Clinical Phenotypes of Castration-Resistant Prostate Cancer

Tian Zhang, MD, and Andrew J. Armstrong, MD, ScM

Dr Zhang is a clinical fellow in hematology/oncology and Dr Armstrong is an associate professor of medicine and an associate professor of surgery in the Division of Medical Oncology at Duke Cancer Institute in Durham, North Carolina.

Address correspondence to:
Andrew J. Armstrong, MD
Associate Professor of Medicine and Surgery
Duke Cancer Institute
Department of Medicine
Division of Medical Oncology
DUMC Box 102002
Durham, NC 27710
Phone: 919-668-4667
Fax: 919-668-7117
E-mail: andrew.armstrong@duke.edu

Abstract: Castration-resistant prostate cancer (CRPC) is defined as prostate cancer that no longer responds to androgen deprivation therapy. At the genome level, CRPC is a heterogeneous disease that is marked by a range of genetic and epigenetic lesions. These lesions differ from patient to patient, but have common pathway-based themes. Clinically, a range of phenotypic presentations or subtypes of CRPC are observed that mirror this underlying heterogeneity as the disease progresses; each phenotype carries a different prognosis and different implications for treatment. In this review, we discuss the clinical subtypes of CRPC based on histology; the presence of metastatic disease and pattern of spread; patient-reported symptoms; and levels of biomarkers, such as serum bone turnover biomarkers, prostate-specific antigen, circulating tumor cell enumeration, and neuroendocrine biomarkers. We then address the potential relationship between these clinical phenotypes (with their underlying molecular subtypes) and therapeutic decision-making and prognosis, as well as ongoing research strategies.

Background

Prostate cancer is the most common noncutaneous malignancy and the second most common cause of cancer-related mortality in men in the United States. In 2013, more than 238,500 new cases of prostate cancer will be diagnosed, and more than 29,700 men will die of the disease.[1] Prostate cancer is a heterogeneous disease: some men require no immediate therapy and may be managed with active surveillance; others can be cured with local therapies; and others present with metastatic dissemination or develop metastatic, lethal disease despite aggressive local therapies.

Recurrent disease is typically treated with initial observation or androgen deprivation therapy (ADT). Over time, however, resistant clones develop an ability to thrive despite reduced levels of testosterone, a development that contributes to the lethality of prostate cancer. The mechanisms of resistance to ADT are molecularly diverse,[2] and include persistent activation of the androgen receptor (AR)

through mutation, amplification, altered coactivators and corepressors, and c-terminal splice variants, as well as the acquired ability to synthesize or use androgenic precursors. These mechanisms imply an ongoing addiction to and dependence on the AR for survival.[3]

Given the continued dependence of the cancer on hormonal signaling in many men, the term *hormone-refractory prostate cancer* has been replaced with *castration-resistant prostate cancer* (CRPC). This change in understanding is exemplified by the recent successes and approvals of novel hormonal agents, such as enzalutamide (Xtandi, Astellas), a small molecule AR inhibitor, and abiraterone (Zytiga, Janssen Biotech), a novel inhibitor of androgen biosynthesis.

Androgen receptor–independent prostate cancer (ARIPC) occurs when additional molecular mechanisms bypass the AR.[2,3] Determining this subtype in the clinic has been a challenge, given the lack of clinical definitions, metastatic biopsy samples, or biomarker definitions. However, this is rapidly changing as metastatic samples and improved preclinical models and assays for AR activity become available for this disease state.

The most common form of ARIPC is neuroendocrine prostate cancer (NEPC), also called anaplastic carcinoma of the prostate, which is characterized molecularly by a loss of AR dependence and a molecular profile of neuroendocrine differentiation with gains of Aurora A kinase and N-myc,[4] as well as loss of Rb.[5] However, additional forms of ARIPC likely exist and remain to be characterized clinically.

Clinically, CRPC can present in a variety of forms, or phenotypes. A phenotype is defined as an observable physical or biochemical manifestation of an underlying genotype. Although phenotype typically is determined by underlying genomic or epigenomic factors, it also may be influenced by external environmental factors. These external factors include prior exposure to therapy, the patient's metabolic profiles, exposure to carcinogens (eg, tobacco), inflammation, and other lifestyle factors.

Clinical subtypes of CRPC reflect those observable manifestations that carry prognostic importance and therapeutic implications, even if the molecular genotype is not known. These phenotypes can be classified according to histology, sites of metastatic disease, symptom burden, and certain blood-based or tissue-based biomarkers (Table).

Evidence is emerging for an evolving molecular genotypic diversity in prostate cancer,[6-8] as well as an overlying epigenomic classification for lethal prostate cancer.[9] These molecular profiles have not yet been clearly linked to clinical phenotype, prognosis, and altered treatment decisions in the clinic, however. For example, predictive biomarkers that suggest a personalized systemic therapy approach are currently lacking in patients with CRPC.

This review will focus on the clinical subsets of CRPC, which have a range of prognoses and treatment strategies, and provide a discussion of how these clinical phenotypes may be linked to the underlying molecular biology of CRPC.

Histologic Subsets of CRPC

Prostate cancer arises from glandular-forming epithelial cells, which typically do not proliferate, but acquire the ability to proliferate abnormally in response to AR stimulation.[10] Dr Donald F. Gleason initially described the Gleason grading system in 1966,[11] and validated the system for prostate cancer prognosis in 1032 men at the Minneapolis Veterans Administration in 1974.[12] In 2005, the International Society of Urological Pathology updated the Gleason grading system to correspond more closely with patient outcome.[13] Gleason grading reflects the degree of nuclear polymorphism, glandular disruption, basement membrane disruption, disease heterogeneity, and dedifferentiation of prostate cancer, so it reflects the phenotype of a number of underlying genomic lesions. The original Gleason score therefore carries prognostic weight for survival, even in the setting of metastatic CRPC (mCRPC).[14,15]

The 4 main histologies of prostate cancer are adenocarcinoma—which is the predominant form—as well as ductal carcinoma, mucinous carcinoma, and anaplastic carcinoma. Additionally, squamous differentiation is a rare but aggressive subtype of prostate cancer that may emerge de novo or following ADT or radiation.[16]

