miRs) that regulate the AR pathway as novel targets for prostate cancer therapy. The tumor suppressor prohibitin (PHB), and AR were shown to reciprocally repress expression of each other via onco-miR-27a. PHB expression was found to be repressed by miR-27a via association with the PHB 3’UTR, and miR-27a expression was activated by androgens and inhibited by treatment with bicalutamide. AR was recruited to the miR-27a promoter and also promoted processing of pri-miR-27a to its mature form. Finally, treatment with a miR-27a-inhibitor suppressed the growth of LNCaP cells in vitro and LNCaP tumor xenografts in mice. These data indicate that AR suppresses PHB expression via upregulation of miR-27a and suggest that targeting miR-27a may be effective in combination with AR-targeting therapies in treating prostate cancer. In addition, a screen of 950 miR inhibitors was performed to identify other AR activity-modulating miRs in LNCaP and LNCaP-derived C4-2 cell lines. An AR-driven luciferase reporter construct was used as a readout of AR activity. Eight miRs were identified to significantly modulate AR activity in both cell lines. One candidate AR-regulating miR was confirmed as a critical positive regulator of AR. Inhibition of the miR reduced AR activity and growth of LNCaP cells and induced apoptosis. Overexpression of the miR enhanced AR activity, promoted invasion in wound healing and matrigel assays and upregulated EMT markers including ZEB2. These data suggest that inhibition of various AR-activatory miRs may have efficacy alone or in combination with AR-inhibitors in the treatment of prostate cancer.

ENGINEERING OF CYCLIC PROTEINS AS NOVEL DRUG DELIVERY PLATFORMS

Oncogenic protein–protein interactions that differ between tumor and normal cells represent promising targets for cancer therapy. 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. Novel cyclic polypeptides have emerged as promising alternatives to linear polypeptides for development into cancer therapeutics.

Dehua Pei (The Ohio State University) discussed the development of cell-penetrating cyclic peptides (CCPPs) that target specific protein–protein interactions as novel cancer therapies. Covalently bonded peptide rings naturally occur in nature and are highly stable compared to linear peptides. These properties along with the ease of manufacture and modification make cyclic peptides appealing for development into therapeutics. Cyclic peptide libraries were screened to identify inhibitors of oncogenes including Pin1 and K-Ras. A large hydrophilic non-membrane penetrating Pin1-targeting cyclic peptide was identified. Exchanging non-interacting amino acids for arginine and adding two additional arginine residues to the peptide ring transformed the Pin1-targeting peptide into a highly cell-penetrant CCPP. A series of CCPPs with cytosolic delivery efficiencies of 14–84%, excellent proteolytic stability, and oral bioavailability in mice were generated for use as scaffolds for the development of therapeutics that target intracellular protein–protein interactions. In comparison, other gold-standard cell-penetrating peptides have cytosolic delivery efficiencies of <5%. CCPPs were found to enter cells via endocytosis followed by escape from the early endosome. CCPPs can be fused with a drug or can have a targeting sequence intercalated into the ring. Additionally, fusion of CCPPs with impermeable cyclic peptides that target a specific protein generated significantly more stable bicyclic peptides that retain membrane-penetrating and protein-targeting properties. Integration of a K-Ras-targeting peptide sequence into a CCPP, followed by modification of various residues resulted in the generation of an 11-amino acid K-Ras-targeting CCPP with high membrane permeability and high K-Ras binding affinity, termed Cyclorasin 9A5. Cyclorasin 9A5 exhibited strong inhibition of K-Ras in vitro by blocking phosphorylation of AKT, MEK, and ERK and promoted apoptosis. Cells expressing a constitutively active myristilated-AKT mutant were highly resistant to Cyclorasin 9A5, supporting that the inhibitor acts upstream of AKT activation, directly on K-Ras. Various oncogene-targeting CCPPs are being further developed in preclinical cancer models.

