

Castration-resistant prostate cancer: potential targets and therapies

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Abstract: The treatment landscape for patients with castration-resistant prostate cancer (CRPC) is undergoing significant changes with the advent of new therapies and multidisciplinary efforts by scientists and clinicians. As activation of multiple molecular pathways in the neoplastic prostate makes it impossible for single-target drugs to be completely effective in treating CRPC, this has led to combination therapy strategy, where several molecules involved in tumor growth and disease progression are targeted by a therapeutic regimen. In the present review, we provide an update on the molecular pathways that play an important role in the pathogenesis of CRPC and discuss the current wave of new treatments to combat this lethal disease.

Keywords: chemoresistance, CRPC, molecular pathways, neoplastic prostate

Introduction

The pathogenesis of castration-resistant prostate cancer (CRPC) in human patients is complex. As yet, no definite mechanism has been proposed that is responsible for driving the progression of disease from a treatable hormone-dependent condition to untreatable CRPC. While several studies point to a progression of CRPC from hormone-dependent phenotype, there are reports suggesting that CRPC and hormone-dependent disease develop simultaneously and independent of each other in patients. This phenomenon could be related to the activation of multiple signaling pathways in tumor tissues. The identification of causative or contributive molecular pathways has high relevance for the development of new target-based therapeutic agents to treat CRPC. In the current review, we discuss several molecular pathways that have been reported to play critical roles in the pathogenesis of CRPC and are being pursued as drug targets.

The androgen receptor (AR) and CRPC Role in etiology of disease

In the majority of prostate cancer (CaP) cases, the AR has been found to be a principal driver of disease progression, although some exceptional CaP cases do exist in which prostatic tumors are not linked to AR signaling.^{1,2} CaP begins as an androgen-dependent disease, which is managed by a series of therapies that suppress AR signaling by androgen depletion and/or the administration of AR antagonists.³ However, the regression of tumor growth brought about by these therapies is usually only temporary and, after a short remission period, prostate tumors often regrow and become resistant to therapy. At this stage, the tumors are described as “androgen independent” or

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“castration-resistant” (ie, CRPC). Recent studies have shown that increased expression of the AR is the most common event associated with CRPC cell growth.⁴ Further, accumulating evidence suggests that during the progression of CaP to CRPC, phenotype could be attributed to the restoration of AR signaling in surviving CaP cells in patients receiving antihormone therapy.⁴

CRPC emergence has been attributed to a variety of mechanisms, including activation by receptor tyrosine kinases from growth factors, loss of cell-cycle regulators, and, in some cases, genomic mutations in the AR allowing response to nonspecific AR ligands.⁵ During CRPC emergence, CaP cells develop a multitude of mechanisms to activate AR.⁶ These include *AR* gene amplification, overexpression of AR co-activators and ligand-independent activation of AR in an androgen-depleted environment.^{7,8} This is based on studies showing that *AR* gene mutation and amplifications resulting in elevated AR protein expression are detected in the majority of metastatic prostatic tissues in CRPC patients.⁹ Consistent with these clinical observations, *AR* gene mutation, amplification, and protein overexpression are commonly observed in the majority of CaP cell lines derived from CRPC patients.⁹ Some prostate tumors are reported to become truly androgen-independent by activating survival pathways that allow the tumor cells to survive in the absence of AR signaling. There is some consensus that even after androgen ablation therapy, a low concentration of androgens released from the adrenal gland and biosynthesized within tumors sustain the AR activation and proliferation of remaining tumor cells in CRPC patients.^{9,10}

The discovery of AR splice variants has given another important dimension to the significance of AR in CRPC.^{11,12} AR splice variants have been observed in CaP cell lines.^{11,12} AR splice variants have been shown to lack a ligand-binding domain (LBD), however, they exhibit higher AR transcriptional activity in CaP cells.¹² The mechanisms through which functionally active AR splice variants (lacking LBD) arise during progression of disease are not well known. Using the rapid amplification of cDNA ends technique, Dehm et al¹³ reported that the splicing of a novel exon within AR intron 2 introduces a premature stop codon upstream of exon 3 in the AR transcript that would encode an AR protein (lacking the second zinc finger of the DNA-binding domain and LBD) if translated. Previously, it has been identified that up to seven different AR splice variants lack the LBD.¹⁴

