

Future perspectives of prostate cancer therapy

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Abstract: We summarize several recent laboratory advances to tackle the problem of tumor-stroma-immune cell microenvironment interaction with the hope of developing and advancing new concepts and therapeutic strategies for prostate cancer therapy by improving bone and soft tissue metastases in prostate cancer patients. Given the emerging enthusiasm for immunotherapy in prostate cancer due to (I) improved understanding of the role of immune cells in the tumor microenvironment, (II) approval by the FDA of an immunotherapeutic drug to treat prostate cancer, and (III) recognition of immunotherapy as a novel approach to treat solid tumors by the Nobel Prize Committee (for discovery of dendritic cells that are used in immunotherapy), the field of tumor immunology is poised for growth in the next decade with the hope of developing new immunomodulatory drugs which will compliment and perhaps eventually replace traditional chemotherapeutic drugs. In this article, we provide a timely review of recent advances in the field of immunotherapy for prostate cancer, lessons learned from successes and failures, the contributory factors in the tumor microenvironment that could be rendered hostile to cancer cells, an exciting area of future research.

Keywords: Prostate cancer; cancer cells; stromal fibroblasts; vascular endothelial cells; immune cells

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Introduction

Prostate cancer is the second most common cause of cancer-related death in American men. Along with surgery or radiation therapy, hormonal therapy is a main mode of treatment. For men with metastatic disease, chemotherapy provides a significant survival advantage. Therefore, new treatment options are being actively pursued to extend the survival of metastatic cancer patients. In this review, we will focus on current advances in therapies that target cancer cells, outline recent advances in our understanding of the tumor microenvironment and its therapeutic implications for advanced metastatic prostate cancer patients and discuss the current therapeutic modalities, highlight new treatment options and offer future perspectives on prostate cancer therapy. We will discuss therapies that target: (I) cancer cells; (II) stromal fibroblasts; (III) vascular endothelial cells; (IV) immune cells and (V) less well-defined population of cells that contribute to the effectiveness of immunotherapy and cancer vaccines.

Targeting prostate cancer cells

Hormonal agents for prostate cancer therapy

Androgen and androgen receptor (AR) are required for normal prostate development and carcinogenesis. Castration-resistant prostate cancer tissues (CRPC) express AR and remain responsive to low levels of androgens. AR mutation, truncation and/or amplification may confer differential ligand and antagonist affinity and specificity. Thus, even low levels of testosterone could still activate the AR and confer the growth and survival advantages for prostate cancer cells. Several studies have demonstrated that low levels of testosterone are present in prostate cancer cells. Mohler *et al.* studied testosterone levels in clinical specimens collected from castrated patients who underwent prostatectomy and found that intratumoral testosterone levels were elevated despite an overall reduction in serum testosterone (1). Intracellular androgen in prostate cancer tissues has demonstrated clinical significance as

treatment with agents that reduce their levels have impacted overall survival for men with castrate-resistant disease. Abiraterone (Zytiga, Janssen Biotech, Horsham, PA) is a selective 17,20 lyase inhibitor, which inhibits the conversion of 17- α -hydroxyprogesterone to androstenedione. This agent was brought to a randomized phase III clinical trial against placebo in men with castrate-resistant disease who had received prior docetaxel. In this study, treatment with abiraterone was associated with a 35% reduction in death from prostate cancer with an improvement in median survival from 10.9 to 14.8 months (2). The survival benefit was observed across all subgroups analyzed, including number of prior chemotherapeutic regimens (one or two), type of progression (PSA versus radiographic), and patients with visceral metastatic diseases. Orteronel (TAK-700, Millennium Pharmaceuticals, Cambridge, MA) is another compound targeting 17,20 lyase and endogenous testosterone biosynthesis. In a phase I/II study of orteronel, of 43 patients with RECIST-evaluable disease, 6 showed a partial response, 23 had stable disease, and 9 showed progression (3). Phase III clinical trials with Orteronel are currently in progress in chemotherapy naïve and post-docetaxel settings. They will evaluate tumor response rate and survival benefit attributed to Orteronel therapy. Unlike abiraterone, it may be possible to administer orteronel without the use of concomitant prednisone given the higher specific inhibition of this agent against CYP17.

MDV3100 (Medivation, Inc., San Francisco, CA) is an orally bioavailable anti-androgen lacking the agonist properties of conventional non-steroidal antiandrogens such as bicalutamide (4). MDV3100 antagonizes AR action by preventing the translocation of the AR from cytoplasmic to nuclear compartment and by inhibiting DNA binding of AR and hence repressed the expression of androgen-regulated genes. In a phase I study of docetaxel-naïve and docetaxel-treated patients, 62% and 51% of patients, respectively, had at least a 50% PSA decline (5). Phase III randomized trials have been completed evaluating MDV3100 in both the pre- and post-docetaxel clinical spaces (1,3,6-10). The results from the completed post-chemotherapy studies (AFFIRM) will be presented in February 2012.

Novel cytotoxic chemotherapeutics to treat prostate cancer

Cabazitaxel (Jevtana, XRP6258, RPR 116258A; Sanofi-Aventis, Paris, France) is a semisynthetic taxane that has been shown to have activity against multidrug-resistant prostate cancer cell lines *in vitro* (11). This preclinical observation led to a randomized trial in patients with CRPC who failed docetaxel-based chemotherapy. Patients were eligible for

study if they had PSA progression, or with soft tissue and/or new lesions on bone scan. In this phase III trial, 720 patients were randomly assigned to receive either cabazitaxel, or mitoxantrone, every 3 weeks. The median survival for patients treated with cabazitaxel was 15.1 months, compared to 12.7 months in those patients treated with mitoxantrone with an overall 30 reduction in death from prostate cancer (12). In order to compare the efficacy of cabazitaxel/prednisone as first-line chemotherapy to the current therapeutic regimen, docetaxel/prednisone, an international randomized study is currently being designed at the mandate of the US Food and Drug Administration (12).

