The evolving biology and treatment of prostate cancer

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Since the effectiveness of androgen deprivation for treatment of advanced prostate cancer was first demonstrated, prevention strategies and medical therapies for prostate cancer have been based on understanding the biologic underpinnings of the disease. Prostate cancer treatment is one of the best examples of a systematic therapeutic approach to target not only the cancer cells themselves, but the microenvironment in which they are proliferating. As the population ages and prostate cancer prevalence increases, challenges remain in the diagnosis of clinically relevant prostate cancer as well as the management of the metastatic and androgen-independent metastatic disease states.

We are on the verge of accusing 1 in 5 men of having prostate cancer.

—H. Ballentine Carter, Prouts Neck, Maine, USA, November 2, 2006

Prostate cancer accounts for 33% of all cancer diagnoses in American men (218,890 in 2007) as well as 9% of cancer deaths in men (27,050 in 2007) (1). Even though mortality has fallen by 25% over the last decade and the 5-year survival rate is approaching 100%, several challenges to decreasing the morbidity and mortality from this disease remain.

Autopsy studies have shown that at the time of death, approximately 70% of men have cancer in their prostate gland, but these cancers are most often not clinically relevant (generally referred to as latent, microscopic, or histologic). It has been estimated that 15%–30% of males over the age of 50 and as many as 80% of males over the age of 80 harbor microscopic, undiagnosed prostate cancer (2). Prostate cancer is currently diagnosed in 1 of 6 men and is treated because it is thought to be clinically relevant; however, it is fatal for only 3% of men. Prostate cancer is generally diagnosed because of an elevated prostate-specific antigen (PSA) level or abnormal digital rectal exam (DRE) (1). PSA is a protein produced by normal epithelial cells of the prostate gland as well as prostate cancer cells. It is present in small quantities in the serum of men without cancer, but is routinely elevated in the presence of prostate cancer and in other benign prostate disorders such as infection, inflammation, and benign prostatic hyperplasia (BPH). Serum PSA as a screening tool sets the upper limit of normal at 4.0 ng/ml; levels above that point identify men who should be considered for prostate biopsy. As a result of PSA screening, most cancers are now discovered while they are still localized to the gland, and overt metastatic disease at the time of diagnosis has become a relatively rare event (1, 3). Unfortunately, PSA does not distinguish which type of prostate cancer a man may have in his gland at a given time — a microscopic cancer that will never cause a problem, a clinically relevant cancer that will cause morbidity and mortality if left in place, or a cancer that is lethal and hence incurable with localized therapy because it has already metastasized to distant organs. Recent studies suggest that prostate cancer is being overdiagnosed 30%–50% of the time (i.e., finding clinically irrelevant cancers); others suggest that we may be treating 20 or more men in order to keep 1 man from dying of prostate cancer (3–7). The current challenge is to identify which men have disease that may be cured with treatment, and which men do not require treatment and therefore should not be exposed to the morbidities associated with therapy.

The goal of PSA screening is to provide a balance between missing clinically important cancers and performing unnecessary biopsies. Unfortunately, the sensitivity of the PSA test at the cutoff value of 4 ng/ml is only approximately 20%, and this has led to the overdiagnosis and treatment of many men with microscopic cancers that may not benefit from local treatment. In addition, approximately another 20% of patients who undergo primary treatment for prostate cancer still develop metastatic disease as a result of dissemination of cancer cells outside of the prostate prior to diagnosis. If the PSA level threshold for diagnosis is lowered to less than 4 ng/ml in an effort to catch these cancers prior to metastasizing, more cancers will be discovered and treated with the intent to cure, but the sensitivity for discovering cancers that may be clinically relevant would decrease even further. Thus the PSA cutoff of 4.0 ng/ml is neither highly sensitive nor is it specific to the diagnosis of clinically relevant, truly localized, prostate cancer. To address these issues, the major medical societies such as the American Cancer Society, the American Urological Association, and the National Comprehensive Cancer Network (NCCN) are changing their suggested screening guidelines (see *American Cancer Society guidelines for prostate cancer screening*) (8).

Tracking a man's PSA level for several years may be very useful in differentiating between lethal and nonlethal cancers. Findings from the Baltimore Longitudinal Aging Study determined that 10–15 years before diagnosis, the PSA levels of men who eventually died of prostate cancer were rising at an exponential rate (6). Men who had

Nonstandard abbreviations used: AR, androgen receptor; BPH, benign prostatic hyperplasia; CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; CXCR, CXC chemokine receptor; DHT, dihydrotestosterone; DRE, digital rectal exam; NCCN, National Comprehensive Cancer Network; PIN, prostatic intraepithelial neoplasia; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; SDF-1, stromal-derived factor–1.

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American Cancer Society guidelines for prostate cancer screening

The American Cancer Society, like all of the medical societies, continues not to recommend routine testing for prostate cancer at this time, but does suggest that men talk to their doctors about the benefits, risks, side effects, and controversies associated with early prostate cancer testing and treatment. A PSA blood test and DRE should be offered annually beginning at age 50 to men who have a life expectancy of at least 10 years (8). In men for whom DRE is an obstacle to testing, PSA alone is an acceptable alternative. Men at high risk, including men of sub-Saharan African descent and men with a first-degree relative diagnosed at a younger age (i.e., less than 65 years) should begin testing at age 45. Men at even higher risk of prostate cancer due to more than one first-degree relative diagnosed with prostate cancer before age 65 could begin testing at age 40, although if PSA is less than 1.0 ng/ml, no additional testing is needed until age 45. If PSA is between 1.0 and 2.5 ng/ml, annual testing is recommended. If PSA is greater than 2.5 ng/ml, further evaluation with biopsy should be considered.

a PSA velocity of 0.35 ng/ml/yr or less at 10–15 years before diagnosis had a prostate cancer–specific survival rate of 92% compared with 54% for men with a PSA velocity greater than 0.35 ng/ml/yr ($P < 0.001$). These findings suggest that PSA velocity may be useful in differentiating among (a) individuals with clinically irrelevant prostate cancers; (b) individuals with clinically relevant prostate cancers who would benefit from primary treatment; and (c) individuals with prostate cancers that have metastasized and who may not benefit from immediate localized treatment. In an effort to capture more clinically relevant cancers, the NCCN has tried to incorporate PSA velocity into its most recent screening recommendations and has also recommended annual screening for younger men with a PSA greater than 0.6 ng/ml (Figures 1 and 2) (9).