Adenocarcinoma accounts for 95% of all prostate cancers.[17] A recent review of rare histologic subtypes of prostate cancer in the Surveillance, Epidemiology and End Results (SEER) database identified an incidence of 61 cases per 10,000 people per year for mucinous carcinoma of the prostate, 49 cases per 10,000 people per year for ductal carcinoma of the prostate, and 35 cases per 10,000 people per year for NEPC.[18] Mucinous carcinoma of the prostate had a 5-year overall survival (OS) rate that was similar to that for prostate adenocarcinoma (75.1% vs 76.5%, respectively).[18] Ductal carcinoma of the prostate had a more aggressive phenotype and had a 5-year OS rate of 61.7%.[18]

In contrast, NEPC has a very poor prognosis, with a 5-year OS rate of only 12.6%.[18] NEPC can arise de novo in the primary setting with a low serum prostate-specific antigen (PSA) level and obstructive symptoms, and often presents with distant metastases at the time of diagnosis. However, the more common type of NEPC arises as an emerging or secondarily resistant subtype that develops castration resistance months to years after the first diagnosis of prostate adenocarcinoma.

NEPC is characterized by tissue and serum overexpression of chromogranin A (CgA) and synaptophysin.[19] Molecular lesions in NEPC include amplification of *Aurora A kinase* and *N-myc*,[4] as well as other molecular

Table. Clinical Phenotypes in CRPC, With Implications for Prognosis and Importance to Clinical Care

Clinical Phenotype	Implications
Neuroendocrine histology	Prognostic for poor overall survival Lacks sensitivity to hormonal therapy Correlated to Aurora A kinase and N-myc amplification May be predictive for Aurora A kinase inhibition
Pattern of spread	Nonmetastatic biochemical recurrence with options for observation, salvage radiation therapy, or hormone therapy Prognostic for survival In order of worsening survival: lymph node–only metastases, bone metastases, visceral metastases (lung, liver)
Pain	Prognostic for survival May be predictive of lack of benefit to sipuleucel-T (Provenge, Dendreon)
Anemia	Prognostic for survival
Performance status	Prognostic for survival
PSA levels	Prognostic for survival Changes can be indicative of improvements in survival after treatment PSA kinetics are prognostic High levels may be predictive of benefit to hormonal therapies and treatment response (AR pathway) Low PSA despite mCRPC may indicate neuroendocrine prostate cancer and lack of benefit to hormonal agents Lower PSA levels may be predictive of benefit with immunotherapy (ie, sipuleucel-T)
Alkaline phosphatase	Prognostic for survival prior to and during therapy May be predictive of response to radium-223 treatment
Lactate dehydrogenase	Prognostic for survival Elevated in neuroendocrine prostate cancer
CTC enumeration	Prognostic for survival CTC declines after treatment are prognostic with a range of therapies Under evaluation as a surrogate biomarker CTC biomarkers may provide predictive information linked to specific therapies

AR, androgen receptor; CRPC, castration-resistant prostate cancer; CTC, circulating tumor cell; mCRPC, metastatic CRPC; PSA, prostate specific antigen.

aberrations such as overexpression of EZH2,[4] loss of Rb,[5] or activation of the PI3 kinase pathway,[20] all of which present potential targets for therapy.

One fascinating aspect of NEPC is the ability of these tumors to revert histologically to adenocarcinoma with loss of neuroendocrine biomarkers during therapy with an Aurora A kinase inhibitor,[4] a phenomenon that mimics the one observed in the reversible transitions of non–small-cell and small-cell lung carcinomas.[21] The fact that the majority of NEPCs arise from previously diagnosed prostate adenocarcinoma suggests that prostate cancer cells have an inherent plasticity; they are able to change histologic subtypes to evade treatment pressures.

NEPC correlates with poor prognosis.[18] A historical series of 21 patients with NEPC at the University of Texas MD Anderson Cancer Center were treated with chemotherapy that was active in small-cell carcinoma of the lung. The median OS was 9.4 months, with a range of 1 to 25 months.[22] In a subsequent phase 2 trial of 120 patients with NEPC, participants were treated with carboplatin and docetaxel (CD) as first-line therapy, followed by etoposide and cisplatin (EP).[23] Primary endpoints included response rates and time to progression with each of these regimens. Of the 74 patients who underwent treatment with both regimens, 50% had a benefit from both regimens, 34% responded to CD but not to EP, 9% responded to EP but not to CD, and 7% did not respond to either regimen. The median OS was 16 months and the median time to progression after responding to first-line CD was 5 months.[23] Despite recent molecular characterization of amplification of *Aurora A kinase* and *N-myc* in NEPC, OS is still limited with current chemotherapy treatment options, and new approaches are needed. An ongoing phase 2 trial of the Aurora kinase inhibitor MLN-8237 is actively accruing patients with NEPC to evaluate drug efficacy and predictive biomarkers.[24]

