Definition, mechanisms of development and current treatments of castration-resistant prostate cancer

Akira KOMIYA, Tomonori KATO, Kenji YASUDA, Hideki FUSE

去勢前立腺がんの定義、機序と現在の治療について

Abstract
Prostate cancer is initially androgen dependent, and androgen deprivation therapy is the first-line treatment for advanced disease. Most patients respond to castration; however, tumors eventually become castration-resistant prostate cancer (CRPC). Nevertheless, androgen and the androgen receptor (AR) remain active, and affect the progression of CRPC. More effective androgen suppression or AR-targeted therapies can be useful to treat such patients. The mechanisms underlying the development of CRPC include AR hypersensitivity, intra-tumoral androgen synthesis, changes in AR ligand specificity due to mutations, ligand-independent AR activation, pathway(s) bypassing the AR and the proliferation of androgen-independent tumor cells. Historically, the primary endpoint of CRPC treatment has been palliation. In 2004, docetaxel became the first chemotherapeutic agent to demonstrate a significant survival benefit. In 2010, cabazitaxel was proven to prolong survival after docetaxel resistance. Also in 2010, Sipuleucel-T was approved as a cell-based cancer immunotherapy, and an adrenal androgen inhibitor, abiraterone acetate, was revealed to prolong the survival of patients with CRPC. In 2013, a second-generation anti-androgen, enzalutamide, was shown to improve the survival of CRPC patients, and radium-223, a bone-targeted agent, was approved for CRPC. Determining how to apply these new agents for each patient in clinical practice is an important issue.

Key words: androgen, androgen-independent prostate cancer, androgen receptor, castration-resistant prostate cancer, hormone-refractory prostate cancer

Introduction
Prostate cancer (PCa) is one of the leading causes of death from cancer in males in Western countries and is the sixth most common cause of cancer mortality in Japanese males.9 PCa is initially androgen dependent, and castration (androgen deprivation therapy, ADT) is the first-line treatment for advanced PCa to suppress androgen action. Most patients respond to this treatment initially; however, the PCa eventually becomes castration-resistant, and is then often subsequently lethal. Nevertheless, even in this castration-resistant state, androgen and the androgen receptor (AR) remain...
active in the progression of PCa. Therefore, the terms, “hormone-refractory prostate cancer” or “androgen-independent prostate cancer” are now thought to be inappropriate; castration-resistant prostate cancer (CRPC) is now being widely used instead, because more effective androgen suppression or AR-targeted therapies can still be used to treat such patients. The definition of CRPC, the mechanisms underlying its development and progression and the progress in the treatment of CRPC are reviewed in this article.

I. The definition of castration-resistant prostate cancer

The precise definition of recurrent or relapsed PCa remains controversial. Several groups have published practical recommendations for defining CRPC. However, the definition of castration-resistant progression has varied among studies. In the guidelines on PCa 2012 (also in 2013) from the European Association of Urology (EAU), the definition is described as follows:

- Castrate serum levels of testosterone (testosterone < 50 ng/dL or < 1.7 nmol/L).
- Three consecutive rises of prostate specific antigen (PSA), 1 week apart, resulting in two 50% increases over the nadir, with a PSA > 2 ng/mL.
- Anti-androgen withdrawal for at least 4 weeks for flutamide and for at least 6 weeks for bicalutamide.
- PSA progression, despite consecutive hormonal manipulations.

*Either anti-androgen withdrawal or one secondary hormonal manipulation should have been done in order to fulfill the criteria for CRPC if patients have been treated with anti-androgens in the context of maximum androgen blockade or step up therapy following PSA progression after failure of luteinizing hormone-releasing hormone (LHRH) treatment.
†Progression of osseous lesions: progression or appearance of two or more lesions on bone scan or soft tissue lesions using RECIST (Response Evaluation Criteria in Solid Tumours), and with nodes > 2 cm in diameter.

These criteria indicate that PSA progression was the main condition required to define progression. In 2014, this definition was changed as follows:

- Castrate serum testosterone < 50 ng/ml or 1.7 nmol/L plus either: Biochemical progression: Three consecutive rises of PSA, 1 week apart, resulting in two 50% increases over the nadir, with PSA > 2 ng/mL.

or

- Radiological progression: The appearance of two or more bone lesions on bone scan or enlargement of a soft tissue lesion using RECIST.

Observation of the response after anti-androgen withdrawal or second- and third-line hormone therapies is important. However, no associated survival benefit has ever been reported for such treatments. In addition, the description of anti-androgen withdrawal is not included in the latest CRPC definition from the EAU. A similar definition of PCa progression is included in the Prostate Cancer Working Group 2.

In the General Rules for Clinical and Pathological Studies on Prostate Cancer (Japan), CRPC is defined as being present when clinical and/or PSA progression is found even if the serum testosterone level is maintained below 50 ng/dL either by surgical or medical castration, regardless of the use of anti-androgens. Therefore, it is necessary to keep in mind that the CRPC definition varies among studies, and may be changed in the future.

II. The mechanisms underlying the development and progression of castration-resistant prostate cancer

Scher et al. classified PCa based on its androgen-dependency and hormone sensitivity according to the tumor response to treatments under various situations. In this scheme, a tumor that is proliferating despite castrate levels of testosterone, and that responds to additional hormonal manipulation is considered to be androgen-independent but hormone-sensitive. A tumor that has not responded to sequential hormonal manipulations is androgen-independent and hormone-insensitive. It is the latter tumors that are typically classified as hormone refractory. When a tumor can be called androgen-independent and hormone sensitive, a decrease in proliferation may be observed in response to adrenal androgen blockade, corticosteroids, anti-androgen withdrawal or other hormonal manipulations.

The potential mechanisms of the castration-resistant progression of PCa are summarized in Table 1. The key issue is that androgen and the androgen receptor are involved in most cases of CRPC; AR amplification is found in about 30% of cases and hyperactivated AR mutations are present in 20-40% of the patients with progression of PCa.