Prostate tumorigenesis

Prostate cancers appear to develop over 20–30 years or more (10, 11). While approximately 5%–10% of prostate cancers are thought to occur on an inherited genetic background that makes the host more susceptible to prostate tumorigenesis, these genes have yet to be identified (12). Prostate cancers, like all carcinomas, arise in differentiated epithelial cells and/or progenitor cells in which embryonic pathways are reactivated through the activation of oncogenes and the loss of tumor suppressor genes, which leads to a growth and survival advantage (13). Whether the process of prostate carcinogenesis is the result of DNA damage that occurs in a differentiated cell or a stem cell, it is the result of a complex interplay of genes, the cellular microenvironment, the macroenvironment of the host, and the environment in which the host resides. Multiple genetic changes have been associated with prostate cancer, and these appear to correlate with microscopic changes in cell structure and gland histology (Figure 3) (14–27).

In classic carcinogenesis models, damaging insults to cells are generally classified into those that cause initiating events (DNA damage that starts cells along a tumorigenesis pathway) and those that cause promotional events (further DNA damage or cell proliferation). Early prostate tumorigenesis appears to be associated with a dysplasia that starts with proliferative inflammatory atrophy (PIA) and progresses to prostatic intraepithelial neoplasia (PIN), which in some cases leads to carcinoma (16). Evidence suggests that these early lesions may be initiated by inflammation that occurs with exposure to different infectious

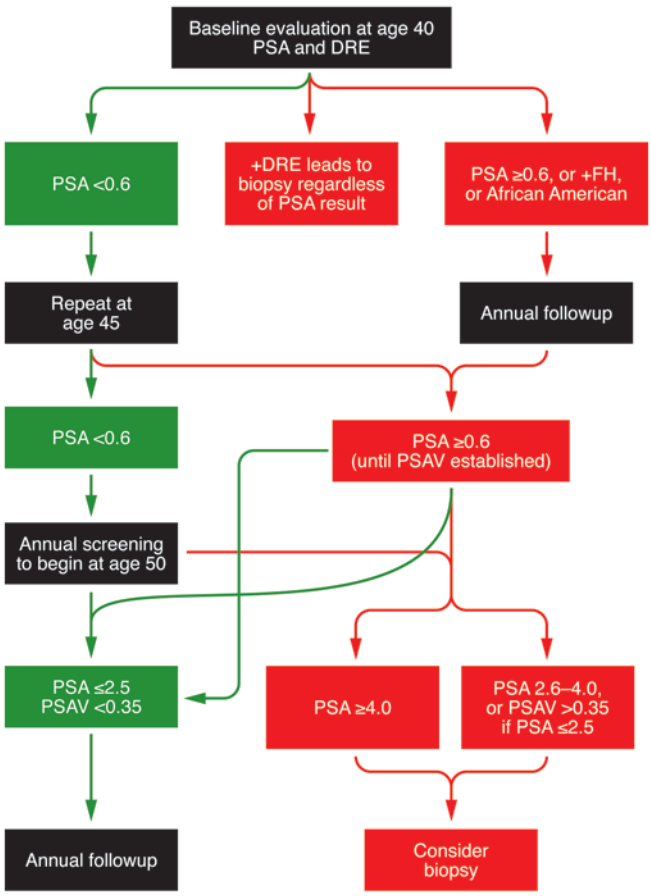


Figure 1

Evolving screening guidelines for prostate cancer detection: NCCN early detection screening guideline. Physicians should initiate a discussion of the risks and benefits of early prostate cancer detection and offer baseline screening with DRE and PSA beginning at age 40. PSA values are shown in ng/ml. Men with PSA less than 0.6 ng/ml at age 40 should repeat screening at age 45; if PSA is less than 0.6 ng/ml at age 45, annual screening should be considered at age 50. If initial PSA at age 40 is 0.6 ng/ml or more, or if the patient has a family history of prostate cancer (+FH) or is African American, annual screening with DRE and PSA is recommended. If subsequent PSA is less than 0.6 ng/ml, the patient can repeat screening at age 45; all others should continue with annual screening. In the annual screening group, men with PSA 2.6–4.0 ng/ml, or whose PSA velocity (PSAV) exceeds 0.35 ng/ml/yr, should be considered for biopsy. Biopsy is highly recommended for any individual with PSA greater than 4.0 ng/ml and for men with positive DRE at any point in the screening process. Note that PSA velocity measurements (shown in ng/ml/yr) should be made on at least 3 consecutive specimens drawn over a period of at least 18–24 months.

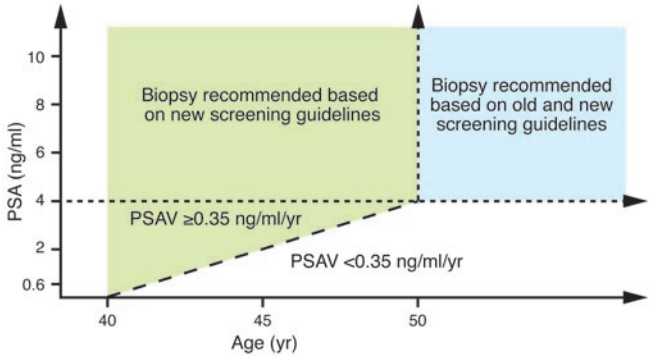


Figure 2
Effect of NCCN guidelines on prostate cancer screening and detection. Screening that starts at age 50 still results in prostate cancers that metastasize prior to detection and are therefore incurable. The initiation of screening starting at age 40, factoring in PSA value and PSA velocity, has the goal of the detection of more clinically relevant cancers and should result in increased sensitivity of PSA as a screening test. The effect on specificity and whether this method will also result in an increase in non–clinically relevant cancers remains undetermined.

agents and/or ingestion of carcinogens (16–20). As premalignant lesions progress to primary cancer, to metastatic cancer, and to androgen-independent cancer, genetic alterations continue to accumulate within the tumor cells (Figure 3) (15–27). In addition to these genetic changes, androgens act as promoters for further growth and proliferation. The androgen testosterone is bound to sex hormone–binding globulin in the circulation (Figure 4). Upon entering prostate cells or prostate cancer cells, testosterone is immediately converted by the enzyme 5α-reductase into its active metabolite, dihydrotestosterone (DHT). After DHT binds to the androgen receptor (AR), the receptor dimerizes and is phosphorylated and is then transported to the nucleus, where it binds to genes with androgen response elements, a process modulated by coactivators and corepressors (28, 29). If a normal prostate epithelial cell is prevented from metabolizing testosterone, it undergoes programmed cell death (i.e., apoptosis).