Further, research has suggested that activation of splice variants of AR could be a possible mechanism

involved in the growth of tumor cells and emergence of the CRPC phenotype in CaP patients post-androgen-ablation therapy.^{12–14} Among all identified AR splice variants, AR-V7, or AR3, has been detected in several human CaP cell line xenografts and normal and malignant human prostate-tissue samples.¹⁴ AR-V7 levels are generally higher in CRPC versus androgen-dependent tumors, and AR-V7 expression in early stage CaP has been associated with a worse prognosis after radical prostatectomy.¹⁴ The significance of AR splice variants in CRPC could be understood from the observation that these variants activate a subset of genes in prostatic tumor cells, which are not activated by full-length AR in the presence of androgens.¹⁴ An important finding in this context is a report by Sun et al showing that AR splice variants activate genes involved in the metabolism of androgens; this therefore suggests that expression of AR splice variants in tumor cells may provide a survival advantage in a low-androgen environment.¹⁵

The AR as therapeutic target in CRPC

The AR has emerged as a promising therapeutic target for the treatment of both androgen-dependent CaP and CRPC, and several strategies to inhibit AR activation have been suggested. These include targeting the AR directly or inhibiting the resources/rate-limiting enzymatic pathways that lead to its activation in CRPC tumors.¹⁶ Several approaches to inhibiting AR expression and activity have been suggested. The classical approach leads to the identification of novel agents that inhibit AR expression or activity in CRPC cells. There is an enormous amount of literature regarding the efficacy of synthetic or natural agents in inhibiting AR expression, activity, or both under laboratory conditions (see Chi et al¹⁶ and Siddique et al¹⁷ and references therein these studies). However, the systemic toxicity, inability to achieve pharmacological doses, and low bioavailability does not permit most of these agents to be pursued beyond the laboratory stage, although some seem very promising. Recently, Siddique et al¹⁷ showed that lupeol, a small natural molecule exhibiting a structural similarity to cholesterol, significantly inhibits the activity of AR in CRPC cells and tumor xenografts. It is noteworthy that lupeol was observed to be highly stable under physiological conditions and exhibited high bioavailability in mice.¹⁷

Accumulating evidence suggests that targeting AR in the late stage of disease could be an ideal strategy, since the AR is highly amplified in this disease stage. In light of this, several agents are being pursued that could specifically

target AR in CRPC at a late stage of disease. Among these, MDV-3100, a potent agent that specifically targets AR in CRPC cells, is in clinical trial and has shown promising results.¹⁸

Further, AR knockdown has been suggested as a promising strategy based on the study in which knockdown of AR expression in CRPC cell lines was observed to inhibit cell proliferation.¹⁹ Several agents have been reported that were observed to decrease the expression of the AR under in vitro conditions (see Snoek et al¹⁹ and references therein). However, none of these agents have so far moved to the translational stage.

