Prostate Cancer Immunotherapy: Beyond Immunity to Curability

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Abstract

Metastatic prostate cancer is the second leading cause of death from cancer in the United States. It is the first prevalent cancer in which overall survival in advanced disease is modestly, but objectively, improved with outpatient delivered dendritic cell–based immunotherapy. More prostate cancer patients have enrolled through Facebook and trusted-site Internet searches in clinical trials for prostate cancer vaccine–based immunotherapy than in immunotherapy trials for lung, breast, colon, pancreas, ovarian, and bladder cancer combined in the past 7 years. Exceptional responses to anti–CTLA-4 treatment have been documented in clinics, and prostate cancer neoantigen characterization and T-cell clonotyping are in their research ascendancy. The prostate is an accessory organ; it is not required for fertility, erectile function, or urinary continence. The true evolutionary advantage of having a prostate for male mammalian physiology is a topic of speculation in seminar rooms and on bar stools, but it remains unknown. Hundreds of prostate lineage-unique proteins (PLUP) exist among the >37,000 normal human prostate lineage-unique open reading frames that can be targeted for immunologic ablation of PLUP$^+$ prostate cancer cells by prostate-specific autoimmunity. This bioengineered graft-versus-prostate disease is a powerful strategy that can eliminate deaths from prostate cancer. Immunologic tolerance to prostate cancer can be overcome at every clinical stage of presentation. This *Cancer Immunology at the Crossroads* article aims to present advances in the past two decades of basic, translational, and clinical research in prostate cancer, including bioengineering B-cell and T-cell responses, and ongoing prostate cancer immunotherapy trials. *Cancer Immunol Res; 2(11); 1034–43. ©2014 AACR.*

Introduction: Prostate Cancer Responds to and Warrants Immunotherapy Research

Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning.

–Sir Winston Churchill

Prostate cancer research is an exciting, compelling, and fundable area of biomedical science (www.pcf.org). Although metastatic, hormone therapy–refractory prostate cancer remains the second most common cause of cancer-related deaths in men after lung cancer in the United States, the years of life lost to prostate cancer have dropped by 40% in the past 20 years (1). In total, six new agents (denosumab, Sipuleucel-T, cabazitaxel, abiraterone, radium 223, and enzalutamide)— each with its own new and different mechanism of action— have been approved by the FDA for treatment of metastatic prostate cancer (mPCA). More new FDA-approved drugs have been used for treatment of mPCA in the past 3 years than in the past four decades. For patients beyond early detection and cure

with surgery or radiotherapy, survival times for men with mPCA have been more than doubled, with a new armamentarium of systemic treatments (Fig. 1; ref. 2).

Metastatic prostate cancer is the first prevalent cancer in which overall survival (OS) in advanced disease is modestly, but objectively, improved with outpatient delivered immunotherapy. Sipuleucel-T is an autologous, GM-CSF–activated, CD54-expressing, prostate-specific acid phosphatase (PAP) peptide–loaded cellular therapy in which three randomized phase III trials showed survival benefits in men with asymptomatic or symptomatic mPCA (3). Whether Sipuleucel-T generates tumor-specific, intratumoral cytotoxic T lymphocytes (CTL) against metastatic castration–resistant prostate cancer (mCRPC) antigens (or works by any of the advertised explanations in the oncology clinical journals) remains unclear. What is clear is that delaying the progression of mCRPC with this cellular immunotherapy does extend survival, does not confer high frequency of complete NCI RECIST criteria remissions, and needs a scientific explanation.

The absence of a mechanism of action to account for an FDA-approved anticancer agent is not that uncommon in oncology. It took 27 years after the curability of metastatic testicular cancer was established with cisplatin to witness a molecular mechanism explaining the action of the drug. In brief, cisplatin "log-kills" tumor cells in the testes that are deficient in the excision repair cross-complementation gene 1, which encodes an enzyme for interstrand cross-link DNA repair (4). More