Prostate carcinomas present as different grades based on a histologic pattern that is scored by the Gleason grading system (30). In this system, the most prominent histologic pattern is assigned a grade of 1–5, and the second most common pattern is assigned another grade; these 2 grades are summed and reported as the total Gleason score. The most common pattern is a Gleason 3, which consists of small glands that have not fused together. A Gleason pattern 4 consists of small glands with fusion. Gleason pattern 5 consists of sheets of anaplastic cells without discernable glands. Generally, prostate cancers with a total Gleason score of 5–7 are considered to be intermediate grade/moderately differentiated and those with a score of 8–10 are considered to be high grade/poorly differentiated. It remains unclear why some cancers present as latent, well differentiated, moderately differentiated, or poorly differentiated; that is, the genetic alterations/molecular events that accumulate between PIN and the formation of different grades of carcinoma have remained uncharacterized.

Recent work by Chinnaiyan and colleagues has led to the discovery of what appears to be an early, critical step in prostate tumorigenesis: a genetic rearrangement that causes a fusion product of *TMPRSS2*, a prostate-specific, androgen-regulated gene, with the *ETS* family of transcription factors (31–34). This fusion of the 3′ end of the *ETS* family member to the 5′ end of *TMPRSS2* leads to the overexpression of an androgen-responsive oncoprotein. The TMPRSS2-ETS fusions have been noted in over 70% of several series of hospital-based prostate cancers, suggesting that it may be one of the most common somatic molecular rearrangements in human cancer (35–37). The most common variant noted occurs via intronic deletion, resulting in the TMPRSS2-ERG 21q22.3-21q22.2 fusion. Evidence suggests that the TMPRSS2-ETS gene fusions first appear in late PIN lesions and are related to an invasive phenotype (35). TMPRSS2-ERG fusion prostate cancer is associated with higher tumor stage and prostate cancer–specific death as well as with specific morphologic features associated with aggressive prostate cancer: blue-tinged mucin, cribiform growth pattern, macronucleoli, intraductal tumor spread, and signet-ring features (34, 36, 37). Efforts are underway to identify these gene fusions in the urine of patients with prostate cancer to improve detection and predict prognosis (36).

Targeting steps in early prostate tumorigenesis for prevention

Men castrated at adolescence (eunuchs) lack traits attributable to male hormones and do not suffer from prostate cancer (38). This practice, while offering a potential cure for virtually all prostate cancer, has understandably not been widely accepted as a standard medical treatment and has left the scientific/medical community searching for other solutions. Because testosterone acts as a tumor promoter in at least early prostate cancer, it can be used as a target for the chemoprevention of prostate cancer.

The phase III Prostate Cancer Prevention Trial demonstrated that treatment with the 5α-reductase inhibitor finasteride for 7 years led to a 25% decrease in the incidence of prostate cancer in men over the age of 50 (39). This medication left potency intact and was generally well tolerated. Unfortunately, when the prostate cancers were analyzed by Gleason score, there were an increased number of high-grade prostate cancers (Gleason higher than 7) found in the finasteride arm than in the placebo arm of the trial. The reasons for this remain unclear, but several possible explanations exist. (a) The higher-grade cancers may be the result of the hormone treatment inducing a more poorly differentiated phenotype. This would mimic what is seen after treatment with androgen-ablating agents in men with hormone-refractory prostate cancer. (b) There was an almost 25% reduction in gland volume in the finasteride-treated group; this volume reduction would increase the likelihood that cancer, especially higher-grade cancer, would be sampled in a biopsy procedure. (c) A potential ascertainment bias may also be involved. Because finasteride decreases the symptoms of BPH and decreases PSA increases caused by BPH, men with persistently elevated PSA levels on finasteride would have an increased probability of harboring prostate cancer compared with men with high PSA levels not on finasteride. The study demonstrated a higher sensitivity of detecting prostate cancer in the finasteride arm, and this increased sensitivity may be responsible at least in part for the increased detection of high-grade cancers in that group (40–43). Men enrolled in this trial are still being followed, and further investigation will hopefully clarify these issues. In the meantime, finasteride has not been widely adopted as a chemopreventive agent for prostate cancer. Other agents that target testosterone metabolism by inhibiting 5α-reductase, such as dutasteride, are currently in clinical trial (44).

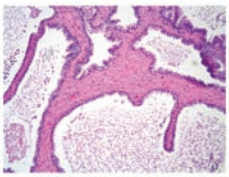
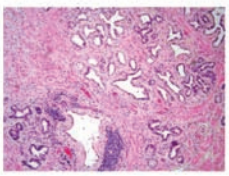
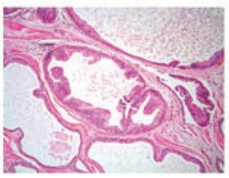
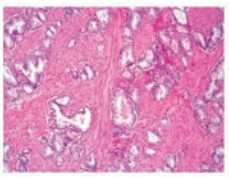
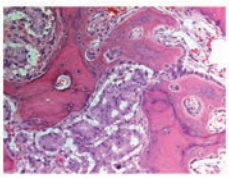
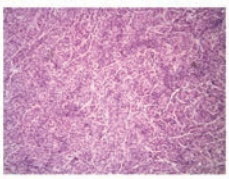
Disease state	Histology	Description
Normal prostate		Large glands with papillary in-foldings that are lined with a 2-cell layer consisting of basal cells and columnar secretory epithelial cells with pale cytoplasm and uniform nuclei Susceptibility genes associated with hereditary prostate cancer *RNASEL*: Regulates cell proliferation through the interferon regulated 2′-5′-oligoadenylate pathway *ELAC2*/*HPC2*: Loss of function of tRNA 3′′ processing endoribonuclease *MSR1*: Macrophase scavenger receptors process negatively charged macromolecules
PIA		Atrophic glands have scant cytoplasm, hyperchromatic nuclei, and occasional nucleoli and are associated with inflammation Susceptibility genes or events *NKX3.1*: Allelic loss of homeobox protein, allowing growth of prostate epithelial cells *PTEN*: Allelic loss of phosphatase and tensin homolog, allowing decreased apoptosis and increased cell proliferation *CDKN1B*: Allelic loss of cyclin-dependent kinase inhibitor p27, allowing increased cell proliferation
PIN		Intermediate to large size glands with proliferative changes contained within the gland and having nuclear abnormalities that resemble those of invasive carcinoma Susceptibility genes or events *GSTP1*: Hypermethylation of the upstream regulatory region inactivates this Pi-class glutathione-S-transferase enzyme, which detoxifies carcinogens Hepsin: Increased expression of this serine protease leads to increased invasiveness and disruption of the basement membrane *AMACR*: Increased expression results in increased peroxisomal b-oxidation of branched chain fatty acids from red meat, thereby increasing carcinogen exposure *TMPRSS2*: Fusion of this androgen-regulated gene with the ETS family of transcription factors in late stages of PIN results in increased breakdown of the extracellular matrix Telomerase: Activation leads to maintenance of telomere length and immortalization of cells
Prostate cancer		Small, irregular glands with cells having abnormal nuclei and nucleoli (large, deep staining) and lacking basal cells Susceptibility genes or events *MYC*: Overexpression leads to cell proliferation and transformation *RB*: Loss of expression leads to cell proliferation and transformation
Metastatic prostate cancer		Nests of cancer cells within the bone Susceptibility genes or events *TP53*: Mutation results in loss of multiple tumor suppressor functions *E-cadherin*: Aberrant expression leads to increased invasive and metastatic phenotype *NM23*: Loss of this NDP kinase leads to increased metastasis *EZH2*: Histone methyltransferase PcG protein whose activation causes repression of genes that suppress invasion and metastasis
Androgen-independent prostate cancer		Cancer cells that grow in an androgen-depleted environment Susceptibility genes or events *AR*: May remain activated through amplification, phosphorylation by other steroids, or non-androgen growth factors *BCL2*: Increased expression leads to protection from apoptosis Stem cells: Potential repopulation by progenitor cells