A. AR hypersensitivity

Masai et al. reported that the percentage of strongly positive cancer cells of the prostate was inversely cor-
related with the grade. Relapsed cells showed a low population of strongly positive cells, irrespective of the grade. These findings mean that the AR still exists even in cells treated by androgen deprivation.\textsuperscript{17} In later studies, Visakorpi’s group reported AR amplification in hormone refractory PCa cells. Comparative genomic hybridization studies showed that amplification of the Xq11-q13 region (the location that encodes the AR), is common in tumors recurring during ADT. They found high-level AR amplification in seven of 23 (30\%) recurrent tumors, but in none of the specimens taken from the same patients prior to therapy.\textsuperscript{18} Hormone-refractory tumors expressed the AR and showed, on average, six-fold higher expression than androgen-dependent tumors or benign prostate hyperplasias (P<0.001). Four of 13 (31\%) hormone-refractory tumors contained AR gene amplification detected by fluorescence \textit{in situ} hybridization. Androgen-independent tumors with gene amplification expressed, on average, a two-fold higher level of the AR than did the refractory tumors without the gene amplification.\textsuperscript{19} AR amplification can also be detected in circulating tumor cells.\textsuperscript{20}

Using microarray-based profiling of isogenic PCa xenograft models, Chen et al. found that a modest increase in AR mRNA was the only change consistently associated with the development of resistance to anti-androgen therapy. This increase in AR mRNA and protein expression was both necessary and sufficient to convert PCa growth from a hormone-sensitive to a hormone-refractory stage, and was dependent on a functional ligand-binding domain.\textsuperscript{21} These findings indicated that AR amplification emerges during ADT by facilitating tumor cell growth in an environment with a low androgen concentration.

B. Intra-tumoral androgen synthesis

When castration therapy is administered, either surgically or with a LHRH agonist, it has been demonstrated that dihydrotestosterone (DHT) can still be detected in locally recurrent and metastatic prostate cancer tissue at levels that are sufficient to activate the AR. The two dominant sources of androgens in males with CRPC are the adrenal androgens, dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S), and \textit{de novo} synthesis by the prostate gland.\textsuperscript{22,23} Nishiyama et al. demonstrated that the DHT levels in prostate tissue after ADT remained at approximately 25\% of the amount measured before ADT. The DHT levels in serum decreased to approximately 7.5\% after ADT. The level of DHT in prostatic tissue before ADT was not correlated with the serum level of testosterone. The serum levels of adrenal androgens were reduced to approximately 60\% after ADT. The source of DHT in prostatic tissue after ADT is thought to involve intracrine production within the prostate, wherein adrenal androgens are converted to DHT. Targeting the DHT still remaining in prostate tissue after ADT may require new therapies to reduce intra-tumoral androgen synthesis.\textsuperscript{24}

Montgomery et al. reported that castration-resistant metastases displayed alterations in genes encoding steroidogenic enzymes, including upregulated expression of FASN, CYP17A1, HSD3B1, HSD17B3, CYP19A1, and UGT2B17, and downregulated expression of SRD5A2 (P<0.001 for all), compared with the levels in primary prostate tumors. Metastatic PCas from androind males were found to express transcripts encoding androgens. 

<p>| Table 1. Mechanisms underlying the development and progression of castration-resistant prostate cancer |</p>
<table>
<thead>
<tr>
<th>Causes of castration-resistance</th>
<th>Androgen dependence</th>
<th>AR-dependent change</th>
<th>Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. AR hypersensitivity</td>
<td>+</td>
<td>+</td>
<td>Amplification of AR; abnormal expression of AR co-factors</td>
</tr>
<tr>
<td>b. Intra-tumoral androgen synthesis</td>
<td>+</td>
<td>+</td>
<td>Production of androgen within tumor cells</td>
</tr>
<tr>
<td>c. Change of AR specificity due to mutations</td>
<td>+/-</td>
<td>+</td>
<td>Stimulation by non-androgens (antagonists); androgen-receptor mutations; abnormal expression of AR co-factors</td>
</tr>
<tr>
<td>d. Ligand-independent AR activation</td>
<td>-</td>
<td>+</td>
<td>Growth factor functions as ligand (IL-6, IGF, EGF, KGF); alternative signaling pathways (STAT3, MAPK, Akt, cSrc); AR splicing variants; abnormal expression of AR co-factors</td>
</tr>
<tr>
<td>e. Bypassing the AR</td>
<td>-</td>
<td>-</td>
<td>Anti-apoptosis; neuroendocrine differentiation; alternative signaling pathways; methylation of the AR promoter</td>
</tr>
<tr>
<td>f. Proliferation of pre-existing androgen-independent tumor cells</td>
<td>-</td>
<td>-</td>
<td>Malignant epithelial stem cells</td>
</tr>
</tbody>
</table>
gen-synthesizing enzymes and to maintain intratumoral androgens at concentrations capable of activating AR target genes and maintaining tumor cell survival. Dillard et al. reported that established androgen-independent PCa cell lines, PC3 and DU145, expressed the mRNA and proteins for scavenger receptor type B1 (SRB1), steroidogenic acute regulatory (StAR) protein, cytochrome P450 cholesterol side chain cleavage (P450scs), 3beta-hydroxysteroid dehydrogenase (3beta-HSD), and other enzymes involved in androgen biosynthesis. Moreover, the expression of all these proteins and enzymes was significantly higher in the androgen-independent derivative of LNCaP PCa cells (C81) than in the androgen-dependent parental cell line (C33).

In serum-free cultures, the androgen-independent C81 cells secreted approximately five-fold more testosterone than C33 cells, as determined by immunoassays of the conditioned media. These cells could also directly convert radioactive cholesterol into testosterone, which was identified by thin layer chromatography. These results show that PCa cells in advanced stages of the disease could synthesize androgens from cholesterol, and hence, are not dependent upon testicular and/or adrenal androgens. In addition, Bertaglia et al. reported that serum testosterone levels lower than the currently adopted cutoff of 50 ng/dL have a prognostic role in patients with PCa receiving LHRH agonists, and are a promising surrogate parameter of LHRH agonist efficacy. Using a receiver operating characteristic curve, it was found that a testosterone value of 30 ng/dL offered the best overall sensitivity and specificity for the prediction of death, with serum testosterone levels <30 ng/mL associated with a significantly lower risk of death (adjusted HR, 0.45; P =0.034). It has been suggested that the maximal therapeutic efficacy in the treatment of CRPC will require agents capable of lowering the androgen level as much as possible, as well as inhibiting the intracrine steroidogenic pathways within the prostate tumor microenvironment.