Pattern of Metastatic Spread

At the time of autopsy, men who have died of prostate cancer are commonly found to have dissemination of their disease in the bone, liver, lymph nodes, and lungs.[25] Men with CRPC can also present without metastatic
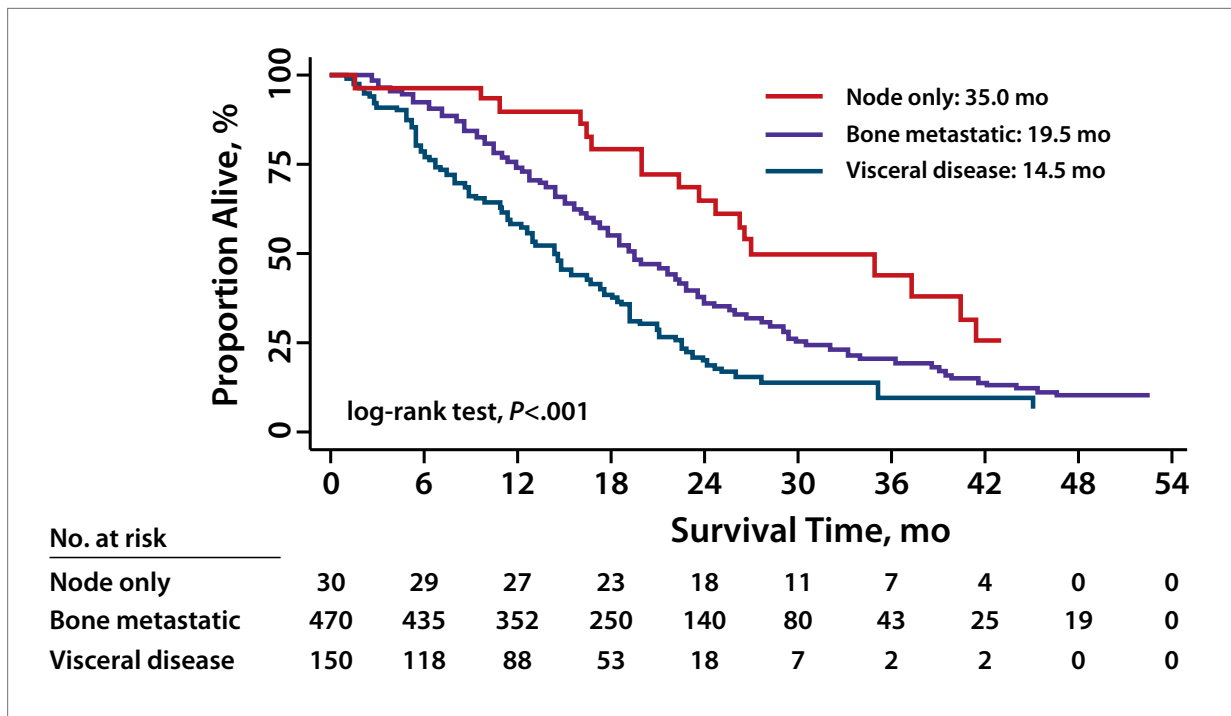
Figure 1. Kaplan-Meier overall survival estimates for patients in the TAX-327 trial as separated by lymph node–only disease, bone metastases, and visceral disease.[26]

Figure from Armstrong AJ, Tannock IF, de Wit R, George DJ, Eisenberger M, Halabi S. The development of risk groups in men with metastatic castration-resistant prostate cancer based on risk factors for PSA decline and survival. *Eur J Cancer.* 2010;46(3):517-525. Reprinted with permission from Elsevier.[26]

disease, which represents a clinical phenotype and disease state that has therapeutic implications. In the TAX-327 (Docetaxel Plus Prednisone or Mitoxantrone Plus Prednisone for Advanced Prostate Cancer) study, in which 1006 men with mCRPC were randomized to docetaxel or mitoxantrone chemotherapy, OS for mCRPC depended on the pattern of metastatic spread (Figure 1).[26] Men with lymph node–only CRPC treated with docetaxel had the best median OS, of 35 months. Men with CRPC that had metastasized to bone (with or without nodal spread) tended to have a poorer prognosis, with a median OS of 19.5 months, and patients with visceral disease had the poorest median OS at 14.5 months (*P*<.0001).[26] Thus, the pattern of metastatic spread carries real prognostic significance, and is reflected in the current Prostate Cancer Working Group 2 (PCWG2) reporting criteria for clinical trials.[27]

Nonmetastatic CRPC represents a relatively common subset of prostate cancer, with a heterogeneous but defined natural history.[28,29] Although no systemic agents are specifically approved for this disease state, several trials of novel agents, such as enzalutamide, are under way. Outcomes in nonmetastatic CRPC, which is typically asymptomatic, are determined by measures of PSA and PSA kinetics. These measures can reliably predict the onset of metastatic disease and the timing of death. The disease of men with

nonmetastatic CRPC has a more indolent natural history, and the goal of therapy in this setting is to delay the onset of symptomatic metastases while also extending life. Thus, nonmetastatic or locally advanced CRPC represents a clear phenotype of CRPC[27] with distinct outcomes.

At the time of prostate cancer diagnosis, up to 10% of patients have regional metastasis to the lymph nodes.[30] Lymph node metastasis seems to occur via collective migration with abnormal lymphangiogenesis, and has been correlated with higher levels of vascular endothelial growth factor C (VEGF-C) and VEGF receptor 3. In preclinical models, this pattern of spread may be associated with a pattern of cell migration called collective sheet migration. In contrast, hematogenous spread relies on the tumor necrosis factor (TGF)-β–driven movement of single cells.[31,32]

Based on historical and retrospective cohort data, patients with metastasis to the lymph nodes have been shown to have long durations of progression-free and disease-specific survival. In an analysis of 3463 consecutive patients at the Mayo Clinic in Rochester, Minnesota, 322 had lymph node disease. The 10-year cancer-specific survival rate for these patients was 83%, and the 10-year progression-free survival rate was 64%.[30]

Despite advances in therapy over the past decade, a recent retrospective analysis in 2013 of 369 men at Memorial Sloan-Kettering Cancer Center with lymph

node metastasis showed that the 10-year cancer-specific survival rate and 10-year rate of freedom from metastasis were 72% and 65%, respectively.[33] Thus, node-only spread of CRPC likely reflects a more favorable prognosis and different underlying pathobiology when compared with hematogenous spread of the disease.

In contrast, the mechanism of sclerotic bone metastasis in prostate cancer may depend on epithelial plasticity. Epithelial plasticity refers to the ability of prostate cancer cells to undergo a phenotypic change to a more mesenchymal or primitive state, invade blood vessels, and disseminate through the blood stream to colonize the bone marrow. This process may be regulated by chemokines, inflammation, activated stroma, and other host- and treatment-related factors.[34] Once the cells gain access to the bone marrow niche, they interact locally with paracrine signaling molecule—such as TGF-β, vascular endothelial growth factor, and receptor activator of nuclear factor κ-B ligand (RANKL)—to acquire a bone-like primitive phenotype that can compete for the hematopoietic stem cell niche,[35] leading to progressive bone marrow failure.[35,36] This plasticity and osteomimicry—the ability to form bone or mimic osteoblastic cellular properties—has been commonly observed in bone metastases and circulating tumor cells (CTCs) of men with CRPC, indicating the importance of molecular pathways that regulate stemness, differentiation, and plasticity in bone metastasis development and treatment resistance.[37-39]