Antiapoptotic agents in prostate cancer

One unique feature of the androgen-independent prostate cancer cells is that the regression of prostate tumors still required an activation of apoptotic machinery. In many cases, AR blocking is capable of inducing apoptosis. Therefore, identifying a cure for prostate cancer requires identification and reversal of the apoptotic avoidance mechanisms, either AR-related or unrelated, responsible for drug resistance and/or newer therapies that bypass the apoptosis-resistance pathways. A number of antisense oligonucleotides targeting several anti-apoptotic genes, including BCL-2, BCL-XL, clusterin, the inhibitors of apoptosis (IAP) family, MDM2, protein kinase C- α , c-raf, insulin-like growth factor binding proteins and the AR, are being tested for potential clinical use in prostate cancer. Clusterin is a proapoptotic protein expressed in prostate, kidney, bladder, ovarian, lung, colorectal, and breast cancers. Clusterin expression increases with Gleason score, and is upregulated after androgen blockade (13,14). Clusterin modulates resistance to androgen blockade, radiation therapy, and chemotherapy. OGX-011 (Custirsen) is an investigational antisense compound that downregulates clusterin expression and enhances apoptotic death of prostate cancer cells (15). Increased apoptotic index of prostate cancer cells have been reported subsequent to clusterin inhibition. OGX-011/docetaxel/prednisone has been evaluated in combination with docetaxel/prednisone in men with CRPC (16). Although there was no difference was observed in time to disease progression (7.3 *vs.* 6.1 months), a superior survival was noted with OGX-011 (23.8 *vs.* 16.9 months) (7,17). In a randomized trial of Custirsen with docetaxel or mitoxantrone in patients who have progressed through docetaxel chemotherapy, the addition of Custirsen was well tolerated and appeared to improve pain response. In the population of men who had previous docetaxel, the addition

of OGX-011 yielded 23% partial radiographic responses by RECIST criteria and PSA declines in excess of 30% in 55% of the treatment arm (17).

Targeting tumor stromal fibroblastic microenvironments in prostate cancer

Tumor-stroma interactions are crucial for normal prostate development and neoplastic prostate progression. It has been demonstrated that fibromuscular stroma and stromal fibroblasts play a regulatory role in prostate development and prostate carcinogenesis. In these studies, urogenital sinus mesenchyme (UGM) or embryonic/adult stromal fibroblasts were shown to drive the growth of UG epithelium (UGE) and prostate cancer (18-22). Using a tissue recombination technique, it has been demonstrated that while UGM derived from AR-negative testicular feminized mice failed to induce prostate morphogenesis and cytodifferentiation, UGM isolated from AR-positive wild-type mice is competent in conferring growth and differentiation signals to UGE tissues by responding to androgen-regulated growth (23-28) and expression of differentiation-related genes regardless of their AR status. Results of these studies in aggregate suggest that AR signaling from the stroma is critical for the development and differentiation of the normal prostate epithelium (19,21). The inductive role of adult prostate stromal fibroblasts, promoting prostate cancer progression, was first demonstrated by our laboratory using cell recombination models (19,20,23-28). Specifically, the progression of prostate cancer from androgen-dependent to androgen-independent state and the acquisition of bone and soft tissue metastatic phenotypes can be achieved through cellular interactions between prostate cancer cells and organ-specific stromal fibroblasts including prostate or bone stromal cells in mice *in vivo* or when co-cultured these interactive cells under three-dimensional (3D) conditions (29-34). These findings, taken together, emphasized the important role of the stromal and tumor microenvironment in prostate cancer progression and hence the rationales for co-targeting tumor and stroma (20,22,34,35).

Stromal cells surrounding the cancer cells, including stromal fibroblasts, endothelial cells, and inflammatory cells in the primary and bone cells at the metastatic sites have been shown to exert directive action on prostate cancer cells by modulating reciprocally cancer cell growth, migration, invasion and metastasis. Impairment of reciprocal stromal or bone cell function and their communication with cancer cells could significantly impact the growth and progression of prostate cancer within the tumor microenvironments. *Table 1* summarizes several co-targeting strategies of cancer-