C. Mutations in the AR gene

Mutations in the AR gene have been detected in about 10–20% of PCa specimens. The frequency of mutations is generally higher in hormone-refractory, metastatic tumors compared with untreated, lower-grade primary tumors. The initial drive to deal with AR gene mutations in PCa originated from a study of the LNCaP cell line, which was derived from a metastatic lesion of the lymph nodes of a patient with PCa. The AR gene of LNCaP cell line contains one mutation at codon 877 (Thr to Ala). The growth of LNCaP cells is stimulated in vitro by a wide variety of substances, including androgens, estrogens, progesterone and several anti-androgens, indicating the broadened ligand responsiveness of AR mutants.

Sack et al. analyzed the crystallographic structures of the AR ligand-binding domain (LBD). The AR LBD is monomeric, possibly because of the extended C terminus of the AR, which lies in a groove at the dimerization interface. The binding of the natural ligand, DHT, by the mutant LBD involves interactions with the same residues as in the wild-type receptor, with the exception of the side chain of threonine 877, which is an alanine residue in the mutant. This structural difference in the binding pocket may explain the ability of the mutant AR found in LNCaP cells (T877A) to accommodate progesterone and other ligands that the wild-type receptor cannot. The same mutation was the most frequently found in clinical practice, especially within the patients who underwent maximal androgen blockade using flutamide.

Given that certain mutations can alter AR ligand specificity, AR mutations might play a key role in ‘anti-androgen withdrawal syndrome’. This phenomenon occurs in a subset of patients with castration-resistant progression. The cessation of anti-androgen medication improves the symptoms and the serum PSA levels decrease, suggesting that the anti-androgen acts agonistically in the tumor cells to promote growth. Our previous study found that in two of four patients who experienced improvement after anti-androgen withdrawal, AR mutations had occurred during anti-androgen treatment. These mutations were identical to that in LNCaP cells (T877A), and were not detected in untreated tumors. Furthermore, it is known that AR mutations such as Trp741Cys or Trp741Leu are activated by bicalutamide.

D. Ligand-independent AR activation

Androgen-independent activation of the AR mediates the castration-resistant progression of PCa in the absence of androgen. By using intracellular signaling pathways involving epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I), keratinocyte growth factor (KGF), interleukin-6 (IL-6) or HER2/neu, phosphorylation occurs in tyrosine residues or serine and threonine residues of the AR, and the AR is then activated without androgens. Several clinical investi-
bodies specific to AR-V7 frequently detected the AR-V7 protein in HRPC specimens, but rarely in hormone-naïve PCa specimens. AR-V7 was localized in the nuclei of cultured PCa cells under androgen-depleted conditions, and was constitutively active, thus driving the expression of canonical androgen-responsive genes, as revealed by both AR reporter assays and expression microarray analysis. In addition, CRPC positive for AR-V7 did not respond to abiraterone or enzalutamide, and the patients showed significantly shorter survival. Therefore, AR variants may be explored as potential biomarkers and therapeutic targets for advanced PCa.

E. Bypassing the AR

1. Neuroendocrine differentiation (NED)

Neuroendocrine (NE) cells exist within prostatic ducts and acinar cells. These cells regulate the differentiation, proliferation and secretion of the prostate. The AR is not expressed in NE cells, and NED is thought to underlie one of the mechanisms by which cells undergo castration-resistant progression, because of its androgen-independence. In the majority of PCa cases, malignant NE cells were also found in tumor tissues to some extent. There is no clear definition of NED; however, a region is defined as having undergone NED if NE markers are positive by immunohistochemical staining. This is a basic feature of the prostate, regardless of whether it is benign or malignant. NE cells express certain peptide hormones or pro-hormones, which affect the target cells by endocrine, paracrine, autocrine and/or neuroendocrine transmission in an androgen-independent fashion. Neuroendocrine markers can be measured in serum or plasma samples. Chromogranin A, neuron-specific enolase and ProGRP have prognostic significance in PCa. Among these, serum chromogranin A is thought to be the most important, and the pretreatment serum levels of chromogranin A are associated with castration-resistant progression.

NED can be a therapeutic target. Ueda et al. demonstrated a mechanism of cross-talk between the IL-6 and AR signal transduction pathways in LNCaP human PCa cells. IL-6 induced several androgen response element-driven reporters that were dependent upon the AR, that increased the phosphorylation of mitogen-activated protein kinase (MAPK) and activated the AR N-terminal domain (NTD). Inhibitors of MAPK and JAK decreased the IL-6-induced phosphorylation of MAPK and activation of the AR N-terminal domain (NTD). Immunoprecipitation and transactivation studies showed a direct interaction between amino acids 234-558 of the AR NTD and STAT3 following IL-6 treatment of LNCaP cells. The activation of the human AR NTD by IL-6 was mediated through MAPK and STAT3 signal transduction pathways in LNCaP PCa cells.

In clinical practice, significant suppression of serum IL-6, through inhibition of androgen-independent activation of the AR, is thought to be one of the mechanisms underlying the effects of dexamethasone therapy in PCa patients with progressive disease. The change in serum IL-6 levels was significantly associated with the response to dexamethasone in the treatment of CRPC.