Patients with CRPC that has metastasized to bone benefit from therapy that targets the bone microenvironment. Relevant agents include bisphosphonates such as zoledronic acid,[40] RANKL inhibitors such as denosumab (Xgeva, Amgen),[41] and radiotherapy in the form of radium-223 (Alpharadin, Algeta).[42] In the phase 3 denosumab trial, 1904 patients with metastatic CRPC were randomized to receive either denosumab or zoledronic acid, with time to first skeletal event as the primary endpoint. The median time to first skeletal event was 20.7 months for patients who received denosumab, compared with 17.1 months for those treated with zoledronic acid (hazard ratio [HR], 0.82; P=.0002).[41]

Radiotherapy is changing the treatment paradigm for patients with CRPC that has metastasized to bone. The US Food and Drug Administration (FDA) recently approved radium-223, a novel radioisotope, for the treatment of patients with CRPC that has metastasized to bone. The ALSYMPCA (Alpharadin in Symptomatic Prostate Cancer) phase 3 trial studied 921 patients who had CRPC with bone metastases but no visceral metastases. Patients were treated either after docetaxel or before docetaxel based on medical fitness or patient refusal of chemotherapy.[43] Patients received either radium-223 (50 kBq/kg intravenously every 4 weeks) for 6 treatments,

or a placebo. Patients who received radium-223 had a significantly improved OS of 14.9 months, compared with 11.3 months in the patients treated with placebo (P<.001), as well as significantly increased time to first skeletal event at 15.6 months compared with 9.8 months, respectively (P<.001).[43] In this trial, there was a suggestion of a greater survival benefit with radium-223 in men with mCRPC who had elevated levels of serum alkaline phosphatase (AP, ≥220 U/L), suggesting that this bone-forming phenotype may have clinical importance in patient selection for bone targeting therapy.

CRPC that has metastasized to visceral organs such as the lungs and the liver often behaves more aggressively than CRPC that has metastasized to lymph nodes and bones (Figure 1). Based on a recent analysis of the TAX-327 study, the prognosis of CRPC that has metastasized to the lungs is more favorable than the prognosis of CRPC that has metastasized to the liver.[44] This finding is reflected in published nomograms in this population, in which liver metastasis confers a strong negative impact on survival.[14,45] A recent abstract at the 2013 American Society of Clinical Oncology annual meeting analyzed outcomes of visceral disease in metastatic CRPC patients treated with enzalutamide in the post-docetaxel AFFIRM (A Study Evaluating the Efficacy and Safety of Investigational Drug MDV3100 in Men With Advanced Prostate Cancer) trial. The authors showed that CRPC that had metastasized to the lungs alone had a median OS of 15.5 months, compared with 7.7 months for those patients with CRPC that had metastasized to the liver (including those with involvement of both the liver and the lungs).[46] Although men with visceral disease do appear to benefit from newer hormonal agents such as enzalutamide and abiraterone, as well as from traditional chemotherapy agents such as docetaxel, patients generally have much lower rates of response and survival once they develop visceral metastases, particularly those to the liver. The finding of visceral metastases has therapeutic implications, given that men with visceral disease are excluded from radium-223 therapy as per the FDA label. Furthermore, current National Comprehensive Cancer Network guidelines do not recommend the use of sipuleucel-T immunotherapy (Provenge, Dendreon) in men with mCRPC and liver metastases given the inherent poor prognosis of these men and exclusion from the IMPACT (Immunotherapy for Prostate Adenocarcinoma Treatment) phase 3 trial of this agent.

Symptom Subsets of CRPC

Pain
Patients with CRPC can present with a multitude of cancer-related symptoms, most notable of which are pain and fatigue owing to anemia. Pain often arises in

the setting of metastatic disease to bone. At its onset, bone pain is often an ache or soreness, which increases in intensity over weeks to months. However, many men can develop metastatic CRPC in the bone with multiple sites of disease in the absence of pain. For example, in the TAX-327 trial, 50% of men with multiple hot spots on their bone scan were without clinically significant pain, whereas 50% of similar men with multiple bone lesions required opiates to control pain, reflecting clear heterogeneity in the clinical manifestations of bone metastases.[14] Pain responses may also occur to systemic therapy in the absence of biochemical responses as measured by PSA, indicating that the benefit of palliative chemotherapy may be independent of immediate changes in PSA.[47,48]

Complications of bone metastases include pathologic fracture, spinal cord compression, and severe pain requiring surgical or radiation oncology intervention. The physiology of these symptomatic complications and pain exacerbations is not clear, and patients may present clinically without pain and yet with a super scan (ie, a bone scan in which all the uptake is in the bones and the kidneys are not visible). However, pain may also be related to osteoblastic progression of disease at a given site, compression fractures due to bone insufficiency and osteoporosis, transient flares during treatment initiation,[48] or the release of humoral factors such as endothelins that may elicit bone pain.[49]

In 2 subsequent analyses of the TAX-327 trial, the symptom of pain at baseline and following treatment was an independent prognostic predictor of OS,[47,50] and is included in current nomograms in this setting.[14,45] These findings were confirmed in a large analysis of NCI cooperative group trials of men with CRPC, in which pain interference was clearly associated with shortened survival (17.6 vs 10.2 months for men with low vs high pain scores.)[51] In TAX-327, men with significant pain at baseline (typically those men taking opioids for pain relief) who had a significant reduction in pain intensity had a median OS of 18.6 months, compared with 12.5 months in patients whose pain did not respond to treatment.[47]

These results have been recapitulated in studies of abiraterone as well, reflecting the ability of novel hormonal agents to alter this clinical phenotype with resultant improvements in prognosis.[52] In addition, pain response led to the approval of mitoxantrone chemotherapy by the FDA, and the prevention of skeletal related events led to the approvals of zoledronic acid and denosumab, reflecting the clinical importance of pain relief and prevention. In addition, durable pain palliation is currently under evaluation as an approvable and quantifiable endpoint in the ongoing phase 3 COMET (Cabozantinib MET Inhibition CRPC Efficacy Trials) studies of the dual VEGF receptor 2/c-Met inhibitor cabozantinib in men with mCRPC. Thus, pain is a clear clinical phenotype associated with survival outcomes, and while the underlying molecular mechanisms regulating pain are diverse and often unknown, therapeutic interventions can alter this manifestation for palliative benefit.