Figure 3
Histologic changes associated with prostate tumorigenesis. For more information, see refs. 21–34. PIA, proliferative inflammatory atrophy; *RNASEL*, 2′-5′-oligoadenylate–dependent RNase L; *AMACR*, α-methylacyl-coenzyme A racemase; *EZH2*, enhancer of zeste homolog 2; PcG, polycomb group. Original magnification, ×100.

Understanding that prostate carcinogenesis occurs as a result of interactions between genes and the environment has led to the development of several other potential chemoprevention strategies that are aimed at preventing DNA damage or the proliferation of premalignant cells (Table 1) (15, 45, 46). Antioxidants, which are believed to prevent DNA damage by oxygen free radicals, are in clinical trials and include pomegranate juice, curcumin, vitamin D, vitamin E, selenium, and lycopene (45–48). The Selenium and Vitamin E Cancer Prevention Trial, a phase III randomized, placebo-controlled trial (32,400 enrolled) of selenium (200 μg/d) and/or vitamin E (400 IU/d) supplementation for a minimum of 7 years and a maximum of 12 years was initiated in 2001 to test the effectiveness of these agents to prevent prostate cancer (49).

It has been known for several decades that men from Asian countries have a much lower incidence of prostate cancer, and one hypothesis behind this observation is their high consumption of antioxidants in the form of naturally occurring estrogens (isoflavones) through the ingestion of soy and green tea (50). One such isoflavone, genestein, has been demonstrated to induce the expression of genes involved in defense against oxidative stress (51). In

