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Review

Metastatic prostate cancer remains incurable, why?

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Abstract Metastatic prostate cancer patients present in two ways—with already disseminated disease at the time of presentation or with disease recurrence after definitive local therapy. Androgen deprivation therapy is given as the most effective initial treatment to patients. However, after the initial response, almost all patients will eventually progress despite the low levels of testosterone. Disease at this stage is termed castration resistant prostate cancer (CRPC). Before 2010, the taxane docetaxel was the first and only life prolonging agent for metastatic CRPC (mCRPC). The last decade has witnessed robust progress in CRPC therapeutics development. Abiraterone, enzalutamide, apalutamide and sipuleucel-T have been evaluated as first- and second-line agents in mCRPC patients, while cabazitaxel was approved as a second-line treatment. Radium-223 dichloride was approved in symptomatic patients with bone metastases and no known visceral metastases pre- and post-docetaxel. However, despite significant advances, mCRPC remains a lethal disease. Both primary and acquired resistance have been observed in CRPC patients treated by these new agents. It could be solely cell intrinsic or it is possible that the clonal heterogeneity in treated tumors may result from the adaptive responses to the selective pressures within the tumor microenvironment. The aim of this review is to list current treatment agents of CRPC and summarize recent findings in therapeutic resistance mechanisms.

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1. Introduction

Prostate cancer (PCa) is the most common cancer and the second and third leading cause of cancer death in American and European men, respectively [1,2]. Approximately 160 000 new diagnoses of PCa and over 29 000 deaths are estimated to occur in the United States in 2018. In 2012, 400 000 European men were newly diagnosed with PCa out of a global 1.1 million new cases [3,4]. Though the reported incidence and mortality rate of PCa in Asian countries including China is much lower than in Western countries, the PCa incidence rate has increased rapidly in China with an annual 12.6% change since 2000 [5]. The 5-year survival rate of non-metastatic PCa is 98.9% (measured between 2005 and 2011), however, the rate in patients with metastatic PCa on initial diagnoses is less than 30% and remains low [2,6,7].

Metastatic patients present in two ways—with already disseminated disease at the time of presentation or with disease recurrence after definitive local therapy. Androgen deprivation therapy (ADT) is given as the most effective initial treatment to patients, however, after the initial response, almost all patients will eventually progress despite the low levels of testosterone in the systemic circulation following a median 18—24 months of ADT [8]. Disease at this stage is termed castration resistant PCa (CRPC). The median survival for CRPC is now in the range of 15—36 months, although exact survival rates vary depending on disease burden once a patient enters the state of castration resistance [9,10]. Terms such as hormone-resistant PCa or hormone-independent PCa were also used to describe this stage, but after it has been demonstrated that androgen receptor (AR) signaling still remains actively supporting the survival and growth of PCa cells, these terms have been largely abandoned [11]. The current official definition of CRPC by the European Association of Urology (EAU) guideline is biochemical progression (three consecutive rises in prostate-specific antigen [PSA] 1 week apart resulting in two 50% increases over the nadir and a PSA $>$2 ng/mL) or radiological progression (appearance of two or more new bone lesions on bone scan or a soft tissue lesion using Response Evaluation Criteria in Solid Tumors [RECIST] in the presence of serum testosterone $<$50 ng/dL or 1.7 nmol/L) [12].

The reasons why PCa progresses to castration resistance are still not fully understood. When mechanisms of resistance were delineated, many novel therapeutic approaches were successfully developed that improved survival. For example, "supracastration" agents were developed once it was understood that ADT could not block all the androgen synthesis in a patient's body despite an obvious drop in serum testosterone level. PCa cells can still get androgen support from an increased synthesis of androgens in the tumor microenvironment or from adrenal androgen precursors. In addition, a series of alterations of AR signaling have been observed, including increased AR expression, *AR* gene mutations (which cause ligand promiscuity and activation by glucocorticoids or even AR antagonists), and also AR variants that are constitutively active in absence of ligands [11,13—15].

Before 2010, the taxane docetaxel had been the first and only life prolonging agent for metastatic CRPC (mCRPC) [16]. The last decade has witnessed robust progress in CRPC therapeutics development. Five new drugs have shown efficacy in improving overall survival, leading to their licensing for the treatment of mCRPC. Abiraterone, enzalutamide and sipuleucel-T have been evaluated as first- and second-line agents in mCRPC patients, while cabazitaxel was approved as a second-line treatment. Apalutamide has recently been approved by United States Food and Drug Administration (FDA) for treatment of patients with non-metastatic CRPC (nmCRPC), which could also be promising for mCRPC treatment in the near future. Radium-223 dichloride (radium-223) was approved in symptomatic patients with bone metastases and no known visceral metastases pre- and post-docetaxel [15,17]. Though the armamentarium now holds many new weapons, we are still facing the challenges of when to choose what weapon and against which enemy. For example, approximately 15%—25% of patients with CRPC do not respond to first-line treatment with the "supra-castration" agents abiraterone or enzalutamide [18].

One of Dr. Donald S. Coffey's most favorite and famous aphorisms was "If this is true, what does it imply?". This philosophy inspires us to keep pursuing the underlying mechanism behind resistance. Despite significant advances, mCRPC remains a lethal disease. What does this imply? Dr. Coffey believed that dissecting tumor heterogeneity was a key to deciphering cancer therapeutic resistance. Resistance to a therapy could be solely cell intrinsic and, therefore, present in a treatment-naïve setting (primary resistance), or it is possible that the clonal heterogeneity in treated tumors may result from the adaptive responses to the selective pressures within the tumor microenvironment (acquired resistance) [19,20]. How to precisely select therapies and arrange them in an appropriate order for a single patient based on the understanding of tumor heterogeneity and resistance mechanisms is one of the most important challenges for current CRPC treatment. The aim of this review is to list current treatment agents of CRPC and summarize recent findings in therapeutic resistance mechanisms.

2. Current treatment patterns for CRPC

As a member of the nuclear receptor superfamily of ligand-activated transcription factors, AR is a key regulator of normal prostate function as well as cancer development. For decades we have known that PCa cell proliferation is inhibited when low serum levels of testosterone and dihydrotestosterone (DHT) are maintained [21,22]. Many studies have revealed that AR regulated genes participate in various cellular processes that contribute to the initiation and progression of PCa [23].