According to Ryan et al,²⁰ a divergence of disease biology may occur in which certain patients develop CRPC disease that continues to rely to some extent on androgen-AR signaling and is thus amenable to therapies that target androgen synthesis or receptor activation. In CRPC tumor tissues, levels of dehydroepiandrosterone, dehydroepiandrosterone-sulfate, and 5-androstenediol exceed the concentrations of testosterone and dihydrotestosterone and can activate wild-type and mutant AR.²¹ In addition, adrenal androgens can be metabolized and converted to dihydrotestosterone in CaP cells.^{21,22} These facts have led to the testing of agents that would indirectly inhibit activation of the AR in CRPC cells by blocking adrenal steroidogenesis with the aim of establishing this strategy as a therapy to treat CRPC.²² In this direction, the clinical efficacy of the antifungal agent ketoconazole (as a blocker of cytochrome P450-17 (CYP) and 11 β -hydroxylase, a rate-limiting enzyme responsible for mediating the conversion of cholesterol to androgens) has been extensively studied (see Hamada et al²³ and references therein). Hamada et al²³ showed that the presence of a polymorphism in the promoter region of the *CYP17* gene is associated with survival in patients with CRPC, regardless of the therapy. In clinical settings, treatment with ketoconazole was observed to lead to a prostate-specific antigen (PSA) decline of >50% in 46%–62% of CaP patients.^{24–26} Further, results of a randomized Phase III trial in men with CRPC demonstrated a PSA response of 50% or greater in 27% of patients treated with ketoconazole; however, no difference in overall survival was observed.^{24–26} It should be mentioned that ketoconazole is not a selective adrenal inhibitor and could lead to adrenal insufficiency.

Recently, a new class of drugs that inhibit AR activity by reducing the availability of androgenic steroids in CRPC patients has been discovered.²⁷ Abiraterone acetate is a potent and selective inhibitor of CYP17 (17-hydroxylase/

C17, 20-lyase) enzyme.²⁷ Abiraterone acetate is capable of reducing androgenic steroid production downstream from CYP17. Phase I and II studies have demonstrated the maximum tolerated dose and safety of abiraterone acetate.^{27,28} Moreover, the results of a recently conducted double-blind placebo-controlled Phase III study of abiraterone acetate and prednisone in chemotherapy-refractory metastatic CRPC patients are promising.^{27,28} The median overall survival for patients treated in the abiraterone/prednisone arm was 14.8 months compared with 10.9 months for the patients in the prednisone/placebo arm (hazard ratio 0.646, $P < 0.0001$). This benefit was observed across all patients, regardless of sites of metastatic disease and number of prior chemotherapy regimens undertaken. It is noteworthy that the abiraterone acetate treatment caused a significant delay in the radiographic progression-free survival in CRPC patients.²⁹

Phosphoinositide-3-kinase (PI3K) and Akt in CRPC disease

Role in emergence of disease

Clinical observations suggest that the loss of AR expression may serve as an alternative means of escaping androgen withdrawal or AR-targeted therapies, possibly through concomitant activation of compensatory signaling pathways. One of the possible survival and proliferative pathways is the PI3K/Akt signaling pathway, which is negatively regulated by the phosphatase and tensin homolog (PTEN) tumor suppressor.³⁰ The PI3K/AKT pathway plays critical roles in mammalian cell survival signaling and is activated in various cancers including CaP. Morgan et al³¹ reported that PI3K/Akt signaling is upregulated in 30%–50% of CaP cases and associated this activation of signaling with the loss of PTEN function. PTEN function loss occurs frequently during human CaP progression, with up to 70% of late stage samples exhibiting loss of PTEN function or activation of the PI3K pathway.^{32,33} Alterations in PTEN expression are associated with a number of clinico-pathologic variables in CaP. It has been determined that loss of PTEN expression correlates with Gleason score and pathologic stage of primary tumors and increases the incidence of development of lymph node metastases.^{32,33} Further, PTEN function loss or activation of the PI3K/AKT pathway has been shown to enhance CRPC disease development,^{32–35} conversely, restoration of functional PTEN activity has been shown to block the growth of PTEN-negative prostate tumors in a xenograft mouse model and restore sensitivity to chemotherapy

(see Carrión-Salip et al,³² Krohn et al,³³ Li et al,³⁴ and Mulholland et al³⁵ and references therein).

