REVIEW ARTICLE

The 23rd Annual Prostate Cancer Foundation Scientific Retreat report

Andrea K. Miyahira | Howard R. Soule

Introduction: The 23rd Annual Prostate Cancer Foundation (PCF) Scientific Retreat was convened October 27-29, 2016, in Carlsbad, CA.

Methods: This event focuses on the latest advances in basic, translational, and clinical prostate cancer research with the greatest promise for advancing our understanding of prostate cancer biology and improving patient outcomes and quality of life.

Results: Themes highlighted at this year’s meeting included: i) targeting DNA repair deficiency in prostate cancer; ii) optimizing the use of Radium-223 and bone-targeting agents; iii) advances in cancer immunotherapeutic approaches; iv) targeting developmental pathways in prostate cancer; v) advances in circulating tumor DNA technology and applications; vi) precision survivorship; and vii) novel treatments and treatment strategies in prostate cancer.

Discussion: This article reviews the key advances discussed at the Retreat for the purpose of disseminating this knowledge to accelerate the development of new treatments and improved outcomes for men suffering with prostate cancer.

KEYWORDS
androgen receptor, diagnosis, prognosis, therapy, tumor biology

1 | INTRODUCTION

The Prostate Cancer Foundation (PCF) held its 23rd Annual Scientific Retreat from October 27-29, 2016, at the La Costa Resort, in Carlsbad, CA. The Annual PCF Scientific Retreat began in 1993 as a small gathering of PCF-funded investigators sharing their latest findings, but has since grown into the leading conference in the world focused on prostate cancer research. The Retreat has had an immeasurable impact on prostate cancer research through broadcasting emerging science with the greatest promise for advancing prostate cancer biology and treatment, and for establishing a highly collaborative culture among prostate cancer researchers that has resulted in cross-disciplinary, cross-institutional, bench-to-bedside team science approaches becoming a prevailing paradigm in the field.

The plenary sessions consisted of 46 oral presentations, a Precision Clinicopathologic Conference panel discussion and 153 poster presentations. Most of the presentations consisted largely of novel, previously unreported data. A wide variety of scientific disciplines were represented, including precision medicine, cancer genomics, cellular and molecular biology, medical oncology, tumor immunology, immunotherapy, molecular pharmacology, drug discovery, drug development, computational biology, survivorship, neurobiology, epigenetics, developmental biology, nuclear medicine, molecular imaging, radiation oncology, pathology, surgery, urology, and clinical trials.

There were 539 invited attendees from 16 countries, 102 academic institutions, 9 medical research foundations, 47 biopharmaceutical companies, 7 other for-profit companies, and U.S. governmental agencies including the NIH, NCI, and Department of Defense. 65% of the speakers were presenting at a PCF Scientific Retreat for the first time.

This article reviews the key advances discussed at the Retreat in order to disseminate this knowledge throughout the research community and promote novel research that will ultimately result in new therapies and improve the lives of men with prostate cancer. A report containing a detailed summary of the presentations and the complete Retreat agenda is available for download at: https://www.pcf.org/c/2016-state-of-science-report/
1.1 Prostate cancer grading beyond Gleason score: 3D, molecular and clinical perspective

Arno van Leenders (Erasmus Medical Centre, The Netherlands) presented an improved Gleason grading system for prostate cancer. While Gleason grading has been used for decades to predict tumor aggressiveness, the system is subjective and suffers from significant inter-observer variability. A refinement to the grading system with more powerful predictive value would be useful. Cribiform growth is a sieve-like pattern observed in some prostate Gleason score 7-10 tumors. The presence of cribiform growth in diagnostic biopsies was found to be an independent predictor of reduced disease-specific survival. In multivariable analysis, the predictive value of biopsy Gleason scores almost disappeared when cribiform status was incorporated into the model. This suggests that the predictive value of Gleason grading is partially dependent on the presence of cribiform growth. Tumors with cribiform growth exhibited genomic alterations consistent with more aggressive prostate cancer, suggesting an association between cribiform morphology and genomic instability. A novel imaging methodology was developed to analyze different tumor growth patterns in 3D. A 3D microscopic analysis demonstrated morphologic and spatial continuity of well-delineated Gleason grade 3 tumor glands with ill-formed, fused and some glomeruloid Gleason grade 4 glands, while cribiform growth appeared a separate entity. These studies suggest that incorporating other architectural features, specifically cribiform growth, into prostate cancer grading will improve prediction of disease outcome and support therapeutic decision-making.

1.2 Circulating tumor DNA as a therapeutic response biomarker and precision medicine tool

Circulating tumor DNA (ctDNA) is rapidly emerging as a promising methodology for measuring tumor burden and therapeutic responses in cancer patients and as an alternative means to interrogate tumor mutations, copy number alterations, and gene rearrangements. Alexander Wyatt, PhD (Vancouver Prostate Centre) presented studies using ctDNA from prostate cancer patients to identify tumor mutations and copy number changes. Clinically informative genomic alterations including loss of tumor suppressor genes and hotspot mutations were observed in ctDNA from castrate-resistant prostate cancer (CRPC) patients progressing on enzalutamide and were concordant with mutations identified in tumor biopsy samples. Androgen receptor (AR) gene mutations and amplifications were common in ctDNA from CRPC patients and were associated with resistance to AR-targeted therapy. These data suggest that ctDNA is a highly feasible and reliable surrogate for interrogating tumor mutations in lieu of tissue biopsy in most patients with CRPC. The fraction of ctDNA in total plasma DNA was highly informative of treatment failure in patients progressing on either abiraterone or enzalutamide in a phase II cross-over trial. ctDNA also enables feasible temporal monitoring of changes in tumor mutations. At the Vancouver Prostate Centre, a liquid biopsy program is being developed to use ctDNA to identify tumor mutations in patients enrolled in various clinical trials.

1.3 Optimizing the use of Radium-223 and bone-targeting agents for the treatment of metastatic prostate cancer

Bone metastases occur in ∼85-90% of metastatic CRPC (mCRPC) patients, and are by far the most common site of prostate cancer metastasis. Elucidating the mechanisms that enable the development of prostate tumors in bone will lead to improved therapeutic strategies for the treatment of men with mCRPC.

Radium-223 is a radioactive calcium-mimetic that is FDA-approved for the treatment of patients with bone-only metastatic prostate cancer. Despite having gained FDA approval, much remains to be learned about the biology and clinical activity of Radium-223 to ensure that this life-prolonging therapy is being used to its full potential. Issues that require further exploration include identifying the optimal dose and duration of Radium-223 treatment, identifying biomarkers to select patients most likely to benefit from treatment, testing therapeutic combinations, determining optimal timing and sequencing of treatment, and elucidating the mechanisms of action. Many of these issues were discussed in detail at a recent Scientific Working Group Meeting on Radium-223 hosted by the Prostate Cancer Foundation, a review of which has been published.

Michael Morris (Memorial Sloan Kettering Cancer Center) reviewed ongoing and planned clinical trials that will address some
of the unresolved questions surrounding the optimal clinical use of Radium-223. FDA approval of Radium-223 was based on results from the phase III ALSYMPCA trial, which demonstrated that treatment with Radium-223 prolonged median overall survival (OS) by 3.6 months. In regards to the optimal dose and duration of therapy, one study addressing this issue is a three-arm extended dosing prospective randomized trial, testing six cycles of Radium-223 at 55KBq/Kg, six cycles at 88KBq/Kg, or 12 cycles at 55 kBq/kg in CRPC patients with symptomatic bone metastases but no known visceral disease. In regards to combination therapeutics that target the tumor itself in addition to targeting the bony compartment, Radium-223 is being tested in combination with systemic therapy including AR-targeted agents and chemotherapy. The ongoing ERA223 trial is testing Radium-223 + abiraterone/prednisone versus placebo + abiraterone/prednisone in chemotherapy-naïve asymptomatic/mildly symptomatic CRPC patients, and is expected to report out its first data in late 2017. The European Organisation for Research and Treatment of Cancer (EORTC) PEACE-III trial is testing Radium-223 + enzalutamide versus enzalutamide alone in patients with rising PSA or bone-only metastases who are undergoing ongoing androgen deprivation therapy (ADT). A completed phase II trial (NCT01106352) conducted by Bayer in collaboration with the Prostate Cancer Clinical Trials Consortium (PCCTC) investigated Radium-223 (50 KBq/kg) + docetaxel at 60 mg/m² versus docetaxel alone at 75 mg/m². Adverse events observed in the combination arm including neutropenia, febrile neutropenia, and leukopenia, were lower than in the docetaxel alone arm. Time to prostate specific antigen (PSA) progression, declines in PSA levels, and declines in alkaline phosphatase levels were greater in the Radium-223 plus docetaxel arm compared to docetaxel alone. Early results suggest that docetaxel plus Radium-223 was safe, feasible, and had favorable treatment effects as assessed by PSA and bone alkaline phosphatase. These results have led to the planning of two phase III trials testing this combination. The PCCTC will conduct a randomized trial in progressive mCRPC testing the same regimen as the phase II trial. The Alliance and NRG Oncology groups will conduct a similar study in patients with metastatic castration-sensitive prostate cancer. This trial was designed in response to the CHAARTED trial, in which the addition of docetaxel at the time of ADT initiation in the metastatic castration sensitive disease state delayed median OS by 13.6 months. Other trials are examining the efficacy of Radium-223 in combination with immunotherapies, chemotherapy, and PARP-inhibitors.