associated stroma, either in the primary tumor or in bone metastases that have been implemented in the clinic for improving the mortality and morbidity of prostate cancer patients. Future research on the specific mediators and cell signaling pathways regulating the reciprocal cellular communication between cancer cells and their immediate microenvironments and circulating factors in cancer and microenvironment cell milieu could further significantly improve our ability to target the progression of cancer and its lethal metastatic progression. For example, it has been established that immortalized stromal fibroblasts or cancer-associated fibroblasts (CAF) adjacent to tumors are morphologically and functionally distinct from normal stromal fibroblasts adjacent to normal epithelium (18,31). These cells exhibit marked differences in gene expression profiles and have been shown to predict the progression of prostate cancer (66). We demonstrated the reciprocal cellular interaction between prostate cancer and CAF or stromal fibroblasts from different zonal origin (31). Using marginally androgen responsive tumorigenic LNCaP human prostate cancer cells, we demonstrated that co-culture of the cancer cells with microcarrier beads previously seeded with prostate or bone stromal cells of the human prostate gland or human bone, under 3D culture system, led to permanent nonrandom genetic and phenotypic changes in both the cancer and the stroma. LNCaP cells derived from these growth conditions became androgen-independent and gained the ability to metastasize. Stromal fibroblasts that interact with cancer cells, also gained increased levels of brain derived neurotropic factor (BDNF), chemokines (e.g., CCL5 and CXCL5), versican, tenascin, stromal cell derived factor-1 (SDF-1/CXCL12), and transcription factors like HIF-1 α . These were validated using clinical tissue or serum samples obtained from prostate cancer patients with bone metastases. Studies from our group and others have demonstrated the role of stromal soluble factors such as VEGF, bFGF, HGF/SF, TGF- β , IGF-1, IL-6 and KGF, interacting with receptors on prostate cancer cells (refs). These studies highlight the bidirectional interactions and co-evolution of tumor-stroma in cancer progression (67). Therapies that target many of the stromal factors have been tested in preclinical models and in clinical trials to eradicate or delay the lethal progression of prostate cancer and other solid tumors to the metastatic phenotype.

Targeting angiogenesis in prostate cancer

Angiogenesis is essential for the growth and dissemination of

Table 1 Summary of pre-clinical and clinical studies in prostate cancer therapy

Agent	Mechanism of action	Clinical status	References
Abiraterone (anti-testosterone)	17,20 lyase inhibitor	Phase II studies completed	(1,2)
TAK-700 (anti-testosterone)	17,20 lyase inhibitor	Phase III trials ongoing	(3)
MDV3100 (anti-androgen)	Prevents androgen receptor translocation	Phase II trials ongoing	(3,6-10)
Cabazitaxel	Cytotoxic anti-microtubule agent	EU approved for CPRC patients	(11,12)
Docetaxel	Cytotoxic anti-microtubule agent	FDA approved for CPRC patients	(12)
OGX-011	antisense compound against clusterin	Phase II clinical trials complete for CRPC patients	(7,13-17)
Bevacizumab	Angiogenesis inhibitor (anti-VEGF antibody)	Phase II clinical trials ongoing for CRPC patients	(36-39)
Aflibercept	Angiogenesis inhibitor	Phase II clinical trials ongoing for CRPC patients	(40)
Cabozantinib	c-Met and VEGFR2 inhibitor	Phase III trials ongoing in bone metastatic patients	(41-43)
Atrasentan	ET-1A inhibitor (Endothelin inhibitor)	Phase II trials ongoing for CRPC patients	(44)
Dasatinib	Src kinase inhibitor	Phase III trials ongoing for CRPC patients	(45-48)
Denosumab	anti-RANK antibody	FDA approved for bone metastatic	(49-52)
Radium-223	alpha-emitter radioisotope	Phase III trials ongoing in bone metastatic patients	(53,54)
Tenascin inhibitors	anti-stromal agent	Clinical trials planned	(55)
Anti- β 2-microglobulin antibody	Blocks activity of β 2-M growth factor	Preclinical trials completed	(56)
AMD3100, NOX-A12, or CCX2066	anti-CXCL12 agents (targeting the stroma)	Clinical trials planned	(57)
CNTO 888	CCL2 chemokine inhibitor	Phase I clinical trials ongoing	(58,59)
Provenge	Immunotherapy (GM-CSF and PAP loaded DCs)	Approved by FDA for CRPC patients	(60-62)
PROSTVAC-VF	Gene therapy to deliver Poxvirus based PSA expression	Phase III trials ongoing	(63,64)
Ipilimumab (anti-CTLA-4 antibody)	Immunotherapy (checkpoint inhibitor)	Phase I clinical trials completed	(65)

prostate cancer cells. The process of blood vessel formation is regulated by complex interactions of vascular growth factors, including VEGF, matrix metalloproteins, and integrins. Inhibition of these proteins that support angiogenesis can block tumor growth as well as inhibit metastasis. Several studies demonstrate that circulating levels of VEGF were increased in patients with CRPC and serve as prognostic markers for patient survival (68). Microvessel density has been found to be increased in patients who have metastatic disease in comparison to those who have clinically localized cancer (36,37). Thus, the tumor vasculature appears to be a rational therapeutic target for men with prostate cancer. Significant work has been undertaken evaluating putative antiangiogenic agents. Early work with thalidomide showed activity as a single agent (38). This work has developed into a series of clinical studies supported by the intramural program of the National Cancer Institute including recent work with a combination of docetaxel, bevacizumab, and thalidomide (39). Bevacizumab, an antibody which blocks the binding of VEGF-A to the VEGF-R, is approved for use in non-small-cell lung and colorectal cancer (69). Other potent anti-angiogenic agents such as sorafenib (Nexavar), sunitinib (Sutent) (70), and aflibercept (VEGF Trap) (40) have shown the potential for benefit in this disease that is still under evaluation.