Julio Camarero (University of Southern California) discussed cyclotides, a class of plant-derived cyclic microproteins that can be used to target intracellular protein–protein interactions. Cyclotides are ~30aa in size, are highly stable, orally bioavailable, and exhibit diverse biological functions. Cyclotides are structured in a circular cysteine knot topology generated by a head-to-tail connection and three intercalated disulfide bridges. Cyclotides contain five hypervariable loop regions that are highly amenable to modification, such as replacement with bioactive polypeptides. Hdm2 is an ubiquitin protein ligase that ubiquitinates p53 and promotes p53 degradation, while blocking this interaction stabilizes p53 and increases p53 levels.
A linear p53/Hdm2-inhibitory peptide was generated based on a modified Hdm2-binding sequence of p53 that outcompetes p53 for binding to Hdm2. However, the linear peptide exhibited poor stability and cellular bioavailability. To generate a more stable and bioactive p53/Hdm2-targeting agent, the p53/Hdm2-inhibitory peptide sequence was grafted onto loop six of the cyclotide MCoTI-I. The modified cyclotide, termed MCoTI-PMI, was produced by recombinant expression in E. coli using a TEV-leading peptide sequence, followed by purification, TEV-cleavage, and reduced glutathione-induced folding to guide native backbone cyclization. The IC50 of MCoTI-PMI for Hdm2 was 30 nM compared with 2,300 nM for WT-p53. MCoTI-PMI exhibited a half-life of 30 hr in human serum and 0.8 hr in mice. Treatment of p53-positive cancer cell lines including LNCaP and MCF-7 with MCoTI-PMI led to p53 stabilization, downregulation of HdmX levels, upregulation of the p53 target p21, and enhanced apoptosis. Cell lines lacking functional p53 including DU145 and PC3 were resistant to MCoTI-PMI. Treatment of mice bearing HCT-116 xenograft tumors resulted in reduced tumor growth and activation of p53 in residual tumor tissue. The efficacy of MCoTI-PMI is being further studied in preclinical models. Cyclotides that target other oncogenes including Ras and HdmX are being developed.

HIGHLIGHTS FROM THE ST. GALLEN ADVANCED PROSTATE CANCER CONSENSUS CONFERENCE

The inaugural St. Gallen Advanced Prostate Cancer Consensus Conference (APCCC) was held from March 12 to 14, 2015, in St. Gallen, Switzerland, to generate recommendations for clinical management of prostate cancer patients in areas where little or no evidence exists. Silke Gillessen (Kantonsspital, Switzerland) reviewed the recommendations generated at the conference. The consensus panel consisted of 41 prostate cancer experts from various disciplines around the world. Prior to the conference, the panel was put through three rounds of generating consensus questions on the most critical topics of unmet need in prostate cancer as part of a modified Delphi process. These questions were discussed and voted on at the conference. Topics included management of castration-naive metastatic prostate cancer; defining castration-resistance; management of non-metastatic 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 reducing risk of skeletal related events; value and use of predictive biomarkers; oligometastatic prostate cancer; and general management of patients. Detailed voting results and discussion thereof can be found in the publication in Annals of Oncology [1]. A few of the key recommendations are summarized here: For asymptomatic or minimally symptomatic CRPC patients, either abiraterone or enzalutamide in addition to ADT was generally recommended as first-line therapy while chemotherapy was generally not recommended. Chemotherapy was recommended by more panelists in the situation of first-line therapy for healthy but symptomatic CRPC patients. Radium-223 was not generally recommended as first-line therapy for symptomatic CRPC patients (with bone but no visceral metastases). Bicalutamide was not recommended for treatment of men progressing on ADT if abiraterone or enzalutamide are available. Treatment with bisphosphonates or denosumab in the dose for reduction of skeletal related events was not recommended for castrate-naive metastatic prostate cancer patients. No recommendation could be reached regarding the optimal dosing and timing of bisphosphonates or denosumab in CRPC patients with bone metastases. It was recommended to measure testosterone levels to determine CRPC, to perform baseline staging examinations that include imaging before starting a treatment for CRPC, and to use imaging and other methods in addition to PSA to monitor treatment responses of CRPC patients. No consensus was reached however on how and when to use novel imaging methods to guide treatment decisions. It was not recommended to stop treatment of CRPC patients based only on a rise in PSA. Patients should be encouraged to enter clinical trials. Non-metastatic CRPC patients were not recommended to be treated with survival prolonging agents outside of a clinical trial. No consensus was reached on diagnosing and treating oligometastatic prostate cancer. In 2017, a second St. Gallen Advanced Prostate Cancer Consensus Conference (www.apccc.org) will be held to address additional topics of critical unmet need in prostate cancer.