Anemia

Fatigue related to anemia has long been shown to be a complicating symptom in a subset of patients with prostate cancer.[53,54] The development of anemia has multiple etiologies, including anemia of chronic disease, ADT, renal disease, disseminated intravascular coagulation, chemotherapy toxicity, blood loss, or bone marrow infiltration of prostate cancer cells. Bone marrow infiltration represents a unique mechanism, as it results from prostate cancer cells acquiring a stem-like phenotype and outcompeting hematopoietic stem cells for the bone marrow niche.[35] Anemia is therefore a harbinger of bone marrow infiltration, which can ultimately progress to bone marrow failure and contribute directly to the mortality of patients.

The presence of anemia in a patient with CRPC is an independent prognostic characteristic for worse prognosis and has been shown by multivariate analysis to be important in all published nomograms for survival in metastatic CRPC.[14,15,45,53,55] The TAX-327 database was used to show the contribution of anemia and its role for prognosis (Figure 2).[14] Median OS was 14.9 months for patients with anemia compared with 21.7 months in patients without anemia, and the presence of anemia is included in nearly all published nomograms and multivariate models of survival in the mCRPC disease state.[14,15,45,55]

Although the use of erythropoietin growth factor has been shown to increase hemoglobin levels and improve quality of life in patients with prostate cancer,[56,57] a Cochrane meta-analysis ultimately showed that erythropoietin increases the risks of hypertension, thrombocytopenia, and venous thromboembolism in patients with solid tumors.[58] Therefore, erythropoietin is not given as a standard treatment to prostate cancer patients unless chemotherapy causes more severe anemia, and then only after an informed consent discussion. Anemia typically improves with therapy directed at the tumor, including chemotherapy and hormonal therapies.

Performance Status

The importance of Karnofsky performance status in determining prognosis has also been well established as an independent prognostic factor in multiple nomograms and multivariate analyses of survival in patients with mCRPC.[14,15,45,55] Performance status is often variable depending on patient presentation and can be affected by a multitude of factors, such as comorbidity and prior medical conditions. It reflects not only symptoms such as pain and anemia, but also emotional stress and mood changes associated with a cancer diagnosis. Thus, functional status is of paramount importance in
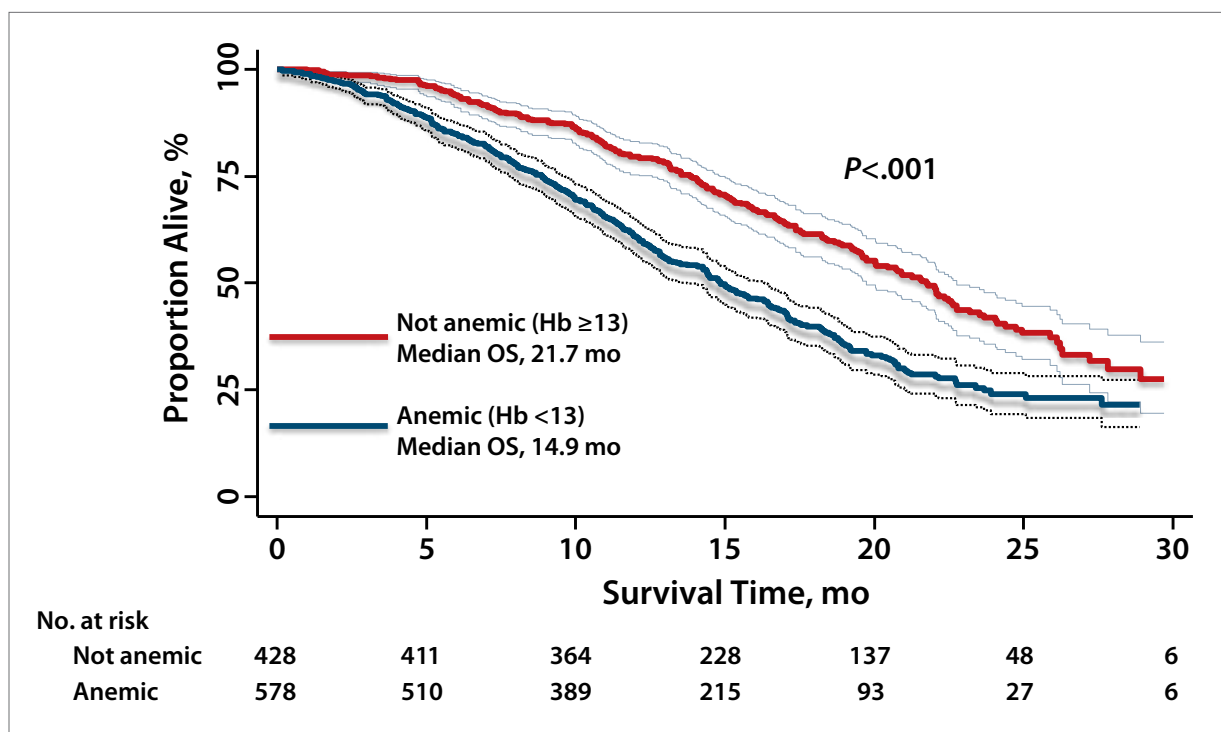
Figure 2. Kaplan-Meier overall survival estimates for patients with metastatic castration-resistant prostate cancer in the TAX-327 trial with anemia (Hb <13 g/dL) vs patients without anemia (Hb ≥13 g/dL).

Data are from Armstrong AJ, Garrett-Mayer ES, Yang YC, de Wit R, Tannock IF, Eisenberger M. A contemporary prognostic nomogram for men with hormone-refractory metastatic prostate cancer: a TAX327 study analysis. *Clin Cancer Res.* 2007;13(21):6396-6403.
Hb, hemoglobin; OS, overall survival.

determining prognosis and also in how aggressive a patient and provider may wish to be in selecting systemic therapies. The abilities of novel hormonal agents such as enzalutamide, abiraterone, and radium-223 to improve survival—even in men with mCRPC who have impaired performance status—are important milestones that have expanded the menu of treatment options for most men.[43,59,60]

Serum Biomarker Subsets of CRPC

A recent review discussed the role of multiple biomarkers in the progression of CRPC, including PSA, AP, lactate dehydrogenase (LDH), albumin, and CTCs.[61] These biomarkers not only can define prognosis, particularly when used as part of a nomogram that considers multiple simultaneous factors, but also can establish clinical phenotypes that relate to different underlying molecular mechanisms of progression and responses to systemic therapies. For example, PSA production is regulated by AR activity and may be an output of AR pathway dependence, whereas low PSA production such as in small-cell prostate cancer or NEPC indicates ARIPC. Bone biomarkers may reflect the burden of bone disease and benefit with bone-targeted agents. This section will review clinically relevant biomarkers that determine the clinical phenotype of men with mCRPC.