Bilateral orchiectomy is a traditional and still accepted cost-effective form of ADT with relatively few side effects [24]. In the 20th century, several androgen-axis targeting agents have been developed as alternatives to surgical castration. Subgroups include androgen biosynthesis inhibitors, agonists of the gonadotropin-releasing hormone (GnRH), and estrogens. Over the years, several chemotherapeutic agents and, in case of bone metastasis, bisphosphonates were applied in a complementary manner to ADT. However, oncologic outcomes remained poor. In 2004 the chemotherapeutic docetaxel was approved for

metastatic PCa, which marginally but significantly improved overall survival. Although the effect was moderate, it was the start of an arms race against CRPC. In a short time, more drugs were approved by the FDA such as GnRH antagonists, a new chemotherapy (cabazitaxel), bone-directed therapies (zoledronic acid, denosumab, radium-223), a new androgen biosynthesis inhibitor (abiraterone), new AR blockers (enzalutamide, apalutamide) and immunotherapy (sipuleucel-T). Table 1 presents an overview of current FDA approved therapies. Many more therapeutic agents are currently under clinical evaluation.

2.1. ADT

2.1.1. Estrogens

Estrogens inhibit testosterone production [21]. In clinical practice it lost popularity decades ago, mainly due to cardiovascular side-effects such as thromboembolic events [25]. In lower doses these events may occur less frequently and treatment costs are low compared to other forms of ADT, however in the currently increasing complexity of the therapeutic mCRPC landscape, it might be too late for a "comeback" into routine clinical practice [15,26–28]. It is argued that the parenteral administration of estradiol bypasses the hepatic first-pass effect and may reduce the risk of adverse effects. In a Scandinavian trial, the use of intramuscular administered polyestradiol phosphate (PEP) in mCRPC patients was found to be equally effective compared to continuous ADT. Although there was no significant increase in cardiovascular mortality in the PEP group compared to the ADT group, there was a significant increase in non-fatal cardiovascular events ($p < 0.05$) [29]. Transdermal estrogen administration is currently being investigated [30].

2.1.2. GnRH analogues

After the start of GnRH analogue therapy, testosterone production briefly increases as a result of stimulation of follicle-stimulating hormone and luteinizing hormone, also referred to as a flare, which can cause symptoms such as pain in men with a heavy disease burden. Through a feedback loop, GnRH receptors are then desensitized and downregulated. Until this effect is established, an AR

Table 1 Approved pharmacologic therapies in PCa.

Agent name	Target	FDA approved year
Chemotherapy		
Estramustine	Anti-tubulin, lowering LH + FSH through competitive GnRH receptor binding	1981
Mitoxantrone	DNA synthesis, DNA repair	1996
Docetaxel	Anti-tubulin, AR signaling disruption	2004
Cabazitaxel	Anti-tubulin	2010
Androgen deprivation therapy		
Ketoconazole	CYP 17 inhibition	Non-FDA approved[a]
Abiraterone	CYP 17 inhibition	2011
Leuprorelin	Lowering LH + FSH through desensitization of pituary gland	1989
Goserelin	Lowering LH + FSH through desensitization of pituary gland	1989
Histrelin	Lowering LH + FSH through desensitization of pituary gland	1991
Triptorelin	Lowering LH + FSH through desensitization of pituary gland	2000
Buserelin	Lowering LH + FSH through desensitization of pituary gland	Non-USA[b]
Abarelix	Lowering LH + FSH through competitive GnRH receptor binding	2003
Degarelix	Lowering LH + FSH through competitive GnRH receptor binding	2008
Cyproterone	Competitive inhibition of AR	Non-USA[b]
Flutamide	Competitive inhibition of AR	1989
Bicalutamide	Competitive inhibition of AR	1995
Nilutamide	Competitive inhibition of AR	1996
Enzalutamide	Competitive inhibition of AR	2012
Apalutamide	Competitive inhibition of AR	2018
Bone health agent		
Pamidronate	Inhibition of bone resorption	1991
Zoledronic acid	Inhibition of bone resorption	2003
Denosumab	RANKL-antibody	2010
Bone-directed agent		
Radium-223 dichloride	Hydroxyapatite osteoblastic bone metastases	2013
Immunotherapy		
Sipuleucel-T	Prostatic acid phosphatase	2010
Pembroluzimab	PD-1 receptor of lymphocytes in MSI-H patients	2017

FDA, United States Food and Drug Administration; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; AR, androgen receptor; CYP, cytochrome P450; RANKL, receptor activator of nuclear factor kappa-B ligand; PD-1, programmed cell death protein 1; MSI-H, microsatellite instability-high; PCa, prostate cancer.

[a] Off-label used in second line, but not approved.

[b] Used outside of USA, but not available in USA, so not FDA-approved.

antagonist, such as bicalutamide, may be prescribed to counter a flare and the associated clinical consequences [31]. GnRH analogues remain the most prescribed first-line therapy for metastatic PCa [12,15,32]. The continuous use of GnRH analogues with an AR antagonist has been termed continuous androgen blockade (CAB).

2.1.3. GnRH antagonists

Abarelix and degarelix antagonize the GnRH receptor, causing a direct inhibitory effect on testosterone metabolism. Therefore, no concomitant use of an AR blocker is necessary with this form of ADT [33].