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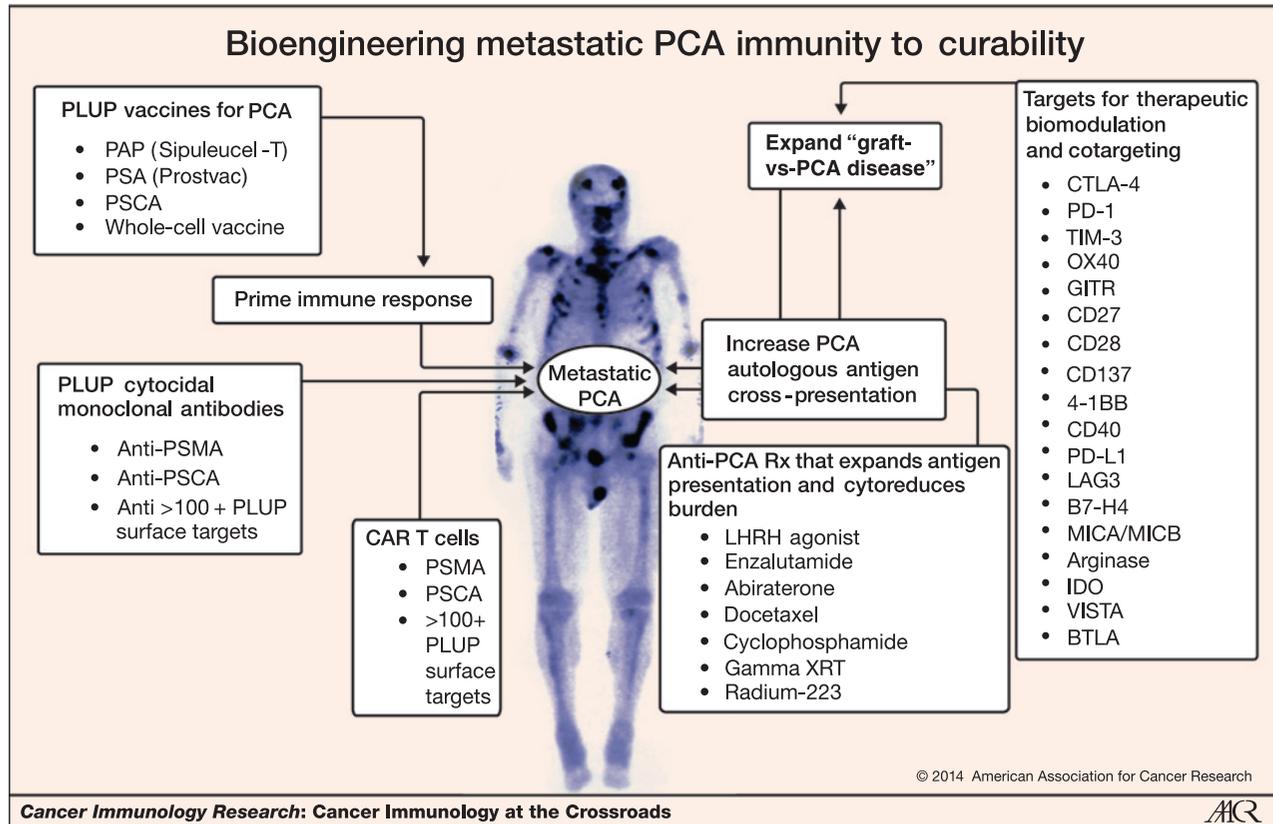
Figure 1. Bioengineering metastatic prostate cancer (PCA) immunity to curability. This diagram shows some of the current therapeutic strategies being developed and applied for the treatment of metastatic prostate cancer. These approaches focus on reawakening and augmenting the host's immunity against prostate cancer cells.

recently, as covered in this journal, the story for the precise mechanism(s) of action of the anti–CTLA-4 antibody ipilimumab in patients with cancer is still evolving beyond its precedent-setting FDA approval in advanced melanoma (5).

Research leading to the development of more effective immunotherapies for mCRPC is urgently needed. Patients are not cured with Sipuleucel-T treatment. A death from mCRPC still occurs every 18 minutes in the United States. Beyond North America, prostate cancer incidence and death rates are rising in India, China, and South America with the extension of life expectancy (6). The prostate cancer research enterprise is responding. A recent Medline search has manifested over 156 phase II clinical trial reports on immunotherapy for prostate cancer, and 5,942 research articles on the immunobiology of prostate cancer. This article aims to present an overview of the immunotherapy research agenda for prostate cancer, addressing three sets of questions: (i) where we have come from, (ii) where we are now, and (iii) what are the new questions.

Where We Have Come from and Where We Are Now

Science must begin with myths and with the criticisms of myths.
                                                              –Karl Popper

The first two decades (1990–2010) of molecular immunotherapy research in prostate cancer were focused fundamentally on dispelling myths. The central 20th-century myth was that prostate cancer in the clinic could "never" respond to immunotherapy. The "Bethesda nihilism myth" was predicated on these axioms: (i) Unlike melanoma or renal carcinoma, immune-mediated spontaneous regressions and abscopal effects in mCRPC never occurred in patients; (ii) the prostate gland was intrinsically nonimmunogenic: peripheral tolerance could never be broken (there are no autoimmune diseases against the prostate, unlike those against the skin or kidney); (iii) older men with mCRPC had preexisting T-cell anergy due to aging that prevented antineoplastic T-cell immune responses; (iv) all prostate cancers were MHC class I$^{low/null}$; and (v) no antitumor activity was seen using recombinant cytokines as single agents, e.g., INFγ or IL2, in small clinical trials. Although evidence existed that men with untreated prostate cancer had active delayed-type hypersensitivity responses to autologous tumor antigens derived from lysates of their own cancers, the data were in small numbers from a single institution's data—buried in the clinical urology literature of the 1970s (7).

"TIL cell-Mafia-Key-Opinion-Leader-Group-Think" of the 1990s, as a wall of doubt on the worthiness of prostate cancer as a subject for immunotherapy research funding, was toppled by a variety of paradigm-shifting preclinical, and subsequent clinical, research translations. Whereas high-dose IL2, IFNα, and INFγ showed minimal activity against advanced prostate

cancer, systemic GM-CSF infusions as monotherapy induced objective PSA responses in a small percentage of patients with mPCA (8). Hurwitz and colleagues (9) demonstrated that the combination of GM-CSF gene–transduced, irradiated tumor cells (GVAX) plus anti–CTLA-4 antibody had curative efficacy in preclinical, poorly immunogenic transgenic models of mCRPC, and the effector cells involved in the antineoplastic immune responses were identical to those raised against the B16 melanoma model. Formal evidence that both T-cell and B-cell tolerance could be broken to prostate tumor–specific antigens in patients by autologous and allogeneic prostate cancer–associated antigens delivered by GM-CSF–activated dendritic cell (DC)–based antigen presentation was demonstrated by Simons and colleagues (10, 11). Vaccinia-based cDNA vaccines encoding prostate-specific peptides showed prolongations in time to progression and OS in advanced disease (12). In 2014, more than 2,500 prostate cancer patients are recruited and evaluated on global immunotherapy clinical trials, supported by NCI-funded extramural Special Programs of Research Excellence (SPORE), contrarian biotechnology start-ups for the potential market share in an FDA-approved indication for mPCA treatment. Translational research support comes from the Department of Defense with funds from Congressionally directed Medical Research Programs (DoD CDMRP), the Prostate Cancer Foundation, and large pharma supporting phase III trials of immunotherapy in advanced prostate cancer.