Recently, altered AR splicing patterns have been identified as a mechanism of PCa progression and resistance to ADT. Several studies have described the synthesis of alternatively spliced transcripts encoding truncated AR isoforms that lack the ligand-binding domain, which is the ultimate target of androgen depletion. Many of these truncated AR isoforms function as constitutively active, ligand-independent transcription factors that can support androgen-independent expression of AR target genes, as well as the androgen-independent growth of PCa cells. Luo’s group reported seven AR variant transcripts lacking the reading frames for the ligand-binding domain due to the splicing of “intronic” cryptic exons to the upstream exons encoding the AR DNA binding domain. AR-V1 and AR-V7 mRNA showed an average 20-fold higher expression in CRPC (n=25) when compared with hormone-naïve PCa (n=82; P<0.0001). Among the hormone-naïve PCa cases, higher expression of AR-V7 predicted biochemical recurrence following surgical treatment (P=0.012). Polyclonal antibodies specific to AR-V7 frequently detected the AR-V7 protein in HRPC specimens, but rarely in hormone-naïve PCa specimens. AR-V7 was localized in the nuclei of cultured PCa cells under androgen-depleted conditions, and was constitutively active, thus driving the expression of canonical androgen-responsive genes, as revealed by both AR reporter assays and expression microarray analysis. In addition, CRPC positive for AR-V7 did not respond to abiraterone or enzalutamide, and the patients showed significantly shorter survival. Therefore, AR variants may be explored as potential biomarkers and therapeutic targets for advanced PCa.
tation-resistant progression in PCa are being investigated. Wu and Huang showed that both the phosphati-
dylinositol 3-kinase-AKT-mammalian target of rapamy-
cin pathway and ERK are activated, but only the for-
ermer is required for the NED of cells. Constitutively ac-
tive AKT promoted NED, and a dominant negative
AKT inhibited it. The activation of AKT by IGF-1 led
to NED, and the NED induced by epinephrine required
AKT activation. The authors of that study also showed
that the AKT pathway is likely responsible for the
NED of DU145 cells.50

Neuroendocrine-derived parathyroid hormone-relat-
ed protein (PTHrP)-mediated signaling through the epi-
dermal growth factor receptor (EGFR) and Src path-
ways contributes to the phenotype of advanced PCa by
reducing the AR protein turnover. PTHrP-induced ac-
cumulation of the AR depended on the activity of Src
and EGFR and subsequent phosphorylation of the AR
on Tyr (634). PTHrP-induced tyrosine phosphorylation
of the AR resulted in reduced AR ubiquitination and
interaction with the ubiquitin ligase COOH terminus
of Hsp70-interacting protein. These events resulted in in-
creased accumulation of the AR, and thus enhanced the
growth of PCa cells at low levels of androgen.51

2. The HGF/cMET pathway
Hepatocyte growth factor (HGF) is a ligand of c-MET.
This ligand-receptor system promotes proliferation, mi-
gration, invasion, metastasis and anti-apoptosis effects
in cancer cells, including PCa. HGF is expressed in stro-
mal cells, and its receptor, c-MET, is expressed in an-
drogen-independent PCa cells (PC-3 and DU145), but
not in androgen-dependent PCa cells (LNCaP).51 There
is a potential link between androgen signaling and
and c-Met expression in PCa cells. For example, it is known
that the AR represses the expression of c-Met in a li-
gand-dependent manner. The AR controls HGF/c-MET
and ADT upregulates this system. Sp1 induces the
transcription of c-Met, and the AR can repress the
Sp1-induced transcription in PCa cells. The AR inter-
feres with the interaction between Sp1 and the func-
tional Sp1 binding site within the c-Met promoter. The
repressive role of androgen signaling on c-Met expres-
sion was confirmed in PCa xenografts. Although the
currently used androgen ablation therapies can repress
the expression of growth-promoting genes that are ac-
tivated by the AR, it may also attenuate the repressive
role of AR on c-Met expression. Therefore, the introd-
tion of therapeutic strategies to inhibit the activation of
the HGF/c-Met pathway may be of benefit when com-
bined with current androgen ablation treatment.53

Yasuda et al. reported that HGF-related serum mark-
ers could be prognostic markers. The serum active
HGF (AHGF) levels were increased in patients with
stage D or D3 compared with stage B disease. In addi-
tion, there were significant differences in the serum
AHGF levels between patients with well-differentiated
and poorly-differentiated adenocarcinoma. Further-
more, the mean serum AHGF/THGF ratio in patients
with stage D3 PCa was significantly higher than that in
patients with stage B PCa.54 Hepatocyte growth factor
activator inhibitor type-1 (HAI-1) inhibits hepatocyte
growth factor activator and matriptase. The expression
of HAI-1 detected by IHC in patients with PCa was
significantly higher than that in the patients with a neg-
avative prostate biopsy. CRPC tumors exhibited signifi-
cantly lower HAI-1 expression than untreated metastat-
ic PCa. The PSA progression-free rate was worse in
patients without HAI-1 expression than in those with
positive HAI-1 expression.55

3. The inhibition of apoptosis
Apoptosis, which is usually induced by ADT in PCa, is
suppressed by the inactivation of tumor suppressor
genes, or the activation of oncogenes and anti-apoptotic
genes. Mutations in TP53 and PTEN are found in a
subset of CRPC.56,57 Bcl-2 causes an anti-apoptotic effect
by inhibiting the activation of caspase required for the
induction of apoptosis. Furuya et al. reported the fre-
quency of bcl-2 protein expression using immunocyto-
chemical staining during the progression of human PCa
from an androgen-sensitive non-metastatic phenotype
to an androgen-independent metastatic phenotype. Five
(17%) of the 30 lymph node metastases from pathologi-
cally disseminated D1 disease and 14 (52%) of 27 bone
metastases from pathologically disseminated D2 disease
expressed detectable bcl-2 protein. These data demon-
strate that there is a statistically significant (P<0.05)
association between the expression of bcl-2 and the pro-
gression of human prostatic cancer cells to a metastatic
phenotype.58