OPTIMIZING TREATMENT OF OLIGOMETASTATIC PROSTATE CANCER

Patients with oligometastatic prostate cancer, in which five or fewer metastatic lesions are present, are known to have improved outcomes compared to patients with overt metastases. Whether these differences are due to unique disease states or are a product of lead time bias, are unclear. Nevertheless, these patients are considered a potentially curable population, if optimal therapeutic strategies can be developed.
Joshua Lang (University of Wisconsin Carbone Cancer Center) presented an overview of the PCF-sponsored Coffey-Holden Prostate Cancer Academy (CHPCA) Meeting, which was held from June 25 to 28, 2015 in La Jolla, California. The CHPCA Meeting convenes ~75 investigators annually to address a selected topic of critical unmet need in prostate cancer in a unique think-tank format, in which talks are limited to 10 min, followed by 20 min of discussion. The 2015 CHPCA Meeting was themed “Multidisciplinary Intervention of Early, Lethal Metastatic Prostate Cancer” and focused on how to best diagnose and treat patients with localized high risk or oligometastatic prostate cancer. Session topics included understanding the unique biology of high-risk and oligometastatic disease, targeting the tumor microenvironment, development and application of novel molecular imaging and molecular biomarkers, and designing optimal multi-modal interventions. Several important topics of discussion are highlighted here.

Treatment of the primary tumor without curative intent in high-risk or metastatic prostate cancer patients has not been a standard practice. However, improved surgical techniques along with evidence that surgical removal or radiation treatment of the primary tumor can prolong survival in these patients even without being curative, has prompted testing of this treatment strategy in a number of clinical trials. The planned TRoMbone clinical trial, discussed by Prasanna Sooriakumaran (University of Oxford, UK), will test the efficacy of treatment as usual versus treatment as usual plus radical prostatectomy in previously untreated oligometastatic patients. Another trial is testing the efficacy of neoadjuvant chemotherapy followed by radical prostatectomy and stereotactic body radiation therapy to metastatic sites in oligometastatic prostate cancer patients. Neoadjuvant treatment of high-risk patients with enzalutamide or abiraterone followed by radical prostatectomy has been shown to be life-prolonging, though not curative in several recently completed trials. Genomic analysis of residual tumor foci in one of these trials identified clonal heterogeneity and de novo treatment resistance as factors limiting the efficacy of this strategy. An ongoing clinical trial in intermediate and high-risk prostate cancer patients is testing the efficacy of maximal neoadjuvant androgen axis blockade with abiraterone plus ARN-509. A novel strategy of using CXCR4-blockade to evict prostate tumor cells from metastatic bone sites followed by treatment with docetaxel to kill mobilized tumor cells will be tested in an upcoming trial at Johns Hopkins University. Another trial being planned at Johns Hopkins University will test the efficacy of combining stereotactic body radiation therapy with ADXS-PSA, a live, attenuated strain of Listeria monocytogenes expressing PSA, in oligometastatic prostate cancer patients.

Gleason Pattern 3 (GP3) tumors are usually considered indolent while GP4 suggests aggressive disease. To understand the biology of aggressive prostate cancer, the genomic aberrations present in adjacent GP3 and GP4 tumors were compared. Many mutations were shared by adjacent GP3 and GP4 tumors, suggesting clonal origin. However, GP4 tumors commonly gained mutations in tumor suppressor genes not present in GP3 tumors, indicating that tumor suppressor gene loss plays a role in progression from indolent to aggressive disease. Mutations or loss of DNA repair genes were generally associated with a higher rate of genomic aberrations. Novel aspects of the tumor microenvironment may be targetable for therapy. For instance, androgens produced by stromal cells may facilitate tumor growth and provide a buffer against the effects of ADT. WNTs secreted by tumor and stromal cells promote tumor growth while inhibiting proliferation and functional activity of anti-tumor T cells. Analyses of tumors treated with Sipuleucel-T found that T cells elicited by the vaccine gathered along tumor peripheries but rarely entered tumor centers. Factors in the tumor microenvironment that contribute to immune suppression need to be understood and resolved in order for immunotherapy to be successful in prostate cancer.