PTEN function loss causes the activation of the PI3K/Akt pathway, resulting in the phosphorylation of the Akt molecule. Phosphorylation of Akt has been used as a surrogate biomarker for studying this pathway. Phospho-Akt levels have been shown to be significantly higher in high-grade prostate tumors than in low- or intermediate-grade tumors. Study by Bedolla et al³⁶ showed that PTEN in combination with phospho-Akt (an activated form of Akt protein), is a better predictor of the risk of recurrence of disease in CaP patients. In this study, 90% of all cases with high phospho-Akt and negative PTEN were recurrent, whereas 88.2% of those with low phospho-Akt and positive PTEN were nonrecurrent.³⁶

Activation of the PI3K/Akt pathway has been associated with ligand-independent activation of the AR and with progression of CRPC phenotype. In addition to promoting cell survival and inhibiting apoptosis, the Akt pathway is reported to regulate cell growth, proliferation, and angiogenesis through the mammalian target of rapamycin (mTOR) pathway, thus activating proliferation genes such as *c-Myc*, cyclin D, nuclear factor kappa B, and vascular endothelial growth factor.³⁷ Activated Akt is reported to phosphorylate the FoxO subfamily of forkhead box family transcription factors, which inhibits transcription of several pro-apoptotic genes, for example, *Fas-L*, *IGFBP1*, and *Bim*.^{38–40} In addition, Akt is reported to modulate cell-cycle progression through its inhibitory phosphorylation of the cyclin-dependent kinase inhibitors, p21^{WAF1/CIP1} and p27^{KIP1} (see Kharaziha et al⁴¹ and Leong et al⁴²) and stabilizing the expression levels of proliferative protein cyclin D1.^{43,44}

The PI3K/AKT pathway as a target for CRPC

Kreisberg et al⁴⁵ conducted a comparative study on Akt activation and established the significance of the Akt pathway in therapy failure in CaP patients. The study reported a significant increase in mean phospho-Akt staining ($P < 0.001$) in the PSA failures versus non-failures: 222.18 ± 33.9 ($n = 37$) versus 108.79 ± 104.57 ($n = 16$). Using the best-fitting multiple logistic regression equation, a 100-point increase in activated Akt (phospho-Akt) levels resulted in a 160% increase in the odds of being a PSA failure. Further, Kaarbø et al⁴⁶ showed that the PI3K pathway is dominant over AR signaling in CaP cells and should be considered in developing novel therapeutic strategies for CaP. The rationale for targeting the PI3K/Akt pathway

in combination therapy comes from data describing constitutive or residual pathway activation in tumor cells that have developed resistance to conventional chemotherapy and radiation (see Kreisberg et al⁴⁵ and Kaarbø et al⁴⁶ and references therein).

Under preclinical conditions, inhibitors of PI3K such as LY294002 and wortmannin diminish signaling to Akt.^{47,48} Although these commercially available inhibitors effectively inhibit PI3K, poor solubility and high toxicity have limited their clinical applications. However, these compounds provide powerful preclinical tools to study the cellular consequences of pathway inhibition.

Perifosine, a lipid-based compound, has been reported to act as a potent inhibitor of Akt and in preclinical settings it inhibits the growth of CaP cells along with Akt activity.⁴⁹ Results of a Phase II clinical study conducted with recurrent CaP patients showed that, although well tolerated, perifosine did not meet prespecified PSA criteria for response as a single agent.⁵⁰ However, 20% of patients had evidence of PSA reduction, suggesting modest single-agent clinical activity. This study suggested that effects of perifosine in combination with androgen deprivation or chemotherapy could show better results than monotherapy in CaP patients.