Similarly, McDonnell et al., immunohistochemically
evaluated androgen-dependent and androgen-indepen-
dent prostate carcinomas for bcl-2 expression. Bcl-2 was
undetectable in 13 of 19 cases of androgen-dependent
cancers. In contrast, androgen-independent cancers dis-
played diffuse, high levels of bcl-2 staining (P<0.01).
These findings indicate that bcl-2 expression is aug-
mented following androgen ablation, and is correlated with the progression of PCa from androgen dependence to androgen independence.\textsuperscript{59} Bcl-2 has previously been addressed as a therapeutic target in hormone-refractory PCa, and antisense therapy was also developed for the MDM2 oncogene, which regulates p53 and various other molecules associated with cancer.\textsuperscript{60~62} In addition, the effects of Bcl-xL of the Bcl-2 family with regard to suppressing apoptosis are very strong. Enforced expression of the Bcl-xL gene dramatically increased the cell proliferation \textit{in vitro} and promoted xenograft tumor growth \textit{in vivo}. Bcl-xL overexpression significantly increased the expression of cyclin D2, which might be responsible for Bcl-xL-induced cell proliferation and tumor growth. Therefore, androgen controls Bcl-xL expression via the AR, and the increased Bcl-xL expression plays a versatile role in the castration-resistant progression of PCa.\textsuperscript{63}

F. Proliferation of pre-existing androgen-independent tumor cells

Coffey and Isaacs at Johns Hopkins University postulated that the androgen independence of PCa was due to a selective growth advantage of preexisting hormone-independent clonal populations of preexisting, hormone-independent stem cells. This mechanism is likely to be completely independent of androgen- and AR-dependent growth stimulation.\textsuperscript{64,65} However, one would rarely expect to encounter this kind of PCa in clinical practice.

III. Treatment of CRPC

The agents for CRPC/HRPC approved by the U.S. Food and Drug Administration are summarized in Tables 2 and 3. The endpoints are largely classified as quality of life or palliation and survival.

A. Agents to improve the QOL

Tannock et al. reported the results of thirty-seven patients with symptomatic bone metastases from prostate cancer that had progressed following earlier treatment with estrogens and/or orchidectomy, who were treated with low-dose prednisone (7.5 to 10 mg daily).\textsuperscript{60} Thirty-eight percent of these patients showed improvement in indices used to assess pain at one month after starting prednisone. The major findings of that study were that: (1) low-dose prednisone may lead to pain relief in some patients with advanced PCa. (2) The pain relief was associated with suppression of adrenal androgens. (3) Measures of pain and quality of life can be used to assess the possible benefits of systemic therapy in patients with metastatic PCa.

Tannock et al. also reported the results of a study comparing chemotherapy with mitoxantrone plus prednisone or prednisone alone for symptomatic hormone-resistant PCa in a Canadian randomized trial with palliation following androgen ablation, and is correlated with the progression of PCa from androgen dependence to androgen independence.\textsuperscript{59} Bcl-2 has previously been addressed as a therapeutic target in hormone-refractory PCa, and antisense therapy was also developed for the MDM2 oncogene, which regulates p53 and various other molecules associated with cancer.\textsuperscript{60~62} In addition, the effects of Bcl-xL of the Bcl-2 family with regard to suppressing apoptosis are very strong. Enforced expression of the Bcl-xL gene dramatically increased the cell proliferation \textit{in vitro} and promoted xenograft tumor growth \textit{in vivo}. Bcl-xL overexpression significantly increased the expression of cyclin D2, which might be responsible for Bcl-xL-induced cell proliferation and tumor growth. Therefore, androgen controls Bcl-xL expression via the AR, and the increased Bcl-xL expression plays a versatile role in the castration-resistant progression of PCa.\textsuperscript{63}

F. Proliferation of pre-existing androgen-independent tumor cells

Coffey and Isaacs at Johns Hopkins University postulated that the androgen independence of PCa was due to a selective growth advantage of preexisting hormone-independent clonal populations of preexisting, hormone-independent stem cells. This mechanism is likely to be completely independent of androgen- and AR-dependent growth stimulation.\textsuperscript{64,65} However, one would rarely expect to encounter this kind of PCa in clinical practice.

III. Treatment of CRPC

The agents for CRPC/HRPC approved by the U.S. Food and Drug Administration are summarized in Tables 2 and 3. The endpoints are largely classified as quality of life or palliation and survival.

A. Agents to improve the QOL

Tannock et al. reported the results of thirty-seven patients with symptomatic bone metastases from prostate cancer that had progressed following earlier treatment with estrogens and/or orchidectomy, who were treated with low-dose prednisone (7.5 to 10 mg daily).\textsuperscript{60} Thirty-eight percent of these patients showed improvement in indices used to assess pain at one month after starting prednisone. The major findings of that study were that: (1) low-dose prednisone may lead to pain relief in some patients with advanced PCa. (2) The pain relief was associated with suppression of adrenal androgens. (3) Measures of pain and quality of life can be used to assess the possible benefits of systemic therapy in patients with metastatic PCa.

Tannock et al. also reported the results of a study comparing chemotherapy with mitoxantrone plus prednisone or prednisone alone for symptomatic hormone-resistant PCa in a Canadian randomized trial with palliation.

Table 2. Agents used for palliation in CRPC/HRPC approved by the U.S. Food and Drug Administration

<table>
<thead>
<tr>
<th>Agents for palliation</th>
<th>Year</th>
<th>Endpoint</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estramustine</td>
<td>1981</td>
<td>QOL</td>
<td>Indicated for the palliative treatment of patients with metastatic and/or progressive carcinoma of the prostate</td>
</tr>
<tr>
<td>Prednisone</td>
<td>1989</td>
<td>QOL</td>
<td></td>
</tr>
<tr>
<td>Mitoxantrone + corticosteroids</td>
<td>1996</td>
<td>QOL</td>
<td>Pain related to advanced hormone-refractory prostate cancer</td>
</tr>
<tr>
<td>Strontium 89 chloride</td>
<td>1993</td>
<td>QOL</td>
<td>Palliation of painful bone metastases</td>
</tr>
<tr>
<td>Samarium 153</td>
<td>1997</td>
<td>QOL</td>
<td>Relief of osteoblastic bone pain in cancer patients.</td>
</tr>
<tr>
<td>Phosphorus 32</td>
<td>1997</td>
<td>QOL</td>
<td>Seldom used – 89Sr and 153Sm are considered safer agents.</td>
</tr>
<tr>
<td>Zoledronic acid</td>
<td>2003</td>
<td>QOL/SRE</td>
<td>The treatment of hypercalcemia of malignancy.</td>
</tr>
<tr>
<td>Denosumab</td>
<td>2010</td>
<td>QOL/SRE</td>
<td>The prevention of skeletal-related events (SREs) in patients with bone metastases from solid tumors</td>
</tr>
<tr>
<td>Denosumab</td>
<td>2011</td>
<td>QOL/SRE</td>
<td>A treatment to increase bone mass in patients who are at high risk of fractures from receiving androgen deprivation therapy (ADT) for nonmetastatic prostate cancer</td>
</tr>
</tbody>
</table>