Newly emerging molecular biomarkers and molecular imaging technologies that may allow earlier detection of prostate cancer and improved assessment of treatment responses were discussed, including PSMA-PET imaging and WB-MRI, which are discussed elsewhere in this article. Overall, 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 overview on the Meeting has been published by the 2015 CHPCA organizing committee [2].

**NEW CONCEPTS IN CANCER IMMUNOTHERAPY**

David Mooney (Harvard University) discussed the development of a novel biomaterial-based cancer vaccine technology. In this strategy, a highly porous biomaterial (poly(lactide-co-glycolide)) tablet the size of an aspirin pill is loaded with GM-CSF, adjuvant such as CpG nanoparticles, and freeze dried tumor cell particles obtained from patient biopsies, and injected subdermally. GM-CSF released from the vaccine attracts DCs into the biomaterial, where they pick up tumor antigens and home to nearby lymph nodes.
nodes to activate anti-tumor T cell responses. The biomaterial then completely biodegrades over time. In mice, over one million DCs could be observed infiltrating the vaccine at a time in a GM-CSF dose-dependent manner. When the biomaterial was loaded with FITC as a model antigen and subdermally injected into mice, DCs carrying FITC could be visualized entering lymph nodes. Lymph node trafficking required loading of the vaccine with CpG and GM-CSF. A single vaccination of mice harboring B16 melanoma tumors prevented tumor growth better than GVAX and 50% of mice that received a second biomaterial vaccination experienced complete tumor regression and long-term survival. Survival of vaccinated mice correlated with increased frequencies of CD8-positive DCs and plasmacytoid DCs at the vaccine site, suggesting that efficacy of the vaccine may be due to the ability of the biomaterial to recruit and activate diverse types of DCs. The efficacy of the TLR9 agonist CpG, TLR3 agonist polyI:C, and TLR4 agonist MPLA as vaccine adjuvants were compared. CpG and polyI:C but not MPLA induced long-term tumor regression. CpG and polyI:C also produced substantially greater numbers of melanoma antigen Trp2-specific splenocyte CD8 T cells, and tumor-infiltrating IFNγ-positive and CD107a-positive CD8 T cells compared with MPLA. The biomaterial vaccine was tested for synergy in combination with anti-CTLA4 or anti-PD1 antibodies using doses which did not achieve long-term regressions for any treatment alone. When treated with the vaccine plus either anti-CTLA4 or anti-PD1 antibodies, over 50% of B16 tumor-bearing mice experienced complete tumor regression and long-term survival. A phase I trial has been initiated at the Dana Farber Cancer Institute to test the vaccine, WDVax, in melanoma patients. No safety concerns have been observed. Patients with disease progression are being studied for mechanisms of vaccine failure. Additional biomaterial vaccine technologies are in development. A highly elastic cryogel has been developed that can be compacted into a needle for subdermal injection where it will regain its original shape within 200 ms. Injectable 80–120 μm mesoporous silica microparticles that spontaneously create a 3D scaffold in vivo are also under development. These microparticles have 7 nm pores that can be loaded with molecules such as cytokines and chemokines, adjuvant, bioactive drugs of interest, and tumor cell particles. 100 μm sized mesoporous silica microparticle-based vaccines recruited millions of immune cells into the vaccine site. Robust serum IgG1 and IgG2a antibody responses were generated after a single vaccination. These novel biomaterial-based vaccination strategies are continuing to be developed and need to be tested in prostate cancer.

