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REVIEW ARTICLE

# New Biomarkers for Selecting the Best Therapy Regimens in Metastatic Castration-Resistant Prostate Cancer

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**Abstract** Prostate cancer is the most common cancer in men. In recent years, several new targeted therapeutic agents for the treatment of metastatic castration resistant prostate cancer (mCRPC) have been developed. These include androgen receptor targeting agents, new taxanes, radium-223, and immunotherapies. In this short review, we provide a summary of clinical and preclinical biomarkers for each of these new treatment strategies, also including new markers currently presented in conference papers only. Moreover, we address the role of these biomarkers in clinical routine with the aim to select best-personalized treatment strategies for patients. Finally, we provide a decision tree for selecting the proper therapy for patients with mCRPC according to the discussed biomarkers.

**Key Points**

In recent years, the landscape of therapy options for the treatment of metastatic castration resistant prostate cancer has greatly expanded

However, there is a lack of biomarkers for selecting the best therapeutic options for each patient

Biomarkers aim to offer personalized treatment strategies to each patient

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## 1 Introduction

Prostate cancer (PCa) is the most common cancer in men in Europe, with the highest incidence in Northern and Western Europe (>200/100,000) [1]. In general, PCa varies from slow-growing indolent tumors to highly aggressive tumors associated with disease-related morbidity and mortality. However, determining which subtypes of PCa are likely to progress to metastatic disease is one of the most important challenges in PCa research.

In general, targeting the androgen receptor (AR), either by direct blocking with AR inhibitors or by suppressing the secretion of testicular androgens using luteinizing-hormone releasing hormone (LHRH) agonists or antagonists, is a standard treatment in locally advanced or metastatic PCa [2, 3]. However, after about 20 to 36 months, AR signaling is reactivated leading to castration resistant PCa (CRPC) associated in most patients with the formation of metastatic lesions (mCRPC), predominantly in bones and lymph nodes [2, 3]. Until some years ago, docetaxel chemotherapy was the sole treatment for this stage of the disease. However, in recent years, next-generation AR targeting agents such as abiraterone and enzalutamide have been developed and are now routinely used in the clinical setting. In addition, cabazitaxel (a next-generation taxane), immunotherapy, as well as radium-223 have been approved for the treatment of mCRPC (www.Uroweb.org). Table 1 summarizes the currently approved substances for the treatment of mCRPC.

On the one hand, the integration of tumor biology into clinical practice led to a more individualized, patient-specific treatment. On the other hand, having so many different new substances available in the treatment landscape of mCRPC, carries the risk of using the wrong substance at the wrong time. Therefore, the identification of appropriate biomarkers to predict patient prognosis and treatment success for each of these new substances is of dire need.

**Table 1** Overview of approved substances in metastatic castration resistant prostate cancer

| Substance | OS (months) | PFS (months) | Approval study | |
|---|---|---|---|---|
| **Docetaxel** (vs Mitoxantrone) | 18.9 vs. 16.5 | not assessed | TAX327 | Post chemotherapy |
| **Abiraterone** (vs Predisone) | 34.7. vs. 30.3 | 16.5 vs. 8.3 | COU-AA-302 | Post chemotherapy |
| **Enzalutamide** (vs Placebo) | 32.4 vs. 30.02 | Not reached vs. 3.9 | PREVAIL | Post chemotherapy |
| **Sipoleucel T** (vs Placebo) | 25.9 vs. 21.4 | 11.7 vs.10 | SIPOLEUCEL-T | Post chemotherapy |
| **Enzalutamide** (vs Placebo) | 18.4 vs.13.6 | 8.3 vs. 2.9 | AFFIRM | Chemotherapy naive |
| **Abiraterone** (vs Predisone) | 15.8 vs. 11.2 | 5.6 vs. 3.6 | COU-AA-301 | Chemotherapy naive |
| **Radium-223** (vs Placebo) | 14.9 vs.11.3 | 3.6 vs. 3.4 | ALSYMPCA | Chemotherapy naive |
| **Carbazitaxel** (vs Mitoxantrone) | 15.1 vs. 12.7 | 2.8 vs. 1.4 | TROPIC | Chemotherapy naive |