CRPC, castration-resistant prostate cancer; HRPC, hormone-refractory prostate cancer; QOL, quality of life; SRE, skeletal-related event
tive endpoints. Palliative responses were observed in 23 of 80 patients (29%) who received mitoxantrone plus prednisone, and in 10 of 81 patients (12%) who received prednisone alone (P=0.01). The duration of palliation was longer in patients who received chemotherapy (median, 43 and 18 weeks; P<0.0001, log-rank). There were no significant differences in the PSA response or overall survival between the groups. Most responding patients had an improvement in quality-of-life scales and a decrease in the serum PSA level. Based on these studies, mitoxantrone plus prednisone and prednisone alone were still used as controls in recent randomized controlled trials for the treatment of CRPC. However, mitoxantrone is not approved for the treatment of PCa in Japan.

### B. Bone-targeted therapies

Strontium-89 chloride, Samarium-153 and Phosphorus-32 are radioactive agents used to relieve painful bone metastases. Strontium-89 acts as calcium mimic and is absorbed by the bone metastases. Lewington et al. conducted a prospective, randomized, double-blind crossover study to examine the efficacy of strontium-89 in pain palliation in patients with advanced prostate cancer metastatic to bone. Dramatic improvement, some improvement, no improvement/deterioration were found in four, four and four patients in the strontium-89 injection group and zero, three and eleven patients in the placebo group. A statistical comparison between placebo and strontium-89 showed clear evidence of a therapeutic response to strontium-89 compared with only a limited placebo effect (P<0.01).

Zoledronic acid and denosumab are agents that can suppress osteoblast and skeleton-related events (SREs), defined as pathological fractures, spinal cord compression, bone pain requiring palliative radiotherapy and orthopedic surgery. SREs deteriorate the patients’ quality of life. Saad et al. studied the effects of a new bisphosphonate, zoledronic acid, which blocks bone destruction, on the skeletal complications in PCa patients with bone metastases. In that study, a greater proportion of patients who received placebo had SREs than those who received zoledronic acid at a dose of 4 mg (44.2% versus 33.2%; P=0.021). The median time to first SRE was 321 days for patients who received placebo, and was not reached for patients who received zoledronic acid (P=0.011 versus placebo). The pain and analgesic scores increased more in patients who received placebo than in patients who received zoledronic acid, but there were no significant differences in disease progression. The infusion of 4 mg of Zoledronic acid every three weeks reduced SREs in PCa patients with bone metastases.

Fizazi et al. conducted a randomized, double-blind study to compare denosumab and zoledronic acid for the treatment of bone metastases in males with CRPC. Denosumab is a human monoclonal antibody against RANKL; it also inhibits osteoclast-mediated bone destruction. The median time to first on-study SRE was 20.7 months with denosumab, compared with 17.1 months with zoledronic acid.

### Table 3. Agents with a survival benefit in CRPC/HRPC patients approved by the U.S. Food and Drug Administration

<table>
<thead>
<tr>
<th>Agents</th>
<th>Year</th>
<th>Endpoint</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Docetaxel</td>
<td>2004</td>
<td>OS</td>
<td>HRPC with prednisone in androgen-independent (hormone-refractory) metastatic prostate cancer</td>
</tr>
<tr>
<td>Cabazitaxel</td>
<td>2010</td>
<td>OS</td>
<td>A microtubule inhibitor used in combination with prednisone, indicated for the treatment of patients with metastatic HRPC previously treated with a docetaxel-containing regimen</td>
</tr>
<tr>
<td>Sipuleucel-T</td>
<td>2010</td>
<td>OS</td>
<td>An autologous cellular immunotherapy indicated for the treatment of asymptomatic or minimally symptomatic metastatic CRPC/HRPC</td>
</tr>
<tr>
<td>Abiraterone acetate</td>
<td>2011</td>
<td>OS</td>
<td>A CYP17 inhibitor indicated for use in combination with prednisone for the treatment of patients with metastatic CRPC who have received prior chemotherapy containing docetaxel</td>
</tr>
<tr>
<td>Abiraterone acetate</td>
<td>2012</td>
<td>OS/rPFS</td>
<td>Used with prednisone for metastatic castration-resistant prostate cancer before chemotherapy</td>
</tr>
<tr>
<td>Enzalutamide</td>
<td>2012</td>
<td>OS</td>
<td>An androgen receptor inhibitor indicated for the treatment of patients with metastatic CRPC who have previously received docetaxel</td>
</tr>
<tr>
<td>Radium 223 dichloride</td>
<td>2013</td>
<td>OS</td>
<td>Used for the treatment of patients with CRPC, symptomatic bone metastases and no known visceral metastatic disease</td>
</tr>
<tr>
<td>Enzalutamide</td>
<td>2014</td>
<td>OS/rPFS</td>
<td>The treatment of patients with metastatic CRPC (who have not received chemotherapy)</td>
</tr>
</tbody>
</table>

CRPC, castration-resistant prostate cancer; HRPC, hormone-refractory prostate cancer; QOL, quality of life; OS, overall survival; rPFS, radiographic progression-free survival
months with zoledronic acid (hazard ratio 0.82; \( P = 0.0002 \) for non-inferiority; \( P = 0.008 \) for superiority). More events of hypocalcaemia occurred in the denosumab group (13%) than in the zoledronic acid group (6%, \( P < 0.0001 \)). Osteonecrosis of the jaw occurred infrequently in both groups (2% vs. 1%; \( P = 0.09 \)). Therefore, denosumab was better than zoledronic acid for the prevention of skeletal-related events, and potentially represents a novel treatment option for patients with bone metastases from CRPC.