Darrell Irvine (Massachusetts Institute of Technology) discussed the development of drug-carrying nanoparticles that can be attached to the surface of T cells in order to deliver autocrine activation signals or to harness the T cell as a vehicle for drug delivery to the tumor microenvironment. A two-step cross-linking chemistry was developed to attach drug-carrying nanoparticles to the cell surface. Nanoparticles coated with maleimide-functionalized lipids were first coupled to free thiols in cell surface proteins, followed by PEGylation. Nanoparticles remained bound to the surface of non-phagocytic cells such as T cells and hematopoietic stem cells for extended periods of time, even following cell division, whereas they were internalized when bound to DCs. When bound to T cells, nanoparticles were efficiently delivered to tumors and did not affect T cells trafficking to tumors in TRAMP mice. In a metastatic B16 melanoma model, when IL-21 and IL-15-containing nanoparticles were coupled to melanoma-specific T cells, massive T cell expansion occurred, T cells were maintained as long-term memory cells and 100% of mice experienced tumor regressions while no T cell expansion or tumor regressions occurred if IL-21 and IL-15 were delivered as separate injections. To generate T cells able to deliver SN-38 chemotherapy-containing nanoparticles to tumor sites, T cells were first expanded in the presence of rapamycin and IL-2 to induce senescence and chemo-resistance while maintaining tumor-homing capacities. In a lymphoma mouse model, when T cells delivered SN-38-nanoparticles, tumor cells were killed with 10-fold less drug at a significantly higher efficacy compared with free SN-38-nanoparticles or SN-38 alone. Overall, these studies demonstrate that nanoparticle “backpacks” allow T cells to deliver autocrine or paracrine therapeutics to targets that are efficacious at far lower doses than can be achieved by traditional systemic delivery.

Ulka Vaishampayan (Wayne State University; Karmanos Cancer Institute) discussed results from clinical trials testing the efficacy of a HER2-targeting bi-specific antibody-armed activated T-Cell (BAT) therapy in breast and prostate cancer. BATs are considered to have a CAR-like activity but are less toxic and less expensive than CAR therapy or bi-specific antibodies. To develop BATs, peripheral T cells are first collected from patients by PBMC apheresis followed by ex vivo expansion with OKT3 (anti-CD3) and IL-2. Expanded T cells are then coated with CD3/HER2-targeting bi-specific antibodies and re-infused into patients. In vitro, HER2-BATs were still able to target HER2-low/negative SUM1315 cells with efficacy comparative to HER2-positive SK-BR-3 cells, indicating little HER2 expression is required for targeting. In a phase I trial in heavily pre-treated...
metastatic breast cancer, some clinical responses were observed in patients with either HER2-positive or HER2-negative tumors. Median overall survival was 57.4 months in patients with HER2-positive tumors, 27.4 months in patients with HER2-low/negative tumors, and 36.2 months for the cohort overall. Therapy was well tolerated with the only toxicities observed being some rigors and infusion reactions. BATs induced cytokine release which may associate with the development of anti-tumor immune responses. HER2-BATs were found to exhibit specific cytotoxicity against prostate cancer cell lines PC3, DU145, and LNCaP in vitro. HER2-BATs secreted GM-CSF, TNFα, IFNγ, and IL-4 when cultured with PC3 cells. These data prompted initiation of a phase I dose escalation trial in prostate cancer. Eight mCRPC patients were administered two infusions per week for 4 weeks of 2.5, 5, or 10 × 10^9 BATs in combination with IL-2 and GM-CSF. Of seven evaluable patients, one partial response was observed with a PSA reduction of >50% for 4 months. Two additional patients had significant PSA decreases and reductions in pain scores. No dose-limiting toxicities were observed. Immune evaluations pre and post-treatment revealed increases in serum Th1 cytokines and in IFNγ-expressing PBMCs in one partial responder and two minor responders. In vitro culture of BATs with ipilimumab significantly enhanced expression of IFNγ, IL-12, and IL-2R and decreased expression of IL-10, suggesting that BATs may synergize with ipilimumab. A phase II trial testing the combination of HER2-BATs with anti-PD-1 is being planned in mCRPC.

ADVANCEMENTS IN MOLECULAR IMAGING FOR IMPROVED DETECTION OF METASTATIC PROSTATE CANCER

Currently, technetium-99m bone scans and computed tomography (CT) are the standards for imaging metastatic prostate cancer for disease detection. However, these imaging modalities suffer from poor specificity and sensitivity, and bone scans are limited in scope, detecting only bone lesions. This can lead to delayed detection of metastases and the inability to accurately assess tumor burden or response (under-treatment and over-treatments). Improved imaging modalities are needed to enable more accurate detection of metastatic disease and thus improve disease staging at presentation and relapse, for optimizing treatment strategies, and to follow treatment responses, particularly in the latter stages of advanced prostate cancer when PSA becomes less reliable. More precise identification of metastatic disease in the setting of biochemical relapse could allow salvage pelvic lymph node dissections or stereotactic body radiation therapy (SBRT) targeted to individual oligo-metastatic sites. In therapy assessment settings, composite biomarkers that combine new imaging modalities with other serum/cellular biomarkers of response should be developed and used to determine disease progression and therapeutic benefit to guide treatment options for patients.