Robert Coleman (University of Sheffield, UK) discussed studies on the mechanisms of prostate cancer cell homing to the bone marrow microenvironment and therapeutic strategies to prevent the outgrowth of dormant tumor cells residing in bone. To determine whether bone metastases are formed by quiescent or actively dividing prostate cancer cells, PC3 cells were labeled with a red lipophilic dye, DiD, sorted into DiD-positive (quiescent) and DiD-negative (actively dividing) populations, and injected into mice. The DiD-positive, mitotically quiescent fraction formed significantly more bone metastases than the DiD-negative fraction, suggesting that dormant prostate tumor cells are more likely to take up residence in the bone and outgrow into bone metastases. To identify the bone marrow sites that dormant tumor cells home to, mice were injected with DiD-labeled cancer cells and imaged by multiphoton or confocal microscopy. Dormant prostate cancer cells were observed to preferentially home to the lateral bone surface, which is rich in osteoblasts. In contrast, the medial bone surface is an osteoclast-rich area, and was not a site highly occupied by dormant tumor cells. These studies suggest that osteoblasts may support prostate cancer cell residency and/or metastatic outgrowth. Short-term treatment of mice with parathyroid hormone (PTH), a hormone which increases osteoblast activity, prior to prostate tumor cell injection, resulted in significantly increased growth of bone metastases, particularly at sites that had low bone turnover prior to PTH treatment. PTH treatment also lowered circulating tumor cell (CTC) numbers, suggesting that activated osteoblasts promote recruitment of CTCs to the bone. Castration, which activates osteoblast activity and bone turnover, also increased the development of prostate cancer bone metastases in mice. The bone metastasis-promoting effect of castration was blocked by treatment of mice with zoledronic acid, which blocks osteoclastic bone resorption and in turn, through coupling mechanisms, inhibits osteoblast activity and bone remodeling. These results suggest that combining bisphosphonates with AR-targeted therapy early in the evolution of the disease before metastases are established may benefit patients with prostate cancer. Treating and preventing bone metastases may best be achieved by therapies that induce and/or maintain dormancy of micro-metastatic bone tumors. In breast cancer trials, adjuvant treatment with bisphosphonates significantly reduced tumor recurrence in bone but not at other sites, and resulted in a reduction in breast cancer mortality, specifically in postmenopausal women. Unfortunately, in randomized clinical trials in prostate cancer, bisphosphonates have not shown effects on the development of metastases, although the number of patients studied without overt metastatic disease are relatively few. Treatment with the osteoclast-targeting agent denosumab, however, extended bone metastasis-free survival by up to 7.2 months in prostate cancer patients with a short PSA doubling time. Longer follow-up on this trial is needed to determine any impact of denosumab on overall survival. Radium-223 treatment has been shown to prevent the formation of bone metastases in mice, suggesting that Radium-223 may be able to target dormant tumor cells. Clinical studies testing Radium-223 in earlier disease settings including patients with high risk disease or experiencing biochemical relapse are warranted. Overall, these studies support further exploration into targeting osteoblasts and/or osteoclasts for the treatment and prevention of bone metastatic prostate cancer.

Nora Navone (The University of Texas MD Anderson Cancer Center) discussed mechanisms of bone-prostate interactions and strategies to therapeutically target the bone microenvironment for the treatment of mCRPC. Clinical studies on Radium-223 have demonstrated that targeting the bone extends overall survival of patients with metastatic prostate cancer. To identify potential therapeutic targets that mediate prostate tumor growth in bone, cell lines and patient derived xenograft (PDX) models were developed from clinical bone metastasis samples. Injection and subsequent growth of these PDXs
into the bones of mice led to the development of osteoblastic bone reactions. Interestingly, when one of these PDXs (MDA PCa 118b) was implanted and grew under the skin of mice, new bone developed at that site and the bone cells were host-derived. These data suggest that prostate cancer cells drive bone development, which may play a role in the predilection of prostate cancer to form metastases in bone. Candidate pathways of interest that mediate prostate cancer interactions with the bone microenvironment include the FGF pathway and the WNT-canonical pathway. FGF9 was found to be overexpressed in prostate cancer cells. Treatment of mice with anti-FGF9 antibodies reduced the ability of prostate cancer cells to form bone metastases. Based on these findings, a clinical trial (NCT00831792) was initiated to test the FGFR-inhibitor dovitinib in mCRPC patients. Approximately 30% of patients treated with dovitinib experienced progression-free survival for more than 4 months, warranting further exploration of this treatment strategy. Clinical responses correlated with changes in bone-specific alkaline phosphatase but not PSA levels, suggesting that alkaline phosphatase but not PSA is a suitable biomarker of response to FGFR-targeted therapy. Similar observations for the utility of alkaline phosphatase but not PSA as treatment response biomarkers have been made in clinical trials for the bone-targeting therapies Radium-223 and cabozantinib. Treatment with cabozantinib has resulted in improvements in bone scans and pain in CRPC clinical trials, suggesting cabozantinib targets the bone microenvironment. Preclinical studies examining cabozantinib treatment for mCRPC have found that c-MET, the supposed target of cabozantinib, was not required for the anti-tumor activity of cabozantinib. These studies confirm other studies that found MET was not an important cabozantinib target in prostate cancer and leave open the question of how cabozantinib affects prostate cancer bone metastases. Preclinical studies have also been conducted to investigate the mechanisms by which Radium-223 exerts its effects against bone metastatic prostate cancer. Treatment of mice bearing bone tumors with Radium-223 increased bone volume and reduced osteoclast numbers in the normal, non-tumorous bones. In the tumor bearing bones, treatment of mice with Radium-223 resulted in an increase in trabecular thickness and connectivity, although effects on osteoclast numbers require further study. These studies demonstrate a clear effect of Radium-223 on the bone microenvironment and have prompted studies to identify the bone and tumor cell cytokines and other secretory factors that mediate these effects. Identification of predictive biomarkers of response to bone-targeted agents will inform the design of clinical trials testing these agents in prostate cancer.

Radiation therapy can induce immunogenic (immune-activating) tumor cell death and therefore may be ideal for use in combination with immunotherapy. James Gulley (National Cancer Institute) discussed preclinical studies and clinical trials testing the efficacy of combining immunotherapies with radiation therapies, including Radium-223. In in vitro studies, Radium-223 treatment slowed the growth of prostate cancer cells but did not result in cell death. However, while the addition of tumor specific T cells to the culture caused some lysis, the combination of Radium-223 and T-cells significantly enhanced tumor cell killing. This effect was found to be due in part, to upregulation of immunogenic cell stress responses in tumor cells following treatment with Radium-223, including expression of the immune-modulatory molecule calreticulin. Blocking of calreticulin on tumor cells significantly abrogated immune-killing, suggesting that calreticulin is an important aspect of anti-tumor immunity and maybe useful as a predictive biomarker of response to immunotherapy following Radium-223 treatment. Palliative levels of the bone-targeting radionuclide Samarium-153-EDTMP (Quadramet) have been shown to upregulate prostate cancer cell expression of immune-regulating molecules including MHC-I, Fas, and ICAM-1, as well as tumor associated antigens, and sensitized cells to immune killing. To test whether Samarium-153-EDTMP synergizes with immunotherapy, a randomized phase II clinical trial (NCT00450619) was conducted that tested Samarium-153-EDTMP with or without the PSA-TRICOM prostate cancer vaccine in bone-metastatic CRPC. Compared with Samarium-153-EDTMP alone, patients who received PSA-TRICOM plus Samarium-153-EDTMP experienced a 2 month extension in median progression free survival (PFS) (1.7 months vs 3.7 months), more frequent declines in PSA, and exhibited more frequent PSA-specific T cell responses (25% vs >60%). Immune cell parameters that associated with patient survival were used to develop an “immunoscore,” which could be used as an immune biomarker to identify the patients that benefitted from the PSA-TRICOM + Samarium-153-EDTMP combination. In murine tumor models, anti-PD1 treatment highly synergized with radiation therapy in preventing tumor growth. Overall these studies provide ample rationale for additional studies examining the efficacy of combining radiation therapy and immunotherapy for the treatment of prostate cancer.