Targeting prostate lineage-unique antigens: monoclonal IgGs and CAR T cells

The prostate is an accessory organ; it is not required for fertility or urinary continence. The true evolutionary advantage of having a prostate for male mammalian physiology is a topic of speculation in seminar rooms and on bar stools (12), and it remains unknown. Hundreds of prostate lineage-unique proteins (PLUP) exist among the 37,000+ normal human prostate lineage-unique open reading frames in EST and SAGE libraries (13). Immunologically ablating PLUP$^+$ mCRPC cells with prostate-specific autoimmunity—"bioengineering graft- versus-prostate disease"—is a compelling strategy to eliminate deaths from prostate cancer. These PLUPs are particularly inviting candidates for anti-PLUP antibody research. Thus far, approaches to immunotherapy have been pioneered on the use of specific anti-PLUP mAbs targeting proteins highly enriched on mCRPC cells. Only two PLUPs have been targeted with high-affinity mAbs: prostate stem–cell antigen (PSCA) and prostate cancer–specific membrane antigen (PSMA; ref. 14). Expression of ectopic PLUP antigens is validated *in vivo* in metastatic sites, such as the bone marrow, liver, lung, dura of the central nervous system, and lymph nodes, where PLUP$^+$ antigens are never expressed. A humanized, high-affinity mAb J591 against PSMA manifests tumor targeting, but achieves only relatively low frequencies of PSA and objective tumor responses (<20%; ref. 14). As a result, the subsequent development of J591 has evolved into radioimmunotherapy with conjugation to ^{177}Lutetium (^{177}Lu-J591) with further effort focused on different cytocidal payloads. This radioimmunotherapy approach has shown short-term antineoplastic

effects manifested by PSA reductions, and it also permits imaging of metastatic sites simultaneously. Clinical trials of ^{177}Lu-J591 combined with hormonal therapy are now being evaluated for OS benefit in patients with mCRPC. In addition to anti-PSMA, PSCA antibodies and minibodies both show impressive affinity, localization, and antitumor effects in chemotherapy and hormone-refractory xenograft models of human prostate cancer (15).

There is early but vigorous work in creating chimeric antigen receptor–modified T cells against PLUPs for treatment of mCRPC. Through multiple funding mechanisms from government, biopharma, institutional funds, and venture philanthropy, proof-of-concept trials using ScFv-CD28-CD3ζ with targeting moieties against PSMA, PSCA, and other PLUPs are in development (C.H. June et al., unpublished data; ref. 16).

Anti-androgens and mutational diversity of prostate cancer antigens

The androgen receptor (AR) is a central transcription factor contributing to progression and death from mPCA. Anti-androgens improve mPCA survival and are the mainstay of treatment of mPCA. Interestingly, anti-androgens also enhance early B-cell development, reverse thymic involution, promote thymopoiesis, inhibit tolerance to prostate antigens, and increase prostate cancer immune effector-cell infiltrates (17–20). Thymic regeneration is observed in both humans and mice following androgen blockade (19). After treatment with anti-androgens, a substantial and specific T-cell response (greater for CD4$^+$ than for CD8$^+$) was observed in both benign and malignant areas within prostatectomy specimens (18, 21). These findings place an emphasis on integrating anti-androgen therapy with novel approaches to induce cytocidal anti–prostate cancer immune responses. Drake and colleagues (21) showed in seminal experiments that naïve prostate-specific CD4$^+$ T cells remain indifferent to prostate model antigens. When transgenic mice develop autochthonous prostate cancers, their naïve CD4$^+$ T cells recognize and traffic to the prostate gland. Recognition, however, was tolerogenic, and the induction of effector cytokines was blunted. Androgen ablation with agents routinely used in the urology clinic abrogated the immune tolerance to prostate model antigens, and effector functions in response to prostate vaccination were observed. These findings underpin a testable working model: Prostate cancer immunotherapy might be effectively used as soon as possible after the first use of anti-androgens (21). A wave of apoptotic prostate cancer cell death facilitating prostate cancer antigen spreading can be shown in murine models, but new biotechnology is required to prove this hypothesis in men with prostate cancer. With whole-genome sequencing of >300 mPCA patient tumors at this writing funded by this foundation, we know that a battery of neoantigens will be discovered for prostate cancer immunotherapy research. Of interest to prostate cancer neoantigen hunters, human prostate cancer, in both primary and metastatic sites, on average can have 3 logs lower open reading frame mutation rates (2/megabase) than those found in advanced-stage melanomas (22).

Single prostate cancer antigen vaccines

Prostvac-VF (Bavarian Nordic) uses another PLUP: the full-length human PSA cDNA. Prostvac is a prostate cancer vaccine regimen comprising a recombinant vaccinia vector prime followed by multiple booster vaccinations with a recombinant fowlpox vector (22). Both vectors contain the transgenes for PSA and three costimulatory molecules (TRICOM): B7.1, ICAM-1, and LFA-3. The PSA-TRICOM vaccine infects antigen-presenting cells (APC) and expresses the transgene products on their surface. Prostvac phase II trials suggested a survival benefit after treatment, especially in patients with more slowly progressing metastatic disease (23). After 3 years, patients who received Prostvac-VF had an OS of 30% versus the control group, which had an OS rate of 17% (12). The Prostvac-VF–treated group also had longer median survival by 8.5 months, and a 44% reduction in death rate in men with minimally symptomatic mCRPC. Increased ELISPOT reactivity to PSA was correlated with extended OS. Prostvac in combination with docetaxel-based chemotherapy regimens is under evaluation in multiple centers.