Whole body magnetic resonance imaging (WB-MRI), as discussed by Anwar Padhani (Mount Vernon Cancer Centre; Institute of Cancer Research, UK), has been shown to be superior to bone scans in detecting metastatic lesions. In one comparative meta-analysis, WB-MRI had a sensitivity of 95% and a specificity of 96%, bone scans had a sensitivity of 76–86% and a specificity of 80–84%, and Choline-PET/CT had a sensitivity of 87% and a specificity of 97% in detecting bone metastases. WB-MRI has numerous advantages including ready availability, no ionizing radiation exposure, and multiparametric imaging ability including the ability to visualize structural and functional tissue attributes of bone marrow. WB-MRI is well suited for repeated imaging thus allowing the identification of heterogeneous responses including mixed treatment responses to targeted therapies, and so enable selective biopsies that seek to find emergent actionable tumor mutations.

Prostate-specific membrane antigen (PSMA) represents an excellent prostate cancer imaging target as expression is largely specific to malignant prostate tissue, it is increased in advanced prostate cancer, and remains highly expressed in AR-independent prostate tumors. Martin Pomper (Johns Hopkins University School of Medicine) discussed PET imaging using 18F-DCFBC and 18F-DCFPYL, a series of small-molecule ligands targeting PSMA. Due to high levels of PSMA expression, high levels of these PSMA-PET imaging agents can distribute in tumors allowing detection of lesions as small as 3 mm. In a pilot study, 18F-DCFPYL uptake (SUV_max) correlated with Gleason score, and four-times more lesions were detected by 18F-DCFPYL PET than bone scans plus CT. 18F-DCFPYL could also identify intra-prostatic disease in high risk patients and could distinguish prostate cancer from benign prostatic hypertrophy. 18F is a choice radionuclide for PET imaging, as it has good imaging properties, high synthesis output, and an established mechanism for distribution throughout the US. 18F-DCFPYL has been shown to deliver clearer images than 68Ga-PSMA PET imaging agents, due to a shorter positron path and a greater amount of imaging agent that can be administered to patients. Clinical trials demonstrating the utility of these agents are underway. At Johns Hopkins University, multiple 18F-DCFPYL PET trials are ongoing, including trials in high risk patients prior
to radical prostatectomy and in post-prostatectomy or post-radiation patients with PSA recurrence (≥0.2 ng/ml) but no visible metastases by bone or CT scans. PSMA is also expressed by tumor neovasculature and 18F-DCFPYL may therefore be useful in imaging other tumor types, as was recently demonstrated in clear cell renal cell carcinoma.

The high-specificity and stability of antibodies makes them ideal biomolecules for in vivo applications. However, antibodies are relatively large, and could take ~1 week before reaching tissues of interest and clearing from the body sufficiently for molecular imaging. Robert Reiter (University of California, Los Angeles) discussed the use of radiolabeled antibody fragments targeting PSMA or prostate stem cell antigen (PSCA) as PET imaging agents (immunoPET).