PSA

PSA testing was approved approximately 20 years ago to screen for prostate cancer, and to track its progression and response to therapies. Although controversial as a tool for prostate cancer screening, the use of PSA to monitor disease recurrence and response to local and systemic therapies is well accepted. PSA levels and PSA kinetics are strongly prognostic across all prostate cancer disease states, including M0 and M1 CRPC.[28,45] Reductions in PSA, such as a decline of least 30% or 50% during treatment, have also been shown to be highly associated with improvements in OS with chemotherapy. Although PSA levels are not a surrogate biomarker, they provide longitudinal data that can be clinically informative.[47] For example, in TAX-327, men who achieved a PSA reduction of greater than 30% during the first 3 months of chemotherapy treatment were found to have a median OS of 21.6 months, compared with 13.0 months for those patients who had less than a 30% PSA decline.[47] This benefit is counterbalanced by the existence of transient PSA rises in 15% to 20% of men with mCRPC in the first 3 to 4 months of chemotherapy.[48] This type of PSA flare does not have prognostic significance, and thus isolated changes in PSA during the early cycles of chemotherapy should not lead to treatment discontinuation.
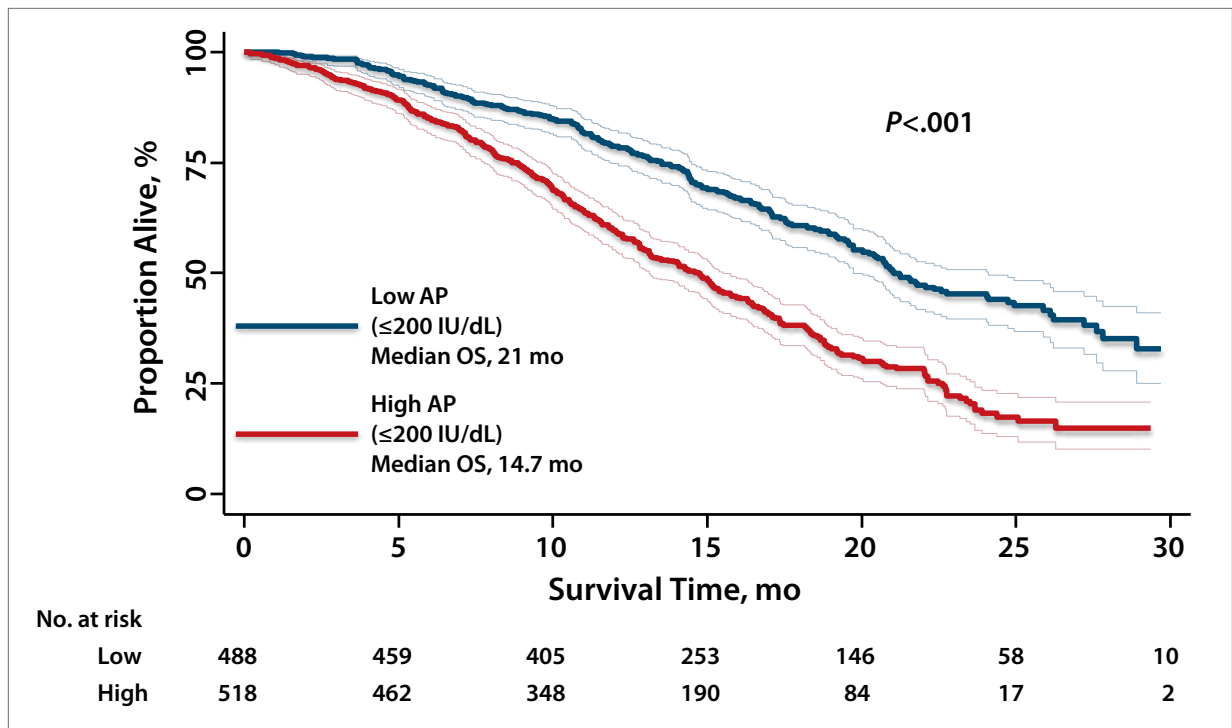
Figure 3. Kaplan-Meier overall survival estimates for patients with metastatic castration-resistant prostate cancer in the TAX-327 trial with alkaline phosphatase elevation (>200 IU/dL) vs patients with normal alkaline phosphatase (≤200 IU/dL).

Data are from Armstrong AJ, Garrett-Mayer ES, Yang YC, de Wit R, Tannock IF, Eisenberger M. A contemporary prognostic nomogram for men with hormone-refractory metastatic prostate cancer: a TAX327 study analysis. *Clin Cancer Res.* 2007;13(21):6396-6403.

AP, alkaline phosphatase; OS, overall survival.

PSA declines in response to hormonal therapies such as standard ADT, as well as with enzalutamide and abiraterone. However, given the frequent disconnect between PSA and radiographic/clinical changes, as well as observations that PSA can drift upward with these agents for long periods without clear clinical implications, using the PSA level alone for decisions related to discontinuing these newer agents is not recommended.[62] In addition, systemic agents such as sipuleucel-T and radium-223 are available that can improve survival without noticeable immediate changes in PSA levels, whereas other agents such as bevacizumab (Avastin, Genentech) and docetaxel combinations can lead to greater PSA declines without improvements in survival.[63,64] Thus, PSA levels can be informative prognostically, they can be tracked longitudinally to update prognosis, and PSA levels themselves may inform underlying tumor biology. Changes in PSA levels need to be considered in the context of the mechanism of action of the agent being studied, however.

