

Novel Therapeutic Approaches to Advanced Prostate Cancer

Andrew J. Armstrong, MD, and Michael A. Carducci, MD

Dr. Armstrong is a second-year Fellow in Medical Oncology at the Johns Hopkins School of Medicine in Baltimore, where Dr. Carducci is Associate Professor of Oncology and Urology and Co-Director of GU and Drug Development.

Address correspondence to:
Michael A. Carducci, MD, Prostate Cancer Research Program, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, CRB 1M88, 1650 Orleans Street, Baltimore, MD 21231; E-mail: aarmstr7@jhmi.edu.

Abstract: Considerable progress in the treatment of advanced prostate cancer was made in 2004 with the approval by the US Food and Drug Administration of docetaxel for the treatment of metastatic hormone-refractory prostate cancer. The survival benefit with docetaxel and prednisone, however, has been modest, on the order of 2–3 months compared with mitoxantrone and prednisone. While docetaxel-based therapy has demonstrated improvement in symptomatic and quality-of-life endpoints, certainly there is a pressing need for improvement in outcomes. A number of novel agents are in basic and clinical development for advanced prostate cancer, some of which are specific to mechanisms that may be important in the development and spread of prostate cancer. Novel approaches including immunotherapy, antiangiogenic compounds, and cell growth and survival pathway inhibitors, as well as targeted cytotoxic compounds, are among the broad categories that will be discussed in this review. Clinical advances in meaningful endpoints such as survival and quality of life are eagerly awaited in large-scale trials of active and rationally designed agents.

In 2004, docetaxel was approved in the United States for use in metastatic hormone-refractory prostate cancer (HRPC), based on the results of 2 large randomized multicenter trials.^{1,2} The overall survival of patients with HRPC has increased over the last several decades from less than 12 months to nearly 20 months and until recently this appeared to be primarily a result of stage migration and lead-time bias from early detection of disease recurrence and not from improvements in cytotoxic chemotherapy.^{3,4} While many patients experience a palliative benefit in terms of pain control and quality-of-life outcomes with docetaxel, the median time to progression is generally less than 6 months. The 2–3 month survival benefits reported in the 2 trials, TAX327 and Southwest Oncology Group trial 9916, were the first demonstrated improvements in overall survival with a cytotoxic agent and confirmed that docetaxel-based chemotherapy is the new standard of care with which to compare novel agents. Currently, over 200 novel therapies are being tested in patients with advanced prostate cancer, both in combination and as single agents.

Keywords

Prostate cancer, immunotherapy, angiogenesis, novel therapies, mTOR inhibitors, vaccines

Table 1. Overview of Rational Target Exploration in Prostate Cancer

Target Pathways	Examples of Agents in Development
Receptor tyrosine kinase	Imatinib, gefitinib, lapatinib, others
Proteasome	Bortezomib
Endothelin-1 axis	Atrasentan
PTEN/PI3 kinase/mTOR pathway	CCI-779, RAD001
Angiogenesis agents	Thalidomide, bevacizumab
Differentiation pathways	Vitamin D analogs, histone deacetylase inhibitors, methylation inhibitors
Apoptosis	Oblimersen sodium
Immunologic	Vaccines, thalidomide, anti-CTLA-4 combinations, lenalidomide, CC-407
Surface antigens	MLN 2704, MDX 070, radioisotope conjugates
Novel cytotoxics	Epothilones, satraplatin, nanoparticle albumin-bound paclitaxel

mTOR = mammalian target of rapamycin; PTEN = phosphatase and tensin homolog deleted on chromosome 10; PI = phosphatidylinositol.

Rational Target Overview

The consideration of potential targets for therapeutic trials of novel agents in prostate cancer requires an understanding of several tenets that are likely to be important in predicting the outcomes of clinical trials. The first is the demonstration of a mutation or functional dysregulation of the target in cancer cells as compared to normal tissue. Simply targeting overexpressed proteins has been less efficient and of lower yield than the specific targeting of novel mutations, which drive the bulk of the tumor growth.⁵⁻⁷ The second is target causality, indicating the importance of the target alone or in combination with other mutations in reproducing the phenotypic findings of prostate cancer. Ideally, there should be evidence from available preclinical models that inhibiting the target leads to tumor regression or quiescence. In prostate cancer, the androgen receptor is one such proposed target, due to the fact that it is mutated or overexpressed in many advanced prostate cancers, prostate cancer is extremely hormone-responsive, and prolonged clinical responses have been observed with androgen withdrawal therapy.⁸ However, it is unclear as to the causality of the hormone receptor in the pathogenesis of advanced or hormone-refractory prostate cancer, and clearly many patients clinically progress despite castrate levels of testosterone and multiple attempts at pharmacologic castration. Thus,

while it is important to continue to consider the androgen receptor in the pathogenesis and treatment of advanced prostate cancer, other targets must be sought and further understood for the rational design of therapeutics. Given the molecular complexity of the prostate cancer cell pathways and our overall current lack of understanding of the driving forces behind prostate cancer development and progression, the inhibition of multiple pathways may be required to produce sustained and clinically meaningful responses.⁹

The major pathways that are under therapeutic investigation in prostate cancer are those involved in growth and survival, chemotherapy and hormonal therapy resistance, angiogenesis, immune surveillance and escape, and stem cell renewal (Table 1). The following sections provide an overview of these pathways as they pertain to prostate cancer, specifically as rational targets, and the approaches that are currently being developed to block these pathways. Novel cytotoxic agents will also be briefly discussed.

