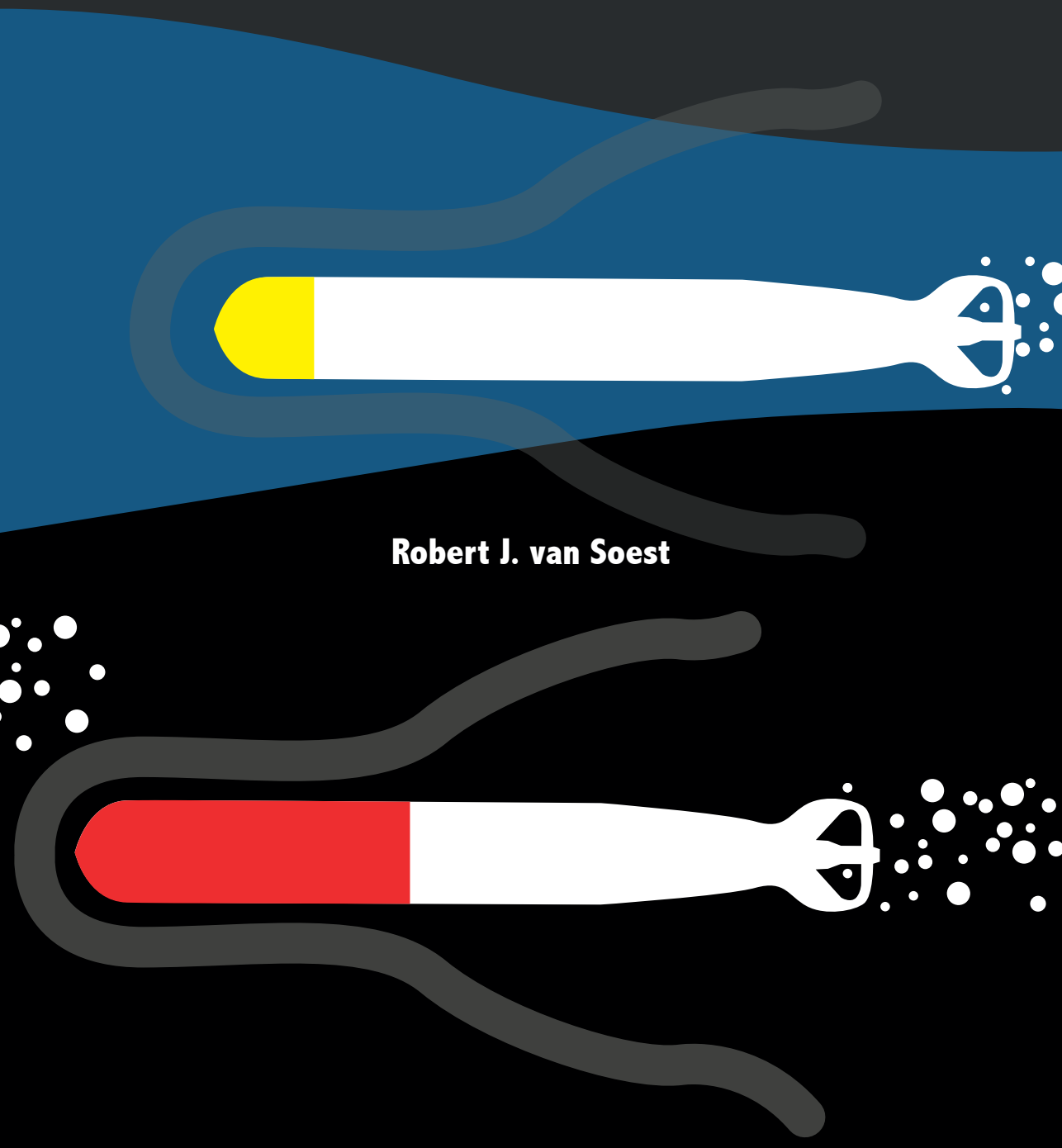
Taxanes and Novel Androgen Receptor Targeted Agents in the Management of Metastatic Castration-Resistant Prostate Cancer



Robert J. van Soest

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TAXANES AND NOVEL ANDROGEN RECEPTOR TARGETED AGENTS IN THE MANAGEMENT OF METASTATIC CASTRATION-RESISTANT PROSTATE CANCER

Taxanen en nieuwe androgeen receptor gerichte middelen in de behandeling van gemetastaseerd castratie-resistent prostaatcarcinoom

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Robert J. van Soest, Ellen S. de Morée, Cora N. Sternberg and Ronald de Wit.

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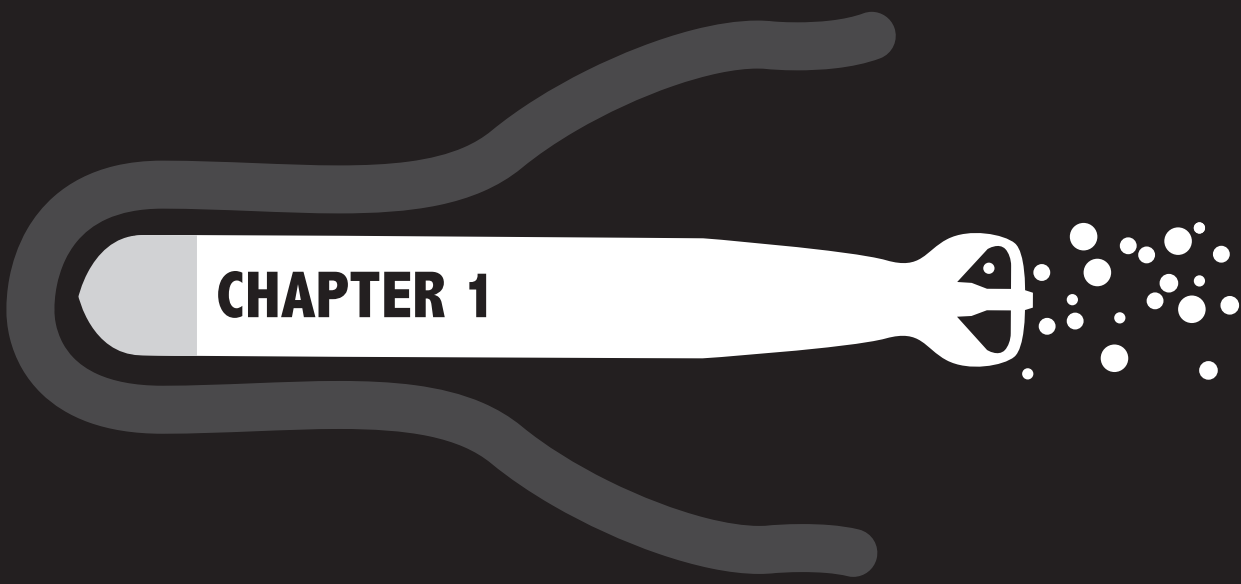