## 2 The Androgen Receptor As Anticancer Target

### 2.1 Abiraterone

Abiraterone is a selective inhibitor of the androgen biosynthesis enzyme cytochrome P450-17 (CYP17), a key enzyme in the production of androgens in the adrenal glands, as well as in tumor tissue. Thus, the drug inhibits adrenal and intratumoral androgen synthesis, which are the main remaining sources of androgens under castration conditions [4]. In April 2011, abiraterone in combination with prednisone was approved for patients with mCRPC after docetaxel chemotherapy. This approval was based on significant overall survival (OS) and progression free survival (PFS) benefits in the clinical phase III COU-AA-301 study (Table 1) [4]. In January 2013, abiraterone was additionally approved for the treatment of CRPC prior to chemotherapy [5] (Table 1). Sub-analyses of the COU-AA-301 study revealed that 14 % of patients did not respond to abiraterone. Thus, the identification of patients who are likely to respond to this kind of therapy is of urgent need [4].

Besides prostate specific antigen (PSA) kinetics and radiographic progression as biomarkers for predicting both therapy response and OS rates [6, 7], our group demonstrated that latent hypothyreosis in the first month after initiating abiraterone therapy is associated with therapy response (PSA declines and radiographic responses rates) compared to patients without any alteration in thyroid parameters [8].

Moreover, circulating androgens (testosterone, dihydrotestosterone, dehydroepiandrosterone, androstenedione, and androsterone) measured in a cohort of 37 patients using high-pressure liquid chromatography were described to predict therapy response, defined as ≥30 % PSA decline at 12 weeks of treatment [9]. In addition, another German group recently demonstrated that dynamic changes of alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and PSA itself during abiraterone therapy are associated with best clinical benefit and OS in bone mCRPC [10].

There is further evidence that the previous duration of response to hormonal therapy (≥12 months vs. < 12 months) is a predictor of sensitivity to next generation AR axis targeted drugs given that these act on the same treatment axis [11]. Changes in the AR itself, including AR mutations, amplifications, or splice variants were intensively studied as potential biomarkers for predicting therapy response: For example, the 878A or L702H AR amino acid changes were associated with resistance to CYP 17 inhibition [12, 13]. Romanel et al. and Azad et al. proposed that both AR gain and AR point mutations were associated with decreased OS, PFS, or resistance in patients treated with abiraterone compared to those with normal plasma AR [13, 14].

Mostaghel and colleagues treated human CRPC xenografts with abiraterone and found an increased expression of full-length AR and truncated AR variants compared to abiraterone naive CRPC xenografts [15]. Copy number variations of the AR and/or CYP17A genes were further associated with early progressive disease, lower PSA decline, and poor ECOG performance status [16].

In recent years, the concept of "liquid biopsies", such as circulating tumour cells (CTC) and cell-free DNA, released from the primary tumor site or from metastases into the circulation have been proposed as promising PCa biomarkers offering the potential for non-invasive characterization of disease and molecular stratification of patients. The advantage of these biomarkers is that they are able to provide diagnostic and prognostic information before treatment, during treatment,

and at progression, including DNA mutations, epigenetic alterations, and other forms of tumor-specific abnormalities such as microsatellite instability and loss of heterozygosity. Danila et al. for the first time showed that < 5 CTC in 7.5 mL peripheral blood of mCRPC patients was prognostic for longer OS under abiraterone therapy [17]. Scher and colleagues were able to show that the number of CTC combined with the LDH level is highly predictive for OS rates in an abiraterone-treated patient cohort [18]. In addition, the AR splice variant ARV7 was isolated from CTC of patients treated with abiraterone [18] and patients who harbored this splice variant had lower response rates compared to ARV7 negative patients. ARV7 was also present in 9 to 15 % of patients who were primary resistant to abiraterone [18]. Moreover, Lin28, a protein that is encoded by the LIN28 gene promotes the development of resistance to abiraterone (and also enzalutamide) by enhancing the expression of AR splice variants such as ARV7 [19].

Despite such promising results, currently ARV7 measurement is not available in daily routine. However, a lot of efforts are ongoing to measure ARV7 in the serum or tissue of patients. In addition, a recent conference paper showed for the first time that the detection of ARV7 in CTCs of mCRPC patients is no absolute exclusion criterion for a response to abiraterone or enzalutamide (*Steinestel et al., NRWGU 2016,* http://dx.doi.org/10.3205/16nrwgu53).