### 1.4 Advances in cancer immunotherapeutic approaches for prostate cancer

Sunil Hingorani (Fred Hutchinson Cancer Research Center) discussed mechanisms and strategies for targeting immune suppression in the tumor microenvironment of pancreatic cancer. Like prostate cancer, immunotherapy has been relatively ineffective in pancreatic cancer. Potential factors that may constrain anti-tumor immune responses in pancreatic cancer include relatively few neo-epitopes (like prostate cancer), physical and cellular barriers conferred by the tumor microenvironment, and expression of immunosuppressive molecules including inhibitory immune checkpoints. Pancreatic tumors have a unique tumor microenvironment, characterized by a desmoplastic response consisting of stromal cells, particularly fibroblasts and immune cells, and a dense extracellular matrix, whereas tumor cells make up a minor fraction. The genetically engineered KPC mouse model (KrasLSL-G12D/+ , Trp53LSL-R172H/+ , Pdx1-Cre) recapitulates many of the clinical, histological, and molecular features of human pancreatic cancer. Examination of immune dynamics in KPC mice revealed that pancreatic intraepithelial lesions (PanIN) were dominated by macrophages and regulatory T cells, while pancreatic ductal adenocarcinomas (PDA) were additionally highly infiltrated by granulocytic myeloid derived suppressor cells (Gr-MDSC). Treatment of KPC mice with Gr-MDSC-depleting antibodies resulted in increased...
tumor infiltration by activated CD8+ T cells, and increased tumor cell apoptosis. Gr-MDSC depletion also resulted in significant tumor stromal remodeling including opening of previously collapsed blood vessels. Thus, targeting Gr-MDSC in pancreatic cancer may activate anti-tumor immune responses and improve drug delivery. Pancreatic cancer typically expresses a low level of neoantigens but does express tumor-associated antigens MUC1, ANXA2, and MSLN. To explore the potential for adoptive immunotherapy to target these antigens, multiple epitopes of MSLN were injected into mesothelin knockout mice to generate MSLN-specific T cells. The TCR was isolated from one of these cell populations and further modified to create enhanced affinity MSLN-specific T cells. Treatment of KPC mice bearing metastatic pancreatic tumors with enhanced affinity MSLN-targeted T cells resulted in some T cell expansion, which was markedly improved with co-administration of a vaccine encoding the optimized MSLN antigen plus IL-2, which stimulates T cell proliferation. Following injection, anti-MSLN T cells were observed to accumulate in tumors, persist, and express activation markers. After 28 days however, anti-MSLN T cells upregulated checkpoint molecules (PD1, Tim3, Lag3, and 2B4), and exhibited reduced expression of IFNγ and TNFα. This suggests that multiple immune checkpoints are activated only after an effector anti-tumor T cell response. In an attempt to prolong anti-MSLN T cell activity, tumor-bearing KPC mice were given serial infusions of anti-MSLN T cells and IL-2. Mice that received serial T cell infusions experienced improved tumor regression and increased survival, suggesting that serial adoptive T cell immunotherapy may be effective in pancreatic cancer. More severe disruption of the tumor stroma was observed in these mice, prompting the question of whether stromal disruption is necessary for pancreatic tumor eradication. Future studies will address this hypothesis.

Haydn Kissick (Emory University School of Medicine) discussed studies characterizing checkpoint and costimulatory molecule expression on tumor infiltrating T cells in order to identify strategies to overcome T cell exhaustion. Costimulatory molecules such as CD28 are required for T cell activation upon T cell receptor (TCR) stimulation with cognate antigen. Checkpoint molecules function to negatively regulate TCR signals and inhibit T cell activation. The balance of these signals changes during different stages of T cell differentiation as a major mechanism regulating T cell activity. Three main populations of CD8+ tumor infiltrating lymphocytes (TILs) were observed in renal cell carcinoma (RCC) samples. CD8+ TILs expressing normal levels of the costimulatory molecule CD28 and low levels of the checkpoint molecule PD1, were termed "standard effectors," as these expression patterns imply these T cells could become easily activated. CD8+ TILs expressing low levels of both CD28 and PD1 were termed "terminally differentiated" T cells. CD8+ TILs expressing very high levels of PD1 and other checkpoint molecules, CTLA-4, TIM3, and TIGIT, as well as markers of having recently proliferated (Ki67), were termed "highly active/exhausted" T cells. The numbers of T cells with the highly active/exhausted phenotype correlated with total numbers of T cells in tumors, implying that this subset arose from activation and proliferation of T cells within the tumor. Overall, T cells within tumors expressed lower levels of costimulatory markers compared with circulating naïve T cells. The proliferative capacity of these three subsets was examined ex vivo in response to stimulation with anti-CD3 + anti-CD28 antibodies. The highly active/exhausted and terminally differentiated T cell subsets did not proliferate in response to TCR and CD28 stimulation, while standard effectors proliferated and differentiated into cells with the exhausted phenotype, gaining expression of checkpoint molecules and losing expression of costimulatory molecules. These observations suggest a stem model of T-cell exhaustion, in which standard effector T cells are recruited into the tumor microenvironment and undergo proliferation until they become exhausted, terminally differentiated and no longer able to further proliferate. A similar "stem-exhaustion" T cell pattern, in which all three T cell phenotypes are present, was observed in tumors from patients with kidney, bladder, and lung cancer. In prostate cancer, however, TILs were largely comprised of T cells with the terminally differentiated (CD28-low, PD1-low) phenotype. Prostate tumors also had relatively low numbers of intratumoral T cells compared with kidney and bladder cancer, and almost no proliferating T cells, compared with ~30% of T cells being Ki67-positive in kidney and bladder tumors. This suggests that a unique mechanism of T cell exhaustion occurs in prostate tumors. CD28-negative T cells lacking proliferative capacity have been observed to accumulate in elderly patients, often against chronic antigens like CMV or EBV. Understanding the unique mechanisms contributing to terminal differentiation of T cells in prostate cancer, and whether any signals can be used to reactivate these cells is vital to enable the effective application of immunotherapy in prostate cancer patients.

Christopher Kloss (University of Pennsylvania) discussed the development of PSMA-targeting, TGFβ-resistant chimeric antigen receptor (CAR) T cells for the treatment of metastatic prostate cancer. These cells were developed based on the hypothesis that resistance to signals from the negative regulatory molecule TGFβ, which is present at high levels in the tumor microenvironment, will enhance the activity of anti-tumor CAR T cells. Human peripheral blood T cells were transfected with a lentiviral vector encoding a dominant-negative version of TGFβRII (dnTGFβRII) and a PSMA-targeting CAR molecule (PBBZ), to generate PSMA-targeting/TGFβ-resistant (dnTGFβRII-T2A-PBBZ) CAR T cells. In vitro, dnTGFβRII-T2A-PBBZ CAR T cells were resistant to immunosuppressive signals from TGFβ and specifically killed PSMA-expressing tumor cells. Additionally, dnTGFβRII-T2A-PBBZ CAR T cells cultured in the presence of TGFβ-secreting PC3 cells proliferated to a greater extent than PBBZ CAR T cells without the dnTGFβRII gene. Both human PBZ CAR T cells and dnTGFβRII-T2A-PBBZ CAR T cells exhibited life-extending anti-tumor activity in NSG mice bearing PC3 xenografts. However, only dnTGFβRII-T2A-PBBZ CAR T cells produced complete tumor regressions at low doses (2.5e6 cells). In vivo persistence of T cells was also greater for dnTGFβRII-T2A-PBBZ CAR T cells than for PBBZ CAR T cells. Mice treated with tumor-eradicating numbers of dnTGFβRII-T2A-PBBZ CAR T cells experienced significant weight loss, which was lethal at higher doses. Studies are ongoing to identify the mechanisms of treatment-associated toxicity. The dnTGFβRII vector has previously been tested for safety in a phase I trial using autologous T cells that...
target Epstein-Barr virus (EBV). Safety has also been previously demonstrated for PBBZ CAR T cells in a phase I trial. Overall, these studies demonstrate that TGFβ-resistant, PSMA-targeted CAR T cells may be effective against prostate cancer. The pre-clinical IND package for dnTGFβRII-T2A-PBBZ CAR T cells has been completed and submitted. Activation of phase I trials are anticipated in 2017.

Ivan Borrello (Johns Hopkins Sidney Kimmel Comprehensive Cancer Center) discussed a novel adoptive immunotherapy strategy using marrow infiltrating lymphocytes (MILs). Previous studies have demonstrated that the bone marrow harbors and maintains high levels of both virus-specific and tumor-specific memory T cells. Anti-tumor MILs have been observed in patients with breast cancer, melanoma, and multiple myeloma. To explore the potential for using patient-derived MILs as a cancer therapeutic, a phase I study was conducted in patients with multiple myeloma (MM). MILs isolated from MM patients exhibited significantly greater proliferative and tumor-killing activity compared with peripheral blood T cells when incubated with MM cells ex vivo. MILs isolated from patients, activated ex vivo, and then delivered to mice bearing MM tumors were able to traffic to tumor sites and promote tumor regression. MILs from MM patients also persisted long-term in mice. Compared with peripheral blood T cells, MILs expressed higher levels of CXCR4, the receptor for the chemokine SDF-1, which is expressed at high levels in bone marrow. T cell clonotyping assays revealed that MILs from MM patients contained more discrete subsets of T cell clones compared with peripheral blood T cells, suggesting that only certain antigen-specific T cell clones take up residence in bone marrow and become MILs. Based on these data, a phase I/II clinical trial testing the therapeutic efficacy of MILs was initiated in MM patients. In this trial, MILs were collected from 25 MM patients via bone marrow aspiration, followed by ex vivo expansion, and reinfusion. In 95% of the patients, myeloma-specific T cells were successfully expanded from an average of 1.6% of total MILs at the time of bone marrow aspiration to an average of ~15% following reinfusion, which persisted at these levels for over one year. Complete responses correlated with having greater numbers of central memory CD8 T cells and fewer effector CD8 T cells among MILs at baseline. Clinical responses were associated with higher numbers of myeloma-specific T cells, memory T cell phenotypes, and persistence of reinfused MILs for over 1 year in the bone marrow. Bone marrow retention of MILs correlated with levels of antigen-specific T cells. MILs isolated from breast and lung cancer patients were also found to have high levels of tumor antigen-specificity. This suggests that tumor antigen-specific T cells are able to preferentially occupy the bone marrow niche. Compared with CAR T cells, MIL therapy has several advantages, including a relatively low toxicity profile, and a naturally occurring wide range of anti-tumor specificity, negating a need for genetic modification. Combining MIL therapy with immunotherapies that target the immunosuppressive tumor microenvironment may enhance the clinical activity of MILs. Treatment with Tadalafil (Cialis), was found to block immunosuppressive nitrosylation of T cells by myeloid derived suppressor cells (MDSCs), decrease numbers of intratumoral MDSCs and increase numbers of intratumoral activated T cells in myeloma and head and neck cancer patients. Whether combining Tadalafil with MIL therapy can enhance the activity of MILs and improve clinical outcomes is currently being explored. Overall, these studies demonstrate that MILs contain a population of potent tumor-specific memory T cells that can be safely obtained and used to treat patients. Studies are underway to explore this novel immunotherapeutic approach in prostate cancer.