These provocative results with a single PLUP antigen catalyzed investment in a multinational, randomized, 1,200-patient phase III trial that has been launched (NCT01322490). Patients with chemotherapy-naïve mCRPC are randomized to one of three treatment arms: Prostvac-VF as monotherapy, Prostvac-VF with subcutaneous administration of GM-CSF, or placebo. OS is the primary endpoint of this large, signal-seeking trial of completely outpatient treatment. The trial has finished accrual, and maturation of data is eagerly awaited.

Combining Prostvac-VF with AR inhibitors (leuprolide, flutamide, abiraterone, and enzalutamide) for micrometastatic disease is also contemplated. The sequence of drug administration may matter in prostate cancer immunotherapy for amplifying antigen presentation, and boosting and sustaining antineoplastic immune responses against weaker PLUP antigens. In a phase II study (NCT-00450463) involving 42 men with micrometastatic CRPC, patients were randomized to receive Prostvac-VF followed by the AR antagonist nilutamide versus nilutamide followed by Prostvac-VF (23). Results from this study indicated a trend toward improved survival in men receiving Prostvac-VF before nilutamide rather than the reverse (6.2 vs. 3.7 years; $P = 0.045$). An ongoing study is testing the hypothesis that an amplified antitumor immune response may augment the effect of taxane-class chemotherapy. A randomized phase II trial (NCT01145508) is allocating 144 men with chemotherapy-untreated mCRPC to receive Prostvac-VF followed by up to 12 cycles of docetaxel chemotherapy, or to receive docetaxel chemotherapy before Prostvac-VF vaccination. There are more than 50 preclinical research publications describing the concept of cancer immunotherapy in combination with taxane-based chemotherapy expanding the depth of tumor log-kill and extending the duration of disease-free progression. However, well-designed clinical studies validating the efficacy of the combined regimen in conferring increased patient survival are needed urgently in prostate cancer oncology, and in solid tumor oncology in general.

Anti–prostate cancer DNA vaccines

An alternative immunotherapeutic approach for prostate cancer–associated antigens uses cell-free DNA plasmids (24, 25). Despite their relative ease of GMP production and less complex regulatory issues, so far these vaccines have been unable to induce a high frequency of NCI RECIST criteria clinical responses or strong immune responses in the clinic. A phase I trial of a PAP-encoding plasmid pTVG-HP that was administered to men with rising PSA after surgery or radiotherapy demonstrated the induction of PAP-specific cytolytic T-cell responses (26), and suggested a slowing of PSA doubling time in a number of treated patients (26). This study provided a provocative insight: a wide and significant variation spanning several months in the time to mount specific immune responses to a defined dose and schedule of PAP antigens; all study patients were of seemingly identical prostate cancer stage and were baseline immunocompetent to common recall antigens (26). This finding supports the concept of "treatment to full immune induction" of immune response (individualized immunotherapy/patient), as opposed to comparing fixed doses and schedules that are typically used for cytotoxic drug development in assessing efficacy. A customized, adaptive-tailored approach to pTVG-P vaccine development is currently under way (NCT00849121). Patients are randomized to either a predetermined vaccination schedule (six doses given every 2 weeks followed by booster vaccinations once every 28 days for 3 months) or the adaptive vaccine regimen, in which the six-dose run-in is followed by biweekly or monthly vaccinations or by three booster vaccinations given once every 28 days based on observed cellular immune responses. The endpoints of this trial are safety, immunogenicity, PSA doubling-time modulation, and 1-year metastasis-free survival.

Cell-based polyvalent vaccines

GVAX-based cancer vaccines have been the subject of significant translational and clinical research activity in the past decade (10, 11, 27–30). The rationale for using cells, either autologous or allogeneic, as the source for antigen presentation to DCs and other antigen-presenting cells (APC), in contrast with a single specific protein or peptide tumor antigen, is that prostate cancer cells provide a source of polyvalency of antigens and eliminate the need to identify specific gene products to target in a particular prostate cancer patient. Polyvalency using multiple tumor antigens can induce immune responses to more than one tumor antigen, thereby potentially overcoming tumor antigen loss, and intratumoral heterogeneity in metastatic sites. Furthermore, activated lymphocytes and serologic responses can be dissected to identify novel tumor antigens or categorize the importance of a response to a particular tumor antigen through the comparison of immune responses before and after vaccination (10, 11, 28, 29).

Early academic phase I and II trials with prostate cancer GVAX showed evidence of induction of T-cell responses and new oligoclonal IgG B-cell responses to prostate cancer–associated antigens but not to PSA or PAP (11). Anti–prostate cancer immunity was observed with vaccinations of autologous and allogeneic prostate cancer GVAX in men with micrometastatic prostate cancer that was (i) hormone therapy naïve,

(ii) chemotherapy naïve, and (iii) before the development of bone metastases (11). Under a corporate sponsorship and design, one global phase III trial of allogeneic GVAX in men with advanced mCRPC failed to detect a large OS benefit with GVAX plus docetaxel chemotherapy versus treatment with docetaxel alone. A second phase III GVAX trial in chemotherapy-naïve patients with better performance status was stopped early due to a shortage of funding before completion of survival analysis, and patient follow-up data were not captured. As a monotherapy, allogeneic prostate cancer GVAX does not increase OS in a dramatic fraction of patients with advanced mCRPC.