2.1.4. Androgen biosynthesis inhibitors

CYP17A1, belonging to the cytochrome P450 family, is found in nearly all steroidogenic tissues. This enzyme has both 17α-hydroxylase activity and 17,20-lyase activity. CYP17A1 inhibitors antagonize the production of testosterone and DHT [34]. Ketoconazole, mainly used as systemic antifungal agent, is a CYP17A1 inhibitor. Due to hepatotoxic side effects and low impact on survival, the off-label use as second-line ADT in PCa has disappeared from clinical practice [32,35]. A new CYP17A1 inhibitor, abiraterone, was FDA approved as first- and second-line therapy in mCRPC [36—38]. The COU-301 trial compared the use of abiraterone and prednisone 10 mg with placebo and prednisone 10 mg in patients with progression after prior treatment with ADT and docetaxel. Abiraterone improved survival compared to placebo (15.8 months *vs.* 11.2 months, respectively). Additionally, time to PSA progression and progression free survival were improved in the abiraterone group [39]. The COU-302 trial evaluated abiraterone with prednisone in patients that showed progression under ADT, but had no prior treatment with docetaxel. Overall survival in the abiraterone group was 34.7 months versus 30.3 months in the placebo group. The most common grade 3—4 adverse events were cardiac disorders (8% in abiraterone *vs.* 4% in placebo group), hepatotoxicity (6% *vs.* <1%) and hypertension (5% *vs.* 3%) [40]. Concomitant administration of prednisone helps to prevent side effects related to accumulation of upstream mineralocorticoids such as hypertension, hypokalemia and fluid retention [41]. The LATITUDE trial studied the addition of abiraterone plus prednisone 5 mg to ADT compared with ADT alone in newly diagnosed high-risk metastatic PCa patients. After 30.4 months ADT plus abiraterone and prednisone significantly improved survival and secondary outcomes [42]. The same improvement has been demonstrated in the STAMPEDE study, although the patients included had different tumor characteristics [43]. With the acceptance of abiraterone as treatment of early stage disease, head-to-head comparisons of drugs and specific combinations and sequences have become even more important.

2.1.5. Androgen receptor blockers

Competitive binding to the AR inhibits the proliferative effect of the AR pathway. There are two types of AR blockers: Steroidal (cyproterone acetate) and non-steroidal, including flutamide, bicalutamide, enzalutamide and apalutamide. Cyproterone acetate was the first steroidal AR blocker used to treat advanced PCa in Europe. It competes with androgens for binding to the AR and has anti-gonadotropic capacity as well. However, many severe adverse events have been observed with the use of cyproterone acetate (cardiovascular complications, liver failure, *etc.*), and that largely limits its clinical use [44,45]. On the other hand, many non-steroidal AR blockers have demonstrated high efficacy in treatment, as well as good drug tolerance.

While bicalutamide, nilutamide and flutamide were utilized in conjunction with GnRH inhibitors and were not approved in the mCRPC setting, newer agents bind AR with greater affinity, changing the landscape of treatment. Enzalutamide, approved as first- and second-line therapy in mCRPC, targets the AR with higher affinity compared to bicalutamide and also inhibits nuclear translocation, DNA-binding and recruitment of coactivators [46]. In the AFFIRM trial, patients that were treated with enzalutamide after progression under chemotherapy had a better overall survival than placebo (18.4 months *vs.* 13.6 months respectively; $p < 0.001$). All secondary endpoints, such as PSA response, soft-tissue response (following RECIST), progression-free survival and time to first skeletal-related event were significantly improved by enzalutamide. In the enzalutamide group five seizures occurred (0.6%). Other adverse events more common in that group were: Fatigue, diarrhea, musculoskeletal pain and hot flashes [47]. The seizures are thought to be caused by inhibition of gamma-aminobutyric acid A (GAB-A) receptors in the brain [48]. Recent *post hoc* analysis of the TERRAIN trial evaluating chemo-naïve mCRPC patients demonstrated that more adverse effects occur in patients older than 75 years compared to younger patients [49]. Elderly patients on enzalutamide had a higher incidence of atrial fibrillation (12.1% *vs.* 0.8%), urinary tract infection (20.7% *vs.* 2.4%), falls (12.1% *vs.* 4.0%) and decreased appetite (15.5% *vs.* 6.4%). Therefore, caution is advised when prescribing AR blockers to patients older than 75 years that are already prone to falls or have cardiovascular comorbidity.

Recently the FDA approved apalutamide, also a second-generation AR antagonist with similar effects as enzalutimide, for nmCRPC. The phase III trial (SPARTAN) compared 806 nmCRPC patients on apalutamide with 401 placebo controls. Non-metastatic was determined as a negative technetium-99m bone scan and negative CT scan. CRPC was defined as PSA-doubling in 10 months or less under continuous ADT (bilateral orchiectomy, GnRH analogues or GnRH antagonists). The primary endpoint was median metastasis-free survival (MFS) [50]. The MFS was 40.5 months in the apalutamide group versus 16.2 months in placebo with a hazard ratio (HR) of metastasis or death of 0.28 (95% confidence interval (CI), 0.23—0.35; $p < 0.0001$). The time to symptomatic progression was longer with apalutamide than with placebo (HR, 0.45; 95% CI, 0.32—0.63; $p < 0.001$). Apalutamide was associated with higher rates of grade 3—4 adverse event than placebo (45.1% *vs.* 34.2%, respectively). Most common were fatigue (30.4% *vs.* 21.1%), rash (23.8% *vs.* 5.5%), falls (15.6% *vs.* 9.0%), fracture (11.7% *vs.* 6.5%), hypothyroidism (8.1% *vs.* 2.0%) and seizure (0.2% *vs.* 0%) [51].

In 2017 phase II results were published of the mCRPC study arm that compared the use of apalutamide with ($n = 25$) and without ($n = 21$) prior abiraterone plus prednisone (AAP). PSA response rates after 12 weeks were 88% for AAP-naïve patients and 22% in patients that were

treated with AAP. Median time to progression was 18.5 and 3.8 months, respectively. While awaiting for phase III results, these data suggested that apalutamide may be effective in early stage CRPC [52].

2.2. Chemotherapy

Cytotoxic therapy has an important role in the treatment of metastatic disease. Benefits include increase in time to progression, symptom relief, and overall survival [53–55]. After docetaxel was approved in 2004, estramustine and mitoxantrone (improved symptom relief only) fell into abeyance. Docetaxel is a taxane that stabilizes microtubules. During the process of mitosis these filaments divide the chromosomes evenly. By binding the β-tubulin dimers, docetaxel prevents the separation of chromosomes, triggering mitotic arrest and subsequent apoptosis [56]. Additionally, docetaxel disrupts AR signaling [57]. The initial response rate to docetaxel is 45%–50% [16,58,59]. In the TAX 327 trial, docetaxel with prednisone prolonged median survival of patients that had progressed under ADT compared to mitoxantrone with prednisone (19.2 months *vs.* 16.3 months, respectively; $p = 0.004$).