1.5 | Targeting DNA repair deficiency in prostate cancer

Mutations in DNA damage repair (DDR) genes such as BRCA1 and BRCA2 are known to promote the development of aggressive forms of breast and ovarian cancer. Recent studies have also implicated DDR genes in prostate cancer, with 20-25% of mCRPC found to harbor bi-allelic somatic and/or germline mutations in BRCA2, BRCA1, and other DDR genes. In prostate cancer, certain DDR-deficiencies such as germline BRCA2 mutations are associated with more advanced disease, including higher Gleason grade, lymph node invasion, the presence of metastatic disease at diagnosis, shorter times to recurrence, and reduced overall survival (OS).

Synthetic lethality is a concept in which two biological events that would be tolerable if occurring separately become lethal for the tumor cell when occurring together. Survival of cells with defects in a biological pathway may be reliant on preservation of a second pathway based on a synthetic lethal effect. Targeting this second pathway can lead to death of the cancer cells while sparing normal cells proficient in the first pathway. For example, mutations in DDR genes such as BRCA1 and BRCA2 have been demonstrated to render cells sensitive to treatment with PARP-inhibitors. These findings have led to FDA approval of PARP-inhibitors olaparib, rucaparib, and niraparib for the treatment of BRCA1/2-deficient ovarian and/or breast cancer. Clinical trials testing PARP-inhibitors in prostate cancer are underway.

Alan D’Andrea (Harvard Medical School) discussed studies on targeting DNA repair in breast and ovarian cancer, the findings of which have implications for prostate cancer. BRCA1/2 are members of the Fanconi anemia (FA) gene family. FA is a rare autosomal recessive disease caused by loss of function mutations in BRCA/FA family genes. FA patients present with developmental defects, bone marrow failure, and cancer susceptibility. FA has been linked to familial breast and ovarian cancer, as female family members who carry single germline mutant-BRCA/FA alleles have increased risk for breast and ovarian cancer. Using FA cells, impaired FANCD2 mono-ubiquitination was identified as a biomarker of FA/BRCA pathway deficiency. Using the FANCD2 mono-ubiquitination biomarker, a study found that 20% of ovarian cancer cell lines were FA/BRCA-deficient. However, ~50% of serous ovarian cancers in the TCGA were found to harbor DDR mutations, primarily in BRCA1 and BRCA2. More studies are needed to determine the true prevalence of germline and somatic DDR deficiencies in breast and ovarian cancer. Defective homologous recombination activity due to deficiencies in BRCA1/2 and other DDR genes can sensitize ovarian cancers to PARP-inhibitors and platinum chemotherapy. However, cisplatin sensitivity does not completely overlap with PARP1-inhibitor sensitivity. Studies are underway to
identify which DDR mutations in ovarian cancer confer sensitivity to platinum chemotherapy and PARP-inhibitors. Different PARP-inhibitors act via different mechanisms, including inhibition of base excision repair, and trapping of PARP-DNA complexes. PARP is also a necessary component in the POL-theta DNA repair pathway which mediates alternative end-joining, and mice lacking both POL-theta and FancD2 are embryonic lethal. These observations have led to studies exploring whether inhibition of POL-theta is a therapeutic option for DDR-deficient or PARP-inhibitor-resistant cancers. Additionally, studies are underway to test therapeutic combinations that can sensitize DDR-proficient ovarian cancer to PARP inhibition. For instance, combinations utilizing inhibitors of CDK, PI3K, or ATR have indicated promise in early studies. PARP inhibitors may increase mutational and neoantigen loads and also increase cell death and inflammation, suggesting that combining PARP-inhibitors with immunotherapy may be effective. Based on this rationale, a clinical trial testing niraparib + pembrolizumab in ovarian cancer has recently been initiated. Resistance to PARP-inhibitors has been observed in ovarian cancer. The contributing mechanisms include somatic mutations causing reversion or restoration of functional BRCA genes, acquisition of hypomorphic BRCA alleles, epigenetic reversion of BRCA1 promoter hyper-methylation, loss of PARP expression, loss of end resection regulation, replication fork stabilization, mutations in the 53BP1/PTIP/ RIF1/Artemis pathway, and drug efflux. These studies have many implications for prostate cancer.

Joaquin Mateo (The Institute of Cancer Research, UK) discussed opportunities and challenges for using PARP-inhibitors in prostate cancer. The TOPARP clinical trial is a multistage academic trial being conducted in the UK to test the efficacy of the PARP inhibitor olaparib in CRPC. TOPARP-A, the first stage of the trial, tested olaparib in unselected mCRPC patients who subsequently underwent tumor biopsies that were assessed for the presence of DDR gene mutations. Of 49 patients evaluated, 16 exhibited objective responses to olaparib, biopsies that were assessed for the presence of DDR gene mutations. Of 49 patients evaluated, 16 exhibited objective responses to olaparib, and 14 of the responders had tumors harboring DDR defects. Mutations were primarily in BRCA2, BRCA1, and ATM. Only two non-responders had tumors harboring biallelic DDR gene defects. TOPARP-B, the second stage in the trial, is ongoing, and is prospectively assessing patients for DDR defects who then receive treatment with olaparib. If efficacy is demonstrated for olaparib in TOPARP-B, then TOPARP-C will be activated, in which unselected patients will be randomized to olaparib versus placebo, followed by assessment of tumor tissues for the presence of DDR mutations. Randomized trials testing all-comers, such as TOPARP-C, are necessary to identify any DDR-proficient patients that can benefit from olaparib. Currently, the significance of many DDR mutations are unknown. Identification of DDR mutations that confer functional DNA repair deficiencies and sensitivity to PARP-inhibitors, versus non-relevant DDR mutations, is necessary to refine the DDR biomarker to assure that PARP-inhibitors are prescribed to the proper patients. Also, better response biomarkers are needed to optimize the drug development process. The utility of circulating tumor DNA (ctDNA) as a biomarker of treatment response is under investigation in trials with PARP inhibitors. Early results from the TOPARP trial have found that a ≥50% decline in ctDNA levels predicted longer progression free survival (PFS) and overall survival (OS) following treatment with olaparib. Functional MRI may also be useful in measuring responses to PARP-inhibitors and is being further evaluated. Ongoing and future clinical trials will optimize the clinical application of PARP-inhibitors for prostate cancer treatment. Whether PARP-inhibitors exhibit synthetic lethality with other therapies in prostate cancer is also being tested in preclinical studies as well as in combination clinical trials. Also of critical importance, is ascertaining the safety and efficacy of combining PARP-inhibitors with abiraterone, enzalutamide, platinum chemotherapy, and other experimental therapies with promise for the treatment of CRPC. Overall, PARP-inhibitors have demonstrated significant promise for the treatment of DDR-deficient CRPC and are anticipated to be the first FDA-approved precision medicine treatment for prostate cancer if ongoing and planned validation trials are positive.

Richard Kennedy (Queen’s University Belfast and Almac Diagnostics) discussed the impact of chemotherapy on breast, ovarian, and prostate cancers that are deficient in the BRCA/Fanconi anemia (BRCA/FA) DNA damage response pathway. Studies in breast and ovarian cancer have found that BRCA1/2-deficiencies can confer sensitivity to treatment with DNA-damaging agents including platinum chemotherapy, etoposide, and bleomycin. Various clinical trials have tested carboplatin, cisplatin, satraplatin, and oxaliplatin in prostate cancer, with 10-70% of unselected patients responding in various studies. In one report, exceptional responses to carboplatin were observed in three DDR deficient mCRPC cases. In contrast, antimicrotubule agents including taxanes may be less effective against BRCA/FA pathway-deficient breast cancer compared with BRCA-normal cancer. In a retrospective analysis of an ovarian cancer dataset, the addition of taxanes to platinum chemotherapy improved PFS in BRCA/FA pathway-proficient patients but did not provide a benefit in DDR-deficient patients. A small unpublished study of 52 prostate cancer patients reported that FA/BRCA-deficiency was a biomarker for poorer OS following treatment with docetaxel monotherapy (median OS 12.4 months versus 24.8 months for FA/BRCA-pathway-proficient patients). However, another study in prostate cancer reported that germline BRCA1/2 mutations did not predict resistance to taxane therapy, although only seven BRCA1/2-deficient patients were assessed. Taken together, these data support the use of platinum chemotherapy in DDR-deficient prostate cancer, while a role for taxanes requires further investigation.