Targeting tyrosine kinases in prostate cancer

The efficacy of receptor tyrosine kinase inhibitors such as sunitinib or sorafenib has been disappointing in clinical trials for prostate cancer. Unlike other therapies, these agents have been associated with prolonged progression-free survival but no potent anti-tumor effect. A receptor tyrosine kinase inhibitor has a unique clinical phenotype that may potentially translate to therapeutic benefit. Cabozantinib (XL184, Exelixis, San Francisco, CA), is an orally available, multiple tyrosine kinases inhibitor. It inhibits activation of the c-MET protooncogene, as well as VEGFR2. In preclinical animal and cell models, cabozantinib exhibited potent dose-dependent cancer growth inhibition and tumor regression against a variety of solid tumors (41,42). Studies with prostate cancer specimens derived from primary tumors as well as bone, lymph node, and soft tissue metastases reveal that 51% of primary prostate cancer tissues expressed c-MET. In particular, osseous metastases from prostate cancers have been found to express significantly more c-MET than even soft tissue specimens (41,42). A 9-arm randomized discontinuation trial of cabozantinib which included patients with metastatic CRPC has been reported (43). In the CRPC arm, of the 168 patients enrolled, 100 were evaluable for response by RECIST (Response Evaluation Criteria in Solid

Tumors). Fifty-five of the 65 (85%) patients with serial bone scans showed complete or partial resolution of lesion as early as 6 weeks after starting therapy. Cabozantinib continues to be evaluated in prostate cancer as Exelixis has planned two phase III studies with this agent in prostate cancer that should begin in 2012- one evaluating pain response, the other evaluating the survival benefit associated with this agent.

Bone-directed targeting for treating prostate cancer bone metastasis

The endothelins (ET-1, ET-2, and ET-3), consisting of 21 amino acids, are expressed by endothelial cells; kidney and intestine; and brain, respectively. This class of peptides is known to control vasoconstriction, mitogenesis, and bone matrix formation with their actions mediated by ET receptors, ET_A and ET_B. The endothelin receptors are expressed in a variety of human tumors, including prostate cancer and osteoblasts. Interaction of endothelins with their receptors results in enhanced cell proliferation, bone matrix synthesis and deposition, and resistance to apoptosis in prostate cancer (41,44). Atrasentan, a specific ET-1_A inhibitor, exhibits anti-mitogenic activity, anti-osteoblastic activity, decreases rates of bone metastases, anti-angiogenesis activity, and blocks nociceptive effects.

Dasatinib, a tyrosine kinase inhibitor that inhibits the Src-family kinases (SFKs) has been studied in CRPC. SFKs are known to play an important role in bone resorption (71) and appear to be upregulated in advanced prostate cancer (45). Work by our group and others have pointed toward SFKs as regulators of metastatic behavior (46). A phase II study in metastatic CRPC demonstrated that 41% of patients have greater than 50% PSA decline with 35% reduction in bone turnover in 46% of patients (47). Bone alkaline phosphatase levels also were decreased in dasatinib-treated patients. Docetaxel was combined safely with conventional docetaxel therapy (48) showing again, potent effects on bone turnover. Based upon the preliminary data, a randomized phase III trial comparing docetaxel/prednisone in 1500 patients with CRPC, either with or without dasatinib was executed. Results from this study are pending and may be available in 2012.

The receptor activator of nuclear factor- κ B (RANK)/RANKL axis has been shown to play a critical role in maintenance of osteoclast and osteoblast function. Given the imbalance of activities between these cell populations in prostate cancer, RANKL has been considered an attractive target for therapy. This has been borne out in preclinical models of prostate cancer metastasis (49). Denosumab (Xgeva, Prolia; Amgen, Thousand Oaks, CA)

is a fully humanized monoclonal antibody that targets RANKL. The FDA initially gave approval for this agent in 2010 for the treatment of osteoporosis related to menopause. Subsequently, denosumab received approval for the treatment of skeletal related events in prostate cancer (50) and other solid tumors (51) and osteoporosis due to hormonal anti-cancer therapies in breast (52) and prostate cancer. Denosumab, targets RANKL axis was shown to delay the onset of bone metastasis and skeletal related events including the relief in the bone pain in men with CRPC who develop bone metastasis. Given the putative impact of RANKL on progression to osseous metastasis, a phase III trial of denosumab was initiated to test the hypothesis that treatment with denosumab would delay the onset of bone metastases in patients who were currently metastasis free (72). This study focused on a population of men at high risk for osseous metastasis (CRPC with serum PSA >8.0 ng/mL and/or PSA doubling time of <10 months). Treatment with denosumab was associated with a 15% reduction in the risk of bone metastasis with a median time to metastasis of 29.5 *vs.* 33.2 months in favor of denosumab.

Radioisotopes, such as strontium-89 (Metastron) and samarium-153-EDTMP (Quadramet), are approved for the palliation of bone pain in men with CRPC (73,74). Radium-223 chloride (Alpharadin; Algeta) is a selective α -emitter that has been evaluated in patients with CRPC. In contrast to the approved isotopes mentioned, improved survival was noted in patients treated with radium-223 when compared to placebo (65.3 *vs.* 46.4 weeks) in a phase II trial (53). As such a formal phase III study (ALSYMPCA) was initiated comparing radium-223 to placebo in men with bone metastases who had previously received docetaxel or were ineligible for docetaxel therapy (54). This trial was closed early by the independent data monitoring committee as criteria for a significant treatment benefit were reached. Treatment with radium-223 was associated with a 30% decrease in prostate cancer related death compared to placebo with median survivals of 14.0 *vs.* 11.2 months in favor of radium-223. Algeta has moved forward with their new drug application with hopes of approval in 2012.