In metastatic but hormone naïve patients, the role of docetaxel in the earlier, advanced stage of PCa was studied in the CHAARTED trial [60]. The effect of ADT was compared with ADT plus docetaxel. Adding docetaxel to ADT in this early stage improved overall survival (57.6 months *vs.* 47.2 months for ADT alone; HR, 0.72; 95% CI, 0.59–0.89; $p = 0.0018$). With additional analysis, improvement in survival was seen only in high-volume disease defined as presence of visceral and/or $\geq$ four bone metastases (51.2 months *vs.* 34.4 months with only ADT; $p = 0.001$). In low-volume disease no survival benefit was observed, however, the study was not powered for these subgroup analyses [61].

The multi armed STAMPEDE study compared standard of care (ADT and optional local RT if applicable) with and without docetaxel. A median overall survival of 81 months with docetaxel was reported versus 71 months with standard of care ($p = 0.022$). Grade 3–5 adverse events occurred in 52% in the docetaxel group versus 32% in the control group [62]. Other recently published data from the STAMPEDE investigators on hormone-naïve and advanced PCa, compared standard of care with prednisone plus either abiraterone or docetaxel. No difference in overall or PCa-specific survival was found. The occurrence of symptomatic skeletal and severe adverse events were similar [63].

Cabazitaxel, another taxane, was approved in 2010 after the TROPIC trial. In mCRPC patients that had ADT and showed progression under docetaxel, it compared cabazitaxel (25 mg/m^2 every 3 weeks) with prednisone versus mitoxantrone with prednisone. Overall survival was 15.1 months on cabazitaxel versus 12.7 months on mitoxantrone. PSA response in the cabazitaxel group was 39.2% *vs.* 17.8%. Common adverse events of cabazitaxel were febrile neutropenia (94%) and diarrhea (47%) [64]. The PROSELICA trial demonstrated non-inferiority of a lower dose, 20 mg/m^2 every 3 weeks. The median survival in the 20 mg/m^2 dose group was 13.4 months versus 14.5 months when on 25 mg/m^2. The lower dose group had worse secondary outcomes, such as PSA response 29.5% versus 42.9% on 25 mg ($p = 0.001$). Grade 3–4 adverse events after low dose were 39.7% versus 54.5% after high dose. Neutropenia occurred in 66.6% in low dose versus 88.6% in high dose [65]. Prior use of abiraterone does not seem to impact the response to cabazitaxel [66]. A phase II trial reported a 34.9% PSA response rate in a 10 mg/m^2 weekly treatment schedule and reported a lower toxicity, 14.2% neutropenia and 35.7% diarrhea [67].

2.3. Immunotherapy

In 2010 the first active cellular immunostimulant, sipuleucel-T, was approved as a first-line therapy for non-to minimally-symptomatic mCRPC [68–70]. Mononuclear blood cells are harvested through leukapheresis. The cells, including antigen-presenting cells, are activated with the antigen prostatic acid phosphatase (PAP) and granulocyte-macrophage colony stimulating factor *ex vivo*. The autologous, activated product is reinfused three times [71]. The IMPACT trial demonstrated a 4.1-month increase in overall survival in the sipuleucel-T group (25.8 months *vs.* 21.7 months in placebo). HR for death in the sipuleucel-T group was 0.78 (95% CI, 0.61–0.98; $p = 0.03$). Grade 3–5 adverse events were equal compared to the placebo-group. Overall events that occurred significantly more frequent included chills, fever and headache [72,73].

The IgG4-antibody pembrolizumab was recently FDA approved for all mismatch repair (MMR) deficient tumors. Although more common in gastro-intestinal and endometrium cancer, literature on MMR deficiency in metastatic PCa suggests an incidence rate of 2%–12% [74–76]. Pembrolizumab inhibits the programmed cell death protein 1 (PD-1) on T-lymphocytes. MMR deficient tumors can activate the PD-1 receptor, which functions as an autoimmune suppressor, thereby causing the immune system to tolerate the tumor [77,78]. For PCa, the outcomes of a phase Ib study reported an overall response rate of 13% [78]. Preliminary data of a phase II trial showed that 20% of mCRPC patients had a PSA decrease when pembrolizumab was added to enzalutamide after progression [77]. Only in one of two exceptional responders was evidence of MMR deficiency found.

2.4. Bone health agents

Bisphosphonates, like pamidronate and zoledronic acid, can prevent complications of bone metastases as they inhibit osteoclast activity. They affect cell survival and cytoskeletal dynamics. A study by Saad et al. [79], demonstrated fewer skeletal-related events (SRE) in mCRPC treated with zoledronic acid compared to placebo (33.2% *vs.* 44.2% respectively, $p = 0.021$). However, a randomized controlled trial ($n = 1\,904$) compared it to the antibody denosumab (approved in 2010), in which the latter showed a delay in on-study SRE (17.1 months *vs.* 20.7 months on zoledronic acid; HR, 0.82; 95% CI, 0.71–0.95; $p = 0.0002$ for non-inferiority; $p = 0.008$ for superiority) [80]. The total numbers of adverse events were equal (both 97%), but the denosumab group had more serious events (63% *vs.* 60%) such as hypocalcaemia (13% on denosumab *vs.* 6% on zoledronic acid; $p < 0.0001$). In this study the

occurrence of osteonecrosis of the jaw (ONJ), a severe side effect of treatment with denosumab or bisphonates in oncologic doses, did not differ significantly between the denosumab group (2%) and the zoledronic acid group (1%, $p = 0.09$). This corresponds with the risk of developing ONJ in oncologic patients in the literature [81]. The therapeutic target of denosumab, RANKL, is an essential protein for formation, function, and survival of osteoclasts. The addition of radium-223 to denosumab seems to improve overall survival 2 months, without difference in adverse events. However, a survival benefit of denosumab alone has not been reported [82–84]. The addition of zoledronic acid to docetaxel has no proven benefit [62]. A retrospective cohort showed that concomitant use of denosumab (42.4%) or zoledronic acid (21.3%) with other relatively new agents is common clinical practice for CRCP. Sipuleucel-T was most often combined with denosumab and cabazitaxel with zoledronic acid [84].