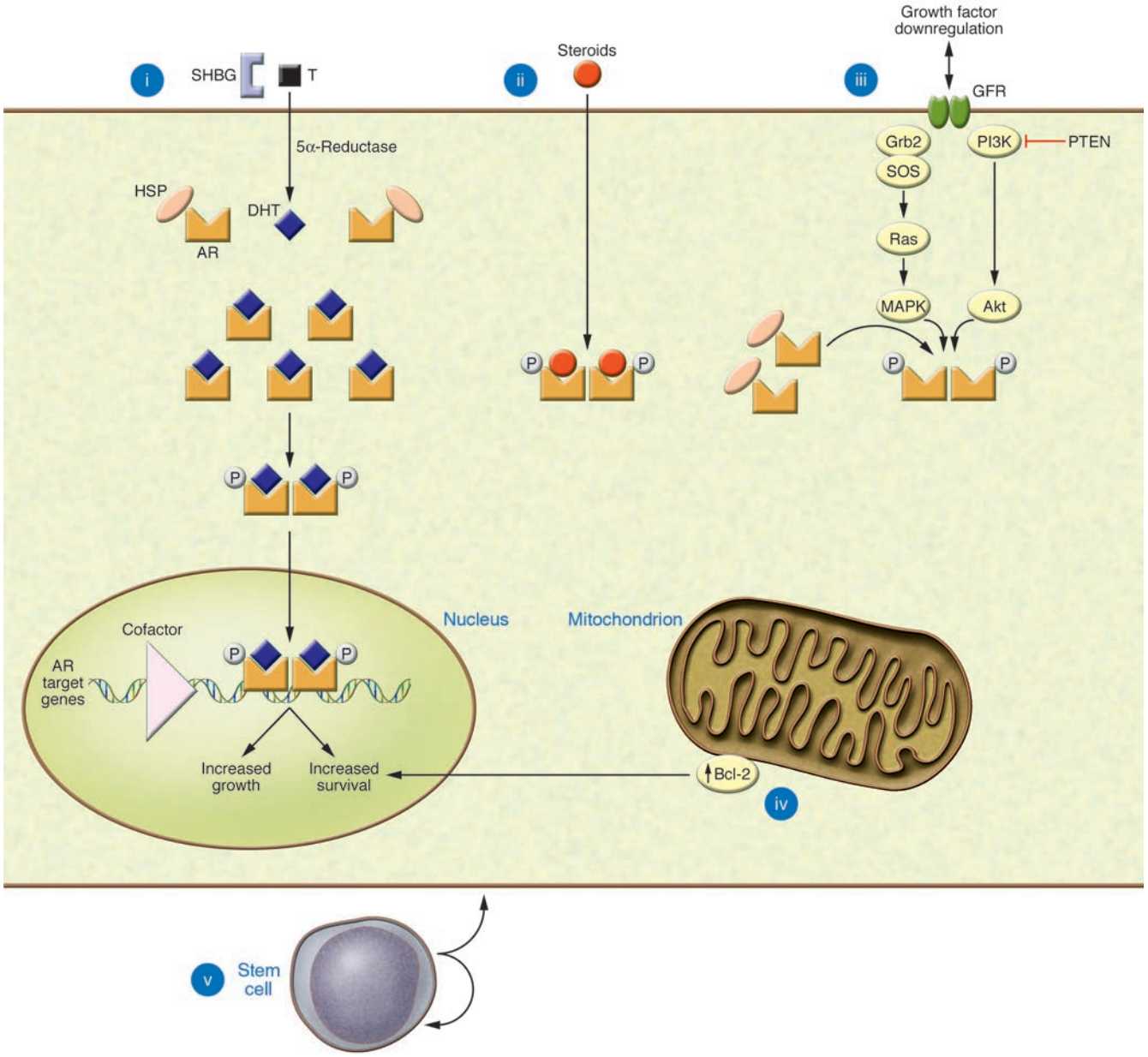


Figure 4

Mechanisms of androgen independence. (i) Amplification. Prostate cancer cells develop the ability to utilize low levels of androgen for survival by increased sensitivity of the AR to testosterone (T), by increased local conversion of testosterone to DHT by 5α-reductase, and by increased numbers of ARs. Once DHT binds to AR, the receptor dimerizes and phosphorylates and is transported to the nucleus, where it binds to androgen-responsive elements of genes. This process is modulated by cofactors that act as coactivators and corepressors and results in increased cell proliferation and survival. (ii) Promiscuous pathway. Nonandrogenic steroid molecules normally present in the circulation, as well as antiandrogens, bind and activate the AR. (iii) Outlaw pathway. AR is activated by phosphorylation by nonhormone growth factors through their tyrosine kinase receptors. (iv) Bypass pathway. Prostate cancer cells develop the ability to survive independent of AR. The best-known bypass pathway occurs through upregulation of the molecule Bcl-2 by androgen-independent prostate cancer cells, which protects them from apoptosis. (v) Stem cell regeneration. Prostate cancer stem cells, which are not dependent on the AR for survival, continually resupply the tumor cell population. SHBG, sex hormone–binding globulin; HSP, heat shock protein; GFR, growth factor receptor; P, phosphate group; PTEN, phosphatase and tensin homolog; Grb2, growth factor receptor–bound protein–2; SOS, son of sevenless. Figure modified from *Clinical Cancer Research* (28).

addition, it has been demonstrated in breast and prostate cancer cells that genistein induces apoptosis and inhibits activation of cell survival genes in the NF-κB and Akt signaling pathways (50). Even though definitive evidence is lacking, many physicians recommend green tea as a preventive measure against prostate cancer.

Other strategies for prostate cancer prevention are also under investigation (Table 1). For reasons that remain unclear, prostate epithelial cells and prostate cancers have high levels of polyamines. These molecules are involved in many biochemical processes including cellular proliferation, cell cycle regulation, and protein

Table 1
Chemoprevention agents for prostate cancer, and their presumed mechanism of action, currently in clinical trial

Agent	Mechanism
Exisulind	Inhibits cGMP phosphodiesterase (55)
Celecoxib	Inhibits COX-2; increases β-catenin (56)
Genistein	Multiple effects including downregulation of AR, ERα, PR, EGFR, and IGF1 (50, 51)
DFMO	Multiple effects caused by inhibited ornithine decarboxylase (52)
Diindolylmethane	Inhibits angiogenesis (54)
Toremifene	Selective ER modulator (53)
Selenium yeast	Antioxidant (49)
Vitamin D analog	Antioxidant (45)
Vitamin E	Antioxidant (49)
Pomegranate	Antioxidant (47)
Lycopene	Antioxidant (48)
Curcumin	Antioxidant (48)
Finasteride	Inhibits 5α-reductase (39)
Dutasteride	Inhibits 5α-reductase (44)

For more information, see ref. 46. ER, estrogen receptor; DFMO, α-difluoromethylornithine; PR, prostaglandin receptor; COX-2, cyclooxygenase-2.

synthesis. As an inhibitor of ornithine decarboxylase, the rate-limiting enzyme in the polyamine synthetic pathway, α-difluoromethylornithine, is being studied as a chemoprevention agent (52). The selective estrogen receptor modulator toremefine is being investigated for its ability to inhibit the evolution of PIN to prostate cancer (53). In addition, 3,3′-diindolylmethane acts as an angiogenesis inhibitor and has been demonstrated to downregulate the androgen receptor and the *AKT* pathway in prostate cancer cells (54). Exisulind, an inhibitor of cGMP phosphodiesterase that induces apoptosis, has been studied extensively alone and in combination with other agents as both a preventive and a treatment for different stages of prostate cancer (55). Multiple nonsteroidal antiinflammatory drugs, including celecoxib, hold promise as chemoprevention agents for cancer, including prostate (46, 56).

Targeting metastatic prostate cancer for treatment

Although metastatic prostate cancer remains an incurable disease at present, therapy can delay progression. The first step in treatment of metastatic disease is to block testosterone-driven proliferation of prostate cancer cells through androgen deprivation therapy with medical or surgical castration. This causes apoptosis in the majority of the prostate cancer cells and leads to a remission in the majority of patients for 18–36 months (28). During that clinical remission, cells that have escaped the requirement of testosterone to grow continue to proliferate, and a castration-independent clone (hormone refractory, androgen independent) of cells emerges as the predominant phenotype. Median survival time for men with androgen-independent disease is approximately 18–24 months (57). Current research is focused on understanding the molecular events that underlie the transition to androgen independence in order to develop new treatment strategies.