DDR-deficiencies can also activate innate immune responses, due in part, to abnormal accumulation of cytosolic DNA, which activates the cGAS/STING pathway, an innate sensor of DNA virus infections. Subsequent activation of the interferon pathway by cGAS/STING ultimately results in upregulation of cytotoxic T cell responses which are then inhibited by immune checkpoint proteins including PD-L1. These findings suggest that DDR-deficient tumors may be more sensitive to immunotherapy, especially checkpoint inhibitors that target PD-L1 or PD1, its receptor on the T cell. In support of this hypothesis, a recent study found higher levels of T cell infiltration, interferon pathway activation, and PD-L1 expression in BRCA-mutant...
breast cancer. Clinical trials testing checkpoint inhibitors in DDR-deficient breast, ovarian, and prostate cancer are underway.

Bruce Montgomery (University of Washington) and Mark Pomerantz (Dana-Farber Cancer Institute) discussed clinical trials testing the use of platinum agents in DDR-deficient prostate cancer. Thus far, not all platinum chemotherapy agents appear to be similarly beneficial in unselected patients. The double-blind, randomized phase III SPARC trial testing prednisone plus either satraplatin or placebo in post-taxane treated CRPC patients found that satraplatin delayed progression but conferred no OS benefit in the population as a whole although a subset clearly did benefit. A phase II trial in unselected patients indicated a modest benefit for carboplatin plus docetaxel in docetaxel-refractory mCRPC. A study at the Dana Farber Cancer Institute tested carboplatin plus docetaxel in 141 mCRPC patients, eight (5.6%) of whom were found to have BRCA2-deficient tumors. Seven of the eight BRCA2-deficient patients experienced a PSA decline following carboplatin/docetaxel, and six experienced PSA declines >50%. Additional DDR gene mutations were identified in four patients, in MSH2, ATM, BLM, and FANCA, and all but the FANCA-mutant case responded to carboplatin/docetaxel. At the University of Washington, three patients with homozygous BRCA2 inactivation and a patient with a germline PALB2 defect and a somatic loss of the second allele in the tumor, who had exhausted all other therapeutic options exhibited exceptional responses to docetaxel and carboplatin. These early studies suggest that DDR deficient tumors may respond to platinum therapies.

While DDR-deficient prostate tumors can respond to both genotoxic chemotherapeutic agents and PARP-inhibitors, head to head comparisons have not yet been conducted. PCF has recently initiated a partnership with the U.S. Department of Veterans Affairs, with the goal of bringing precision medicine clinical trials to U.S. veterans. Montgomery and Matthew Rettig (University of California, Los Angeles) are leading the first of these clinical trials, POPCAP1 (Precision Oncology Program in Cancer of the Prostate), which will screen veterans with CRPC for germline DDR mutations and enroll carriers into phase II and III carboplatin/docetaxel or rucaparib trials. Non-carriers will receive standard of care therapy and may be evaluated for somatic DDR mutations. While these trials will not determine the relative efficacy of platinum agents versus PARP inhibitors, they will aid in positioning these therapies for best use in the clinic and provide more robust data for randomized studies to come. The relative costs and toxicities of these therapies also need to be considered when making treatment decisions.

Colin Pritchard (University of Washington) discussed recent findings on mismatch repair (MMR) gene mutations and microsatellite instability (MSI) in prostate cancer. Hypermutated phenotypes have been observed in 16% of colorectal cancer and 22% of endometrial cancer in the TCGA database. In both these tumor types, MSI is most commonly caused by promoter silencing of MLH1. A subset of ultramutated tumors that exhibit MSI but may or may not be MMR-deficient have been found to harbor mutations in POLE. These observations have led to recommendations that all colorectal cancer patients, regardless of age or family history, receive genetic testing for inherited and somatic MMR gene mutations. Recent studies have identified a subset of prostate cancers that display hypermutated phenotypes. Hypermutated phenotypes were observed in 10 of 103 (9.7%) patients in a study analyzing tumors from an autopsy series at the University of Washington and a series of LuCaP xenografts. Hypermutated prostate cancers were found to harbor double somatic or germline (Lynch syndrome) mutations in the MMR genes MSH2, MSH6, MLH1, and PMS2. MSH2 and MSH6 mutations were often structural rearrangements accompanied by loss of protein expression. Using data from the BROCA cancer risk gene panel next generation sequencing (NGS) assay, a method termed mSINGs was developed that could accurately identify prostate cancers with the MSI phenotype based on the fraction of unstable loci. mSINGs identified four of 150 CRPC patients in the PCF International Dream Team cohort with hypermutated phenotypes, all exhibiting MSI and MMR mutations. These four patients all had double somatic mutations (none with Lynch syndrome). Whether prostate cancer is a part of the Lynch syndrome spectrum was recently examined in a study following 188 male Lynch syndrome patients. Prostate cancer incidence was found to be fivefold higher in Lynch syndrome patients compared to controls. Lynch syndrome patients that developed prostate cancer predominiantly had MSH2/6 mutations, which differs from Lynch syndrome patients that developed colon cancer. Two prostate tumors from this cohort were available for analysis, both of which were found to exhibit MSI. To more definitively delineate the prevalence of MMR mutations in prostate cancer patients, tumors from 682 metastatic prostate patients were assessed for mutations in 20 DDR genes known to be associated with increased cancer risk. In this cohort, 11.8% (82/692) of patients were found to have deleterious germline mutations in 16 DNA repair genes, and 0.6% (4/692) had pathogenic or likely pathogenic mutations specifically in MSH2, MSH6, MLH1, and PMS2. In the TCGA dataset, which consists largely of primary prostate cancer, 3/499 (0.6%) cases exhibited germline MMR gene mutations, which is comparable to the metastatic disease cohort. At least one of the primary tumor cases had a documented MSI/hypermutated phenotype. Larger cohorts are needed to more definitively assess the prevalence of MMR mutations and MSI in prostate cancer patients at different disease stages. In a prospective validation series, 6 of 98 patients with high risk localized disease or mCRPC patients (6%) were found to harbor MMR-mutated tumors, one of which was a Lynch syndrome patient. Five of the six MMR-deficient prostate tumors in this cohort exhibited a ductal morphology. The relationship between different MMR mutations and clinical outcomes is being examined in these patients. Overall, these studies have found that ~3-10% of prostate tumors exhibit a hypermutated phenotype largely due to MMR deficiency.

MMR-deficient colorectal cancers are characterizedly sensitive to checkpoint immunotherapy likely due to the generation of higher levels of anti-tumor T cells in response to the markedly higher load of tumor neoantigens. In a study at Oregon Health and Science University, 3 of 10 enzalutamide-resistant mCRPC patients treated with pembrolizumab exhibited exceptional responses. One of two responders with available tumor tissue was found to exhibit MSI.
Another exceptional responder to pembrolizumab identified at the University of Washington was found to harbor a tumor with a MSI phenotype and a MSH6 mutation with loss of heterozygosity (LOH). Ongoing studies are evaluating whether MMR deficiency predicts for prostate cancer patients who will respond to checkpoint immunotherapy.

1.6 | Identification of a prostate cancer subtype that requires treatment with platinum chemotherapy

Ana Aparicio (The University of Texas MD Anderson Cancer Center) discussed the use of platinum agents in a subset of patients with aggressive variant prostate cancer (AVPC) defined either by clinical or molecular characteristics. Small cell prostate cancer (SCPC) is a non-adeno-carcinoma form of CRPC with atypical morphological and clinical features that can include lack of AR expression, earlier development of visceral metastases, and sensitivity to platinum chemotherapy. A phase II clinical trial was conducted to test the efficacy of platinum chemotherapy in SCPC and aggressive variant prostate cancer with SCPC-associated clinical features. Patients selected for this trial had “clinical AVPC” defined by at least one of the following seven features; SCPC morphology: visceral metastases only; lytic bone metastases; bulky lymph node or primary tumors; low PSA levels relative to tumor volume; neuroendocrine markers and increased serum carcinoembryonic antigen (CEA) or lactate dehydrogenase (LDH) levels; or primary castration-resistance. Over 80% of patients with clinical AVPC exhibited sustained responses (>4 cycles of therapy) to carboplatin plus docetaxel, suggesting the clinical AVPC classification system may serve as a biomarker for identifying patients who may benefit from platinum chemotherapy. In this study, patients with a bulky primary tumor experienced the worst clinical outcomes. This supports the view that treatment of the primary tumor may benefit metastatic CRPC patients and not only patients with oligometastatic disease. A prospective, randomized clinical trial is being conducted at MD Anderson to evaluate the addition of definitive treatment of the primary tumor to best systemic therapy in patients who present with metastatic prostate cancer, regardless of the extent of disease (NCT01751438). To identify a molecular signature for AVPC, samples from clinically defined AVPC patients and PDX models were analyzed by genomic sequencing and histological staining for SCPC-associated markers. Clinically defined AVPC tumors were found to harbor ≥2 concurrent genomic alterations in Tp53, RB1, and/or PTEN more frequently than unselected CRPC. To determine whether this molecular signature identifies patients who will benefit from platinum chemotherapy, a phase I/II clinical trial was conducted which prospectively stratified patients into clinical AVPC, molecular AVPC subtypes, and “typical adenocarcinoma” subtypes lacking these characteristics. Patients were then randomized to receive either cabazitaxel alone or cabazitaxel + carboplatin. In the overall mCRPC cohort, the addition of carboplatin to cabazitaxel prolonged PFS. Patients with clinical AVPC had a slightly greater benefit from the addition of carboplatin than patients without clinical AVPC. However, analysis of tumor samples, available from a subset of the participants, showed that those with molecular AVPC were significantly more likely to benefit from the addition of carboplatin than molecularly defined non-AVPC patients. To further strengthen the predictive value of the molecular AVPC signature, the investigators are analyzing ctDNA and CTCs from all participants. These data suggest that the molecular AVPC criteria will significantly aid in identifying patients who will not only benefit from platinum chemotherapy, but will do worse without it. Preliminary studies have also found that tumors with the molecular AVPC signature are enriched for DDR gene mutations. Based on these and other observations, a clinical trial has been initiated to explore the role for PARP-inhibitors in molecular AVPC. Patients with molecular AVPC will receive cabazitaxel plus carboplatin for six cycles, followed by randomization to observation vs olaparib maintenance. Studies are also being initiated to explore the role for immunotherapy in AVPC patients. Dissemination of the findings that molecular AVPC tumors are a clinically distinct prostate cancer subset that require treatment with platinum chemotherapy is critical.