2.5. Radionuclide therapy

Radium-223 dichloride is an α-particle emitter. It selectively targets areas of bone turnover, *i.e.* bone metastases, as it gets built into hydroxy-apatite as a calcium substitute. The emitted high energy α-particles radiate within 100 μm and induce irreversible DNA double-strand breaks [85,86]. In 2013 the ALSYMPCA-trial compared placebo versus radium-223 in addition to standard of care. The included castration-resistant patients had two or more bone symptomatic nodes and no visceral metastases. The radium-223 group had an improved overall survival of 3.6 months (14.9 *vs.* 11.3) with a HR of 0.70 (95% CI, 0.58–0.83; $p < 0.001$) [87]. The effect of radium-223 on overall survival compared to placebo was more outspoken without prior docetaxel-use, but in both groups a significant effect was seen. There were more adverse events after docetaxel use (62% after docetaxel, 54% no prior docetaxel). Events were mostly hematological side effects, specifically thrombocytopenia occurred more often after prior usage of docetaxel (9% *vs.* 3%) [88].

3. Therapeutic resistance in CRPC

3.1. Mechanisms of resistance to new generation hormonal therapies

As introduced above, abiraterone acetate can effectively block CYP17A1, inhibiting androgen synthesis, whereas enzalutamide, as a potent AR inhibitor, can reduce nuclear translocation of the AR complex and subsequent DNA binding. If primary resistance is defined as a treatment failure within the first 3 months after initiation, the primary resistance rates of abiraterone and enzalutamide in CRPC patients are about 15% and 25%, respectively. Acquired resistance typically develops after 9–15 months of treatment with either agent [18]. Generally, the mechanisms of abiraterone and/or enzalutamide resistance can be summarized in three main categories: AR driven mechanisms, AR bypass mechanisms and AR independent mechanisms (Table 2).

3.1.1. AR driven mechanisms

In some CRPC patients treated by abiraterone and/or enzalutamide, AR signaling is still playing an active role in supporting PCa cell survival that can be up-regulated by: 1) Androgen biosynthesis, 2) AR amplification/overexpression and stabilization, 3) AR mutation, and/or 4) AR splice variants.

3.1.1.1. Androgen biosynthesis up-regulation. While effective, abiraterone is not able to completely block all the serum precursor steroids such as DHEA-S [89]. Therefore, tumor cells can increase the ability to metabolize steroid precursors downstream of CYP17A1 as a strategy to survive androgen deprivation. It has also been observed that, as an adaptive response to androgen blockage, a series of genes encoding enzymes that are involved in androgen biosynthesis are upregulated under the treatment of new generation hormonal therapies. *CYP17A1* gene is two-fold overexpressed with treatment of abiraterone in CRPC xenografts [90]. Intratumoral expression of CYP17A1 was also found to be increased in biopsy samples from patients treated with abiraterone [91]. DHT can be synthesized from 5-α-androstenedione instead of testosterone through the activity of the enzyme AKR1C3, which is found to be overexpressed in both abiraterone- and enzalutamide-resistant PCa cells [92,93]. Some studies showed that *ERG* gene rearrangement is associated with response to abiraterone [94,95]. The ERG-fusion protein TMPRSS2-ERG was found to be co-expressed with AKR1C3 in CRPC, and upregulates its expression [96]. The ERG-AKR1C3-AR feed-forward loop can contribute to drug resistance by maintaining androgen synthesis. Recently, Xiao et al. [97] found that liver receptor homolog-1 (LRH-1), a nuclear receptor originally characterized as an important regulator of some liver-specific metabolic genes, had a high expression level in CRPC xenograft models. It can promote *de novo* androgen biosynthesis by its direct transactivation of several key steroidogenic enzyme genes, elevating

Table 2 Mechanisms of resistance to new generation hormonal therapies.

AR driven mechanisms	AR bypass mechanisms	AR independent mechanisms
● Androgen biosynthesis up-regulation	● By glucocorticoid receptor	● Neuroendocrine differentiation
● AR amplification/overexpression	● By progesterone receptor	● Crosstalk with other pathways
● AR stabilization		
● AR mutation		
● AR splice variants		

AR, androgen receptor.

intratumoral androgen levels and reactivating AR signaling in abiraterone-treated CRPC tumors.

3.1.1.2. AR amplification/overexpression and stabilization. The *AR* gene is located on chromosome Xq11—12. AR is a 110-kDa nuclear protein that contains 918 acid residues and binds the androgen response element (ARE) [98]. Increased copy number and expression of AR may increase AR responses to low androgens levels. About 80% of CRPC patients showed high levels of AR expression [99]. A study showed that in CRPC patients who had disease progression during treatment with abiraterone, enzalutamide or other therapy, 45% had AR overexpression. It was demonstrated that AR overexpression was much more common in the enzalutamide group compared to the abiraterone group (53% *vs.* 17%) [100]. Another study showed that high level of AR expression before treatment was related to poor prognosis when treated with enzalutamide [101]. One mechanism that may contribute to AR overexpression is the upregulation of retinoic acid receptor-related orphan receptor γ (ROR-γ), which can promote AR expression by recruiting the AR coactivators SRC-1 and SRC-3 [102]. On the other hand, at low androgen levels, HER2 and HER3 may play a role in stabilizing the AR and promote binding to ARE. It has been demonstrated that HER2 stabilizes AR protein through PI3K/AKT signaling. Also, enhanced treatment responses were observed in xenograft models blocking HER2 using lapatinib in combination with abiraterone [103,104].

3.1.1.3. AR mutatio. Clonal selection of tumor cells can determine the expansion of both AR somatic mutations and aberrant transcription. *AR* mutations occur in approximately 12%—48% of CRPC patients receiving enzalutamide or abiraterone [99,105]. Romanel et al. [106] found that outcomes with abiraterone were much better in patients who had the wild-type *AR* gene than in those who had *AR* mutations by looking at circulating tumor DNA (ctDNA). In a recent study, Wyatt et al. [105] also found that the detection of AR amplification and heavily mutated AR in ctDNA from patients treated with enzalutamide was associated with worse progression-free survival. There are three different types of mutations in the ligand-binding domain (LBD) of AR that are relatively well studied. The first is the F876L mutation that was found both in cell lines and patient samples treated with enzalutamide that could convert enzalutamide and apalutamide from an antagonist into a partial agonist [107—109]. With this mutation, these two agents are actually activating AR instead of inhibiting it. The antiandrogen withdrawal syndrome (AAWS) is defined as a further significant (>50%) reduction in PSA values after the discontinuation of antiandrogen therapy, which can be explained by AR mutations shifting the antiandrogen activity from antagonist to agonist. Several studies have reported AAWS after discontinuation of abiraterone or enzalutamide [110—112]. The second one is the L701H mutation that results in activation of the AR by glucocorticoids such as prednisone [113]. As mentioned above, since prednisone is recommended to be given with abiraterone, this mutation may contribute to the resistance to abiraterone. The third one is the mutation of T877A and T878A that causes AR activation by progesterone [91,113]. Since serum level of progesterone is increased during treatment with abiraterone, this mutation may play a role in abiraterone resistance [114]. Recently, Liu et al. [115] performed molecular dynamics simulations to generate an ensemble view of the dynamic properties and binding mechanism of enzalutamide with wild type (WT)/mutant ARs. They found that helix 12 (H12), which lies on the top of the AR LBD like a cover, plays an important role for the function of enzalutamide. Enzalutamide will act as an AR antagonist when its C-ring locates near to H12, however, it will become an agonist when the C-ring is near to helix 11 or the Loop 11—12.