Molecular therapeutics to co-target prostate cancer and cancer-associated bone cells

Cancer-host interactions play a fundamental role in directing cancer plasticity, progression, responsiveness, and resistance to treatments such as hormone therapy, chemotherapy and radiation therapy. We recommend that

future development of novel therapies should focus on the cancer-host interactions. This may improve treatment efficacy since the tumor-associated microenvironment may be protective to cancer cells, preventing the regression or apoptosis of treated tumors. Targeting only the cancer cells may not be sufficient since cancer cells and their associated stroma co-evolve. The field of tumor-stroma biology has expanded our understanding of cancer as more than a single cell disease. Rather, cancer development and progression involves reciprocal interaction and co-evolution between cancer cells and host stroma with reactive oxygen species, soluble growth factors, chemokines, cytokines and extracellular matrices serving as the key mediators. We and others have shown that cancer cells and their associated stroma are remarkably plastic and capable of expressing genes mimicking the tumor microenvironment. These new understandings of cancer-stroma interaction raise the possibility of co-targeting not only the cancer cell component but also cancer-associated stroma, and blocking not only autocrine but also paracrine cell signaling. Further expansion of our understanding of tumor-stroma biology could lead to the successful development of more effective animal models to study the mechanisms of prostate cancer metastases. This will be a novel step toward the discovery of more effective therapeutic interventions for prostate cancer metastases through the interruption of cancer-stromal fibroblasts, cancer-bone, cancer-endothelium, cancer-stem cell, cancer-nervous system and cancer-immune system communications (55,66,67).

Targeting the epithelial to mesenchymal transition (EMT) in prostate cancer

EMT is a highly conserved process where polarized immotile epithelial cells transition to motile mesenchymal cells. EMT is commonly associated with cancer migration, invasion and metastasis. The common feature of EMT is the loss of E-cadherin and an increased expression of vimentin and N-cadherin. In cancer, EMT could facilitate cancer aggressive behavior by infiltrating surrounding tissues and metastasize to soft tissues and bone. EMT can be enhanced by the augmentation of specific growth factor/growth factor signaling and hence can be targeted by growth factor receptor signaling or at the level of downstream cadherin-switch such as antibody against N-cadherin to prevent the switch between E-cadherin to N-cadherin (75). In prostate cancer, EMT has been described as a notable feature of the androgen-independent prostate cancer (ARCaP_E/ARCaP_M, C4-2/C4-2B, and PC-3) cell models and was confirmed in clinical

specimens and circulating tumor cells (CTCs) harvested from patients (32,56,66,76,77). RANKL is a potent paracrine factor for osteoclastogenesis and bone resorption. Under physiologic conditions, RANKL, expressed by osteoblasts, stimulates osteoclast maturation and bone resorption through the surface RANK receptor-expressing osteoclasts. We previously demonstrated that mesenchymal metastatic human prostate cancer cells (ARCaP_M cells) express higher levels of functional RANKL, capable of promoting osteoclast maturation (78). Interestingly, RANKL-derived from cancer cells can also promote the transition of ARCaP_E cells, with an epithelial phenotype, to express a mesenchymal phenotype, like those of ARCaP_M cells, thus suggesting autocrine function of RANKL in the induction of EMT. In experimental human xenograft and cell models, RANKL is a biomarker associated with EMT (78). Since RANKL is also expressed by the cells in the tumor microenvironments, such as osteoblasts; B- and T-cells, we observed that RANKL can also promote MET in androgen-sensitive LNCaP cells in both autocrine and paracrine manner, and drive their bone and soft tissue metastases through an activation of downstream c-MET signaling. We confirmed that this action of RANKL in promoting EMT and downstream c-MET is highly relevant in both experimental and human prostate cancer towards their development of CRPC phenotype (79) (and Chu *et al.*, poster presentation at the Cancer-induced bone disease meeting, November 30- December 3, 2011, Chicago, IL, USA). In addition to RANKL, prostate cancer cell lines and clinical samples are shown to secrete soluble factors such as β 2-microglobulin (β 2-M). This protein is not only responsible for driving EMT and bone metastasis of human prostate cancer cells but also in human breast, renal and lung cancer cells. The resulting ARCaP_M cells had high levels of the mesenchymal markers such as vimentin, N-cadherin and Snail and exhibit 100% incidence of bone metastasis in an intracardiac injection model. β 2-M interacts with its receptor, hemochromatosis (HFE) protein, to modulate iron responsive pathways in cancer cells. Inhibition of either β 2-M or HFE results in reversion of EMT (56,80,81). These results demonstrate the role of β 2-M in cancer metastasis and lethality. Thus, β 2-M and its downstream signaling pathways are promising prognostic markers of cancer metastases and novel therapeutic targets for cancer therapy. Preclinical studies in both immune-compromised and immune-intact mouse models of prostate cancer revealed anti- β 2-M monoclonal antibody significantly reduced tumor burden of primary tumors and bone metastasis ((56) and unpublished data). Currently,

humanization of anti- β 2-M is underway with the goal of initiating phase I clinical trials in prostate cancer patients with bone metastases. As such we propose that the addition to β 2-M-targeted therapy to RANKL inhibition may be an effective way to treat skeletal metastasis in human prostate cancer.

Targeting immune microenvironment of prostate cancer

Impairment of immune cell function in the cancer microenvironment is believed to be an important step in tumor progression. It is hypothesized that co-targeting of immune cells in addition to cancer cells will lead to better killing of cancer cells. Recent studies highlight the tumor-promoting role of myeloid and lymphoid cells in the progression of solid tumors, linking inflammation and cancer (82-91). Though studies from the last century reported that mononuclear cells infiltrate solid tumors, it took several years to establish that such cells are causally involved in tumor progression. This became possible due to the discovery, phenotypic and functional characterization of a variety of subsets of T cells, B cells, macrophages and dendritic cells facilitated by discovery of novel markers and use of cutting edge technologies including flow cytometry.