C. Agents to improve the overall survival

Docetaxel was the first agent demonstrated to prolong the overall survival in CRPC patients. This agent was approved in 2004 and still plays an important role in the treatment of CRPC. The mechanism of action of taxanes seems to involve not only microtubule stabilization and inhibition of tubulin function, but also both AR nuclear localization and signaling inhibition. There were two important trials that established the efficacy of docetaxel. One was TAX 327 conducted by Tannock et al. They showed that, compared with the patients in the mitoxantrone group, those in the group given docetaxel every three weeks had a hazard ratio for death of 0.76 (\( P = 0.009 \) by the stratified log-rank test) and those given weekly docetaxel had a hazard ratio for death of 0.91 (\( P = 0.36 \)). The median survival was 16.5 months in the mitoxantrone group, 18.9 months in the group given docetaxel every three weeks and 17.4 months in the group given weekly docetaxel. Among these three groups, 32%, 45% and 48% of patients had at least a 50% decrease in the serum PSA level, respectively (\( P < 0.001 \) for both comparisons with mitoxantrone). When given with prednisone, docetaxel (given every three weeks) led to superior survival and improved response rates in terms of pain, the serum PSA level and the patient quality of life, as compared with mitoxantrone plus prednisone.

The other trial was the SWOG9916 trial conducted by Petrylak et al. The median overall survival was longer in the group given docetaxel and estramustine than in the group given mitoxantrone and prednisone (17.5 months vs. 15.6 months, \( P = 0.02 \) by the log-rank test). PSA declines of at least 50% occurred in 50% and 27% of patients, respectively (\( P < 0.001 \)), and objective tumor responses were observed in 17% and 11% of patients with bidimensionally measurable disease, respectively (\( P = 0.30 \)). The improvement in median survival of nearly two months by docetaxel and estramustine, as compared with mitoxantrone and prednisone, provided support for using this approach in patients with metastatic, androgen-independent prostate cancer.

In 2010, another taxane, cabazitaxel, became available. Cabazitaxel is a novel tubulin-binding taxane drug with antitumor activity in docetaxel-resistant cancers. In the TROPIC study, de Bono et al. reported the results of a comparison of the efficacy and safety of cabazitaxel plus prednisone with those for mitoxantrone plus prednisone in patients with CRPC with progressive disease after docetaxel-based treatment. The median survival was 15.1 months in the cabazitaxel group and 12.7 months in the mitoxantrone group (as a control). The hazard ratio for death of the patients treated with cabazitaxel compared with those taking mitoxantrone was 0.70 (\( p < 0.0001 \)). Treatment with cabazitaxel plus prednisone had significant clinical antitumor activity, improving the overall survival in patients with metastatic CRPC whose disease has progressed during or after docetaxel-based therapy. Two ongoing trials (FIRSTANA and PROSELICA) are now being performed to evaluate two different doses of cabazitaxel (20 and 25 mg/m²) in the pre- and post-docetaxel settings to assess whether dose reduction, often required because of myelotoxicity, affects the therapeutic response.

One immunotherapy, sipuleucel-T, was approved based on the results of the IMPACT study in 2010. Sipuleucel-T is an active cellular immunotherapy, a type of therapeutic cancer vaccine, consisting of autologous peripheral blood mononuclear cells, including antigen-presenting cells, that have been activated ex vivo with a recombinant fusion protein (PA2024). PA2024 consists of a prostate antigen, prostatic acid phosphatase, that is fused to granulocyte-macrophage colony-stimulating factor, an immune cell activator. Sipuleucel-T has shown evidence of efficacy for reducing the risk of death among patients with metastatic CRPC. In the sipuleucel-T group, there was a relative reduction of 22% in the risk of death compared with the placebo group (hazard ratio, 0.78; \( P = 0.03 \)). This reduction represented a 4.1-month improvement in median survival (25.8 months in the sipuleucel-T group vs. 21.7 months in the placebo group). Among patients with PSA assessments after baseline, reductions of at least 50% on two visits at least four weeks apart were observed in eight of 311 patients (2.6%) in the sipuleucel-T group, compared with two of 153 patients (1.3%) in the placebo group. The use of sipuleucel-T prolonged the overall
survival among patients with metastatic CRPC, but no effect on the time to disease progression was observed, and the effects on PSA were limited.

Abiraterone acetate is an oral steroid derivative used to suppress androgen production within the testes, adrenal glands and prostate by irreversibly inhibiting CYP17 (P450c17; C17, 20-lyase and 17-a-hydroxylase). Abiraterone acetate affects both the classical and back-door pathways of androgen biosynthesis. Using this agent, the serum testosterone level was reduced below the limit of detection. In the COU-AA-301 study, de Bono et al. evaluated whether abiraterone acetate prolonged the overall survival among patients with metastatic CRPC who had received chemotherapy. The overall survival was longer in the abiraterone acetate-prednisone group than in the placebo-prednisone group (14.8 months vs. 10.9 months; hazard ratio, 0.65; P<0.001). All secondary endpoints, including the time to PSA progression (10.2 vs. 6.6 months; P<0.001), progression-free survival (5.6 months vs. 3.6 months; P<0.001) and PSA response rate (29% vs. 6%, P<0.001), favored the treatment group.

Furthermore, in the COU-AA-302 study, Ryan et al. reported the efficacy of abiraterone acetate in CRPC patients who had not received previous chemotherapy. The co-primary endpoints were the radiographic progression-free survival and overall survival, which was based on a consensus among European experts in mCRPC to be appropriate primary endpoints for phase III clinical trials of CRPC. The median radiographic progression-free survival was 16.5 months with abiraterone-prednisone and 8.3 months with prednisone alone (hazard ratio for abiraterone-prednisone vs. prednisone alone, 0.53; P<0.001). The overall survival was improved with abiraterone-prednisone (median not reached, vs. 27.2 months for prednisone alone; hazard ratio, 0.75; P=0.01) but did not cross the efficacy boundary. Abiraterone-prednisone showed superiority over prednisone alone with respect to the time to initiation of cytotoxic chemotherapy, opiate use for cancer-related pain, PSA progression and the decline in performance status. Therefore, abiraterone acetate has a survival benefit for both chemotherapy-naïve and resistant CRPC patients.