One downstream target of the PI3k/Akt pathway is the mTOR kinase, which is activated in PTEN-negative tumors.⁵¹ Preclinical studies suggested that targeting mTOR could reverse the growth-promoting effects of the PI3K/Akt pathway in CaP cells (see Majumder et al⁵¹ and references therein). Several agents (such as everolimus and deforolimus) directed at Akt/mTor pathways have been developed and are in preclinical and clinical trials to treat CaP. Several in vitro and in vivo studies conducted with transgenic mice expressing human Akt have shown that prostate tumor development could be inhibited with the treatment of everolimus, an oral mTOR inhibitor.⁵¹ With these promising results at preclinical stage, everolimus as a therapeutic agent has moved to Phase I and II clinical trials. The results of a pharmacodynamic study conducted in CaP patients suggested that mTOR inhibition due to everolimus intake could be detected in prostate tumor tissues.^{52,53} Further, everolimus was tested in combination with docetaxel in a Phase I study using fluorodeoxyglucose positron emission tomography imaging as a pharmacodynamic endpoint.⁵⁴ The combination was found to be tolerable (everolimus 10 mg daily + docetaxel 70 mg/m² every 3 weeks) caused a decrease in fluorodeoxyglucose-avidity PSA levels.⁵⁴ Although mTOR inhibitors have little single-agent activity in advanced CRPC, the combination of these agents with

docetaxel is an attractive option given their ability to reverse chemotherapy resistance in CaP cell lines.

Janus kinase (JAK)/signal transducer and activator of transcription (STAT) 3 and CRPC

Role in pathogenesis of disease

STAT3, a component of the JAK/STAT signaling pathway, can be activated by tyrosine phosphorylation as p-STAT3 and is involved in the regulation of cellular growth, survival, and oncogenesis.⁵⁵ The JAK/STAT pathway is activated constitutively in CaP cells.⁵⁶ The significance of STAT3 in the emergence of CRPC disease was ascertained from a report showing that levels of activated STAT3 are significantly higher in AR-negative prostate tumor cells (Du145 and PC3) than in AR positive.⁵⁶ STAT3 is stimulated by cytokines, particularly interleukin-6 (IL-6).⁵⁷ The signal transduction of IL-6 involves the activation of JAK tyrosine kinase family members, thus resulting in the activation of STAT3. Studies by Chen et al⁵⁷ and Lou et al⁵⁸ have shown that IL-6-induced AR activity requires the activation of STAT3 that ultimately influences survival, proliferation, apoptosis, angiogenesis, and invasive potential of prostatic tumor cells. Constitutively active STAT3 is reported to increase motility of prostate epithelial cells and promote prostate tumorigenesis.

A study conducted with prostatic tissue biopsies from 52 patients with localized CaP and 80 patients free of CaP showed that p-STAT3 could be utilized to detect CaP with initial negative biopsies.⁵⁵ This study suggested that p-STAT3 could be a potential adjunctive tool for a current CaP diagnostic program and developed as a diagnostic biomarker that can predict the onset of CaP up to 40 months earlier than currently used diagnostic approaches.⁵⁵ Liu et al⁵⁹ reported that levels of p-JAK-1(Tyr1022/1023) and p-STAT-3(Tyr705) are positively correlated with Gleason score and clinical stage of patients with CaP. Importantly, CaP patients exhibiting lower p-STAT and p-JAK levels exhibited increased recurrence-free survival rates.⁵⁹ Moreover, for patients treated by definitive or hormonal therapy, the recurrence-free survival rates in those with lower p-JAK-1 ($P < 0.001$ and 0.012 , respectively) and p-STAT-3(Tyr705) expression ($P < 0.001$ and 0.015 , respectively) were significantly higher than in those with higher expression.⁵⁹ This study showed that p-JAK-1-(Tyr1022/1023) and p-STAT-3(Tyr705) levels in needle-biopsy specimens are prognostic factors for CaP.⁵⁹ The JAK/STAT3 pathway has been reported to be induced by elevated

levels of circulating IL-6 in the serum of patients, which in turn results in the activation of AR activity.^{60,61}

The JAK/STAT pathway as a target for CRPC

Growing evidence has shown that the STAT3 could be an effective molecular target for CRPC therapy.⁶² A report from the National Institutes of Health Molecular Libraries Program has suggested that the identification of novel compounds that selectively inhibit STAT3 activity may lead to useful tools to reduce cell proliferation, inflammation, and chemotherapeutic resistance.⁶³ Blockade of STAT3 expression is reported to suppress proliferation of CaP cells in vitro and prostate tumorigenicity in vivo.