1.7 | Targeting developmental pathways in prostate cancer

Evolutionarily conserved pathways such as WNT and Notch that regulate development and stem cell activities have emerged as important effectors in tumorigenesis. Understanding the biology of these pathways in prostate cancer may reveal novel therapeutic strategies.

Stuart Aaronson (Icahn School of Medicine at Mount Sinai) discussed the role of canonical and non-canonical WNT signaling pathways in cancer. Triggering of the canonical WNT pathway stops the degradation of β-catenin, which is then able to turn on the expression of genes involved in proliferation, migration, cell fate specification, and other developmental activities. The non-canonical pathway proceeds through a β-catenin-independent pathway to exert its effects. The WNT pathway is commonly mutated and plays a role in many cancer types, particularly colorectal cancer. Autocrine production of WNT ligands have been observed in human cancers including non-small cell lung cancer and sarcomas. Hot spot mutations in β-catenin have been found in ~2% of primary prostate cancer in the TCGA series. WNT pathway mutations were identified in 27 of 150 (18%) of mCRPC patients in the PCF International Dream Team cohort. These included mutations in APC (8%), hot spot β-catenin-activating mutations (4%), RNF43 or ZNRF3 inactivating mutations mutually exclusive with APC (4.7%), and RSP02 gene fusions (1.35%) which correlated with RSPO2 overexpression. The effect of targeting WNT in prostate cancer deserves further exploration. Experimental WNT-targeting strategies include WNT ligand and WNT receptor antagonists, which target both canonical and non-canonical WNT signaling, DKK or LRP ligand traps, antibodies targeting LRPs, Frizzled extracellular domains, and small molecule inhibitors of porcupine and tankyrase. Some of these agents have entered early stage clinical trials in various tumor types. However, caution needs to be exercised when targeting the WNT pathway due to its critical role in gastrointestinal tract homeostasis and other tissue stem cell and developmental pathways.
Carlos Moreno (Winship Cancer Institute at Emory University) discussed the role for SOX4 in WNT signaling in prostate cancer. SOX4 is a transcription factor that is expressed in stem, progenitor, and transit-amplifying cells, and is essential for normal development. SOX4 is overexpressed in a number of cancer types and may drive EMT and metastasis. SOX4 was also found to be overexpressed in prostate cancer samples, with expression being highest in metastatic tumors. Overexpression of SOX4 in RWPE-1 cells promoted cancer stem cell and metastatic activities. To determine the role for SOX4 in prostate cancer, a mouse model was created that allows conditional deletion of both SOX4 and PTEN in prostate cells. Prostate-specific deletion of PTEN resulted in the development of invasive prostate tumors. The additional deletion of SOX4 in PTEN-deficient prostate cells blocked the progression of lesions beyond the high grade prostatic intra-epithelial neoplasia (HG-PIN) stage. Deletion of PTEN promotes prostate tumorigenesis by releasing the brakes on the oncogenic AKT pathway. SOX4 was found to be upregulated in PTEN-deficient murine prostate tumors and PTEN/AKT-mutant human prostate cancer samples, and was required for AKT activation resulting from PTEN-deletion. SOX4 expression in murine prostate tumors was blocked by treatment with AKT pathway-inhibitors but was not affected by inhibitors of AR or MEK pathways. These results indicate that SOX4 and AKT function in a positive feedback loop that drives the progression of prostate cancer. SOX4 transcriptional targets include genes with developmental roles, including WNT and Notch pathway components. SOX4 was also found to stably bind to β-catenin in a Wnt3A-dependent manner and promote β-catenin transcriptional activity. Studies in mice found that β-catenin activity was enhanced in PTEN-deficient prostate cancer cells, but that this enhancement required the presence of SOX4. Collectively, these studies suggest that SOX4 plays a critical pro-tumorigenic role by driving both PI3K/AKT and β-catenin pathways and may be an ideal therapeutic target in prostate cancer.

Michael Kahn (University of Southern California) discussed the potential of pharmacologically targeting the interaction between β-catenin and CREB-binding protein (CBP) to prevent the self-renewal of cancer stem cells. The WNT pathway is involved in both proliferation and differentiation processes in stem cells. These divergent outcomes appear to be controlled by β-catenin choosing to interact with one of the two Kat3 transcriptional coactivators CBP or p300. β-catenin-CBP complexes promote maintenance of pluripotency/multipotency, while β-catenin-p300 complexes promote the initiation of cellular differentiation. A small molecule WNT phenotypic screen initially identified ICG-001. It was subsequently determined that ICG-001 was a strong binder of the N-terminus of CBP and a selective inhibitor of the β-catenin-CBP interaction. ICG-001 treatment blocked the growth of triple-negative breast cancer mammospheres in matrigel culture assays and reduced cancer stem cell side-population and aldehyde dehydrogenase (ALDH)-positive populations observed by flow cytometry. Leukemic cells treated with ICG-001 prior to subcutaneous engraftment in mice grew initially but subsequently failed to engraft in secondary recipients after tail vein injection, whereas the vehicle treated cells readily engrafted and the mice all died within 50 days. This suggested that the leukemia stem cells required for engraftment had been eliminated by ICG-001. Treatment of established leukemia in mice with ICG-001 alone had essentially no effect, however significant synergy was observed when ICG-001 was combined with VDL (vincristine, dexamethasone, and L-asparaginase) treatment in a mouse model engrafted with a patient-derived B-ALL. Furthermore, ICG-001 treatment ameliorated the toxicity of the VDL therapy. In a CML engraftment study, one 28-day course of ICG-001 + nilotinib completely eliminated the disease and the mice lived as long as their control littermates that had never been engrafted or treated, whereas with nilotinib alone, there was a significant extension of life span compared to saline control, however, the leukemia returned and all the mice died. These studies suggest that antagonizing the β-catenin/CBP interaction can eliminate cancer stem cells via forced differentiation without damaging the endogenous somatic stem cell population and may have therapeutic efficacy in patients. A second generation β-catenin/CBP antagonist, PRI-724, has been developed and has demonstrated a very acceptable toxicity profile in phase la clinical trials. A maximum tolerable dose was not achieved while raising the dose level from 40 to 1280 mg/m² and a dose dependent reduction of the biomarker survivin/BIRC5 was observed in CTCs at all dose levels. A phase Ib clinical trial testing PRI-724 in solid tumors and a phase 1b/ Ila trial in leukemia has been initiated as has a phase Ib study for hepatic fibrosis.

Li Xin (Baylor College of Medicine) discussed the role of Notch signaling in prostate cancer metastasis. The Notch pathway regulates lineage commitment pathways including basal versus luminal cell development of prostate epithelial progenitor cells. Notch suppresses prostate basal epithelial cell differentiation in cooperation with TGF-β, and promotes proliferation and anoikis-resistance of prostate luminal epithelial cells in cooperation with NF-κB. Notch pathway ligands and receptors are both upregulated in advanced prostate cancer. However, the role for Notch in prostate cancer has been controversial as different studies have found both tumor-suppressive and pro-tumorigenic roles for Notch. In a study by Xin and colleagues, higher expression of a Notch signature score composed of major Notch ligands, receptors and downstream targets, was found to correlate with poorer PSA recurrence-free survival and disease-specific survival in two independent prostate cancer datasets, supporting a pro-tumor role for Notch. In one of these datasets, expression of Notch inversely correlated with PTEN expression, suggesting that Notch may cooperate with PTEN-loss/AKT-activation in driving prostate cancer. To study the role for Notch in PTEN-deficient prostate cancer, a transgenic mouse model was created in which the Notch intracellular domain (Notch-ICD), an activated form of Notch, is overexpressed in prostate cells. These mice were crossed with prostate-specific PTEN-deficient mice. PTEN-deficiency alone resulted in slowly progressing prostate tumors. PTEN-deficient/Notch-ICD mice experienced a morbid increase in abdominal girth due to enlargement of the seminal vessels, vas deferens, and epididymis, but not the prostate itself, which led to significantly reduced survival times. Notch hyperactivity was found to both drive proliferation and promote cell death by augmenting DNA damage and p53 activation.
Notch-ICD/PTEN-deficient mice developed primary tumors in both prostate and seminal vesicle tissue, and had increased distal metastases that could have originated from either primary site, based on gene expression patterns. Moreover, Notch-ICD/PTEN-deficient mice that had seminal vesicles removed still developed metastases, directly demonstrating that Notch hyperactivity can drive the development of metastatic prostate cancer. Notch-ICD expression was found to induce EMT by driving the expression of EMT genes including FoxC2 and Snail. Knockdown of FoxC2 expression by shRNAs in a metastatic prostate cancer cell line established from lung metastases in a Notch-ICD/PTEN-deficient mouse attenuated the capacity of the cells to form lung metastases upon transplantation into mice, suggesting that FoxC2/EMT are required for Notch-driven prostate cancer metastasis. Overall, these studies suggest a role for Notch in driving prostate cancer progression and support investigations into how best to therapeutically manipulate the Notch pathway.