An approach to enhance the efficacy of polyvalent prostate cancer antigen presentation has been explored in prostate cancer. Early preclinical studies demonstrated that anti–CTLA-4 activity could be enhanced when combined with GVAX (9). In a poorly immunogenic melanoma mouse model, the combination of ipilimumab with GVAX resulted in the eradication of established melanoma tumor burdens, whereas each treatment alone had minimal efficacy (31).

A phase I dose-escalation trial of biweekly intradermal, allogeneic prostate cancer GVAX injections and monthly ipilimumab included 12 mPCA patients in a dose-escalation cohort (0.3–5 mg/kg of ipilimumab) and a subsequent 16 mPCA patients in an expansion cohort treated with a dose of 3 mg/kg of ipilimumab (32). Among all 28 patients, 7 patients (25%) that received ipilimumab (3 mg/kg or 5 mg/kg) had a $\geq$50% decline in PSA from baseline. Of the 22 patients treated with 3 mg/kg or 5 mg/kg of ipilimumab, 5 (23%) had confirmed partial PSA responses with a median duration of 12 months (range, 2–21 months; ref. 32). A strong association between the PSA response and immune-related adverse events (irAE) was noted; all 12 patients in the dose-escalation cohort that had an irAE, including grade 2/3 hypophysitis and grade 3 alveolitis, also manifested PSA responses. Seven patients in the dose-escalation cohort and 8 patients in the expansion cohort had at least stable disease on bone scans, and 2 patients in the escalation cohort had clear regression of metastases. Data from a serologic analysis of recombinant CDNA expression libraries indicated that prostate cancer patients with increased IgG reactivity against PSMA had significantly increased survival. Patients with a PSMA-specific antibody response had a median OS of 46.5 months [95% confidence intervals (CI), 30.2–62.8], whereas patients without a PSMA-specific antibody response had a significantly shorter median OS of 20.6 months (19.0–22.2; $P = 0.028$). These pilot data suggest that anti-PSMA immunity may contribute to the efficacy of GVAX plus ipilimumab combination therapy and that PSMA seroreactivity is a possible biomarker for clinical benefit from many different approaches to targeting PSMA as a PLUP.

DC-based immunotherapy

The FDA approval of Sipuleucel-T, an autologous, PAP antigen–directed cell-based immunotherapy, in which leukapheresed, CD54-expressing APCs are infused into patients, was a game changer. It was the first therapeutic vaccine in oncology to show a survival advantage in a prevalent cancer (3). Sipuleucel-T did not have a large repertoire of traditional published reports of preclinical murine experiments. It was developed

and received FDA approval based on "bioengineered immunotherapy logic": GM-CSF–activated autologous, leukapheresed, APCs enriched for cells with DC markers + highly expressed cytosolic PLUP = anti–prostate cancer immunotherapy. In the more than 37,000 unique transcripts in the normal prostate transcriptome, PAP is abundantly expressed, and the polypeptide was used as the vaccine antigen. In a 512-patient phase III study (NCT00065442), men with minimally symptomatic or asymptomatic mCRPC who received Sipuleucel-T showed a 4-month increase in survival compared with patients that received placebo (25.8 vs. 21.7 months; HR, 0.78; $P = 0.03$). Surprisingly, no difference was found in other measurable endpoints of quantitative reduction in total tumor burden, including PSA responses or bidimensional, radiographic reductions in bone and soft-tissue metastases. Furthermore, the *in vivo* mechanism of action resulting in the Sipuleucel-T treatment benefit is not well understood. Patients in the pivotal trial (3) had higher antibody titers against PAP postvaccination (>1:400) and lived longer (even if their cancer was progressing) than those randomized to receive placebo. However, a survival difference could not be detected between patients in the Sipuleucel-T group who had T-cell proliferation responses to PAP peptides and those who did not.

How do GM-CSF–activated CD54-expressing cells cross prime against tumor-associated antigens, and does this result in T-cell infiltration in metastatic sites following Sipuleucel-T vaccination? These remain important and unsettled mechanism-of-action questions. To this end, Sipuleucel-T is being piloted in the neoadjuvant context in a phase II study enrolling 40 men who are scheduled to undergo subsequent radical prostatectomy (NCT00715104). Patients are receiving three infusions of Sipuleucel-T before radical prostatectomy, with the primary study endpoint being histologic analysis of immune effector cell responses in resected prostate glands, compared with those in core biopsy specimens collected pretreatment. Neoadjuvant prostate cancer immunotherapy translational research extends to assessing amplification of antitumor immune responses by combining immunotherapy with androgen-deprivation therapy. Results from neoadjuvant clinical studies support this combinatorial approach as androgen ablation induces dense immunologic infiltrates into the prostate gland (21, 33).

Questions with urgent clinical pragmatism are being asked in the clinic. For example, when should immunotherapy and combination regimens be used for treatment? What are the optimal timings and durations of the outpatient use of AR-directed therapies (e.g., abiraterone, enzalutamide, and ARN504) that cause apoptotic release of peptide antigens surrounding treatment with Sipuleucel-T vaccinations? Specifically, when in the clinical course of mPCA does prostate cancer immunotherapy induce cures or dramatically extend survival? Even though these studies may take years (prostate cancer can progress very slowly compared with micrometastatic lung, colon, pancreatic cancer, and melanoma), the "most minimum," micrometastatic tumor burden settings seem very compelling now for both investigational and FDA-approved immunotherapy approaches. Potentially for more than 50,000 prostate cancer patients annually in North

America, neoadjuvant therapy may be beneficial before primary therapy (surgery or radiotherapy) for high-grade disease diagnosed locally that has a molecular certainty of coexisting regional or distant metastatic spread.