Growth and Survival Pathways

While a prostate cancer stem cell has yet to be conclusively demonstrated, prostate cancer clearly progresses from an androgen-dependent tumor with features similar to the luminal differentiated glands of the prostate to a hormone-refractory tumor that has features of adult stem

cells, including antiapoptotic mechanisms, chemotherapy resistance, and reliance on non-hormonal signaling pathways. The exact pathways that advanced prostate cancers are reliant on remain to be determined, but candidates include hedgehog signaling, PI3 kinase/Akt signaling, mitogen-activated protein (MAP) kinase signaling, or a combination of these. Activation of the hedgehog-gli developmental pathway has been demonstrated in prostate cancer metastases and in preclinical models, and inhibition of this pathway in the laboratory has led to durable, complete responses, including the inhibition of prostatic epithelial regeneration following androgen withdrawal.¹⁰ Inhibitors of this pathway, such as cyclopamine analogs, are in preclinical development. Table 2 lists the most advanced agents in current clinical development; these agents will be further discussed below.

PI3 Kinase/Akt Pathway

In advanced prostate cancer, loss of the tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome 10) occurs in over 50% of metastatic lesions and in approximately 20% of localized tumors.^{11,12} Mutation in or loss of even a single allele of PTEN correlates with advanced Gleason score, stage, chemotherapy resistance, and other features of advanced prostate cancers.¹¹ PTEN is a negative regulator of the PI3 kinase/Akt cell survival pathway, and advanced prostate cancers frequently have elevated levels of phosphorylated (activated) Akt.^{13,14} The Akt pathway is involved in signal transduction from multiple cell surface receptors, including the insulin receptor, epidermal growth factor receptor (EGFR), insulin-like growth factor receptor (IGFR), platelet-derived growth

factor receptor (PDGFR), and interleukin (IL)-6R, and likely functions as a cellular sensor for nutrient and growth signals.¹⁵ In addition to promoting cell survival through the inhibition of apoptosis, the Akt pathway regulates cell growth, proliferation, and angiogenesis through the mammalian target of rapamycin (mTOR) pathway and the facilitated translation of several downstream signals such as c-myc, cyclin-D, and vascular endothelial growth factor (VEGF; Figure 1).^{13,16} Restoration of functional

Table 2. Novel Growth Factor and Cell Cycle Inhibitors in Clinical Development for Prostate Cancer

Target	Agent and Current Phase of Clinical Evaluation
PDGF receptor	Imatinib and docetaxel, phase II
Proteasome	Bortezomib and docetaxel, phase II
EGF receptor	Gefitinib/erlotinib and docetaxel, phase I-II
Akt/PI3 kinase/mTOR	CCI-779 and RAD001 with docetaxel, phase I-II
Endothelin receptor	Atrasentan with (HRPC) or without (rising PSA only) docetaxel, phase III

EGF = epidermal growth factor; HRPC = hormone-refractory prostate cancer; mTOR = mammalian target of rapamycin; PDGF = platelet-derived growth factor; PSA = prostate-specific antigen.

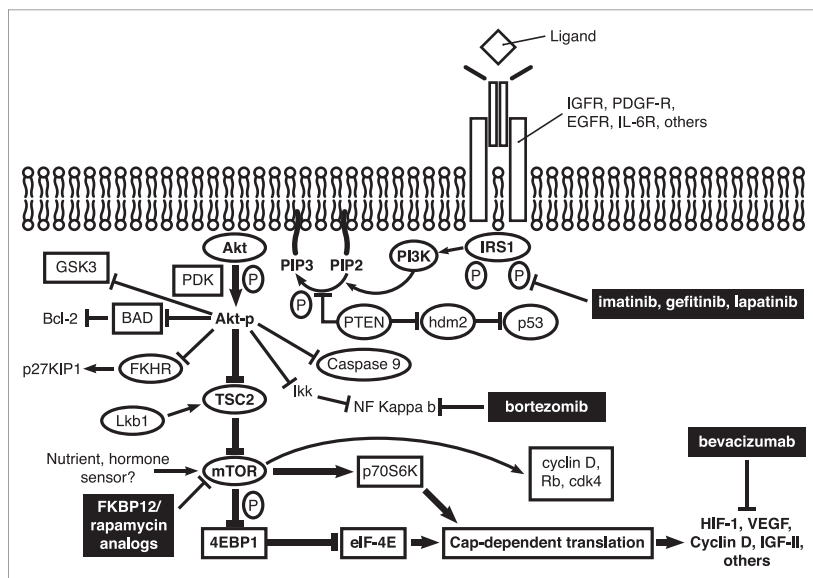


Figure 1. The PI3 kinase/Akt/mTOR pathway in human prostate cancer, with select targets for therapy in current clinical development. Arrows represent protein pathways that involve activation/phosphorylation while T-bars represent inhibitory signals. Some pathways are intentionally abbreviated for the purposes of simplicity.