Alkaline Phosphatase

The osteoblastic bone biomarker AP is variably elevated in men with bone metastases from mCRPC, and serum levels of AP are independently associated with death from prostate cancer.[14,15] Elevations in AP and declines with therapy were first described by Huggins and Hodges in their initial seminal paper on the hormonal dependence of prostate cancer.[65] In the TAX-327 trial, only 60% of the men with multiple bone metastatic lesions had elevations in AP, indicating that the release and detection of AP in the serum is a clinical phenotype independent of the burden of bone metastases and that elevation in AP alone does not reliably predict bone metastases. The TAX-327 trial also showed that patients with elevated AP levels above 200 IU/dL demonstrated a median OS of 21 months compared with 14.7 months for those patients with AP levels below 200 IU/dL (Figure 3).[14] In addition, AP may decline with effective systemic therapy, such as docetaxel, often with a transient initial rise, and these declines in AP are also prognostically important.

Although highly prognostic, elevations in bone markers (such as AP) or osteolytic biomarkers (such as C- or N-telopeptide levels) also change in response to therapy; these bone markers may predict benefit from systemic therapies with not only bone-targeted therapies but also docetaxel chemotherapy.[66,67] Men with mCRPC who presented with high levels of bone AP were found to have increased benefit with treatment from radium-223.[42]

In the phase 3 trial of radium-223 described earlier, there was a significant difference in median OS of patients who had baseline AP of greater than 220 U/L treated with radium-223 compared with those treated with placebo (HR, 0.62; 95% CI, 0.49-0.79; *P*<.001).[43] This study shows that high AP levels may be predictive of benefit in OS from radium-223 treatment,[43] but more prospective trials are needed to study this potential mechanism. Likewise, declines in AP with radium-223 have been shown to be favorably prognostic for survival.[68] Similar findings of bone biomarker changes after treatment have been described with zoledronic acid and denosumab (Xgeva, Amgen), and these changes are also highly associated with outcomes.[67] Thus, AP and bone biomarker elevations clearly signify an important clinical phenotype that reports on the bone metastatic biology in men with mCRPC. Further studies are needed to investigate the sources (tumor vs tumor microenvironment) of AP, the mechanisms leading to bone biomarker elevations and changes, and the clinical implications of these predictive changes in response to systemic therapies.

Lactate Dehydrogenase

LDH is an enzyme that is important in glucose metabolism; it is elevated in many tumors during aerobic glycolysis. Elevations in LDH are common in men with mCRPC and are highly and independently prognostic, implying that LDH levels provide a clinical phenotype of disease aggressiveness and poor survival.[15] In renal cell carcinoma, LDH elevations are prognostic but also may be predictive of the benefit of mammalian target of rapamycin (mTOR) inhibition, suggesting that serum LDH levels may provide an insight into tumor biology and the sensitivity to agents that target tumor metabolism, such as PI3K/Akt/mTOR pathway inhibitors.[69]

In a retrospective analysis of the Cougar 301 trial of abiraterone with prednisone vs prednisone alone, a biomarker panel composed of serum LDH and CTC enumeration at 12 weeks was highly prognostic and was able to identify men with mCRPC at very high risk for early mortality in the post-docetaxel setting. These data reflect the importance of LDH as a validated prognostic biomarker and its clinical value in measuring its changes over time. Although CTC enumeration alone was not a sufficient surrogate for survival, the combination of LDH and CTCs provided a higher level of surrogacy, which may be relevant for the evaluation of drug activity in clinical trials and in estimating survival in the clinic.[90]

In patients with NEPC, elevated serum levels of LDH and low levels of albumin have been shown to correlate with poorer disease-specific survival. One series of patients at MD Anderson Cancer Center showed that those who have high LDH and low albumin levels had a median disease-specific survival of 4.1 months, compared with 13.1 months for all patients.[71] Thus, serum LDH elevation is a highly prognostic clinical phenotype in men with mCRPC and suggests an aggressive disease course.

CgA and Synaptophysin

NEPC often secretes CgA and synaptophysin, both of which are detectable in serum and tissue. These proteins are normally produced by neuroendocrine cells. They are often found to be elevated in peripheral blood and can be monitored during the course of treatment for patients with NEPC.[23] Elevated CgA levels in serum were shown to be independently prognostic in men with mCRPC.[72] It is unclear what specific level of CgA elevation denotes NEPC, however, and it is also unclear whether CgA elevations are predictive of the benefit with alternative systemic therapies, such as platinums. Thus, serum neuroendocrine markers can be useful for defining NEPC, but the prospective study of these biomarkers for disease characterization and treatment decision-making has lagged.

Circulating Tumor Cells

In addition to these serum biomarkers, the detection of CTCs with the CellSearch CTC Test (Janssen) provides independent prognostic information in men with mCRPC.[73] This assay captures tumor cells in peripheral blood by attaching magnetic beads to antibodies against epithelial cell adhesion molecule (EpCAM) to select and capture cells that express EpCAM. These EpCAM-positive cells are differentiated from white blood cells using immunofluorescence stain for CD45 and then enumerated manually. Using the CellSearch assay, CTCs can be found in peripheral blood in men with prostate cancer.

The initial study in CRPC showed that patients with at least 5 CTCs per 7.5 mL of blood had decreased OS when compared with patients who had less than 5 CTCs per 7.5 mL of blood.[73] The median OS for the unfavorable group was 11.5 months, compared with 21.7 months for the favorable group (*P*<.0001).[73] The CTC test was found to change prior to PSA declines, and may provide an improved assessment of response to treatment compared with using PSA alone.[73] Declines in CTC after treatment are highly prognostic as well, indicating that prognosis can be updated based on CTC enumeration. CTC production and detection thus represents a clinical phenotype that provides independent prognostic information. However, many men do not have detectable CTCs despite metastatic CRPC progression, indicating that improved methods to detect CTC are needed. In addition, for prostate tumors that generally do not secrete PSA, such as NEPC, there are case reports of patients with normal PSA levels but elevated numbers of CTCs and poor clinical course.[74]

In subsequent analyses of CTCs, patients seem to have a variable expression of EpCAM CTCs. Patients with metastatic disease to the brain or with triple-negative breast cancer seem to lose expression of EpCAM and therefore lose the ability to capture cells using current capabilities.[75-77] We found that men with mCRPC commonly coexpress epithelial and mesenchymal/stem cell biomarkers, indicating that other biomarkers may be useful to detect different CTC phenotypes.[37] In addition, patients with visceral metastases and mCRPC tend to have lower than expected numbers of CTCs but poor overall prognosis.[78] We and others have found that CTC levels in men with CRPC provide an independent association with survival and are at times disconnected with AP or PSA levels or pain, indicating that this phenotypic clinical presentation of CTC elevation cannot be predicted based on other biomarkers.[78,79] A great deal of research remains to be done for better understanding and characterization of CTCs, to enable more sensitive detection of CTCs in the clinical setting. In addition, the real promise of CTC research lies in the ability to use CTC biomarkers as a window into the underlying tumor biology of the patient in real time, thus providing direct predictive biomarkers linked to specific therapies. A variety of factors, including CTC AR status, phosphatase and tensin homologue (PTEN) loss, and whole genomic RNA and DNA methods, are being evaluated to discover biologic targets for therapy and understand the mechanisms of drug resistance in CRPC.