The TMPRSS2-ERG gene fusion resulting in ERG overexpression has been described in around 50 % of PCa and is a very early event in tumorgenesis. Recently, Attard et al. demonstrated that ERG fusion secondary to the deletion of 21q22 and increased copy number of fusion sequences correlated with increased response to abiraterone compared to those cancers with no ERG fusion [20]. On the other hand, Danila et al. showed in 2011 that the TMPRSS2-ERG fusion measured in CTC was not predictive for therapy response under abiraterone therapy [21].

The tumor suppressor phosphatase and tensin homolog (PTEN) is involved in PCa growth and progression by acting as a negative regulator of the PI3K/ AKT pathway, thereby controlling cell proliferation, tumor growth, and metabolic actions. In 2015, Ferraldeschi et al. identified in a cohort of 144 patients that the loss of expression of PTEN (determined by immunohistochemistry and FISH analyses on FFPE sections of tumor tissue) correlates with worse survival and prognosis in patients treated with abiraterone in the post-chemotherapy setting [22].

Neuroendocrine differentiation could represent another important source of new biomarkers in CRPC patients. Chromogranin A is a glycoprotein primary expressed in neuroendocrine cells. A retrospective study including 48 patients treated with abiraterone in the post-chemotherapy setting identified chromogranin A increase (>3 times than the upper normal level) as a significant marker for PFS and OS [23]. In addition, Heck et al. recently showed that both chromogranin A and neuron-specific enolase (NSE) correlated with shorter PSA-PFS, clinical or radiographic PFS, and OS in patients treated with abiraterone [24].

### 2.2 Enzalutamide

Enzalutamide is an AR inhibitor indicated for the treatment of patients with mCRPC with or without previous docetaxel therapy. The regulatory approval is based on two randomized, double-blinded, placebo-controlled phase III trials in patients with mCRPC pre-(PREVAIL) and post-(AFFIRM) chemotherapy with docetaxel, leading to prolonged OS, PFS, and delayed time to first skeletal event compared to the control groups [25, 26] (Table 1). Enzalutamide itself competitively inhibits androgen binding to the AR and, in contrast to abiraterone, prevents AR nuclear translocation and DNA binding. Like abiraterone, enzalutamide is approved for patients with asymptomatic or mild symptomatic mCRPC [27]. In addition, recent results from the TERRAIN study further support the use of enzalutamide combined with LHRH therapy in patients with asymptomatic or mild symptomatic mCRPC [28]. However, again there is a lack of biomarkers, early and reliably predicting treatment success.

As with abiraterone, the previous duration of response to initial hormonal therapy with LHRH agonists or antagonists is a predictor for an increased sensitivity to enzalutamide in patients with mCRPC [11]. Similar to abiraterone, the AR and its variants play a central role as biomarkers: The presence of the AR splice variant ARV7 measured in bone biopsies from patients with treated with enzalutamide is associated with primary resistance to the drug [29].

Furthermore, ARV7 was isolated from CTC of patients treated with enzalutamideand it was found that ARV7 positive patients had lower response rates compared to ARV7 negative patients. In addition, ARV7 was present in 9–15 % of patients who were primary resistant to enzalutamide. However, there also are data showing that ARV7 is able to converse from positive to negative or vice versa during therapy [30]. In a cell culture model, NF-kB2/p52 downregulation in enzalutamide treated PCa cells reduced ARV7 expression and thus increased the sensitivity to the drug [31]. In 2014, an ARV7 inhibitor was developed aiming to overcome the resistance caused by ARV7 inhibition, which is currently under clinical investigation [32]. Reviewing these findings, one can speculate that active AR variants are important key mechanisms of resistance in enzalutamide therapy. However, in addition to AR splice variants, the missense AR mutation F876L in the ligand binding domain of the AR has been shown in a preclinical model to be associated with primary resistance to enzalutamide by converting enzalutamide into a partial agonist in PCa cell lines [33]. Interestingly, one study found that AR mutations are more commonly found in patients treated

with enzalutamide compared to those treated with abiraterone [14]. Moreover, Azad et al. showed in cell free DNA (cfDNA) of patients treated with enzalutamide that AR amplifications are associated with therapy resistance [14]. Further, Efstathiou et al. analyzed 60 bone marrow biopsies of patients treated with enzalutamide and found that AR overexpression as well as CYP17 expression (>10 %) correlated with therapy benefit [29]. In addition, a recent genomic analysis on plasma of patients with CRPC found an increase in glucocorticoid-sensitive AR L702H and promiscuous AR T878A in patients with prior enzalutamide treatment [34]. This finding is of clinical importance as abiraterone, but also docetaxel and carbazitaxel are administered together with glucocorticoids. Moreover, an antitumoral effect of glucocorticoids per se has been shown in numerous preclinical and clinical studies.