Engineered antibody fragments including minibodies (80 kDa scFv-C(H)3 dimers) and diabodies (55 kDa scFv dimers) retain antigen-specificity and affinity, yet allow next day or same day imaging, respectively. An 124I-labeled PSCA-targeting minibody (A11) was developed and demonstrated to be effective in imaging tumor burden in mice bearing intratibal LAPC-9 xenografts, allowing visualization of responses to MDV3100. A phase I trial testing A11 in in prostate, bladder, and pancreatic cancer is ongoing. A 89Zr-labeled PSMA-targeting minibody (IAB2M) was engineered from the J591 antibody. IAB2M was compared to bone scans and FDG-PET in a first-in-man phase I/IIa trial in metastatic prostate cancer. Twenty-eight patients were imaged by the three modalities, resulting in detection of a total of 393 metastatic lesions. IAB2M detected 81.7% of all bone lesions detected and 85.9% of all soft-tissue lesions detected. A total of 65 bone and 32 soft tissue lesions were detected on IAB2M only. A total of 19 lesions (9 bone and 10 soft tissue) that were not unanimously detected by all three imaging modalities were biopsied for pathology to determine true positive and negative lesions. Two bone lesions detected by bone scans and FDG-PET but not IAB2M were found to be negative on pathology. IAB2M had an overall positive predictive value of 89.5%. An ongoing phase II study is comparing the diagnostic performance of conventional imaging versus IAB2M-PET/CT versus PET/CT with 18F-capromab pendetide, a PET probe made with a different PSMA-targeting antibody, in detecting lymph-node metastases pre-prostatectomy. Specificity and sensitivity will be determined by histological examination of resected lymph nodes obtained during prostatectomy. In six patients examined thus far, IAB2M-PET detected 6/11 tumor-infiltrated lymph nodes out of 48 total lymph nodes, while no positive lesions were detected by either conventional imaging or 111In-capromab pendetide. Specificity of IAB2M was 97.3%, with only one false positive called in 37 negative lymph nodes imaged.

Fluorescently labeled diabodies are being developed to image tumors during surgery. In a prostatectomy study in mice, zero of nine mice administered a Cy5-labeled PSCA-targeting diabody and operated on under white light plus fluorescent light resulted in no positive margins while 8/8 mice operated on under white light only had positive margins. Approximately 20% of prostatectomy patients end up with positive surgical margins, which are more common in patients with high grade tumors. This critical technology would allow surgeons to achieve full resection of primary prostate tumors with negative margins while preserving normal structures.

Heterogeneity in treatment responses of different metastatic lesions in an individual patient are common, as different lesions can respond or progress and new lesions can form concurrently. Robert Jeraj (University of Wisconsin Carbone Cancer Center) discussed development of a Quantitative Total Bone Imaging (QTBI) method for studying heterogeneity of treatment responses in prostate cancer patients. For QTBI, anatomic images from CT are combined with functional imaging, typically from PET or MRI, and the patient is reimaged several times over the course of therapy. The QTBI algorithm is able to accurately count lesions and differentiate close but separate lesions in order to quantify the unique change in each lesion over time. With harmonized protocols, 18F-NaF-PET/CT was found to be ~95% reproducible across three institutions (University of Wisconsin Carbone Cancer Center, Memorial Sloan Kettering Cancer Center, and the National Cancer Institute), indicating this technology can be reliably used in the clinic to image bone metastatic prostate cancer. 18F-NaF PET/CT scan-based QTBI was used to study metastatic bone lesions in prostate cancer patients undergoing treatment with docetaxel or AR-targeted therapy in order to identify imaging biomarkers of response and progression. Total disease burden at the end of treatment was the QTBI parameter most predictive for progression-free survival. In 45 patients examined to date, concurrent responding, progressing, and newly developing metastatic lesions occurred regardless of whether the patient was considered an overall good, bad, or mixed responder. In another ongoing PCF-funded study in CRPC patients being treated with enzalutamide, the most responsive and nonresponsive lesions as identified by 18F-NaF-PET/CT QTBI was biopsied and genomically profiled to identify mechanisms of response as well as imaging biomarkers that predict biology. An additional phase II clinical trial is testing the utility of 18F-NaF-PET/CT QTBI in measuring treatment responses to

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enzalutamide and guiding time on treatment in bone-
metastatic prostate cancer patients. It is important to
note that $^{18}$F-NaF-PET is a measure of bone metabo-
lism and increased activity is also seen in areas of
inflammation, post-traumatic, and degenerative
changes, requiring careful analysis of corresponding
areas on CT images to characterize PET uptake as
malignant or benign. Clinical validation of $^{18}$F-NaF-
PET/CT QTBI as a biomarker for disease progression
is underway.

While each of the discussed emerging imaging
technologies provides significantly improved specific-
ity and sensitivity over current standard bone and CT
scans, they are also significantly more costly. The
effect these technologies have on improving patient
outcomes will need to be demonstrated in clinical
trials before these technologies can be widely
adopted.