Targeting the androgen-independent prostate cancer cell

As a prostate tumor becomes androgen independent, multiple alternative cellular pathways, some involving the AR and others bypassing it, support tumor cell growth (Figure 4) (28, 29). These

Table 2
Agents and their presumed targets currently in clinical trial for the treatment of prostate cancer

Cell type	Target	Sample agents
Prostate cancer cell	Aberrant growth factor receptor activation	EGFR: gefitinib (66); PDGFR: imatinib (70); IGF1R: A12 (71); receptor tyrosine kinase: BAY 43-9006 (69); IL-6: CNTO328 (68)
	Bcl-2	AT101 (73)
	Microtubules	Ixabepilone halichondrin (79–81)
	DNA replication	Satraplatin (77)
	Histone deacetylase	Vorinostat (78)
	Proteasome	Bortezomib (64)
	Hsp90	17-AAG (60)
	Clusterin	OGX-011 (76)
	mTOR	Rapamycin analogs (75)
	? Proliferation	Calcitriol, DN-101 (82)
Osteoblast	Endothelin-1 receptor	Atrasentan, ZD-4054 (106)
Osteoclast	Pyrophosphate	Zoledronic acid, samarium, strontium (100–102)
	RANKL	Denosumab (103)
	SRC	Dasatinib (104, 105)
Endothelial cell	VEGF	Bevacizumab, VEGF-TRAP (108, 112)
	VEGFR	Sunitinib, vatalanib, sorafenib (109–112)
	$\alpha_v\beta_{3/5}$ Integrin	Cilengitide (114)
	Permeability	Dimethylxanthenone (113)
Immunologic activation	Macrophages	CNTO888 (97)
	T cells (CTLA-4)	MDX-010 (119)
	Dendritic cells	Sipuleucel-T (117); GVAX (118)
	Cell antigens	MUC-1 antibodies (120), PSMA (J591 conjugates) (121, 122)

CTLA-4, CTL-associated antigen–4; Hsp, heat shock protein; mTOR, mammalian target of rapamycin; MUC-1, Mucin 1; RANKL, receptor activator of NF-κB ligand.

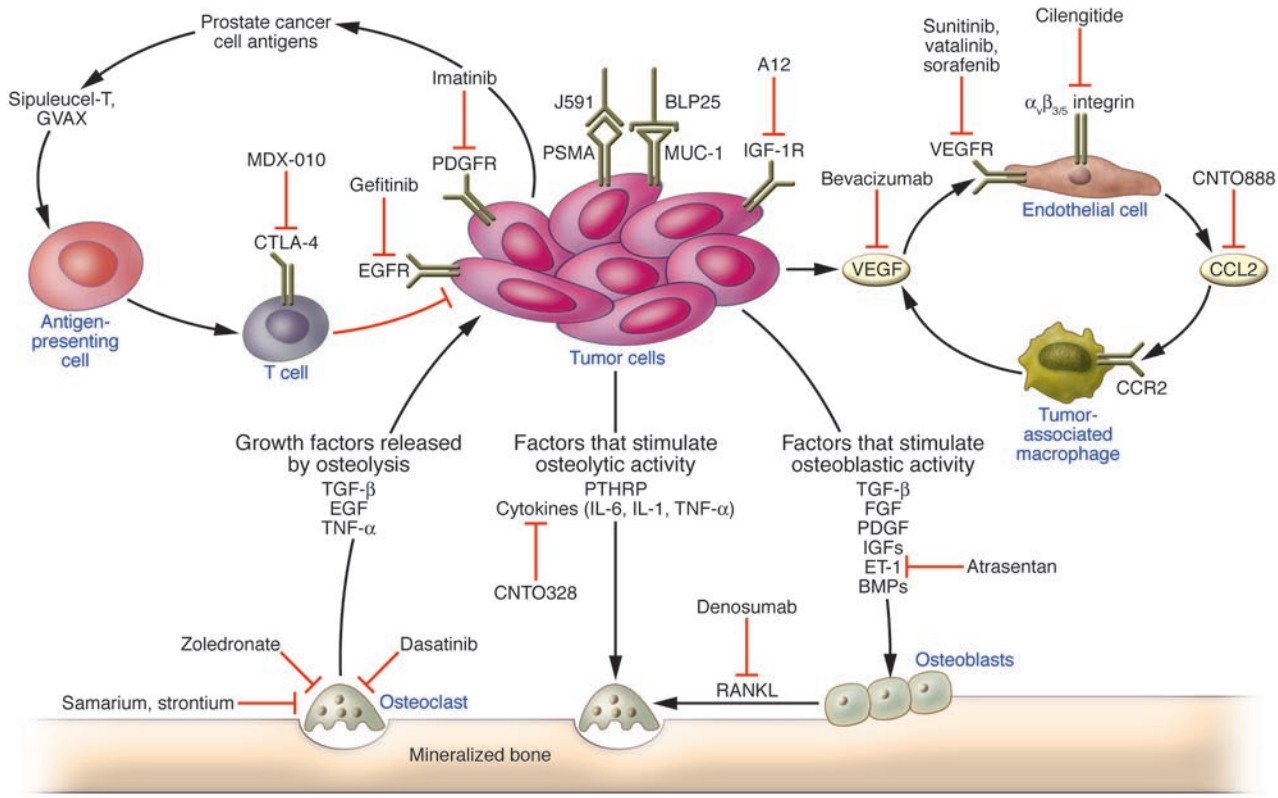


Figure 5

Selected therapies in development for prostate cancer directed against prostate cancer and host cell interaction in the bone microenvironment. Tumor cells alter the bone microenvironment by stimulating osteoclasts parathyroid hormone–related protein (PTHRP), IL-6, IL-1, and TNF-α and by stimulating osteoblasts endothelin-1 (ET-1), FGF, PDGF, IGFs, TGF-β, and bone morphogenic proteins (BMPs). Osteolysis, in turn, releases TGF-β, TNF-α, and EGF, stimulating cancer cell proliferation. Receptor activator of NF-κB ligand (RANKL) expression by osteoblasts binds to the RANK receptor, promoting osteoclast formation and function. Therapies targeting bone metastases include those that target the prostate cancer cells themselves by inhibiting growth factor receptors or by targeting cancer cell antigens (Mucin 1 [MUC-1], BLP25 liposomal vaccine; and PSMA; J591 antibody conjugates). Alternatively, several therapies target the supporting host cells. Zoledronate, denosumab, dasatinib, CNTO328, samarium, and strontium all target osteoclast function to inhibit osteolysis. Atrasentan targets the ET-1 receptor on osteoblasts. Antiangiogenesis drugs target the endothelial cell and include bevacizumab, which targets VEGF, and cilengitide, which targets $\alpha_v\beta_{3/5}$ integrins. Immunotherapy approaches include inhibiting the infiltration of tumor-associated macrophages by inhibiting CCL2, prolonging T cell response by inhibiting the inhibitory receptor CTL-associated antigen–4 (CTLA-4) using the antibody ipilimumab (MDX-010), and stimulating antigen-presenting cells through vaccines such as GVAX and Sipuleucel-T. CCR2, CC chemokine receptor 2.

alternative pathways include: (a) amplification of the AR with associated hypersensivity to lower levels of DHT; (b) broadening of the specificity of the AR to other hormone molecules (receptor promiscuity); (c) activation of the AR through phosphorylation by nonhormone kinases (outlaw pathways); (d) activation of growth through pathways that are independent of the AR (bypass pathways); and (e) repopulation of the tumor through androgen-independent stem/progenitor cells.