From the preclinical point of view, our own data showed that enzalutamide resistant cell lines express high levels of full-length AR and AR variants. Moreover, we found that AR gene amplification is one mechanism of increased AR expression in enzalutamide resistant cells [35]. Apart from the AR, Arora et al. published that the induction of glucocorticoid receptor expression is a common feature of enzalutamide resistance, which can be conferred by the glucocorticoid receptor antagonist dexamethasone [36]. Thus, one can assume that the glucocorticoid receptor may bypass the need for AR by activating a subset of AR target genes, thereby promoting PCa progression.

Moreover, enzalutamide induces autophagy in CRPC cell lines by AMPK activation and mTOR suppression. An animal model was able to show that the combination of enzalutamide and autophagy inhibitors such as metformin significantly reduced tumor growth compared to the control group [37]. FOX-A1 is a transcription factor controlling AR chromatin deposition and AR transcription [38]. FOX-A1 regulates AR-V activity and cell proliferation as was demonstrated in a preclinical model using mRNA and CHIP analyses in PCa cell lines (LnCAP, 22RV1) [37]. Additionally, the impact of enzalutamide treatment on PDL1/2 status in dendritic cells was studied in a CRPC mouse model and found that enzalutamide resistance was associated with higher expression of PDL-1. Clinical findings confirmed that enzalutamide treatment increases PDL1/2 dendritic cells in comparison to treatment naive patients [39]. Further studies investigating the impact of PDL status in patients treated with both enzalutamide and abiraterone are ongoing.

## 3 Taxane Based Chemotherapy

### 3.1 Docetaxel

Until 2012, chemotherapy with the microtubular depolymerization inhibitor docetaxel was the primary treatment for patients with mCRPC. Thus, a multitude of clinical and molecular markers have been established for predicting therapy response. For example, high Gleason score, lymph node metastasis, visceral metastasis, and normal hemoglobin levels are known to predict PSA response after docetaxel [reviewed in 40]. In addition, ALP and hemoglobin, which are used in clinical routine are associated with therapy response to docetaxel [41].

Different patient cohorts have shown that CTC count is an early predictor for therapy response under docetaxel chemotherapy [42, 43]. For example, one study found that CTC count status is an early independent predictor for treatment response, PFS, and OS only 3 weeks following treatment initiation with docetaxel, whereas continuous CTC counts were an inconsistent surrogate marker in mCRPC patients [44]. In 2015, Scher et al. published a biomarker panel combining the number of CTCs and LDH. They observed that elevated CTC count (>5 cells in 7.5 mL blood) in combination with elevated LDH levels at week 12 during therapy with docetaxel was associated with inferior survival times compared to low CTC number and LDH levels [18]. Further, AR cytoplasmic localization in CTCs correlated with clinical response to taxane chemotherapy [45]. In addition to CTC, circulating miRNA has been proposed as a potential early predictor for docetaxel chemotherapy outcome in a patient cohort including 97 metastatic CRPC patients [46].

Another group isolated peripheral blood mononuclear cells from 20 patients with response to docetaxel therapy and detected KLK3, PCA3, and TMPRSS2-ERG in patients who responded to docetaxel while these markers were not detectable in the control group (healthy volunteers) [47]. In 2015, our group was able to show that cell free DNA (cfDNA) concentration before therapy is a useful marker for PSA decline and survival in patients undergoing docetaxel therapy [48]. Reigg et al. demonstrated in a patient cohort of 50 patients that TMPRSS2-ERG expression in PCa tissue correlated with lower PSA-PFS ($p = 0.02$) to docetaxel [49]. In 2012, it has been shown for the first time that cellular communications via exosomes may partly explain docetaxel resistance at least in a prostate cancer cell system [50].