Most of the human solid cancers develop in immune intact human beings. The progression of tumors from low-grade, localized disease to metastasis involves an interaction between the tumor cells and the host immune system. Most of our studies performed with human prostate cancer cell lines in laboratories use immune-deficient athymic nude mice (which lack T cells), SCID mice (lacking B and T cells) or NOD-SCID mice (lacking B, T and NK cells). These immune-deficient mice have allowed human prostate cancer xenografts to grow, greatly facilitating pre-clinical studies of targeted cancer therapies. Given the recent evidence that a vast majority of solid tumors are infiltrated by immune cells that facilitate tumor growth (rather than suppressing the tumor growth), it is imperative to understand the biology of these immune cells in the context of the tumor microenvironment.

Role of T lymphocytes in prostate cancer

Evidence supports a close link between inflammation and prostate cancer have come from epidemiological studies which indicate that prostate cancer is more common in populations with more baseline inflammation (92). Both CD4⁺ and CD8⁺ T cells are present in prostate glands.

CD4⁺ T cells include both T helper 17 (T_H17) and regulatory T (T_{Reg}) cell populations. Prostate-infiltrating CD8⁺ T cells in humans are non-functional and do not upregulate activation markers. T cells surrounding cancer cells may upregulate negative inhibitory molecules that suppress their anti-tumor activity (93). T cells may become anergic or undergo apoptosis due to reactive oxygen species generated by cancer cells. Though T cells surround prostate cancer, increasing evidence suggests that either they exhibit suppressive properties (Tregs) or they become non-functional (CD8⁺ T cell), thus allowing prostate cancer to grow. Overall, research from human and mouse models supports a model where evolving tumors generate T cells with an anti-cancer potential but, in the absence of some intervention, such T cells exist in a non-functional or anergic state (86,94,95).

Role of macrophages in prostate cancer

Tumor associated macrophages (TAM) influence diverse processes such as angiogenesis, tumor cell proliferation, and metastasis during tumor progression and thus play a pro-tumorigenic role (96). TAMs have been shown to play a key role in tumor growth and spread. Macrophages secrete growth factors, including platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and transforming growth factor (TGF)-beta, as well as cytokines such as TNF-alpha and IL-1 that have been shown to promote metastatic spread in several animal models of tumors. In a variety of tumor types including prostate cancer, the amount of TAM has been associated with poor prognosis (97). One of the mechanisms involved in TAM-enhancement of cancer cell invasion involves a paracrine loop in which epidermal growth factor (EGF) produced by TAMs increases the invasiveness and migration of neighbouring breast cancer cells that express the EGF receptor (EGFR). Cancer cells in turn express CSF1, which acts as a potent chemoattractant and chemokine for CSF1R-expressing TAMs. This reciprocal cross-talk can be blocked by either EGFR or CSF1R antagonists, resulting in a decrease in migration and invasion of both cancer cells and macrophages (98).

Myeloid-derived suppressor cells

Another bone marrow-derived myeloid cell type (BMDC), which may share a common progenitor with TAMs, is the Myeloid-derived suppressor cells (MDSCs). MDSCs suppress the adaptive immune response by blocking the functions of CD4⁺ and CD8⁺ T cells, in part through arginase

and nitric oxide production, by expanding the regulatory T cell pool, and by inhibiting NK cell activation (99). MDSC levels are increased in the bone marrow, blood and spleen of cancer patients and tumor-bearing mice, and their accumulation is associated with tumor growth and malignant progression. Disruption of transforming growth factor- β (TGF- β) signalling, through *Tgfr2* deletion, was also shown to increase MDSC homing to tumors in a spontaneous mammary cancer model, an effect that was mediated through the SDF1-CXCR4 and CXCL5-CXCR2 (also known as IL8RB) chemokine axes (100-102). In prostate cancer, MDSCs are recruited to the bone and at the primary sites (103). Clinical trials are being planned to target CXCL12 chemokine in CRPC (57). Another cytokine secreted by prostate cancer cells which recruit myeloid suppressor cells include CCL2 against which blocking antibodies are being tested for its therapeutic utility in solid tumors (58,59).

Mesenchymal stem cells

Another cell type that resides predominantly in the bone marrow, although is not of haematopoietic origin, is the mesenchymal stem cells (MSCs). MSCs are multipotent cells that differentiate into osteoblasts, chondrocytes and adipocytes. MSCs are found in large numbers in primary tumors and, MSCs have been proposed as a cellular vehicle to deliver anti-cancer drugs into the tumor.

Immunotherapy for prostate cancer: Current success and future challenges

Recently, the FDA approved the first immunotherapy-based approach (sipuleucel-T, Provenge; Dendreon Inc) for treat patients with asymptomatic metastatic prostate cancer (60-62). In general, immunotherapy approaches seek to utilize the host immune cells to attack the underlying cancer. Despite a long history of negative clinical trials, in a definitive trial powered for overall survival, sipuleucel-T was associated with a 23% decrease in prostate cancer mortality despite the absence of alternation of progression free survival. This finding has created significant interest in this advancing area of cancer research. Due to a variety of mechanisms by which cancer cells evade immune surveillance, cancer therapy has for years centered on chemotherapy. These toxic chemicals are designed to be more lethal to the rapidly dividing cancer cells than on normal tissue. Unfortunately, normal cells often are killed along with the malignant cells. Professor Ralph

Steinman of Rockefeller University, a leading mind in cancer immunotherapy, identified the dendritic cell- a unique and important part of the immune cascade. For this Prof. Steinman was posthumously awarded the 2011 Nobel Prize in Medicine (63,104-107). The dendritic cell is one of the initial workhorses or sentinels of the immune system, processing foreign materials such as viruses and then presenting them to cytotoxic T cells that are activated in turn to attack the foreign antigens. Dr. Steinman isolated his dendritic cells, exposed them to his pancreatic cancer cells, and thus instructed his T cells to recognize those tumor antigens. The prognosis for the type of pancreatic cancer Dr. Steinman is only 4 months but Dr. Steinman survived for more than 4 years since he was first diagnosed with the disease (107). This immunotherapy regimen while not curative may have prolonged his life.