Molecular Subtypes of CRPC

CRPC has been well characterized by a number of molecular genotypes, without a clear direct correlation to clinical phenotypes or prognosis. Genomic analysis has shown that many CPRCs have ETS family gene fusions (eg, *TMPRSS2-ERG*)[80,81]; loss of tumor suppressors such as *PTEN*,[82] p53, and Rb[5]; activation of the PI3K[20] and Ras pathways; and amplification of *AR* and *C-myc*.[83-85]

Whole genomic analysis performed in 7 prostate cancers has identified numerous chromosome rearrangements and gene fusions, with a median of 90 rearrangements per cancer genome.[80] The most frequent gene fusions in prostate cancer appear to be gene rearrangements involving members of the ETS transcription factor family (especially *ERG*) fused with a partner, which is regulated by the AR (especially *TMPRSS2*).[81] The prognostic impact of the *TMPRSS2-ERG* fusion is not well defined, with mixed results from several retrospective cohorts of patients after prostatectomy that had biochemical recurrence as a primary outcome.[86-88] Fusion appears to promote invasion in active surveillance; after prostatectomy and in CRPC, the presence of these fusions does not play a clear role in determining outcomes.

Many molecular alterations occur upon the progression of CRPC. A recent genomic analysis of 57 prostate tumors showed a coordinated evolution of genetic alterations, which was termed chromoplexy.[85] Through genomic analysis of CRPC, androgen signaling is often found to be affected through various mechanisms, including point mutations in the *AR* gene, *AR* gene amplification, or splice variants of AR.[83,84,89] Several groups recently identified multiple mutations in cofactors that interact with AR, including MLL2, FOXA1, UTX, and ASXL1-3.[7,80,90] Serine protease inhibitor Kazal type 1 *(SPINK1), CDK12*, Ras/Raf, and *SPOP* mutations are also found in many prostate tumors,[7,80,90] indicating clearly distinct genotypes in prostate cancer that lack ETS family fusions. Studies linking these genotypes to clinical phenotypes, therapeutic interventions, and outcomes are lacking, however, and should be the subject of future research.

The loss of the tumor suppressor genes such as *PTEN*,[82] activation of the PI3K/Akt pathway,[20] Ras pathway activation, and loss of *RB1* or *TP53* also appear to be important in CRPC.[85] These molecular aberrations have the potential to be predictive of agents such as PI3K inhibitors or cell cycle checkpoint inhibitors (for Rb wild-type patients) in the clinic. The clinical phenotypes of these mutated CRPCs have not been fully elucidated, and assays to determine these aberrant pathways are under development in parallel with drug development.

Beltran and colleagues recently showed gene amplification of *AURKA* and *MYCN* in 40% of NEPC[4]; these same amplifications were only present in 5% of prostate adenocarcinomas. This has led to the development of Aurora A kinase inhibitor therapy for patients with neuroendocrine CRPC, which is now in phase 2 clinical trials. These tumors frequently have loss of Rb and overexpression of the epigenetic regulator EZH2, indicating their genomic complexity and the likely need for combination treatment.[5]

In addition, CTCs have also been used to investigate the genomics of CRPCs.[83] Namely, Magbanua and colleagues recently used a novel isolation technique of fluorescence activated cell sorting to isolate prostate CTCs from 9 patients and found gains in 8q, loss in 8p, and amplification of the *AR* gene.[83] These mutations suggest that CTCs have the potential to provide genomic information on a patient's tumor in real time without the need for an invasive metastatic biopsy.

Although there is a great deal of emerging data on CRPC genotypes, more work remains to be performed to catalog and correlate these genotypes with the important clinical phenotypes, including histology, pain, anemia, PSA levels and AR activity, and elevated bone AP levels in CRPC, in addition to outcomes after specific local or systemic therapies. These studies will necessitate prospective

biopsies or circulating tumor biomarker studies with correlative analyses to identify genotype-phenotype links and the relevance to disease lethality.

Conclusions

Men with mCRPC present with a wide range of clinical phenotypes. For example, many men receive early ADT prior to the development of metastases and progress through a nonmetastatic CRPC disease state, whereas other men who present with metastatic disease have a larger degree of tumor burden. There is some concern that the reduction in PSA screening under current recommendations may alter the clinical presentation of men at diagnosis, with a reduction in diagnoses of low-risk tumors but perhaps an increase in high-risk or disseminated disease at presentation.

Despite the presence of bone metastases, some men have pain and/or high levels of AP, whereas others have prolonged periods without symptoms and/or normal levels of bone biomarkers. Different presentations of these heterogeneous clinical phenotypes of mCRPC lead to varying natural histories of disease in terms of survival and response to specific therapies. Patients who present with lymph node–only metastases have improved survival when compared with those with bone or visceral involvement, whereas men with hepatic metastases tend to fare worse than those with pulmonary and bone metastases. In addition, patient-reported symptoms and signs such as pain, anemia, and performance status, as well as elevated serum biomarkers such as PSA and AP, are independently prognostic of survival. Thus, there is a high level of both phenotypic and genomic diversity in men with CRPC, reflecting the complexity of the disease but also the importance of conducting studies linking this biology to patient outcomes and presentation. Although we have an understanding of the many genetic and epigenetic lesions that occur in men with metastatic CRPC, much work remains to be done to translate the discoveries of these molecular alterations into clinically actionable findings.

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