In contrast to abiraterone and enzalutamide treatment, ARV7 positive patients did not have increased response rates to taxane therapies (docetaxel and cabazitaxel) [51]. However, other studies have shown that ARV7 and ARV567 expression in serum attenuates cytotoxicity of taxane chemotherapy, respectively [52, 53].

Numerous studies showed that angiogenesis and vascular damage such as endothelial cells significantly contribute to tumor initiation and progression in various tumor entities. Thus, endothelial cells represent an important therapy target and are candidates for biomarker research. The number of circulating endothelial cells has been shown to be prognostic for the response to docetaxel. Recent studies further identified

miRNAs as important regulators for epithelial to mesenchymal transition (EMT), a key step in tumor initiation and progression. In 2012, we were able to show in a preclinical study that EMT causes docetaxel resistance mediated by reduced expression of miR-200c and miR-205 [54]. Furthermore, miR-34a has been shown by another group in a cell culture model to be an intracellular and exosomal predictive biomarker for response to docetaxel therapy [55]. Currently, verification of preclinically identified miRNAs is ongoing in a variety of clinical studies.

Cell culture models showed that docetaxel resistant PC3 cells (isolated from bone metastases) express higher levels of cytokines and that interleukins such as IL-1ra, IL-1ß, IL-4, IL-6, IL-12, IFNγ, and MIC1 are able to predict chemotherapy response [56]. In addition, another group identified MIC1 as well as AGR2 as biomarker for docetaxel resistance [57]. Furthermore, Chen et al. demonstrated that the testicular nuclear receptor 4 (TR4) enhance chemoresistance to docetaxel [58], while Crea et al. showed that overexpression of the BMI1 oncogene is predictive for poor prognosis in different PCa cell lines (DU145 brain metastases and LNCaP lymph node metastases) [59].

### 3.2 Cabazitaxel

Cabazitaxel (Jevtana®) is a novel taxane that showed activity in docetaxel resistant tumors. In a large phase III multicenter study, cabazitaxel showed an OS benefit in patients with mCRPC and progressive disease during docetaxel therapy compared to mitoxantrone chemotherapy (TROPIC trial) [60]. (Table 1).

Concerning the biomarker profile of cabazitaxel, in addition to PSA [61] and PSA doubling time [62], interestingly, severe neutropenia during treatment is associated with a significant survival benefit compared to patients without neutropenia [63]. In addition, it has been shown in a multicenter senior adults program that neutrophil count <4000/mm$^3$ before cabazitaxel administration is associated with grade 3 neutropenia and/or neutropenic complications [64].

As with docetaxel treatment, Reigg et al. demonstrated in 22 cabazitaxel treated patients a prognostic impact of TMPRSS2-ERG expression in PCa tissue [49]. However, in contrast to abiraterone, enzalutamide, and docetaxel, response to cabazitaxel was found to be independent of the presence of AR-V7 splice variant [51].

In general, the neutrophil–lymphocyte ratio (NLR) has been shown to have a prognostic role in several tumor entities including renal cell cancer. Concerning mCRPC, it has been shown in patients treated within the TROPIC trial that conversion from high (≥3) to low (<3) NLR was associated with improved survival, lower PSA values and better RECIST responses [65]. One recent preclinical study found that DNA methylation of pro-apoptotic and cell-cycle regulatory genes may contribute to cabazitaxel resistance and pre-treatment with 5-azacytidine may restore sensitivity to cabazitaxel in Du145 prostate cancer cells (brain metastases) [66].

## 4 Immunomodulatory Agents

In general, immunotherapeutic approaches modulate immunostimulatory pathways, thereby maintaining and prolonging the activity of antigen-presenting cells as well as enhancing cytotoxic T-cell-mediated tumor regression. Sipuleucel-T (Provenge®) is a therapeutic cancer vaccine prepared by extracting peripheral blood mononuclear cells cultured ex vivo with PA2024 recombinant fusion protein and then re-infused into the patient. Sipuleucel-T was associated with longer OS (median 25.8 vs. 21.7 months) (Table 1), although it had no effect on time to disease progression or PSA, so that the identification for biomarkers for therapy monitoring represent an important issue [67].

Data from the phase III IMPACT and phase II ProACT studies showed evidence that antigen spread may occur after Sipuleucel-T treatment, indicating the immune response evolves over time to target multiple prostate antigens [68, 69]. Moreover, a potential correlation between increased eosinophils and OS has been suggested [70]. Meanwhile, at least in Europe Sipuleucel-T is no longer available.