A second attractive context in which an experimental immunotherapy can be tested is in a biochemical-recurrence state, characterized by an abnormal elevation of PSA indicative of new disease activity after the prostate cancer primary has been resected or irradiated with curative intention. These patients represent an ideal setting for immune-based approaches, because after resection of the primary tumor the disease burden is at the lowest in log number ($<1 \times 10^9$ cells), and theoretically the corresponding prostate cancer tumor burden–dependent mechanisms of immune suppression are also at the lowest level. Because such biochemical PSA-relapse patients are often treated with androgen-deprivation therapy for a limited course, one attractive approach is to follow androgen ablation with anti–prostate cancer immunotherapy to eradicate residual prostate cancer cells or to contain their progression in the absence of androgen.

A significant body of preclinical literature supports the hypothesis that anti-androgen targeting of prostate cancer cells induces and augments antitumor immunity and vaccine efficacy. However, the optimal regimen of anti-androgen therapy with immunotherapy is unresolved, in particular, whether immunotherapy should be delivered as priming before androgen ablation, as autologous antigens are shed from apoptosis of prostate cancer cells; or should immunotherapy be administered after androgen ablation, as a boost for autologous antigen release. To answer this question clinically, a 60-patient randomized phase II trial has been initiated (NCT01431391) in which a standard three-dose course of Sipuleucel-T is administered either 6 weeks before or 12 weeks after a 12-month course of androgen-deprivation therapy in men with biochemically recurrent prostate cancer at high risk of metastatic progression. The primary objective of this study is to assess PAP-specific effector T-cell and B-cell responses in an effort to define which sequence generates the most robust antitumor immune response. This study also compares PSA progression and metastasis-free survival in the two treatment arms.

Immunoevasion and new targets of treatment opportunity

Prostate cancer evades host immune responses resulting in approximately 29,000 deaths per year in the United States. While peritumoral $CD8^+$ cells are often seen, robust tumor-infiltrating lymphocytes (TIL) are rarely observed in primary prostate cancer tumors or in AR^+, PSA^+, $HIF1^+$ mCRPC biopsies of metastases (34). When heavily pretreated mCRPC patients have preterminal tumor burdens on bone scans (greater than 6 metastatic sites), a significant percentage of these patients have TCRζ loss in their peripheral blood T cells (35). In contrast, TCRζ is not downregulated in tumors with a high Gleason grade at diagnosis (Simons, unpublished data). $CD4^+$ prostate-infiltrating lymphocytes (PIL) in patients show a dearth of Th2-IL4–secreting cells and appear more skewed toward a $FoxP3^+$ T-regulatory cell (Treg) and Th17 phenotypes

(36, 37). However, between diagnosis and death from prostate cancer, *in situ* $CD8^+$ T cells in the human prostate cancer microenvironment are metabolically intact, but apparently they are refractory to activation even for *allo-responses* (ref. 36; C. Drake, personal communication). Although $CD8^+$ cells can infiltrate and traffic to prostate cancer tumors, there are TCR distal blocks for anti-neoplastic $CD8^+$ killing. Dissecting these blocks and then trying to drug each block individually and in cotargeting strategies are at the vanguard of the prostate cancer immune-oncology research agenda.

Studies of intracellular mechanisms of induction of $CD8^+$ T-cell indifference to prostate cancer–associated antigens have expanded into analyses of epigenetic alterations modulated by the tumor and its microenvironment on $CD8^+$ T cells, and the impairment of kinase signaling pathways. Whether fibroblast activation protein-α^+ carcinoma-associated fibroblasts (FAP^+CAF) mediate intraepithelial T-cell exclusion effects in prostate cancer, which could be actionable with CXCR4 inhibitor AMD3100, is an area of intensive and pressing investigation (38). Compelling published data are lacking on the effects of the following protein targets on reversing the immune and distal T-cell defects in prostate cancer: CD27, 4-1BB, CD40, PD-L1/PD-1, PD-L1/B7.1, LAG3-CD223/TIM3, B7-H4, MICA/MICB, Arginase, IDO, VISTA, and BTLA. Preliminary data with anti–PD-1 antibody monotherapy in a study of <20 mCRPC patients do not yet have a "get ready for a plenary session with before and after CT scan responses" that phase I/II melanoma or non–small cell lung cancer trials produced. However, trials combining anti–PD-1 antibodies with ipilimumab and prostate cancer vaccines are planned for 2015. Most recently, research with OX40 ligation in combination with CTLA-4 blockade showed antitumor activity in a nonimmunogenic murine model of CRPCA, which has been predictive of ipilimumab clinical activity in mCRPCA (39). Anti-OX40 ligation in combination with ipilimumab for mCRPC is slated to enter the clinic soon.