PTEN activity or inhibition of mTOR activity has been shown to inhibit the growth of prostate cancer xenografts lacking PTEN and restore chemotherapeutic and, potentially, hormonal sensitivity.¹⁷⁻²⁰

Rapamycin is a natural compound isolated by Sehgal on Easter Island (also known as Rapa nui), and is derived from soil samples containing the bacterium *Streptomyces hygroscopicus*.²¹ It was initially discarded as an antifungal agent due to its immunosuppressive properties but was later revived as a potent immunosuppressive agent for use in solid organ transplantation. Its antiproliferative properties and antitumor activity in National Cancer Institute (NCI) cell lines led to its clinical development in cardiology as a means of preventing stent restenosis and in oncology where a wide variety of tumors were found to exhibit sensitivity to this agent and an analog, CCI-779 (Temozolimus, Wyeth).^{22,23} Phase I trials of CCI-779 have demonstrated safety and sufficient target inhibition at a wide range of doses, from 25 mg/m² to 250 mg/m² weekly and at a 5-times-daily schedule.^{24,25} No immunosuppressive effects were seen with the most widely studied delivery schedule of CCI-779, given as a 30-minute weekly infusion. Likewise, a phase I study of an oral rapamycin analog, RAD001 (Everolimus, Novartis), given once weekly, demonstrated similar safety with sustained target inhibition occurring at doses above 20 mg.²⁶ Phase II trials of the mTOR inhibitors CCI-779 and RAD001 are in development. Pharmacodynamic approaches correlating clinical responses to pretreatment levels of activated Akt and post-treatment downregulation of well-validated downstream targets such as S6 kinase and 4EBP1 levels should aid in these agents' rational development.^{27,28} Reviews of this emerging pathway and potential resistance mechanisms in prostate cancer have been published extensively.^{12,23,29,30}

Toxicities with rapamycin and rapamycin analogs have been predictable and often not dose-related, and include maculopapular rash, hypertriglyceridemia, allergic reactions, mucositis, and thrombocytopenia.²⁴⁻²⁶ The ideal combination of these active agents with docetaxel, given their ability to induce apoptosis when given in combination with chemotherapy, is being investigated in phase I and II trials.¹⁹ It is conceivable that this form of therapy will be best suited to those patients with advanced prostate cancer who also have demonstrable activation of the Akt pathway, either as a result of PTEN mutation or loss, or other genetic alterations. However, upstream pathways that are left untouched by mTOR blockade, such as the apoptotic regulators BAD, caspase-9, and nuclear factor (NF)-kappa B and the cell cycle inhibitors p53 and p27KIP1 (Figure 1), may prove to be important targets as well in prostate cancer.

EGFR/PDGFR Pathway

The rapid development in the last several years of small molecule tyrosine kinase inhibitors has yielded impressive results in various types of cancers, from non-small-cell lung cancer (NSCLC) in nonsmokers, chronic myelogenous leukemia (CML), and gastrointestinal stromal tumors (GIST).^{5,7,31} Demonstration of response in these tumors has usually correlated with mutation in the target tyrosine kinase, such as EGFR, BCR/ABL, and c-kit, respectively. In these cases, the target mutation has likely played a central role in the pathogenesis of these tumors. In prostate cancer, however, no such mutation has yet to be identified, and indeed, early trials of single-agent tyrosine kinase inhibitors such as gefitinib (Iressa, AstraZeneca) have been disappointing.

EGFR is overexpressed in 40–80% of prostate cancer cells and overexpression may be more common in African American men with prostate cancer.³² Despite preclinical data indicating a correlation of EGFR expression with Gleason score and androgen independence, single agent clinical experience has been disappointing but not altogether unexpected. The EGFR tyrosine kinase inhibitor gefitinib was tested in 2 phase II trials in HRPc and found to have little to no activity, with no significant prostate-specific antigen (PSA) declines in about 100 patients.³³⁻³⁵ Gefitinib resistance may be related to over-activity of the PI3 kinase/Akt pathway in prostate cancer, and thus combinations of agents that target multiple pathways may be more beneficial.³⁶ Indeed, dependence on the Akt pathway has been demonstrated in EGFR-mutated NSCLC cell lines.³⁷ Further trials of combination EGFR or dual kinase inhibitors with chemotherapy or other novel agents are in development.

Prostate cancer cells express high levels of PDGFR, and this signaling pathway utilizes the PI3 kinase/Akt pathway, which has been implicated in prostate cancer progression. A phase I study of imatinib (Gleevec, Novartis), a small molecule PDGFR tyrosine kinase inhibitor used to treat CML and GIST, has been conducted in advanced prostate cancer, alone and in combination with weekly docetaxel.³⁸ The combination of imatinib 600 mg and docetaxel 30 mg/m² weekly for 4 out of 6 weeks demonstrated a greater than 50% PSA reduction in 8 of 21 patients (38%), with several durable (>18 month) responses.³⁸ A randomized trial of this combination sponsored by the NCI Prostate Cancer Specialized Program of Research Excellence (SPORE) clinical consortium is underway.