Amplification of the AR is common in androgen-dependent disease and is likely secondary to either gene amplification as a result of mutation or through selective pressure of the androgen-depleted environment, causing the death of cells with fewer ARs and the clonal expansion of cells with more ARs (58, 59). Potential methods to target continued activation of ARs include the development of better antiandrogens that competively bind the AR, inhibiting AR dimerization by blocking the dissociation of AR from the heat shock protein Hsp90 using geldanamycin (17-AAG), altering proteasome degradation of AR, and inhibiting cofactor binding to the AR (Table 2 and Figure 4, pathways i and ii) (60–64).

The phosphorylation and subsequent outlaw activation of the AR by deregulated growth factors and their downstream signal transduction kinase pathways, including IGF, keratinocyte growth factor, PDGF, EGF, and IL-6, are being targeted in clinical trials using antibodies or small-molecule kinase inhibitors (Table 2 and Figure 4, pathway iii; refs. 65–71).

Androgen-independent prostate cancer cells have been demonstrated to frequently upregulate antiapoptotic molecules including Bcl-2, allowing them to bypass their need for androgens for cell growth and survival (Table 2 and Figure 4, pathway iv) (72–74). Anti–Bcl-2 agents include AT101, which binds to the BH3 domain of Bcl-2 (73). Inactivation of the tumor-suppressor gene *PTEN* with subsequent activation of Akt is also a frequent event in androgen-independent prostate cancer cells, and this is being targeted through the inhibition of mammalian target of rapamycin (75). The cytoprotective gene *Clusterin* has been silenced using antisense oligonucleotide OGX-011 and is the subject of ongoing phase II clinical trials (76).

Multiple agents are under clinical development for androgen-independent prostate cancer that inhibit cancer cell proliferation

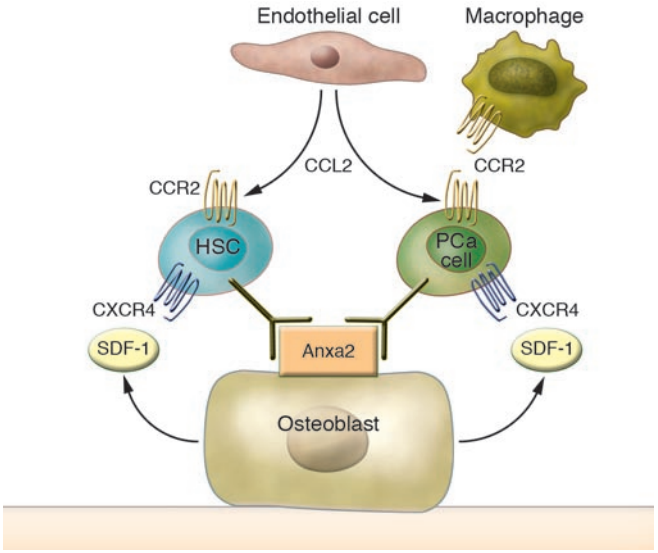


Figure 6
Prostate cancer mimicry of HSC/progenitor cell homing mechanisms. The metastatic process of prostate cancer cells (PCa cells) is functionally similar to the migrational, or homing, behavior of HSCs to the bone marrow. Numerous molecules have been implicated in regulating HSC homing, participating as both chemoattractants and regulators of cell growth. Endothelial cell–derived factors such as CCL2 act as chemoattractants and growth factors for HSCs, tumor-associated macrophages, and prostate cancer cells. Osteoblasts produce the chemokine SDF-1 (CXCL12), which further guides both HSCs and prostate cancer cells into the marrow through their expression of the CXCL12 receptor CXCR4. Both HSCs and prostate cancer cells use the cell surface protein annexin II (Anxa2) on both endothelial cells (not shown) and osteoblasts as a dock/lock mechanism into the bone microenvironment. Conceptually, prostate cancer cells act as parasites of the HSC niche by coopting HSC chemokines and attachment sites to initiate a cascade of events that result in the osteoblastic metastases observed in prostate cancer patients.

by interfering with DNA replication or mitosis. A phase III trial of satraplatin, an oral platinum that inhibits replication through the formation of DNA adducts, has demonstrated activity as a second line chemotherapy for patients with androgen-independent disease (77). The increased understanding of the relationship between DNA and chromatin structural proteins has led to the development of histone deacetylase inhibitors such as suberoylanilide hydroxamic acid (vorinostat), which interferes with chromatin unfolding and subsequent gene activation (78). Antimicrotubule agents including ixabepilone and halichondrin are also under active study (79–81). Vitamin D acts as an antiproliferative agent through a variety of poorly characterized mechanisms, and high-dose calcitriol, DN-101, has demonstrated activity in androgen-independent disease and is the subject of ongoing clinical trials (82).

Another potential mechanism for survival in the androgen-depleted environment is the presence of prostate cancer stem cells that continually regenerate a heterogeneous tumor cell population that is observed in androgen-independent patients despite therapy (Figure 4, pathway v) (14). A small population of cells that are $CD44^+\alpha_2\beta_1^{hi}CD133^+$ and do not express AR has been identified within prostate tumors and is thought to be composed of prostate cancer stem or progenitor cells (14). Although prostate-specific agents have not been identified, multiple therapeutics are being developed for clinical investigation, including inhibitors of *Hedgehog*, *Notch*, and *Bim1*, developmental genes that have been identified as activated in multiple stem populations (83, 84).

Targeting therapy to bone metastases

Bone metastases are the major cause of morbidity, and ultimately mortality, for men with metastatic prostate cancer (85, 86). The interaction of prostate cancer cells with the bone microenvironment has been described as a vicious cycle in which prostate cancer cells interact with both osteoclasts and osteoblasts in a complex interplay resulting in osteoblastic metastases (Figure 5) (85–87). Prior to the establishment of this vicious cycle, data suggest that the presence of the primary tumor can have positive and negative effects on the successful migration and growth of cancer cells at distant sites. Primary tumors appear to act in an endocrine fashion to alter the marrow environment and prime it for the arrival of metastatic cells by creating a premetastatic niche (88). Factors such as hypoxia and inflammation promote the release of factors resulting in the mobilization of bone marrow–derived endothelial progenitor cells and hematopoietic progenitor cells that circulate to distant sites and dictate the localization of metastatic spread of the tumor cells (88–91). Alternatively, primary tumors can also produce growth-inhibitory cytokines such as angiostatin, which suppress the growth of metastases (92, 93).