Ipilimumab monotherapy and swinging for the fences

More than a decade ago, anti–CTLA-4 mAbs demonstrated activity against poorly immunogenic, hormone-refractory prostate cancer models in mice. In the clinic, ipilimumab as monotherapy has a real but low frequency (>0% and <10%) of objective antitumor activity in mCRPC. With the FDA approval of ipilimumab for melanoma, clinical research with ipilimumab in prostate cancer has received greater visibility. Ipilimumab as a monotherapy has been evaluated in men with prostate cancer in a set of phase I and II dose-seeking studies. This agent has some activity in advanced prostate cancer, as judged by PSA response rates of 10% to 20% and objective response rates of 5% to 10% (40, 41). Slovin and colleagues (40) reported a study of 50 patients who received radiotherapy for bone metastases in an effort to generate autologous antigen presentation, and who then received 10 mg/kg of ipilimumab × 4 doses; 8 of the 50 patients had PSA responses of >50%, and 1 patient experienced a complete response.

A randomized, placebo-controlled phase III trial was conducted to evaluate the safety and efficacy of ipilimumab in

mCRPC as a prerequisite for FDA approval (42). A global clinical trial of 799 patients who were refractory to docetaxel chemotherapy has generated new questions. All patients received at least one dose of radiotherapy to bone metastases. The idea was to induce autologous antigen presentation in the bone marrow compartment before ipilimumab treatment; patients were randomly and evenly assigned to the experimental and control arms in an intention to treat. Patients in the experimental arm received maintenance ipilimumab every 12 weeks, and those in the control arm received placebo every 12 weeks. The 1-year and 2-year survival rates for ipilimumab versus placebo treatment were 47% versus 40%, and 26% versus 15%, respectively. The trial showed a median survival improvement of only 1 month, and failed statistically by 0.003 to meet the primary endpoint of a survival improvement; median OS was 11.2 months for ipilimumab versus 10 months for placebo (HR, 0.85; 95% CI, 0.72–1.00; $P = 0.0530$; ref. 42). Median progression-free survival was 4 months in the ipilimumab group and 3 months in the placebo group. This was statistically significant as a secondary endpoint, but it is clinically inconsequential. There was a 1% incidence of treatment-related deaths, and significant GI toxicities were similar to those in melanoma.

Provocatively, Kwon and colleagues (42) found that ipilimumab-treated mCRPC patients with visceral metastases in the lung and liver (23% of all enrollees) had a median survival of 5.7 months versus 14.4 months for patients with osseous and lymph node metastases. This result led them to search for targets in the visceral tumor microenvironment, including the neuronally transdifferentiated AR^+ mCRPC cells that deflect CD8 and natural killer (NK) cell–mediated killing. Study 095 CA184-095 (NCT01057810) is an ongoing phase III randomized double-blind trial comparing the efficacy of ipilimumab at 10 mg/kg versus placebo in patients with only osseous prostate cancer metastases. This study enrolls healthier patients with better performance status who have not received prior docetaxel chemotherapy. It is imperative to see whether there is a 5% to 15% "tail" of durable mCRPC patient survival that is now predicted for ipilimumab treatment.

Additional trials are focused on using ipilimumab in men with earlier stages of life-threatening prostate cancer. One phase II trial combines ipilimumab with androgen ablation in the neoadjuvant setting. Preliminary data from this study indicate meaningful, histologically confirmed pathologic responses in prostatectomy specimens from several patients (43), although a control arm of androgen ablation alone was not included in this trial. A phase II trial (NCT01377389) was launched recently to test the combination of androgen deprivation and ipilimumab in 48 men with newly diagnosed hormone therapy–naïve metastatic prostate cancer. The primary endpoint is an undetectable PSA level after 7 months on the study. Another phase II study (NCT01498978) will test the combination of ipilimumab and anti-androgens in 30 men with mPCA who have not achieved an undetectable PSA level after at least 6 months of hormonal therapy; this trial aims to study whether the addition of ipilimumab to the treatment of these patients can drive PSA to undetectable levels.

Studying Exceptional (but Low-Frequency) Prostate Cancer Patient Responders

Study of "exceptional" responses at the molecular level with the volunteerism of prostate cancer patients has been overwhelmingly hypothesis generating. Graff and colleagues (44) reported studies on an mCRPC patient who is in his sixth year of a complete clinical prostate cancer response to treatment with only three cycles of ipilimumab after experiencing autoimmune hepatitis. The patient mounted IgG responses to 11 candidate tumor-associated antigens, in particular to 3-hydroxyisobutyryl-CoA hydrolase (HIBCH; ref. 44).

Using next-generation deep sequencing to measure the frequency of individual rearranged TCRβ genes, Cha and colleagues found that mCRPC patients who were "exceptional survival responders" following ipilimumab monotherapy maintained high-frequency and high-avidity T-cell clonotypes (45). Ipilimumab treatment increased TCR diversity in mCRPC patients as manifested by the number of unique TCR clonotypes, and the repertoire continued to evolve over treatment. Increased mCRPC patient survival was associated with maintenance of T-cell clonotype stability over a period of months. They also found that preexisting, primed CD8 T cells are maintained with ipilimumab monotherapy. Interestingly, maintenance of TCR clonotype frequency was similarly predictive for extended survival in a cohort of stage IV melanoma patients treated with ipilimumab (45). Although the maintenance of TCR clonotypes over time needs to be validated prospectively, these results open the way for pre- and post-ipilimumab clonotype research in men with mCRPC. This includes characterization of antigens recognized by high-avidity TCR clones that are present at baseline from autologous antigen presentation, and which are clearly detectable in a strong minority of mCRPC patients. A new science of defining PLUP antigens involved with increased mCRPC survival after anti–CTLA-4 therapy can be predicted (45). At this writing, immune responses within more ipilimumab–prostate cancer "extreme responders" are being studied for the involvement of immune responses to mutated gene products, using a combination of mutational analyses, whole-genome sequencing, and measurement of circulating effector cell responses.