Prostate cancer immunotherapy seems promising and extends the mean survival of metastatic patients by an average of 4 months. Sipuleucel-T (Provenge), the first immunotherapeutic agent approved for the treatment of CRPC, is a dendritic-cell vaccine that is produced *ex vivo* from dendritic cells harvested from the patient in the clinic, which then are transported to a local GLP facility where the dendritic cells are loaded with a recombinant granulocyte macrophage colony-stimulating factor/prostatic acid-phosphatase fusion protein (61). These *in vitro* activated cells are reinfused into a patient. Side effects are modest, including fatigue, fevers, and chills at the time of infusion. Three randomized phase III trials comparing sipuleucel-T to placebo have been performed in patients with metastatic CRPC. In all three studies, those patients who were randomized to the placebo arm received a frozen dendritic-cell product at progression. Although the primary end point of progression-free survival was not met in either of the first two randomized trials, a survival benefit of 3-4 months was observed. The third randomized trial evaluating sipuleucel-T, involved patients (n=512) randomly assigned on a 2:1 basis to receive sipuleucel-T or placebo. A median survival benefit of 4 months was observed in favor of the patients receiving sipuleucel-T. At 3 years after study entry, 32% of patients treated with sipuleucel-T are alive compared to 23% of patients treated with placebo (60,62,64,108).

Vaccine based therapies are being currently under trials in CRPC. Two randomized trials using the allogeneic vaccine G-VAX viral vectors have failed to demonstrate a survival benefit (60,64,65,108). These viral vectors can mimic natural infection and, thus, boost the immune response. The viral vectors of poxvirus family have been used to deliver tumor (PSA) antigens as well as other

immunomodulatory factors. A clinical trial was performed in 125 asymptomatic minimal CRPC patients who received either PROSTVAC-VF (Bavarian Nordic, Kvistgaard, Denmark) or control viral vectors. Although progression-free survival was similar in both groups, patients treated with PROSTVAC-VF had an 8.5-month improvement in median survival (24.1 *vs.* 16.6 months in control patients) (109). A randomized phase III trial is underway evaluating the role of this vaccine in asymptomatic CRPC (64).

The clinical trials of sipuleucel-T demonstrated a statistically significant and clinically meaningful improvement in overall survival in patients with mCRPC (64). However, none of these studies showed a concomitant improvement in progression-free survival. When traditional cytotoxic therapies are evaluated, progression-free survival is considered as a critical endpoint to assess the efficacy of therapy. This apparent disconnect between *progression-free survival* and *overall survival* while comparing immunotherapy versus conventional therapy can be explained in many ways. Unlike chemotherapy drugs, the primary target of immunotherapy based drugs is not the tumor itself but the immune system which targets the tumor. It may take few weeks to few months to mount a clinically significant immune response following immunotherapy. However, a vaccine or immunotherapy induces what is called long-lived memory cells which persist in the human body in the lymphoid tissues for years with the potential to continuously generate cytotoxic T cells to act against tumors, resulting in a slowing of the tumor growth. This process is well documented in vaccines that target infectious diseases. For example, vaccines against pox viruses confer lifelong immunity due to persistence of memory B cells and memory T cells. In a tumor, there is a turnover rate of tumor cells which is influenced by tumor cell division, antitumor immune response, combined with factors introduced into the tumor environment (e.g., conventional therapies). An effective anti-tumor immune response may alter the tumor growth equilibrium so that more tumor cells are killed by the immune system. This effect takes time and may not translate into immediate goals of short-term (within 3-4 months) improvements in progression-free survival, but may be long-lasting and overall survival may ultimately follow. Additional approaches to measure intermediate endpoints are the need of the hour to measure the efficacy of immunotherapy-based drugs.

Checkpoint inhibitors

Cytotoxic T-lymphocyte antigen 4 (CTLA4) is a cell-

surface receptor expressed on the surface of helper T cells and interaction of CTLA-4 with its ligand downregulate T-cell responses. Ipilimumab is a fully human monoclonal antibody which attenuates negative signals provided to T cells through the cell surface molecule CTLA-4, thereby blocking a negative checkpoint. Blocking the negative checkpoint leads to activation of T cells which would then kill cancer cells. This antibody has been evaluated in patients with metastatic melanoma and demonstrated an improvement in survival of 4 months. Ipilimumab may be very effective in CRPC. In contrast to vaccine or dendritic-cell based therapy, decline in PSA levels have been observed with this antibody therapy (110). Recent phase III clinical trial data demonstrate that ipilimumab prolongs survival in patients with melanoma, and 2 phase III overall survival trials are investigating the activity of ipilimumab in patients with mCRPC (111-114). The first trial combines a single 8 Gy dose of radiation with either ipilimumab or placebo in the post-docetaxel space, and the second evaluates the activity of ipilimumab versus placebo in chemotherapy-naïve patients.