3.1.1.4. AR splice variants (AR-Vs). Many alternatively spliced AR-Vs lack the C-terminal LBD, but retain the transactivating N-terminal domain, leading to constitutive activation in the absence of ligands [116]. AR-V7 and AR-V567 are the most common variants found in CRPC patients. The expression level of AR-V7 is higher in mCPRC patients (15%) compared to hormone naïve ones, and it can increase under the use of either abiraterone (55%) or enzalutamide (50%), which implies that both primary and acquired resistance to these agents could be associated with AR-V7 [117—119]. Antonarakis et al. [120] first reported the analysis of AR-V7 with a circulating tumor cell (CTC) assay to predict the response of abiraterone or enzalutamide. The results demonstrated a significantly worse response and treatment outcome in patients who harbored AR-V7 in their CTCs. Later, it was shown that it is feasible to detect AR-V7 mRNA transcript in whole blood without the CTC enrichment step, and AR-V7 protein level by immunohistochemistry (IHC) in patient biopsy samples and CTCs [121—123]. Positive AR-V7 status or high expression level was associated with worse treatment outcomes in all these studies. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling has been implicated in mediating resistance to enzalutamide by increasing AR-Vs expression in PCa cells [124,125]. Downregulation of NF-κB/p52 can regain the sensitivity to enzalutamide. A recent study identified AR-V-preferential binding sites (ARVPBS) and a series of genes preferentially transactivated by AR-Vs in CRPC cells by integrated chromatin immunoprecipitation coupled sequencing (ChIP-seq) and RNA sequencing (RNA-seq) analysis [126]. They found ARVPBS exclusively overlapped with AR binding sites in castration-resistant tumors in patients and AR-V preferentially-activated genes were upregulated in abiraterone resistant patient specimens. It still remains unclear if AR-Vs directly contribute to resistance or simply are an indicator of a tumor under stress as it tries to evolve around androgen inhibitors.

3.1.2. AR bypass mechanisms

The glucocorticoid receptor (GR) has been identified as a crucial mechanism to bypass AR blockade by abiraterone or enzalutamide [127,128]. Because of the shared response elements with AR in multiple gene targets, GR is able to drive expression of those genes independent of AR. Arora et al. [127] found that GR expression was upregulated in 30% of patient tumors after 8-week

enzalutamide treatment compared to only 10% before therapy, and higher levels of the GR protein were associated with worse outcomes. A recent study also showed that GR is significantly increased upon long-term abiraterone or enzalutamide treatment in the majority of preclinical models, and patients with high GR experience shortened progression-free survival [129]. As glucocorticoids (like prednisone) are currently widely used in combination with docetaxel, cabazitaxel, and abiraterone to treat mCRPC patients, simultaneous application of GR antagonists is being studied. Similarly, the progesterone receptor (PR) and AR have 88% sequence homology in the LBD and share response elements in multiple gene targets [130,131]. Since both serum levels of progesterone and expression of PR are increased in mCRPC patients, and it has been shown that high PR expression in PCa cells is an independent poor prognostic factor, PR has been identified as another potential mechanism to bypass AR blockade by abiraterone or enzalutamide.

3.1.3. AR independent mechanisms

Neuroendocrine PCa (NEPC) is increasingly recognized as a subset of CRPC that demonstrates resistance to both abiraterone and enzalutamide by shedding its dependence on the AR pathway and acquires histological features of neuroendocrine differentiation (NED) [132—134]. In newly diagnosed PCa, the percentage of NEPC is <2%. However, 20%—25% of patients with mCRPC treated with next generation hormonal therapy relapse with tumor cells that have at least partial features of NED [135]. NEPC is recognized by the expression of biomarkers such as chromogranin A (CHGA), neuron-specific enolase (NSE) and synaptophysin (SYP), while not expressing luminal prostate differentiation markers such as PSA [136—138]. Because of this, NEPC is often suspected in patients with increasing disease burden despite low or moderately rising PSA levels. Recently, Wang et al. [139] observed high levels of NSE and CHGA in enzalutamide-resistant xenografts. The study demonstrated a positive feedback loop between NED in CRPC and tumor-associated macrophages (TAMs). Enzalutamide can elevate high mobility group box 1 (HMGB1) levels and enzalutamide-induced HMGB1 expression can facilitate TAM recruitment and polarization and drive NED via β-catenin stabilization. Interleukin-6 (IL-6) secreted by HMGB1 can augment NED and directly promote HMGB1 transcription by STAT3.

Several pathways other than AR signaling have been suggested to play a role in abiraterone or enzalutamide resistance. Many studies have confirmed that the PI3K-AKT-mTOR signaling pathway is strongly related to PCa progression [140,141]. AR and PI3K/AKT pathways are involved in reciprocal feedback regulation, which means inhibition of one pathway will activate the other, providing a potential mechanism of resistance to AR inhibitors [141]. Both preclinical and early clinical data demonstrated that the combination use of AKT inhibition and abiraterone/prednisone may improve the treatment outcome, particularly in those with PTEN loss [141,142]. Wang et al. [143] recently published whole-exome sequencing and RNA-seq data from metastatic lesions before initiating abiraterone/prednisone in mCRPC patients. They found that genes in the Wnt/β-catenin pathway were more frequently mutated in non-responders and mRNA expression of cell cycle regulatory genes was increased in non-responders. Interestingly, Wnt/β-catenin signaling and therapeutic resistance have also been reported in many other cancer types. Another recent study by Pal et al. [144] identified the transforming growth factor β (TGFβ) and cyclin D1 (CCND1) signaling pathways as significantly upregulated in drug resistant CTCs in mCRPC patients who received abiraterone or enzalutamide.