## 5 Radium-223 (Alpharadin)

Radium-223 (Xofigo®) emits alpha particles that lead to a high frequency of DNA double-strand breaks in adjacent tumor cells, resulting in a potent cytotoxic effect on bone metastases. After evidence of clinical efficacy in the ALSYPMCA trial (Table 1), radium-223 was approved for the treatment of patients with CRPCA and symptomatic bone-metastasis in 2014 [71]. To the best of our knowledge, skeletal tumor burden on baseline fluoride PET/CT has been reported as the only predictive biomarker of OS and the risk of an SRE in patients treated with radium-223 [72]. Another study *(NCT02346526)* investigating the impact of CTC numbers for predicting therapy response to radium-223 is currently recruiting patients (www.clinicaltrials.gov). A conference paper presented at the 2015 ESMO congress including data from our own group identified ALP as well as the ECOG performance status as predictive factors for therapy response to radium-223 (*O'Sullivan J, ESMO congress 2015, abstract number 2561*).

**Table 2** Overview of biomarkers predicting treatment success of approved substances in metastatic castration resistant prostate cancer

| Symptomatic mCRPC | | | Asymptomatic/mild symptomatic mCRPC | | |
|---|---|---|---|---|---|
| **Docetaxel** | **Cabazitaxel** | **Radium-223** | **Abiraterone** | **Enzalutamide** | **(Sipoleucel T)** |
| PSA | PSA | Skeletal tumor burden | PSA | PSA | Antigen spread |
| ALP/LDH | PSA doubling time | ECOG status | ALP/LDH | AR mutations | Eosinophils |
| Hemoglobine | TMPRSS2-ERG | ALP | Circulating Androgens | AR amplifications | |
| PCA3 | Neutophile-lymphocyte ratio | | Hypothyreosis | AR overexpression | |
| TMPRSS2-ERG | DNA methylation status | | AR mutations | ARV7 splice variant | |
| KLK-3 | | | AR amplifications | Glucocorticoid receptor | |
| Ciculating tumor cells | | | ARV7 splice variant | Fox A1 | |
| Circulating fDNA | | | ERG | PDL 1/2 | |
| AR cytosplasmatic location | | | LIN 28 | | |
| miRNA 34a, 200c, 205 | | | PTEN | | |
| Interleukin 1,6,12, INFy, MIC 1 | | | Chromogranin A | | |
| Epithelial-mesenchymal transition | | | NSE | | |
| BMI1 | | | | | |
| TR4 | | | | | |

PSA= Prostate specific antigen
AR = Androgen receptor

## 6 Conclusion and Future Directions

In recent years, the landscape of therapies in mCRPC has expanded greatly. Therefore, biomarkers are necessary to identify those patients who are likely to respond or to be resistant to a certain therapy at the earliest opportunity. Some biomarkers, like the ARV7 splice variant are assessed in much detail and on their way to clinical routine. Beyond the AR as a primary target of biomarker research, genomic alterations like the TMPRSS2–ERG fusion gene, transcription factors, or DNA repair genes have been identified in serum, urine, or tissue as biomarker candidates using new molecular profiling strategies. Table 2 provides a summary of specific biomarkers for each of the available substances as decision support for selecting the best personalized treatment for each single patient. For the future, new mCRPC drugs and consequently new biomarkers are under development. For example, loss of BRCA 1 and 2, ATM mutation, FANCA deletion, CHEK2 deletion as well as HDAC1/2 aberrations have been described as biomarker candidates for therapy response in patients treated with a new PARP inhibitor olaparib leading to improved individual treatment options in mCRPC patients [73].

Moreover, a recent genome-wide DNA methylation analysis revealed the presence of epigenetic markers important for disease progression as marker of prognosis. Hypermethylation of ETV1 and ZNF215 predicted disease progression despite androgen deprivation, while hypermethylation of IRAK3 and ZNF215 were independent markers of prognosis. Thus, epigenetic silencing of the mentioned genes could be novel molecular markers for the prognosis of advanced PCa [74].

In conclusion, also in 2016 efforts to validate and expand current biomarker research are important with the aim to offer personalized treatment strategies to each patient.

**Compliance with Ethical Standards**

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