1.8 Novel treatments and treatment strategies in prostate cancer

Joshua Lang (University of Wisconsin Carbone Cancer Center) discussed the potential for TROP-2 as a biomarker and therapeutic target in prostate cancer. TROP-2 is a transmembrane protein that was initially identified as a marker of prostate cancer cells with stem-like characteristics. TROP-2 expression has now been identified in primary and metastatic prostate cancer. TROP-2 expression in CRPC does not appear to be modulated during treatment with abiraterone and is co-expressed with AR-V7, indicating TROP-2 may be a good therapeutic target in advanced CRPC. An assay using VERSA, a novel microfluidic CTC capture technology, was developed to capture TROP-2-expressing CTCs from CRPC patients and assess phenotypic signatures, genomic alterations, and gene expression patterns. TROP-2-positive CTCs were identified in 73% of CRPC patients and found to express AR, AR-variants, NKX3.1, and synaptophysin, a marker of neuroendocrine differentiation. These studies suggest TROP-2 may be a viable therapeutic target in prostate cancer at various disease stages including pre-chemotherapy or post-abiraterone/enzalutamide. Clinical trials testing the efficacy of the TROP-2-targeting antibody drug conjugate (ADC), IMMU-132, in prostate cancer are being planned. IMMU-132 has previously been tested in phase I/II trials and was found to be well-tolerated and have promising efficacy in various solid tumors. IMMU-132 has achieved FDA breakthrough status for triple-negative breast cancer based on a trial in 58 heavily pre-treated patients, two of whom achieved complete responses, while 18 achieved partial responses, and 23 achieved stable disease. This data support the potential of this agent for men with prostate cancer.

Fahri Saatcioglu (University of Oslo) discussed the potential for targeting IRE1, a regulator of the unfolded protein response (UPR), for the treatment of prostate cancer. The UPR is a stress response pathway regulated by the endoplasmic reticulum (ER), which can promote either cell survival or cell death mechanisms under different conditions. IRE1, an unfolded protein sensor, is a regulator of cell survival pathways activated by the UPR, and may thus be a target for abrogating the UPR pathway to promote cancer cell death. Studies in prostate cancer identified a correlation between the expression of AR and UPR-regulated genes. The expression of IRE1 and UPR target genes were found to be enhanced by androgens and directly regulated by AR. Analyses of clinical gene expression datasets and clinical samples indicated that IRE1 expression increases throughout prostate cancer progression. Knockout of IRE1 using siRNA in prostate cancer cell lines prevented cell growth in vitro and tumor growth in xenograft models. Treatment of prostate cancer xenograft models with toyoacamycin, a small molecule inhibitor of IRE1, significantly delayed tumor growth. These data suggest that IRE1 plays a pro-tumor role in prostate cancer and warrant further studies testing the efficacy of therapeutically targeting IRE1.

Hongwu Chen (University of California, Davis) presented studies examining targeting of the orphan nuclear receptor ROR-γ/ROR-C in prostate cancer. ROR-γ was found to be aberrantly expressed in advanced prostate tumors. Additionally, genomic alterations in ROR subfamily members were identified in clinical prostate cancer specimens. ROR-γ, ROR-A, and ROR-B were mutated in 6%, 3%, and 2%, respectively, in a study of 150 mCRPC samples sequenced by the PCF International Dream Team, and in 36%, 6%, and 23%, respectively, in a study of 107 mCRPC and neuroendocrine prostate cancer (NEPC) prostate cancer samples sequenced by Beltran et al (Nature Medicine, 2016). Knockdown of ROR-γ in prostate cancer cell lines prevented cell proliferation and induced apoptosis. Novel ROR-γ antagonists (XY018) identified using structure-guided screening approaches displayed potent activity in vitro, specifically against AR-positive prostate cancer cell lines, but were ineffective against AR-negative prostate cancer cell lines. ROR-γ antagonists suppressed AR signaling as measured by expression of AR regulated genes and AR ChIP-seq. The expression of both full length-AR and AR-variants were inhibited by ROR-γ antagonists. ROR-γ was found to directly regulate the expression of AR by binding to a novel ROR response element (RORE) in the AR gene body. Deletion of the AR RORE resulted in significantly reduced AR expression, demonstrating that ROR-γ is also required for AR expression. Treatment of mice bearing C4-2B prostate cancer xenografts with the ROR-γ antagonists led to inhibition of tumor growth and caspase 3/7-mediated apoptosis. The antagonist also potently inhibited the growth of enzalutamide-resistant xenograft models. No overt toxicity was observed in mice treated with the ROR-γ antagonists with the exception of a reduction in white adipose tissue. This is consistent with studies indicating a role for ROR-γ in lipid metabolism and growth of adipose tissues. No effects were seen on other androgen-sensitive organs, including the testes and prostate. Work is ongoing to develop orally bioavailable ROR-γ antagonists with improved pharmacokinetics, potency, and target specificity that can be advanced to clinical trials.

Laura Saunders (AbbVie-StemCentrx LLC) discussed the potential for using a novel Delta-Like Protein 3 (DLL3)-targeted ADC, Rovalpituzumab Tesirine (Rova-T) for treating NEPC. DLL3 is a
dominant antagonist of the Notch pathway, and acts by sequestering Notch receptors at the Golgi apparatus, thereby preventing cell surface translocation and activation by extracellular Notch ligands. Repression of Notch and upregulation of ASCL1, a transcriptional activator of DLL3, promote the differentiation of lung stem cells into a neuroendocrine lineage. Loss of both TP53 and RB1 and elevated ASCL1 expression induce the development of neuroendocrine lung tumors. DLL3, which is normally expressed in the Golgi during development, is highly expressed on the cell surface in small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma of the lung (LCNEC). These studies suggested that targeting DLL3 may be a promising therapeutic strategy for SCLC and led to the development of Rova-T, which delivers a PBD toxin to DLL3-expressing cells.

Preclinical studies demonstrated that Rova-T eliminated tumor-initiating cells in SCLC and LCNEC tumor xenograft models and led to sustained tumor regression. In contrast, treatment with standard of care cisplatin/etoposide did not achieve these effects. In a phase 1a/b dose escalation trial in 2nd and 3rd line SCLC, responses were observed particularly in patients whose tumors expressed higher levels of DLL3 and who received active doses of Rova-T (0.2-0.4 mg/kg). At least two DLL3-high patients who received three doses of Rova-T remained alive and progression-free for over 2 years without any further treatment, and are continuing to be followed. In patients with ≥50% DLL3-positive tumor cells, Rova-T outperformed confirmed response rate and OS rates for 2nd line topotecan and 3rd line conventional chemotherapy observed in other clinical trials. The most common grade 3 treatment-related adverse events were thrombocytopenia (12%), serosal effusions (11%), and skin reactions (8%). This data supports a biomarker-directed phase II clinical trial in SCLC. DLL3 is also highly expressed in melanoma, small cell bladder cancer, neuroendocrine pancreatic cancer, neuroendocrine colorectal cancer, medullary thyroid cancer, and glioblastoma. DLL3 was also found to be highly expressed in NEPC, but was not expressed in benign prostate tissues or prostate adenocarcinoma, suggesting that DLL3 may be a therapeutic target in NEPC. Ongoing studies are evaluating DLL3 expression throughout the expression of DLL3 and factors including neuroendocrine marker expression, Notch activity, AR activity, NEPC-associated genomic alterations, PSA levels, and clinical outcomes. These results have led to the initiation of biomarker-guided phase II studies, including a basket trial enrolling patients with DLL3-positive solid tumors from over eight indications including NEPC. Future studies will evaluate the efficacy of Rova-T earlier in the prostate cancer disease course and in combination with other treatments.

Gregory Verdine (Fog Pharmaceuticals, Inc.) discussed a novel technology that enables targeting of currently undruggable intracellular cancer drivers. Cell-penetrating mini-proteins (CPMPs) are synthetic, helical peptides that have been hyper-stabilized with a hydrocarbon or amino “staple.” Theoretically, CPMPs can be developed against any protein containing helix-interacting domains. Lead compounds have been developed that are able to target HDM2/ HDMX, β-catenin, Notch, and Ras. A HDM2/HDMX-targeting CPMP is currently in phase I clinical trials. A Notch-targeting CPMP, SAHM1, was demonstrated to antagonize Notch-regulated gene expression in vitro and exhibit anti-tumor efficacy in a murine acute T cell leukemia model. These and other CPMPs are undergoing further preclinical development at Fog Pharmaceuticals to identify compounds suitable for clinical trials.