Enzalutamide (MDV3100) is a second generation non-steroidal anti-androgen developed by Tran et al. in 2009. Enzalutamide binds to the AR with greater relative affinity than the clinically used anti-androgen bicalutamide, reduces the efficiency of its nuclear translocation and impairs both DNA binding to androgen response elements and the recruitment of coactivators. It is orally available and induced tumor regression in mouse models of CRPC. Of the first 30 patients treated with enzalutamide in a Phase I/II clinical trial, 13 of 30 (43%) showed sustained declines (by >50%) in the serum concentrations of PSA. Then, the AFFIRM trial was conducted as a phase 3, double-blind, placebo-controlled trial by Scher in the setting of CRPC after chemotherapy. The median overall survival was 18.4 months in the enzalutamide group versus 13.6 months in the placebo group (hazard ratio for death in the enzalutamide group, 0.63; P<0.001). The superiority of enzalutamide over placebo was shown with respect to all secondary endpoints, including the proportion of patients with a reduction in the PSA level by 50% or more (64% vs. 2%, P<0.001), the soft-tissue response rate (29% vs. 4%, P<0.001), the QOL response rate (43% vs. 18%, P<0.001), the time to PSA progression (8.3 vs. 3.0 months; hazard ratio, 0.25; P<0.001), the radiographic progression-free survival (8.3 vs. 2.9 months; hazard ratio, 0.40; P<0.001) and the time to the first SRE (16.7 vs. 13.3 months; hazard ratio, 0.69; P<0.001). Seizures were reported in five patients (0.6%) receiving enzalutamide. Therefore, enzalutamide was proven to significantly prolong the survival of patients with metastatic CRPC after chemotherapy, but is associated with a risk of seizures.

In 2014, the results of the PREVAIL trial to develop new treatment options for patients with metastatic PCa who had not received chemotherapy, in whom the disease has progressed despite ADT were reported. This study was conducted by Beer et al. as a double-blind, phase 3 study. The co-primary endpoints were the radiographic progression-free survival and the overall survival, which were the same as in the COU-AA-302 trial. The rate of radiographic progression-free survival at 12 months was 65% among patients treated with enzalutamide, compared with 14% among patients receiving placebo (81% risk reduction; hazard ratio in the enzalutamide group, 0.19; P<0.001). A total of 626 patients (72%) in the enzalutamide group, as compared with 532 patients (63%) in the placebo group, were alive at the data-cutoff date (29% reduction in the risk of death; hazard ratio, 0.71; P<0.001). The benefits of enzalutamide were shown with respect to all secondary endpoints, including the time until the initiation of cytotoxic chemotherapy (hazard ratio, 0.35), the time until the first SRE (hazard ratio, 0.72), the complete or partial soft-tissue response rates (59% vs. 5%), the time until
Assessments of all main secondary efficacy endpoints also showed a benefit of radium-223 compared with placebo. Radium-223 was associated with low myelosuppression rates and few adverse events.

D. The current management of CRPC in the era of multiple agents that can prolong survival

As shown above, during the past decade, multiple options for the treatment of CRPC that can achieve not only QOL improvement, but also survival benefit, have been introduced. Important clinical questions remain unclear; including which agent is the best, or what the best order or sequence is for these agents. Currently, there are no available data to prove the superiority of any of these new drugs compared to the others. Each study has had different patient eligibility criteria and background. Recently, updated clinical practice guidelines for CRPC recommend choosing agents based on the history of prior chemotherapy (docetaxel), the performance status, state of metastasis and the patients’ symptoms.6,84,85) A summarized CRPC guideline from the American Urological Association is shown in Table 4.84) The statements are presented in the guideline as Standards, Recommendations or Options. When sufficient evidence existed, the body of evidence for a particular treatment was assigned a strength rating of A (high), B (moderate) or C (low). In the absence of sufficient evidence, additional information was provided as Clinical Principles and Expert Opinions. There are

<table>
<thead>
<tr>
<th>Index</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symptoms</strong></td>
<td>-</td>
<td>mild</td>
<td>+</td>
</tr>
<tr>
<td><strong>Metastasis</strong></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Prior docetaxel</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>P.S.</strong> (good)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Standard (Level A, B)</strong></td>
<td>-</td>
<td>abiraterone+P (A)</td>
<td>docetaxel (B), sipuleucel-T (B)</td>
</tr>
<tr>
<td><strong>Recommendation (Level C)</strong></td>
<td>observation with continued ADT (C)</td>
<td>abiraterone+P (C)</td>
<td></td>
</tr>
<tr>
<td><strong>Options</strong></td>
<td>first-generation anti-androgens (flutamide, bicalutamide, nilutamide) (C)</td>
<td>first-generation anti-androgens (flutamide, bicalutamide, nilutamide) (C)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>first generation androgen synthesis inhibitors (ketoconazole+steroids) (C)</td>
<td>ketoconazole+steroids (C)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mitoxantrone (B)</td>
<td>observation (C)</td>
<td></td>
</tr>
<tr>
<td><strong>Expert opinion</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Should not offer, recommendation (Level C)</strong></td>
<td>chemotherapy (C)</td>
<td>-</td>
<td>estramustine (C)</td>
</tr>
<tr>
<td><strong>Expert opinion against</strong></td>
<td>chemotherapy (C)</td>
<td>immunotherapy (C)</td>
<td>sipuleucel-T (C)</td>
</tr>
</tbody>
</table>

P.S., performance status; P, prednisone; ADT, androgen deprivation therapy; Ra-223, radium-223
Conflicts of interest
The authors have nothing to disclose in association with this study.

References
5) Crook J.M., O’Callaghan C.J., Duncan G. et al. : Inter-
29) Steketee K., Timmerman L., Ziel-van der Made A.C.: Broadened ligand responsiveness of androgen re-
24