Differentiation Therapy

The ability to terminally differentiate tumor cells into benign cells that lack the ability to proliferate, invade, and metastasize has been a long sought-after novel

approach to cancer therapy. Its clinical utility has been best demonstrated in acute promyelocytic leukemia with all-*trans*-retinoic acid, but other agents with the ability to differentiate neoplastic cells are in development. Beer et al³⁹ have demonstrated clinical activity of one such agent in prostate cancer, the calcitriol analog DN101. While the exact mechanisms are unknown, vitamin D analogs may have differentiation, antiproliferation, and chemosensitizing properties making them promising and well tolerated agents in combination with chemotherapy. Case-control studies have shown that 1,25-dihydroxyvitamin D levels were inversely associated with prostate cancer development, and preclinical models have demonstrated the ability of 1,25-dihydroxyvitamin D to upregulate androgen-responsive genes and induce differentiation and apoptosis of prostate cancer cell lines.^{40,41} A phase II trial of weekly docetaxel (36 mg/m² on day 2) and high-dose oral calcitriol (0.5 µg/kg on day 1) given 6 out of 8 weeks demonstrated PSA responses in 30 of 37 patients (80%) and measurable responses in 8 of 15 (53%), with a median time to progression of 11.4 months and median survival of 19.4 months.³⁹ Given these favorable results, the phase III randomized ASCENT trial was recently completed and results are forthcoming.

Other differentiation strategies include inhibitors of histone deacetylase and DNA methyltransferase, enzymes that are responsible for the silencing and repression of gene expression, and are in early clinical development. Histones maintain DNA in a closed, coiled configuration, and this activity is mediated by a reversible acetylation process in which acetylation of lysine residues on select histone tails favors transcription.⁴² Aberrant hypermethylation and gene silencing of specific promoter regions in prostate cancer has been described, such as the antioxidant enzyme GST-PI1, and may represent a common mechanism of tumor suppressor loss.⁴³ The rationale behind use of these agents is their ability to induce broad gene re-expression in preclinical models of prostate cancer, in addition to the multiple effects that have been seen on prostate cancer cell lines, including induction of apoptosis, upregulation of p21WAF1 signaling, cell cycle arrest, and reduced DNA synthesis.⁴⁴ However, the specificity of these agents for those proteins lost in prostate cancer is unclear, and single-agent activity has been limited in early phase I trials.⁴⁵ Novel combinations of agents that inhibit histone deacetylation or DNA methylation with cytotoxic agents or other novel compounds such as the differentiating agent 13-*cis*-retinoic acid are an active area of clinical investigation.⁴⁶

Endothelin-1 Axis

While the endothelin (ET) receptor may seem an unlikely target in prostate cancer given its predominantly vascular

role, ET-A receptors are overexpressed in prostate cancer and higher plasma endothelin levels have been shown to correlate with tumor stage, grade, and metastases.⁴⁷⁻⁴⁹ Endothelin-1 is a potent vasoconstrictor and one such antagonist (bosentan [Tracleer, Actelion]) has been developed for the treatment of pulmonary hypertension; however, the endothelin family (ET-1, -2, and -3) has also been shown to have multiple effects on cellular physiology and paracrine signaling in prostate cancer. Endothelin-1 is known to influence cell growth via the MAPK pathway and is co-mitogenic with additional growth factors such as IGF-I and -II and it has been shown to regulate apoptosis, perhaps through its interactions with the bcl-2 and PI3K/Akt pathways.⁴⁷⁻⁴⁹ ET-1 also has been shown to regulate angiogenic and osteoblast activity and is likely involved in the paracrine signals between osteoblasts and prostate cancer cells that regulate the development of painful bony metastases in prostate cancer.⁴⁸⁻⁵¹ Thus, the endothelin axis is a rational target for the interference of prostate cancer and bone stromal interactions.⁴⁸⁻⁵¹

Atrasentan (Xinlay, Abbott Labs) is a highly selective ET-A receptor antagonist and is the most clinically developed agent of this class in prostate cancer.⁵² In dose-ranging trials of this agent, the 10-mg dose was found to prolong time to progression (TTP) in the fully evaluable subset of men with metastatic HRPC by 67 days when compared to placebo (196 vs 129 days, respectively, $P=.021$).^{50,53} Adverse events with atrasentan were mild and related to vasomotor reactions, including headache, rhinitis, flushing, and peripheral edema. Favorable effects were seen in markers of bone deposition and resorption, which led to its further clinical development.