3.2. Chemoresistance

Chemoresistance to the taxanes still remains an area that is poorly understood and underexploited in the treatment of PCa.

3.2.1. Multi drug resistance transporters

Docetaxel and cabazitazel binding to free tubulin takes place in the cytoplasm and adequate intracellular concentrations are important to stabilize the microtubules [57]. Membrane proteins transport docetaxel from extra to intracellular, and *vice versa*. Downregulation of influx transporter activity or upregulation of efflux transporters can play a crucial role in taxane efficacy.

3.2.1.1. Efflux transporters. Multidrug resistance (MDR) transporters of the adenosine triphosphate binding cassette (ABC) family drive chemoresistance. These transporter proteins can promote the efflux of multiple drugs, *e.g.* docetaxel, causing intracellular concentrations and subsequent efficacy to decrease. Higher expression of ABC transporters is related to disease progression [145—148]. P-glycoprotein (P-gp) is a well-known MDR protein and is encoded by the *mdr1/ABCB1* gene. Docetaxel is a known ligand for the P-gp and this knowledge aided the development of cabazitaxel that has a lower affinity for P-gp. P-gp itself has gained interest as a potential therapeutic target [149]. Preclinical data suggest that ABCB1 expression might play a key role in cross resistance of cabazitaxel after docetaxel and inhibition of ABCB1 resensitizes cell lines to cabazitaxel [150].

3.2.1.2. Influx transporters. The organic anion-transporting polypeptide (OATP) is an *SLCO*-encoded membrane protein, and that can transport androgen and medication [151]. In PCa, *SLCO* genes are highly expressed and genetic variants (*SLCO1B3* and *SLCO2B1*) are associated with worse outcomes [152]. de Morree et al. [153] determined intracellular concentrations of docetaxel and cabazitaxel in patient-derived xenograft mouse models to further evaluate the role of *SLCO* in mCRPC. *SLCO1B3* was significantly downregulated in a docetaxel-resistant tumor, while overexpression was related to higher intracellular concentrations, suggesting that loss of *SLCO1B3* may drive drug resistance through decreased influx.

3.2.2. Apoptotic escape

The cytotoxic effect of taxanes relies on the cell's response to mitotic arrest and proceeding to apoptosis [56].

However, if the cell survives an oncogenic effect may occur. The BCL-2 family consists of pro- and anti-apoptotic proteins. Bcl-2 is an inhibitor of apoptosis. Expression of this mitochondrial membrane protein is associated with cell survival [154]. Yoshino and others [155—157] discussed how upregulation of the anti-apoptotic Bcl-2 protein may predict poor outcomes of PCa and may predict response to taxanes. Additionally, phenotypic variants can prevent activation of the apoptotic pathway. For example, altered forms of the binding site can counter the effect of docetaxel on the tubules. The microtubules are not stabilized and mitotic arrest followed by apoptosis will not occur. High expression of the tubulin isomer βIII is correlated with impaired docetaxel-binding and worse outcomes in patients on docetaxel therapy [155,158,159].

3.3. Resistance against other therapies

Overall, resistance to PCa therapies remains poorly understood. Since data suggest that prior therapies may affect the response to secondary treatment, *i.e.*, cross resistance, the field needs to better understand how the order and duration of each therapeutic affects downstream treatment [17,160]. In addition, there is an increasing appreciation that the tumor microenvironment plays a critical role in how a tumor responds to a therapeutic attack [161].

3.3.1. Mechanisms of resistance to immune checkpoint inhibitors

The past few years has witnessed rapid clinical progress in the field of immune checkpoint inhibitors. Blocking the cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and/or the programmed cell death ligand 1 (PD-1/PDL-1) pathway using monoclonal antibodies has resulted in unprecedented rates of long-lasting treatment responses in patients with different cancer types by releasing negative regulators of immune activation that limit antitumor responses [162—164]. Several early clinical trials demonstrated that mCRPC seemed to have an intrinsic resistance to immune checkpoint blockade [165,166]. Recently, trials applying checkpoint inhibitors in selected mCRPC patients or in combination with other drugs have started to show favorable results in survival improvement [77,78].

A recent review summarized three main mechanisms of resistance to immune checkpoint inhibitors: 1) Insufficient generation of antitumor T-cells (lack of sufficient or suitable neo-antigens, impaired processing or presentation of tumor antigens and impaired intratumoral immune infiltration), 2) inadequate function of tumor-specific T cells (impaired interferon γ signaling, metabolic/inflammatory mediators, immune suppressive cells and alternate immune checkpoints), and 3) impaired formation of T-cell memory (severe T-cell exhaustion and T-cell epigenetic changes) [167].

Many studies have found that the intratumoral T-cell infiltration of PCa is generally low, which may be one of the intrinsic resistance mechanisms [168]. Lack of suitable neo-antigens and alterations in antigen processing and/or presentation is associated with impaired antitumor immune response. Mutational burden is a tumor-intrinsic feature correlated with antitumor immune response. Tumor types with high mutation burdens, such as melanoma, lung cancer and urothelial cancer, are among those with highest response rates to checkpoint inhibitors [169,170]. For the same reason, DNA-MMR deficiency leading to microsatellite instability (MSI) is associated with enhanced response to PD-1 blockade [171]. Unfortunately, MMR deficiency is present in only 2%—12% of mCRPC patients [74—76].

Myeloid-derived suppressor cells (MDSCs) are known to play important roles in tumor immune evasion [172]. It has been shown that the abundance of circulating MDSCs correlates with PSA levels and metastasis in PCa patients [173,174]. Last year, Lu et al [175], demonstrated that robust synergistic responses could be achieved in mCPRC mouse models when immune checkpoint blockade (anti-CTLA4 and anti-PD1) was combined with MDSC-targeted therapy (cabozantinib and BEZ235), while the single use of either one of them engendered only modest efficacy.