Markus Warmuth (H3 Biomedicine, Inc.) discussed rationale and advances in targeting the RNA spliceosome for the treatment of human malignancies. Spliceosome genes have been found to be commonly mutated in human cancer, including both gain and loss-of-function mutations, and result in aberrant RNA splicing. Recurrent mutations have been observed in core spliceosome components including SF3B1, U2AF1, SRSF2, and ZRSR. In essentially all cases, only single-copy alterations have been observed, implying that slightly altered splicing is beneficial for cancer cells but further splicing aberrations could not be tolerated. This suggests that splicing mutations may be therapeutically exploited as a cancer treatment strategy that would leave normal cells unharmed. A number of naturally occurring lead compounds with spliceosome-targeting activity were identified and further optimized using medicinal chemistry. H3B-8800 is an orally bioavailable SF3B1-inhibitor that exhibited highly selective killing of SF3B1-mutant cells in vitro and inhibition of SF3B1-mutant tumor cells in mice. H3B-8800 was found to exhibit its effects by promoting the retention of short GC-rich introns, a feature common in splicing genes, and led to altered splicing of mRNA encoding for splicing components. Prolonged treatment of HCT116 colon carcinoma cells with H3B-8800 led to the outgrowth of therapeutically resistant tumor clones harboring acquired mutations in SF3B1 and PHF5A. Examination of the 3D structure of the spliceosome revealed these mutations occurred in regions that interact with mRNA, and indicate mechanisms of resistance to H3B-8800. In prostate cancer, resistance to enzalutamide and abiraterone can involve AR-V7 expression or NEPC transdifferentiation, both processes that involve splicing-related mechanisms. Prostate cancer cells treated with a splicing modulator downregulated the expression of AR-V7 and AR-target genes. These data imply that targeting splicing may be an effective strategy for treating or preventing enzalutamide and abiraterone-resistance in prostate cancer. Ongoing studies are identifying splicing modulators that can selectively target certain types of splicing events. Promising splicing modulators are being further optimized in preclinical studies in preparation for clinical trials.

1.9 | Precision survivorship

Alicia Morgans (Vanderbilt University Medical Center) introduced the concept of precision survivorship, an approach to patient care that uses a patient’s unique characteristics including clinical factors, biomarkers, and genetics or molecular markers to predict cancer treatment-related complications. Prostate cancer treatments have been linked to bone, metabolic, cognitive and cardiovascular complications in observational studies. Prospective, randomized trials are needed to assess and validate treatment-related complications, develop biomarkers to identify patients at risk, and address the biology
underlying complications to enable to development of preventative therapy. For example, previous PCF-supported studies identified an association between hormonal therapy and bone complications in prostate cancer patients, which prompted the application of bisphosphonates and RANK ligand inhibitors for treating skeletal-related adverse events.

Charles Ryan (University of California, San Francisco) reviewed studies examining the relationship between ADT and the onset of cognitive disorders. AR is widely expressed in the brain and plays a neuro-protective role, decreasing β-amyloid levels and tau phosphorylation, which are associated with dementia and Alzheimer’s disease, and increasing neuronal viability. Testosterone and DHT have been observed to decline rapidly in the aging brain, while low brain testosterone levels have been observed in men with mild cognitive impairment. A study examining cerebral morphometry in prostate cancer patients found that the brain decreased in size after 6 months of ADT. Another study examining 14 cognitive measurements in prostate cancer patients found that immediate span of attention, visual-spatial ability, and executive function were moderately impaired after 12 months of ADT. The earliest and most affected cognitive function was visual-spatial ability, which is the ability to perceive and assess objects in a 3D space and is tested by tasks including the ability to envision the rotation of geometric objects. An observational study examining long-term effects of ADT found that after 12 months of ADT, the fraction of patients exhibiting cognitive impairment increased from 40% (baseline) to 55-60%. However, the men examined were of advanced age and it is unknown what the effects of aging alone were in this study. A retrospective study conducted by analysis of diagnosis codes in patient medical records identified an association between diagnosis of Alzheimer’s disease and treatment with ADT for 7 years or longer. A similarly conducted study identified an association between earlier diagnosis of dementia and treatment with ADT for 2 years or longer. These studies suggest that low androgen levels brought on by age or treatment with ADT may increase the risk for cognitive impairment, including Alzheimer’s disease. Moreover, individuals with low baseline androgen levels may be more susceptible to cognitive impairment during treatment with ADT. The promoter of AR contains a highly polymorphic number of CAG repeats which have been shown to regulate the expression levels of AR, with fewer CAG repeats being associated with increased AR expression. Whether the number of CAG repeats in the AR promoter influences the onset of Alzheimer’s disease is not yet clear, as different studies have reported conflicting results. Increased baseline levels of β-amyloid and/or apolipoprotein E, which are risk factors for dementia, may also increase risk for cognitive impairment in ADT populations. Approximately 40% of men have one of these risk factors, while ~5% have both. Androgen supplementation has been unsuccessful as a treatment for Alzheimer’s disease, suggesting that androgens may be protective from brain injury but are unable to drive regeneration of already damaged brain tissue. Randomized, prospectively conducted clinical trials are urgently needed to validate whether ADT does increase risk for cognitive impairment in prostate cancer patients, and to identify alternative treatment strategies that reduce risk for cognitive impairment without increasing risk for prostate cancer progression, in at-risk individuals.

To address the need for a prospective clinical trial evaluating the effects of androgen-targeted therapy on cognitive outcomes, Alicia Morgans is initiating the PCF-supported COGnitive Effects of Androgen Receptor (AR) Directed Therapies for Advanced Prostate Cancer (CaP) (COGCaP) trial. In this trial, men with mCRPC without dementia or prior chemotherapy will be randomized to receive abiraterone versus enzalutamide and assessed for outcomes including cognitive function. Cognitive function will be evaluated by a neuropsychologist and a computer-based cognitive assessment at baseline, 3, 6, and 12 months following the initiation of treatment. Blood oxygen level dependent (BOLD)-functional magnetic resonance imaging (fMRI), diffusion tensor imaging (DTI), and arterial spin labeling (ASL) MRI will be performed at baseline and 3 months to examine functional and structural changes in the brains of patients and suggest mechanisms of cognitive impairment that can be studied further. Additionally, single nucleotide polymorphisms (SNPs) will be assessed in this cohort to identify any genetic polymorphisms that correlate with cognitive outcomes. Clinical centers participating in this study will include Northwestern University (Morgans), Vanderbilt University (Morgans and Kelvin Moses) and University of Southern California (Tanya Dorff).

1.10 | Progenics PSMA-targeted DCFPyL research access program

\(^{18}\)F-DCFPyL is a PSMA-targeted radiotracer that has shown significant promise in early trials as a positron emission tomography (PET) imaging probe for staging primary and metastatic prostate cancer. For \(^{18}\)F-DCFPyL PET to gain FDA approval and become a standard of care imaging option, definitive clinical trials are needed to both demonstrate specificity and sensitivity, and show that knowledge gained from \(^{18}\)F-DCFPyL PET imaging leads to beneficial changes in patient treatment plans and clinical outcomes. The phase II/III Osprey trial is one such study, and will assess the diagnostic performance of \(^{18}\)F-DCFPyL PET/CT for detecting primary, locally recurrent, and distant metastatic prostate cancer.

Mark Baker (Progenics Pharmaceuticals) announced a new Progenics initiative which will provide DCFPyL at no or low cost to prostate cancer researchers for investigational studies. Researchers can be from either an academic institution, a non-profit organization, or a for-profit company, and must be able to hold an IND and conduct sponsored clinical trials. Researchers may either obtain \(^{18}\)F-radiolabeled DCFPyL from Progenics, or obtain the DCFPyL precursor and perform radiolabeling at their own GMP facility. Researchers must agree to an MTA-like agreement with Progenics to receive DCFPyL. As part of this agreement, researchers must dose DCFPyL as specified by Progenics and share data including images and minimal clinical information in a cloud-based, open access database. This initiative opened in January 2017 to attendees of the PCF Retreat, and is now available to investigators recommended by PCF-supported investigators. At the same time, Progenics has made a web-based version of the Bone Scan Index freely available to PCF-supported
investigators. The Bone Scan Index performs an automated analysis and indexing of disease burden using bone scans from metastatic prostate cancer patients and is FDA-approved. It is hoped that this initiative will accelerate the basic science and clinical studies required to clinically validate the utility of $^{18}$F-DCFPyL PET/CT and gain FDA approval.

ACKNOWLEDGMENTS

We would like to express our deepest gratitude to all of the speakers who took the time to attend and present at the 23rd Annual PCF Scientific Retreat and for their thoughtful review of this manuscript. The PCF “State of Science Report,” which includes a more detailed summary of each presentation as well as the Retreat agenda can be downloaded at: https://www.pcf.org/c/2016-state-of-science-report.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

REFERENCE


How to cite this article: Miyahira AK, Soule HR. The 23rd Annual Prostate Cancer Foundation Scientific Retreat report. Prostate. 2017;9999:1–14. https://doi.org/10.1002/pros.23366