ASCORBIC ACID IN PROSTATE CANCER TREATMENT

Ascorbic acid (vitamin C), when administered at
high doses intravenously (I.V.), has indicated promise
for the treatment of cancer patients in phase I and II
clinical trials. The average prescribed dose is 28 g
every 4 days which reaches therapeutic levels of
>10 mM in plasma for several hours. At these high
concentrations, ascorbic acid acts as a pro-oxidant,
contributing to the formation of $\text{H}_2\text{O}_2$ in the tumor
microenvironment and leading to DNA damage and
ATP depletion, resulting in tumor cell death. At the
same time, ascorbic acid is neutralized in the blood
and therefore benign to normal cells. Few toxicities
have been reported, with 1.2% of patients experienc-
ing fatigue and rare cases of phlebitis and kidney
stones. Orally administrated ascorbate however, is
poorly bioavailable and can reach only $\sim$0.2 mM, far
below effective therapeutic levels. At 10 mM in vitro,
ascorbic acid completely inhibited the growth of PC3
cells and almost completely killed DU145 cells, indi-
cating efficacy against prostate cancer. High dose I.V.
ascorbic acid has also been demonstrated to reduce
toxicities associated with paclitaxel chemotherapy in
ovarian cancer patients and may increase survival.

Based on these studies, Channing Paller (The
Sidney Kimmel Comprehensive Cancer Center at
Johns Hopkins) is initiating a randomized phase II
clinical trial comparing docetaxel plus placebo (I.V.
fluid) versus docetaxel plus I.V. ascorbic acid (1 g/kg,
three times per week) in metastatic CRPC patients.
Primary endpoints will be % PSA change from
baseline to 12 and 24 weeks and maximal PSA change,
and impact on chemotherapy-related toxicities. Key
secondary outcomes include radiographic progres-
sion-free survival, safety, quality of life, the need for
dose reductions of docetaxel, and proportions of
patients experiencing fatigue, nausea, bone pain,
and anorexia. Laboratory correlates will be measured
including pharmacokinetics of ascorbic acid and
docetaxel, isoprostane levels as a marker of oxidative
stress, and levels of AR and AR-Vs in CTCs. The trial
will activate in early 2016 and will be conducted at
Johns Hopkins University and partnering sites includ-
ing Thomas Jefferson University and Karmanos Can-
cer Center.

Jeffrey Karp (Harvard: Brigham and Women’s
Hospital; Harvard Medical School) discussed the
development of amphiphile-based nanofiber gels as
therapeutic delivery vehicles for targeting ascorbic
acid directly to tumors. The material consists of
hydrophobic and hydrophilic segments joined by
an enzyme-cleavable bond. In solution, the material
self-assembles into a nanofiber gel with the hydro-
phobic groups pointing inward. This allows entrap-
ment of hydrophobic drugs. The drugs can then
be released by specific enzymatic cleavage of the
amphiphile. For example, a hydrogel carrying
the anti-inflammatory corticosteroid dexamethasone
was previously developed and demonstrated supe-
rior efficacy in treating mice with experimental
colitis at lower doses compared with free dexameth-
ason. An amphiphile containing ascorbic acid was
used to make gels that encapsulate docetaxel.
Docetaxel-carrying ascorbic acid-nanofiber gels
exhibited long-term stability and enzyme respon-
siveness, being specifically released in the presence
of prostate cancer cells compared with normal cells.
In a murine 4T3 breast cancer model, dye-loaded
ascorbic acid-nanofiber gels delivered I.V. preferen-
tially accumulated in tumors compared with other
tissues. These studies suggest that amphiphile gels
can passively target tumors and that tumor cells
specifically express the cleavage enzymes necessary
for drug-release. These properties will likely lend to
reduced systemic exposure and lower effective
therapeutic doses. Preclinical studies assessing tox-
icity, pharmacokinetics, and efficacy of these gels
in murine prostate cancer models are ongoing.

ACKNOWLEDGMENTS

We thank all of the speakers for presenting their
invaluable work at the 22nd Annual Prostate Cancer
Foundation Scientific Retreat and for their comments
on this manuscript. We apologize to those whose
studies could not be included in this review. A more
in-depth summary of each presentation and the
Retreat agenda can be downloaded at: http://www.
pcf.org/2015retreatreport.
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