Keeping in view the significance of STAT3 as a potential target for therapy, various inhibitors have been developed to target various molecular steps in the STAT3 activation pathway.^{63,64} The potential STAT3 inhibitors include ML116, tyrphostin AG490, ursolic acid, cucurbitacin I, sanguinarine, antisense and decoy oligonucleotides, dominant negative STAT3 protein, and various small molecule inhibitors.^{41,64-67} Kim et al⁶⁶ showed that an extract of *Chrysanthemum indicum* containing bioactive compounds such as sudachitin, hesperetin, chrysoeriol, and acacetin suppressed constitutive STAT3 and JAK1/2 activation, and induced apoptosis in CRPC cells. Further, Kwon et al⁶⁷ reported that piceatan-nol, a polyphenol detected in grapes and red wine, inhibits the invasive potential of CaP cells by decreasing IL-6/STAT3 signaling and Kharaziha et al⁴¹ reported the suppression of STAT3 by sorafenib in CRPC cells. This study showed that the combination of sorafenib with Bcl-2 antagonists (ABT737) might constitute a promising therapeutic strategy against CRPC.⁴¹

Notch and CRPC Relevance of notch in CaP

The notch signaling pathway comprises receptors, ligands, transcriptional complex components in the nucleus, and downstream genes, which are widely conserved.⁴² Notch signaling is mediated by its receptors and ligands.^{42,68} Four notch receptors, NOTCH-1, -2, -3, and -4, and at least five notch ligands, Jagged-1, Jagged-2, Delta-1, Delta-3, and Delta-4, have been identified in humans.^{42,68} Notch receptors interact with their ligands and undergo a series of proteolytic cleavages by the presenilin complex that is essential gamma-secretase activity. This results in the generation of an intracellular domain, which acts as a transcriptional co-activator (see Leong et al⁴² and Rizzo et al⁶⁸ and references therein). The notch signaling pathway

is reported to play important roles in several biological processes, including embryonic development, adult tissue homeostasis, and tumorigenesis.^{42,68} Recent studies have established an association between aberrant notch signaling and the development of several tumor types.^{42,68}

Notch signaling is reported to be required for normal prostate development; however, its role in CaP development is not fully understood due to conflicting reports.^{69,70} Belandia et al⁷¹ showed that NOTCH-1 signaling represses AR signaling and regulates prostate tumor cell motility. While some studies, such as that of Shou et al⁷² have reported that notch signaling acts as a tumor-suppressing pathway in CaP cells, the majority of studies have reported the tumor-promoting role of notch in CaP cells. However, this conflict could be linked to the consequences of aberrant notch signaling, which in turn depends on cell context, dose, and timing. Martin et al⁷³ showed that the notch ligand Jagged-1 is regulated by androgens in CaP cells. Using CRPC cells (Du145 and 22Rn1) and human prostate tumor specimens, Whelan et al showed that loss of NOTCH-1 signaling promotes prostate tumor development through its role as a regulator of the PTEN tumor suppressor gene.⁷⁴ Reports suggest that the notch ligand Jagged-1 is highly expressed in prostatic tumor cells in human CaP patients who have disease recurrence after radical prostatectomy.⁷⁴ Santagata et al⁷⁵ reported an association between the activated notch signaling and recurrence of disease as CRPC phenotype in human CaP patients. A growing body of evidence suggests that neuroendocrine differentiation of prostatic tumors plays a role in the development of CRPC.