Summary and Questions

When you get to a fork in the road, take it.
    –Yogi Berra

Forks ahead abound along the research road map for prostate cancer. Fortunately, mPCA patients in unprecedented numbers want to take the journey with investigators. They are seeking enrollment through Facebook and Internet sites daily for immunotherapy trials, and many have expressed willingness to volunteer for research leukapheresis, repeated metastatic site biopsies, and consent to rapid molecular assessment at autopsies (46). Between 2007 and 2014, more men with prostate cancer have enrolled in immunotherapy clinical trials than patients with advanced lung, breast, and colon cancers combined. In addition to this patient momentum, North

American and European academic urologists, radiation oncologists, and medical oncologists in consortia supported by the Prostate Cancer Foundation have all been fast adapters of the concept that clinical trials of neoadjuvant and adjuvant immunotherapy for locally advanced prostate cancer and mPCA have as high a scientific priority as any approach in genitourinary oncology.

Permutations for strong signal-seeking phase II trials with correlative scientific aims have expanded to include a blockade of multiple immune checkpoints administered alone or in combination with anti-androgens, defined mCRPC antigen-priming vaccines, docetaxel chemotherapy, and radiotherapy, to optimize antigen presentation and spreading. Given the burden of prostate cancer suffering and death, and the large pharmaceutical market, these future prostate cancer immunotherapy trials feature favorable scientific and financial incentives for coinvestment by biopharma, foundations, and the NIH. Currently, despite leadership torpor at the NCI in responding entrepreneurially and courageously to the seismic potential of immunotherapy research to change cancer patient curability in multiple chemotherapy refractory common cancer types, even the NCI is new exploring private–public partnerships models that support innovation in "signal seeking" phase II immunotherapy trials.

Some conclusions from the two decades of prostate cancer immunotherapy studies lead to new questions, which include:

1. Preexisting tumor burdens of >10^6 cells are not cured experimentally in genetically engineered murine prostate cancer systems, and they represent only 0.1% to 0.01% of the tumor burden in prostate cancer patients, who likely would have a PSA biochemical recurrence following surgery or radiotherapy. Therefore, what are the roles of coreceptors and factors such as CD27, 4-1BB, CD40, PD-L1/PD-1, PD-L1/B7.1, FAP, LAG3-CD223/TIM3, B7-H4, MICA/MICB, arginase, IDO, VISTA, and BTLA, and can they be bioengineered for greater immune-mediated log-kill of mCRPCA tumor cells?

2. DC and APC antigen loading of autologous and allogeneic antigens can mount immune responses against prostate cancer–associated antigens regardless of the disease stage. However, the majority of prostate cancer patients, including those with survival benefit and slower disease progression, succumb to the disease. What is the mechanism that extends survival without eliciting NCI RECIST criteria of partial and complete responses on scans?

3. In greater than 95% of patients, the principal sites of early distant prostate cancer metastases are the hematopoietic stem cell niches of the bone marrow that colonizes a bone metastasis. How is mPCA tumor-associated antigen presentation augmented in bone marrow colonization and spread, for example, by the newly approved bone-seeking alpha particle Radium 225, or by agonists of APC activation, such as anti-CD40 and FLT3L, *in vivo* and *ex vivo*?

4. Human prostate cancer expresses an inviting list of PLUPs for CAR T-cell research, of which PSMA and PSCA are only 2 of >1000+ PLUPs that are thus far under investigation. What are the optimum PLUP antigens for antineoplastic IgGs and T-cell CARs, especially when only the cell surface of the PLUP domain has been characterized and cloned into vectors?

5. RECIST criteria assessment of antitumor responses for prostate cancer immunotherapy cannot account for the extended survival of prostate cancer patients in the absence of high frequencies of complete and partial responses. Besides T-cell clonotype stability after ipilimumab treatment, what are the mechanisms for improved survival that might become actionable diagnostics for prostate cancer patients?

6. Tumor survival factors are keys in the prey–predator relationship that prostate cancer must manage against T-cell, B-cell, and NK-cell responses in the primary tumor site, and in metastatic vascularization and tissue matrix. What are these factors? What are the gene products essential for each pathway to immune evasion, and how can they be therapeutically thwarted?

7. How are preexisting high-avidity CD8 clonotypes generated stably, what are the prostate cancer–associated antigens, and what do they contribute in a minority of mCRPC patients who are benefiting and are now being seen weekly in the clinic at this writing (56)?

For basic immunologists, bioengineered murine models of prostate cancer have been gratifyingly predictive of signals that can be manipulated in prostate cancer immunotherapy trials in the clinic. Even the most nonimmunogenic, fastest-growing, hormone-refractory murine and rat models of prostate cancer can be rendered immunogenic in preexisting small tumor burdens, and can be cured experimentally with (i) GM-CSF–gene transduced irradiated vaccines; (ii) GM-CSF activated DCs and antigen loading; and (iii) anti–CTLA-4 inhibition and autologous tumor antigen presentation *in vivo*, and now with newer permutations of the addition of other checkpoint inhibitors, all of which can be studied in models that fairly well mirror much of the clinical reality. Efforts to define the functions of new molecules involved in the T-cell, B-cell, and NK-cell responses to prostate cancer tumor antigens from men with mPCA, and then to unleash and calibrate them, in real time in the clinic, will define the next decade of principles and practice of bioengineering prostate cancer immunity into cure.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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Prostate Cancer Immunotherapy: Beyond Immunity to Curability

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