Combination therapies

The standard of care for men with mCRPC includes docetaxel and prednisone. It is time we consider combinations of chemotherapy and immunotherapy as well. Frequent administration of doses of docetaxel in combination with immunotherapy may be a rational approach. However, studies that combines vaccine with higher doses of docetaxel (chemotherapy) and prednisone (anti-inflammatory drug) leads to immunosuppression wherein immune-cells are depleted by this approach. One way to overcome this problem might be to administer immunotherapy first followed by chemotherapy to avoid the immunosuppressive effects of chemotherapy. This approach will also facilitate a proinflammatory microenvironment in which tumor-cell killing by chemotherapy can be boosted by cytotoxic T cell mediated tumor killing. Although overall survival has been the only endpoint to demonstrate clinical benefit in clinical trials of vaccine in prostate cancer, it is possible that combination studies of therapeutic vaccines with other modalities may lead to earlier discriminatory endpoints, such as time to progression or PSA response, which could accelerate clinical trials for improved personalized oncology. It is imperative to consider cancer vaccines or immunotherapeutic approaches at the earlier stages of disease in prostate cancer and it can also be considered as an ideal adjuvant therapy post-surgery or

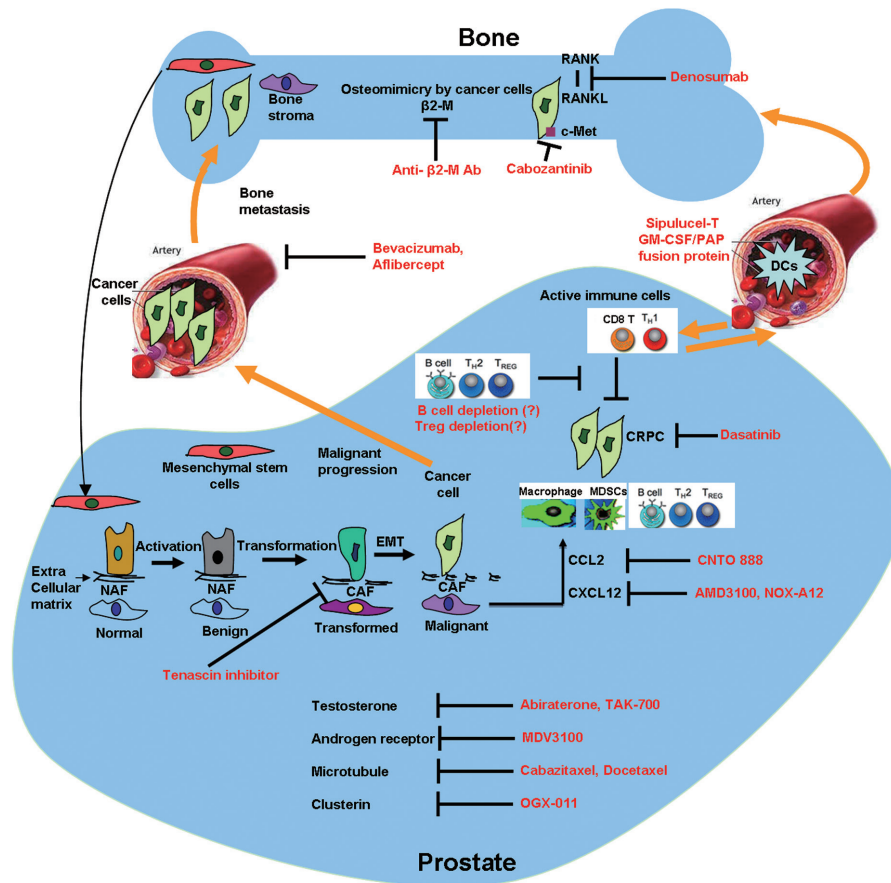


Figure 1 Diagrammatic illustration of therapies that target primary prostate tumor cells, CRPC, cells (stromal/endothelial/immune cells) and soluble factors in tumor microenvironment of the prostate and the bone.

-radiation in which presumably the bulk of the tumors have been removed and a smaller cluster of tumor cells may reside at the metastatic niche.

Summary and Conclusions

We have summarized the current approved treatments, ongoing clinical trials and preclinical studies in *Table 1* and *Figure 1*. Prostate cancer patients with metastatic disease are treated with androgen ablation therapy. These patients respond efficiently with improvement in bone pain, regression of soft-tissue metastases, and decreases in serum PSA levels. After a period of two years, nearly all patients progress to the castrate-resistant state. Until 2004, these patients were treated for symptoms with chemotherapeutic agents, such as mitoxantrone combined with prednisone, as well as isotope therapy or external-beam radiation therapy for painful bone metastases. Two new agents were approved by the FDA in 2010, cabazitaxel

(chemotherapy) and sipuleucel-T (immunotherapy), with abiraterone approved in 2011 all further boost the choice of drugs to treat this deadly bone disseminating disease (1,6-8). The primary endpoints for these drugs are vastly different as we discussed in detail earlier. The need of the hour is to research on exploration of novel biological markers to determine the appropriate drug to use in a given situation. Clearly, future studies, and eventually clinical practice, will need to incorporate newer imaging methods to track cancer cells, biological markers in blood, bone marrow, and circulating tumor cells, to determine the treatment efficacy of individual agent, or combination of hormonal agents, chemotherapy, immunotherapy and/or radiation therapy.

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Footnote

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