In the phase III trial, 809 patients with metastatic HRPC were randomized to placebo or 10 mg of oral atrasentan, with the primary clinical endpoint being TTP. Although TTP with atrasentan was not found to be statistically significantly different from placebo in the intent-to-treat analysis, several secondary endpoints indicated clinical activity, including improvements in quality-of-life and pain scores, and reductions in the rise of laboratory markers including alkaline phosphatase and PSA.⁵⁰ A robust meta-analysis of pooled phase II and III data of similarly treated patients with similar baseline characteristics increased the power of the original planned analyses and demonstrated a significant increase in TTP ($P=.013$), which translated to a 19% reduction in risk of disease progression. The median pain-free duration was found to be prolonged in this meta-analysis by 100 days, and patients taking atrasentan remained pain-free for a longer period of time than those taking placebo (224 vs 127 days, respectively).⁵⁰

These results clearly point to biologic activity of the endothelin axis in modulating osteoblastic metastases,

but underscore the difficulty in studying bony lesions and measuring bony progressive disease. Abbott has filed for US Food and Drug Administration (FDA) approval for atrasentan based on these results, and a large phase III trial of atrasentan in asymptomatic men with a rising PSA after local therapy is ongoing.⁵⁰ Another ET-A receptor antagonist, ZD4054 (AstraZeneca), is in early phase II clinical development at this time.⁴⁹

Proteasome Inhibitors

The ubiquitin-proteasome degradation pathway is an essential component of normal cellular machinery for the processing and recycling of proteins, including those active in cell-cycle progression, checkpoint regulation, and cell death.⁵⁴⁻⁵⁶ Through the specific utilization of ubiquitin ligases directed at unique proteins, cellular machinery components are targeted for destruction, including proteins that may be important to prostate cancer progression, such as the cyclin E-cdk2 inhibitor p27KIP1, and an inhibitor of NF-kappa B, IKK.⁵⁷ Targeted inhibition of this cellular wastebasket machinery may be advantageous due to the combined inhibition of multiple targets involved in the promotion of cell cycle progression, tumor growth, and resistance to cell death.

Bortezomib (Velcade, Millennium), a boronic acid derivative, was the first proteasome inhibitor to be tested and approved for use in humans, specifically in multiple myeloma.⁵⁶ Its use as a single agent in solid tumors, including 48 patients with HRPc, has been investigated in a phase I trial. The maximum tolerated dose of intravenous bortezomib was 1.6 mg/m² weekly; the agent was well tolerated with adverse events including hypotension, fatigue, diarrhea, and nausea.⁵⁵ PSA responses were modest with single-agent use, with only 2 patients demonstrating significant PSA declines and 2 patients demonstrating partial clinical responses.

In a phase I/II trial of bortezomib and docetaxel in patients with HRPc, bortezomib was given at 1.3 mg/m² on days 2 and 9 with docetaxel given at 40 mg/m² on days 1 and 8. Combination therapy resulted in significant PSA declines in 8 of 22 patients (36%) and partial clinical responses in 2 of 12 patients (17%) with measurable disease.⁵⁸ Given that many of these patients had progressed on taxanes, this regimen may represent an improvement over docetaxel alone and thus further trials of combination therapy are being pursued.

Apoptosis Pathways

While androgen ablation induces apoptosis of prostate cancer cells, over time this effect is diminished as antiapoptotic machinery, including *bcl-2*, is activated.⁵⁹ Overexpression of *bcl-2* has been found to correlate with progression to the hormone refractory state and may

also correlate with resistance to chemotherapy as well.⁵⁹ Interestingly, taxanes have been shown to inhibit *bcl-2* phosphorylation directly.⁶⁰ Novel compounds such as the antisense oligonucleotide oblimersen sodium (Genasense, Genta) that target *bcl-2* are in phase I-II trials in combination with cytotoxic therapy.⁶¹ Phase I studies of oblimersen as a single agent in advanced prostate cancer have not demonstrated clinical benefit despite the successful targeting of *bcl-2*.⁶²

Oncolytic viruses have also been exploited as a form of prodrug delivery for the induction of selective apoptosis in prostate cancer cells.^{63,64} Oncolytic adenovirus CV787 utilizes prostate-specific enhancer and promoter sequences to localize the lytic cycles to the prostate or to deliver proapoptotic messages, such as Bax.⁶⁵ Single-agent and combination trials of these cytoreductive gene therapy approaches remain in clinical development.⁶⁶

Immunotherapy

Entraining the host immune system to reject its own developing tumor has been a long sought-after goal of cancer therapy since the original theories of immune surveillance were proposed more than 30 years ago by Paul Ehrlich, Sir MacFarlane Burnet, and Lewis Thomas.^{67,68} Active immunotherapy with vaccination against tumor antigens has been pursued in many different cancer models with a variety of techniques, including dendritic cell-based therapies; novel adjuvants such as bacillus Calmette-Guerin (BCG), granulocyte-macrophage colony-stimulating factor (GM-CSF), or viral carriers; single-antigen or whole-cell vaccines; and genetically modified tumors.⁶⁹ More recently, as our knowledge of danger signals and the link between the innate, adaptive, and regulatory immune response increases, combination therapies using co-stimulatory molecules, CTLA4 blockade, Toll-like receptor agonism, and intracellular viral or bacterial mediators have been developed.⁶⁹⁻⁷²

In prostate cancer, several vaccine strategies have moved forward into rational clinical development. These include the Provenge (Dendreon) autologous prostate acid phosphatase (PAP)-loaded dendritic cell vaccine, the GVAX (Cell Genesys) allogeneic recombinant whole-cell vaccine, the Prostavac-VF recombinant vaccinia/fowlpox PSA vaccine, and the BLP25 MUC1 liposomal vaccine. While each vaccine strategy is designed to stimulate the immune system to overcome toleragenic prostate cancer cells, there are important differences in their approaches, summarized in Table 3.⁷³⁻⁷⁶ Provenge and GVAX are currently in phase III trials of men with HRPc and will be discussed briefly.