3.3.2. Cross resistance

According to the approved drug list (Table 1) it seems that even if an mCRPC patient suffers disease recurrence during or after first-line treatment, he will still have many options. However, it is still unknown if the deployment of subsequent treatments may be negatively affected by initial treatment choice. It has been reported that cross resistance exists between abiraterone and enzalutamide. This means that if one is used as first-line treatment and fails, then the other's efficacy will largely be decreased in second line [176,177]. The rate of response to abiraterone after treatment with enzalutamide is less than 10%, whereas the response rate for enzalutamide after abiraterone is 15%—30%. Though few studies have proved underlying mechanisms of this cross resistance, AR mutation and AR-Vs acquired during the first-line treatment may contribute to the resistance of second agent.

Both abiraterone and enzalutamide have a cross resistance effect on docetaxel [178]. However, interestingly, though both docetaxel and cabazitaxel are taxanes, cabazitaxel seems to retain its antitumor activity in mCRPC progressing after docetaxel, abiraterone or enzalutamide [179]. Preclinical evidence has also shown impaired efficacy of docetaxel in abiraterone- and enzalutamide-resistant cell lines. The potential mechanism is that the taxanes inhibit tubulin-dependent AR nuclear translocation [180]. A recent preclinical study used docetaxel-resistant cell lines to test response to cabazitaxel [150]. They found that docetaxel resistance conferred cross-resistance to cabazitaxel via increased expression of ABCB1, a drug efflux pump. Inhibition of ABCB1 function by the small molecule inhibitor can resensitize taxane-resistant cells to cabazitaxel treatment.

4. Summary and strategies to overcome the resistance

Currently, the diverse heterogeneity of CRPC still results in lethal disease. Though PCa becomes resistant to every known therapy, many new agents and strategies are under investigation. For example, seviteronel (VT-464) is a non-steroidal 17, 20-lyase inhibitor that has higher selectivity for the inhibition of 17, 20-lyase over hydroxylase. Compared to abiraterone, the interference with

corticosteroid production is reduced [181]. Darolutamide (ODM-201) is an AR antagonist with higher affinity to the AR than enzalutamide or apalutamide. It can inhibit mutated AR (AR F877L, H875Y/T878A, and F877/T878A mutants) associated with enzalutamide resistance [182]. EPI-506 (ralaniten acetate) is a first-in-class small molecule transcription inhibitor of the AR N-terminal domain that just passed phase I and phase I-II trial last year [183]. Preclinical studies have demonstrated its activity against both full length and resistance-related AR species, including AR-V7. BRD4 is a bromodomain and extraterminal (BET) family protein that is a critical AR coregulator. Preclinical studies have demonstrated that BRD4 expression is associated with patient outcome and BET inhibitors can reduce AR splicing and AR-V7 expression by regulating alternative splicing, abrogating AR signaling and inhibiting growth of CRPC patient derived models [184,185]. Bipolar androgen therapy (BAT) is based on the hypothesis that a low testosterone environment and AR overexpression will induce vulnerability of CRPC cells to supraphysiological levels of androgens that can inhibit growth and promote cell death [186,187]. Testosterone injections and concurrent ADT are used to obtain rapid cycling between extremes of high and low levels of testosterone. A phase III study applying BAT and enzalutamide to mCRPC patients is ongoing. The frequency of germline DNA repair alterations in unselected men with advanced PCa is about 12%. The poly (adenosine diphosphate ribose) polymerase (PARP) inhibitor olaparib (AZD-2281) has been shown to offer high response rates in patients who no longer responded to standard treatments for mCRPC and have defects in DNA repair genes [188—190]. Immune checkpoint inhibitors are in multiple trials now, especially among mCRPC patients with DNA MMR deficiency and/or MSI-high. Prostate specific membrane antigen (PSMA) is a transmembrane glycoprotein overexpressed in PCas. The degree of PSMA expression positively correlates with tumor stage and is significantly increased in mCRPC. Lutetium-177 [^{177}Lu]-PSMA-617 (LuPSMA), is a small molecule inhibitor that binds with high affinity to PSMA. The short-range 1 mm path length of the β-particle emitted by ^{177}Lu enables effective delivery of radiation to the cancer while minimizing damage to surrounding normal tissues. Several clinical trials have demonstrated that this novel therapy is well tolerated and effective with 45%—57% of patients achieving a PSA decline of 50% or more [191—193].

Besides innovating new therapeutics, exploring the combination and sequential use of existing agents is also of great importance. Many promising clinical trials are ongoing, which aim to compare two agents head-to-head or identify a certain combination or sequence of agents. For example, there are clinical trials investigating the efficacy of enzalutamide with or without abiraterone and prednisone (NCT01949337) and enzalutamide with or without atezolizumab (NCT03016312) in mCRPC patients. In CRPC patients with bone metastasis, enzalutamide with or without radium-223 (NCT02194842) and abiraterone with or without radium-223 (NCT02043678) are being tested [160,194]. A consensus on mCRPC drug sequencing was recently made by 61 multidisciplinary cancer physicians and scientists from 21 countries [195]. Fig. 1 demonstrates an example of sequenced therapies for a patient with CRPC in 2018.

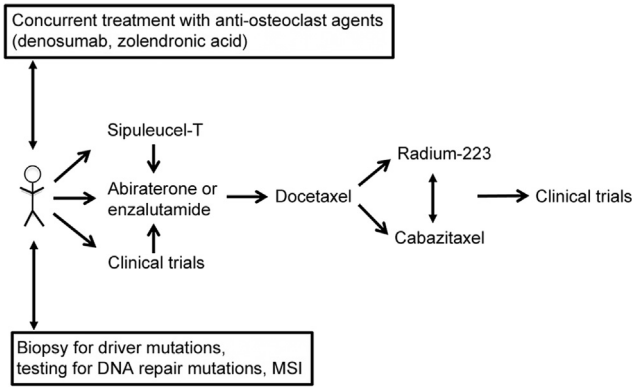

Figure 1 A potential sequence of therapies for mCRPC patients in 2018. In mCRPC, the first-line treatment options include: Abiraterone, enzalutamide, sipuleucel-T or docetaxel. Additional options include: Bone anti-resorptive therapy with denosumab or zolendronic acid, and immunotherapy with pembroluzimab after DNA sequencing for DNA-MMR and MSI. If symptomatic bone metastases without organ involvement are present, radium-223 can be added. Cabazitaxel can be chosen as a second-line chemotherapy. MMR, mismatch repair; MSI, microsatellite instability; mCRPC, metastatic castration-resistant prostate cancer.

Author's contribution

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Conflicts of interest

The authors declare no conflict of interest.

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