The notch pathway as a target for CRPC

Since elevated notch activity has been shown to be a contributing factor along with perturbations in other critical signaling pathways in several cancer types, a growing consensus among researchers is that therapeutic inhibition of notch signaling could be widely applicable, either alone or in combination with other chemotherapeutic approaches.⁷⁶ The utilization of notch as a target for cancer therapy could be considerably prudent, depending on extremely rigorous pre-clinical studies in specific tumor types. Several studies have provided evidence about the utility of notch signaling as a therapeutic target in CaP cells.^{77,78} Studies by Wang et al⁷⁷ and Bin Hafeez et al⁷⁸ have shown that targeting downregulation of NOTCH-1 and its receptor results in the inhibition of CaP cell growth, migration, and invasion. Further, Whelan et al demonstrated that reactivation of NOTCH-1 could inhibit the invasive potential of CRPC cells.⁷⁴ The emerging complexity

of notch in CaP can be ascertained from the report showing that targeted downregulation of NOTCH-1 has been shown to inhibit growth of metastatic PC-3 cells.⁷⁸

It has been suggested that the targeting of distinct growth factor pathways may be effective for eradicating CaP cells with stem cell-like features, thus improving the efficacy of current antihormonal treatments and chemotherapies.⁷⁹ Therefore, the targeting of molecules that either regulate or mediate notch signaling is being suggested as an ideal strategy.⁷⁹ Kong et al have shown that notch signaling is regulated by micro-RNA-200 in CaP cells.⁷⁹ This study showed that reactivating miR-200 causes a reduction in NOTCH-1 activity, thus inhibits the prostatosphere-forming ability of CaP cells.⁷⁹ Ligand-dependent activation of notch requires cleavage by gamma-secretase complex (see Stein et al⁸⁰ and references therein). Gamma-secretase inhibitors have been tested for their anticancer potential in preclinical and clinical trials for various cancer types.⁸⁰ Recent studies have shown that several natural compounds mimic gamma-secretase inhibitors and thereby inhibit CRPC cell growth by inhibiting gamma-secretase (see Kim et al⁸¹ and references therein). Whether targeting gamma-secretase would be beneficial in CRPC patients is not yet clear due to the conflicting studies regarding role of notch in CaP. At present, a National Cancer Institute-sponsored clinical trial (Phase II) is underway to test gamma-secretase inhibitor RO4929097 alone and in combination with bicalutamide for treating CaP patients who previously underwent definitive local therapy for prostatic adenocarcinoma in humans.^{80,82} The primary results of this randomized Phase II study show that bicalutamide decreased PSA by more than 50% in 12/16 patients and RO4929097 was well tolerated.⁸⁰

The immune system and CRPC

Rationale for immunotherapy

Immunotherapy is a form of biological therapy that exploits the immune system to delay or inhibit malignant growth either by targeting tumor-associated antigens or by disrupting molecular pathways that promote tumor growth. A better understanding of the role of immune activation in CaP and the availability of prostate tissue-specific tumor antigens has facilitated the development of several immune-based therapeutic approaches. These approaches either involve therapeutic cancer vaccines designed to elicit antitumor T cell responses or monoclonal antibody drugs with intrinsic immunologic properties. The rationale for immunotherapy in CaP is based on several observations, including: presence of several altered self-antigens,

glycoproteins, and glycolipids, which are overexpressed on CaP cells; a long history of disease/disease burden, which could be amenable to treatment; and intrinsic low proliferative index of most chemoresistant prostatic tumors (see Vieweg⁸³ and references therein). In addition, multiple clinical trials have demonstrated that the majority of CaP patients can mount a vigorous antitumor response despite their disease status.⁸³ It is noteworthy that active or passive immunotherapy does not rely on high cell proliferation, and can be directed against any gene product expressed by CaP cells. Further, because CaP is commonly a slowly progressing disease, multiple vaccinations or boosting is possible, thereby allowing sufficient time to develop a potent antitumor response. Recent studies have shown that there is considerable synergy between cancer vaccination and hormonal ablative therapy, which, in combination, boosts the expansion of vaccine-induced effector cells.^{83–85} Therefore, active immunotherapy against CaP may be most efficacious when administered after androgen ablation.⁸³