Provenge is a vaccine derived from CD54(+) dendritic cells, the major antigen-presenting cells, which

Table 3. Comparison of Selected Current Prostate Vaccine Strategies

Vaccine Type	Development Phase	Delivery	Schedule	Specific Comments
Allogeneic GM-CSF secreting vaccine (Prostate GVAX)	Phase II/III randomized vs docetaxel	Intradermal	Every 2 weeks × 13 doses after prime dose	AAV retroviral gene transfer, injection site reactions, multiple injections, GM-CSF secreted locally. LNCaP and PC-3 cells are allogeneic source material.
Vaccinia/fowlpox PSA vaccine (Prostvac-VF)	Phase II randomized vs placebo	Intradermal	4 vaccines every 6 weeks	No nuclear integration, no systemic shedding of virus. Injection site reactions and hyperglycemia, no autoimmunity. Uses 3 co-stimulatory molecules with PSA: LFA3, ICAN-1, and B7.1 GM-CSF given systemically.
Autologous PAP-loaded dendritic cell vaccine (Provenge)	Phase III randomized for those with Gleason score ≤7 asymptomatic HRPC vs placebo	Intravenous	Every 4 weeks × 3 doses	Autologous CD54(+) dendritic cells, requires PAP positivity. Requires pheresis, individual vaccines. Immunogenic and safe in phase II trial.
BLP25 MUC1 liposomal vaccine	Phase II single-arm open label	Subcutaneous	Weeks 0, 2, 5, 9	Liposomal mucinous carcinoma-associated glycoprotein being studied in men with rising PSA after radical prostatectomy.

AAV = adeno-associated virus; GM-CSF = granulocyte-macrophage colony-stimulating factor; PAP = prostate acid phosphatase; PSA = prostate-specific antigen.

are pheresed from individuals and processed with the recombinant fusion protein PAP and GM-CSF. PAP was chosen based on its prostate cell membrane localization and the success of preclinical models using it to generate prostate-specific immune responses and autoimmune prostatitis.⁷³ In a phase II study of 31 patients receiving every-3-month infusions and a final 24th-month boost, 3 patients experienced a PSA decline greater than 50%.⁷³ This led to a large randomized, placebo-controlled phase III trial in 127 asymptomatic men with metastatic HRPC (PAP positive). At the most recent 3 year update, a statistically significant improvement in survival was seen regardless of original Gleason score despite earlier reports of survival benefits being limited to an unplanned subgroup of men with tumor Gleason scores of 7 or less.^{77,78} While preparation and production of large scale quantities of individually tailored vaccine may be difficult, this vaccine was well tolerated, with minimal infusion-related fever and rigors being the predominant adverse events. Immunologic correlates are forthcoming but were initially reported to be more robust in those with more well-differentiated tumors, suggesting that those at highest risk may not obtain as great a benefit.⁷⁹ A larger confirmatory phase III trial is underway in men with metastatic HRPC

who have a Gleason score of 7 or less, and the FDA has designated fast-track status to this vaccine.

Prostate GVAX is supported by the demonstration of improved immune mediated tumor rejection in mouse models of melanoma when an irradiated tumor vaccine expressed GM-CSF as compared to other transduced cytokine adjuvants.⁸⁰ Given that GM-CSF likely facilitates the maturation and activation of dendritic cells, further work extrapolated these findings in mouse models of prostate cancer with results showing prolonged survival and tumor regression.⁸¹ In a phase I study, autologously derived vaccines were generated and expanded in 8 of 11 patients and T-cell responses were generated in 7 of these 8 following vaccination every 21 days until supply depletion.⁸² No clinical responses were observed, and the inherent lack of feasibility of this approach led to the pursuit of allogeneic vaccine strategies using similar technologies. A phase II study of prostate carcinoma cell lines (PC-3 and LNCaP) virally transduced to secrete GM-CSF and lethally irradiated was conducted in 34 patients with metastatic HRPC.⁷⁴ At the highest doses of vaccine (300 million cells), trends toward improved TTP were seen without any observed dose-limiting toxicities or autoimmune phenomena. Median survival in this trial

was 26 months, historically very favorable. A further evaluation of 80 patients with metastatic HRPc treated at higher doses demonstrated 1 partial PSA response and improvement in markers of bone turnover, with survival analysis still ongoing.⁸³ A phase III trial of GVAX versus docetaxel in minimally symptomatic men with metastatic HRPc is currently open for enrollment.