Prostate cancer cells that successfully metastasize to bone marrow hijack several properties exhibited by normal host cells that traffic through the circulation and bone marrow. For example, prostate cancer cells mimic hematopoietic stem/progenitor cells by upregulating the expression of stromal-derived factor–1 (SDF-1; also known as CXC chemokine ligand 12 [CXCL12]) receptor CXC chemokine receptor 4 (CXCR4), resulting in chemoattraction to the SDF-1 secreted by the osteoblasts (94, 95) (Figure 6). Moreover, SDF-1 signaling through CXCR4 triggers the adhesion of prostate cancers to bone marrow endothelial cells and osteoblasts by activating CD164 and $\alpha_v\beta_3$ integrins (94–96). Similarly, prostate cancer cells mimic monocytes by responding to CC chemokine ligand 2 (CCL2; also known as monocyte chemoattractant protein–1) secreted by bone marrow endothelial cells (97). In conjunction with prostate cancer cells using the chemokines of hematopoietic cells for homing and traffic to the marrow, it appears that the cancer cells also target the adhesive-localization molecules used by hematopoietic cells. One such molecule is annexin II. Blocking annexin II binding prevents stem cell engraftment and prostate cancer homing to the marrow (98). A key implication of these data is that soluble and insoluble factors produced in the marrow play a crucial role in the osteotropism of prostate cancer to bone (87, 99).

While the mechanisms that result in osteoblastic metastases rather than the osteolytic metastases found in most cancers that spread to bone remain obscure, the recognition that metastatic lesions are complex systems involving a supporting framework of multiple host cells has allowed the development of several strategies that target these complex tumor cell–microenvironment interactions as well as the signal transduction pathways of other cells important to the development of metastases (Table 2 and Figure 5). The most successful of these strategies to date has been the use of the bisphosphonate zoledronate in patients with androgen-independent prostate cancer (100). Bisphosphonates, as analogs of pyrophosphate, inhibit osteoclast maturation and function.

Even though prostate cancer bone metastases are osteoblastic, osteoclasts are active in bone remodeling and are critical targets for interruption of the vicious cycle. Zoledronate has been demonstrated to decrease skeletal-related events in men with androgen-independent prostate cancer (100). The radionucleides samarium and strontium also bind to pyrophosphate, releasing presumably lethal radiation to all of the cells in the bone microenvironment (101, 102). Treatment with these agents leads to significant pain control in the majority of patients with osseous metastases. Another approach under active investigation is the interruption of the osteoblast/osteoclast axis through inhibition of the osteoprotegerin receptor/receptor activator of NF-κB ligand axis by denosumab (103). Dasatinib is a tyrosine kinase inhibitor that targets the src pathway and acts as an osteoclast inhibitor with activity in prostate cancer (104, 105). Atrasentan and ZD-4054 are endothelin-1 inhibitors that interrupt osteoblast function and proliferation and are currently in clinical investigation (106).

Prostate cancer metastases, even those in the bone marrow microenvironment, require blood vessels for growth (107). Antiangiogenic strategies are being actively pursued using several different paradigms of inhibition, including a current phase III trial of the combination of docetaxel with the anti-VEGF antibody bevacizumab in men with advanced prostate cancer (108). The interaction of VEGF with its receptors can also be blocked with antibodies that bind to the VEGFRs or with kinase inhibitors (Table 2) (109–112). Dimethylxanthenone acts as a vascular disrupting agent by increasing cell permeability (113). Another strategy uses EMD 121974 (cilengitide), the inner salt of a cyclized pentapeptide containing the amino acid sequence RGD, which blocks the sprouting of blood vessels into the extracellular matrix and consequently restricts tumor growth (114).

Inhibition of prostate cancer growth through the enhancement of the immune response of the host is also being pursued with a variety of strategies (Table 2 and Figure 5) (115, 116). The infiltration and proliferation of prostate cancer tumor by tumor-associated macrophages induced by CCL2 is the subject of a planned clinical trial with an anti-CCL2 antibody, CNTO888 (Table 2) (97). Sipuleucel-T (APC8015; Provenge) is an immunotherapy that exposes autologous dendritic cells to a recombinant fusion protein of prostatic acid phosphatase. These activated cells are then reinfused into the patient (117). GVAX is an immunotherapy in phase III clinical trials that is composed of 2 irradiated prostate cancer cell lines that have been genetically modified to secrete GM-CSF (118). Blockade of the T cell–inhibitory receptor CTL-associated antigen-4 (CTLA-4) augments and prolongs T cell responses and is under active clinical investigation to elicit antitumor immunity (119). Antigens present on prostate cancer cells offer attractive targets for immune therapy. Antibodies targeted against Mucin 1 and other glycoproteins that are overexpressed on multiple epithelial cancers, including prostate, are the subject of multiple clinical trials (120). Prostate-specific membrane antigen (PSMA) is an antigen expressed on the surface of prostate cells as well as the neovasculature of multiple tumor types (121, 122). Radiolabeled anti-PSMA monoclonal antibody J591 trials using the radiometals yttrium-90 and lutetium-177 have demonstrated manageable toxicity, excellent targeting of soft tissue and bone metastases, and efficacy in multiple preliminary trials.

Continued therapeutic development based on an evolving understanding of the biology of prostate cancer

The advances in prostate cancer therapy have been based on known molecular targets and biologic rationale. Unfortunately, prostate cancer remains the second leading cause of cancer death in American men (1). Better use of the PSA and PSA kinetics should increase the sensitivity of detection of clinically relevant cancers while decreasing the diagnostic rate of clinically insignificant cancers. In the realm of metastatic disease, multiple new therapeutic strategies are entering the clinic, directing therapy not only at the cancer cells themselves, but also at the microenvironment in which those cancer cells proliferate. This can serve as a paradigm for multiple other cancers in the future. Furthermore, the evolving understanding that early in metastasis, prostate cancer cells act as parasites of the HSC niche by coopting bone marrow chemokines and attachment sites to initiate osteoblastic lesions will drive future therapeutic development for advanced prostate cancer.

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