The immune system as a target for CRPC therapy

For CRPC patients, a variety of monoclonal antibody-based approaches are currently in development. A widely used approach is the utility of unconjugated antibodies that target and kill cancer cells by either enhancing complement fixation or initiating antibody-dependent cell-mediated cytotoxicity. Several surface proteins have been tested as markers and targets for immunotherapy in CaP disease (see Moffatt et al⁸⁶ and references therein). These include mucins MUC-1, -2, Globo H, prostate stem-cell antigen, PSA, and prostate-specific membrane antigen.^{87,88} Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a small secreted cytokine that binds to specific cell-surface receptors and activates phagocytic macrophages and antigen-presenting dendritic cells.⁸⁷ The therapeutic effect of recombinant GM-CSF as an anticancer agent has been studied in several models. Published studies have reported that autologous or allogeneic prostate tumor cells transfected to produce GM-CSF, demonstrating the generation of immune response (see Zhang et al⁸⁷ and van den Eertwegh et al⁸⁸ and references therein). Recently, antibodies such as MDX-010 (ipilimumab) that block human cytotoxic T lymphocyte-associated protein 4 have entered human Phase I and II clinical trials in CaP patients.⁸⁴ In clinical settings, MDX-010 has been tested in several CaP patients, either as monotherapy or in combination with chemotherapy and vaccines. All these studies revealed

that intravenous administration of MDX-010 is safe and elicits clinical activity (see Thakur et al⁸⁹ and Drake and Antonarakis⁹⁰ and references therein).

Several cancer vaccines are being tested in clinical trials for their efficacy in treating CRPC. These include sipuleucel-T, DCVax[®] (Northwest Biotherapeutics, Bethesda, MD), GVAX (BioSante Pharmaceuticals Inc., IL), and PROSTVAC[®] (Bavarian Nordic, Kvistgaard, Denmark). Sipuleucel-T is a cellular immunotherapy product that consists of an enriched population of a patient's peripheral blood mononuclear cells, which are exposed *in vitro* to a fusion protein comprised of GM-CSF and prostatic acid phosphatase.^{90,91} These peripheral blood mononuclear cells, which include a large proportion of antigen-presenting cells, are then infused back into the patient to stimulate antitumor T-cell responses.^{90,91} Three Phase III sipuleucel-T trials enrolled CRPC patients and reported an increase of 4.1–4.3 months in median overall survival compared with placebo. Estimated survival at 36 months was 32%–33% in the active arms versus 15%–23% in the placebo arms.^{90,91} However, early results of combination therapy with docetaxel have not demonstrated benefits of sipuleucel-T use following chemotherapy.^{90,91} DCVax prostate vaccine consists of autologous, monocyte-derived dendritic cells loaded with a recombinant prostate-specific membrane antigen and has recently advanced to Phase III clinical testing in CaP patients.⁹² GVAX vaccine is composed of allogeneic CaP cells genetically engineered to secrete GM-CSF.⁹³ PROSTVAC vaccine consists of a recombinant vaccinia viral expression cassette engineered to contain a copy of the human PSA and surface receptors such as intercellular adhesion molecules.

Conclusion

CaP is a heterogeneous disease with a number of different genetic/pathophysiologic clusters. Future directions to treat CRPC patients will include custom tailoring of therapies designed on the basis of specific molecules or molecular signatures expressed by tumors that could be used as potential targets for therapeutic agents. The utilization of gene array technology (to gather information about the genes driving the growth of tumor of a selected patient) is expected to play a big role in future CRPC therapies.⁸³ Currently, neither a method for determining the homogeneous versus heterogeneous nature of tumor nor a method for the prevention of the development of CRPC phenotype from dependent androgen-dependent CaP cells is available; therefore, these should be critical areas for extensive future study.⁹⁴ In addition, newer agents are needed to target the

more than 95% of CaP cells within a given metastatic site that are not immediately proliferating.

Disclosure

The authors declare no conflicts of interest in this work.

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