Angiogenesis Targets

The directed formation of tumor microcirculation and recruitment of blood vessels from established vasculature represents a novel target for cancer therapy. As tumors outgrow their own supply of nutrients by diffusion, an angiogenic switch is activated, resulting in the proliferation of endothelial cells, accelerated delivery of nutrients, and tumor proliferation.⁸⁴ The inhibition of tumor proangiogenic factors without affecting normal host vasculature may selectively prevent tumors from progression beyond this critical threshold. Prostate cancer, like many other solid tumors, requires blood vessel formation for the development of metastases and for clinical progression, and elevated levels of the potent angiogenic molecule VEGF have been shown to correlate with advanced clinical stage and survival.^{85,86} While VEGF certainly has other properties that may be important, such as immunomodulatory activity and reductions in capillary permeability and interstitial fluid pressure, it is likely a rate-limiting step in the formation of new blood vessels.⁸⁷⁻⁸⁸ The initial proof-of-concept clinical trial was in metastatic colorectal cancer, where the addition of bevacizumab (Avastin, Genentech), a monoclonal antibody to VEGF, to 5-fluorouracil and irinotecan (Camptosar, Pfizer) prolonged survival by several months.⁹⁰

In a retrospective study of archived serum samples from a Cancer and Leukemia Group B study (CALGB 9480), VEGF levels were independently associated with survival in prostate cancer.⁸⁵ Similarly, antibodies to VEGF have slowed prostate xenograft growth rates, especially in combination with chemotherapy.^{91,92} These observations led to the phase II trial CALGB 90006, which added bevacizumab to docetaxel and estramustine in men with metastatic HRPc. Bevacizumab was given at 15 mg/m² on day 2, estramustine as 280 mg orally 3 times daily on days 1–5, and docetaxel at 70 mg/m² on day 2 every 3 weeks. Prophylactic dexamethasone and low-dose warfarin were also given.⁹³ Among 79 treated patients, a greater than 50% PSA decline was seen in 81%, median TTP was 9.7 months, and overall median survival was 21 months. These favorable results have led to a phase III trial of bevacizumab with docetaxel in HRPc that will omit estramustine given its well-described cardiovascular risk. Bevacizumab is also being tested in conjunction

with dendritic cell vaccination in a phase II trial as a test of the ability of VEGF inhibitors to promote dendritic cell maturation.⁹⁴

Thalidomide (Thalomid, Celgene) was originally developed in the 1960s as a treatment for morning sickness and subsequently linked to teratogenic effects resulting in dysmyelia, or limb shortening. While the exact mechanism of teratogenesis remains unproven, the metabolites of thalidomide have been shown to inhibit angiogenesis through multiple potential mechanisms, included inhibition of proangiogenic signals such as VEGF, basic fibroblast growth factor, IL-6, and tumor necrosis factor (TNF)-alpha.^{95,96} Additionally, thalidomide has been shown to have T cell co-stimulatory activity and is thus felt to be an immunomodulatory agent as well.⁹⁶ For these reasons, thalidomide has been evaluated in prostate cancer with promising early results. Single-agent trials have yielded durable but overall low response rates on the order of 18% for PSA declines despite the use of high doses of thalidomide.^{95,97} In a randomized phase II trial of weekly docetaxel and low-dose thalidomide versus docetaxel alone, 53% of 75 patients experienced a greater than 50% PSA decline, with progression-free survival of 5.9 months and overall 18 month survival of 68%.⁹⁸ While this trial was underpowered to detect a difference against the standard arm, thalidomide's clinical activity and manageable toxicity has led to the development of further more potent thalidomide analogs for combination therapy.

Clinically significant deep vein thrombosis developed in 12 of the first 43 patients in the phase II trial, but after the institution of prophylactic low molecular weight heparin, no thromboses were recorded.⁹⁸ In addition, sedation, neuropathy, constipation, and fatigue have been dose-limiting side effects in these trials. Thus, further trials of thalidomide are likely to use prophylactic anticoagulation and lower thalidomide doses in combination with docetaxel.

Newer thalidomide analogs with immunomodulatory features have been developed that lack the neurotoxicity of thalidomide but retain many of the T-cell modulatory functions, antiangiogenic properties, and even direct pro-apoptotic functions.^{96,99} Second-generation compounds lenalidomide (Revlimid, Celgene) and CC-4047 (Actimid, Celgene) cause significantly more potent TNF-alpha inhibition than the parent compound, and clinical testing with these agents in phase II trials has begun.

Monoclonal Antibodies and Targeted Cytotoxic Agents

Several compounds in early clinical development in prostate cancer include those that use monoclonal antibody

technology and tumor-associated antigen specificity to target prostate cancer cells or enhance drug delivery to prostate cancer cells. Phase II testing is underway for several of these agents, including antibodies to PSMA (prostate surface membrane antigen), MUC-1, and CTLA-4 (cytotoxic lymphocyte-associated antigen-4). Passive immunotherapy with monoclonal antibodies may lead to cell death through a variety of mechanisms, such as antibody-dependent cellular cytotoxicity, complement fixation, and T and B cell idiotype networks. Given the cell surface expression of PSMA and the over-expression of this antigen on prostate cancer cells, PSMA represents a potential target for these monoclonal antibodies and in fact is currently in clinical use for imaging purposes (ProstaScint scan).¹⁰⁰

Conjugates to anti-PSMA antibodies in clinical development include the cytotoxin maytansinoid (DM1) and the radioisotopes lutetium-177 and yttrium-90. The anti-PSMA humanized murine monoclonal antibody J591 is 1 such antibody in development and has shown clinical and biochemical responses in phase I trials in advanced prostate cancer using both radioconjugated and unconjugated approaches.^{101,102} In particular, a conjugate of J591 to yttrium-90 was administered to 29 patients with HRPC and showed a maximum tolerated dose of 17.5 mCi/m², additionally demonstrating selective targeting to metastatic sites and prolonged PSA (>8 months) and measurable disease responses in 2 patients.¹⁰² Also derived from J591 is MLN 2704, which utilizes a conjugated microtubule destabilizing agent maytansinoid (DM1) as a form of targeted cytotoxicity.¹⁰³ It is currently in phase I–II studies given as a monthly injection for HRPC. Finally, an antibody to MUC-1, a mucin protein on the cell surface of prostate cancer cells that may be important in regulating cell survival, has undergone phase I trials in combination with paclitaxel, another microtubule-targeted agent.¹⁰⁴ Its tolerability and feasibility with demonstrated targeting of bone lesions has also led to the initiation of a phase II study.

While perhaps best suited for discussion in the vaccine section, antibodies to CTLA-4 are also in clinical development in conjunction with vaccination. CTLA-4 is a co-stimulating molecule for B7 that is involved in attenuating activated T-cell responses. The inhibition of this molecule may enhance T-cell activation, and when used in combination with prostate-specific vaccination, may enhance the immunogenicity and autoimmunity induced by vaccination.¹⁰⁵ CTLA-4 blockade in conjunction with GM-CSF is being assessed in early phase I trials, and further dose-escalation trials with vaccination strategies are in development with the goal of targeted immunotherapy without the autoimmunity seen in other clinical models such as melanoma.^{106,107}

Novel Cytotoxic Agents

While a full discussion of novel cytotoxic agents is beyond the scope of this discussion, 2 such classes of agents including epothilone analogs and satraplatin (Spectrum-Pharm) will be discussed briefly. The epothilones are a class of microtubule-targeting cytotoxic agents in development for second-line and relapsed HRPC therapy, and are derived from the myxobacterium *Sorangium cellulosum*.¹⁰⁸ In preclinical models they have demonstrated a wide range of clinical activity, including taxane resistant models. While sharing a common mechanism of action with the taxanes, they are apparently not susceptible to P-glycoprotein-induced drug efflux.¹⁰⁸ One such agent, the epothilone-B analogue BMS-247550 (Ixabepilone, Ingenta), has been studied in a phase II trial of men with HRPC.¹⁰⁹ Initial results demonstrated a 34% PSA response and 30% objective response, with a progression-free survival of 8 months—comparable those seen with docetaxel-based therapy. Use of these drugs may be limited by dose-limiting neurotoxicity similar to that seen with the taxanes.^{109,110} The use of BMS 247550 versus mitoxantrone and prednisone in taxane-resistant HRPC is being investigated currently by Rosenberg et al in NCI protocol 6046.¹¹¹ Other novel formulations of taxanes, such as the nanoparticle albumin-bound paclitaxel formulation abraxane (Abraxis Oncology), remain untested in prostate cancer but of emerging interest.

Finally, the oral platinum drug satraplatin is under investigation as second-line chemotherapy for HRPC. While it does not likely represent any mechanistic advance in management, satraplatin's tolerability, ease of administration, and preliminary efficacy may make it suitable for those patients who have progressed on therapy. An underpowered phase II trial of satraplatin given orally for 5 days at 100 mg/m² in combination with prednisone showed non-significantly improved overall survival and TTP compared to prednisone alone in HRPC, and a larger phase III trial, the SPARC (Satraplatin in Refractory Cancer) trial has been initiated for relapsed patients.¹¹²

Conclusions

The development of emerging therapies for prostate cancer has required a continual reassessment of the rational targets and the molecular biology underlying prostate cancer development, progression, and therapeutic resistance. As prostate cancer is a heterogeneous disease with widely varying PSA doubling times, survival rates, Gleason scores, hormone sensitivity, and chemosensitivity, a key to the design of trials of these novel agents is to adequately define in advance the patient population that is most likely to benefit from target inhibition, and that those

patients at greatest risk for progression be considered first for targeted therapies. Given the cytostatic nature of many biologic agents, novel trial designs that take into account endpoints such as prolonged stable disease, time to progression, and tissue pharmacodynamic responses are important to fully understand their clinical benefit. It is essential that urologists and both radiation and medical oncologists continue to develop multidisciplinary collaboration in advancing high priority clinical trials.

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