INTRODUCTION

Urologists have traditionally been the primary caregivers for patients with prostate cancer and patients were referred to medical oncologists only in very late stages. With the advent of docetaxel chemotherapy, this pattern has drastically changed. This has led to better cooperation among physicians and important phase III studies which have shown a survival advantage not only with docetaxel, but also with cabazitaxel chemotherapy, novel hormonal therapies, immunotherapy and novel radiation therapy. Oncologists are highly skilled in administering chemotherapy. With more than two decades of experience with taxanes in a variety of solid tumors, in-depth knowledge and understanding of potential drug-drug interactions, dose modifications, strategies for dealing with patients with medical comorbidities and toxicities has been attained. Since most patients remain with their medical oncologist during the later stages of their disease, post-docetaxel registrations of abiraterone and enzalutamide have in most cases been spearheaded by medical oncologists, with similar arguments about potential drug- drug interactions and handling of toxicities. Oncologists have primarily though not exclusively been involved in the drug development of novel hormonal therapies in the post-docetaxel setting. As these hormonal agents become more widely used prior to chemotherapy, both urologists and medical oncologists will most likely be more intimately involved in their administration.
The right treatment sequence and the most optimal choice for an individual patient still requires further research and development. In this chapter, we have eluded to various predictive factors for benefit with abiraterone and with docetaxel that may impact the treatment choice. In addition, there is an increasing concern about the effectiveness of taxanes post- new generation AR inhibiting drugs. Whoever treats patients with castration-resistant prostate cancer should be encouraged to evaluate their patients in a multidisciplinary team approach.

MITOXANTRONE

In 1996 Tannock et al. reported on a phase III study involving 161 patients with metastatic castration resistant disease who were randomized to mitoxantrone 12mg/m^2 every 3-weeks plus prednisone or prednisone alone [1]. Pain response was the primary endpoint and this was achieved in 29% of patients treated with mitoxantrone, compared to 12% of patients treated with prednisone (p=0.01). Despite superior pain response rates, mitoxantrone did not impact overall survival (OS) which was 12 months in both treatment arms, p=0.27). A trial comparing mitoxantrone plus hydrocortisone versus hydrocortisone alone was conducted by the Cancer and Leukemia Group B (CALBG) to evaluate OS. No survival benefit was observed in this study, although there was a small but significant increase in time to disease progression in the mitoxantrone arm [2]. Based upon these results, the Food and Drug Administration (FDA) approved mitoxantrone as palliative

chemotherapy in patients with castration-resistant prostate cancer. Consequently, mitoxantrone became the control arm in the two pivotal phase III trials investigating docetaxel in patients with mCRPC. Anthracyclines and more specifically mitoxantrone were the standard for cytotoxic chemotherapy until the introduction of docetaxel in 2004 and treatment of men with metastatic castration-resistant prostate cancer (mCRPC) was primarily driven by symptom palliation.

DOCETAXEL

Microtubules are the main target of taxanes, which bind to a specific binding site on the tubulin β-subunit. Taxanes suppress microtubule dynamics by promoting tubulin assembly and stabilizing microtubules, blocking mitosis at the metaphase/anaphase transition, which results in cell death [3-5]. It has been recently shown that AR transport is facilitated by microtubules and the motor protein dynein. By interfering with microtubules, taxanes also inhibit AR nuclear transport, a known mechanism of antitumor activity in mCRPC [6-8] (Figure 1).

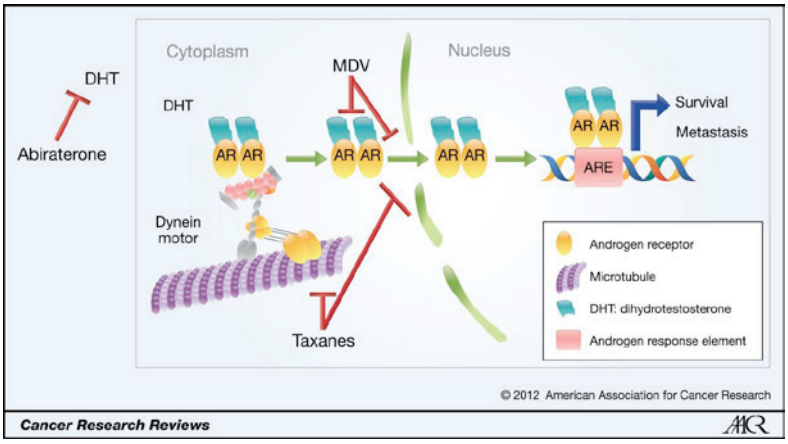

Figure 1. Adapted from Thadani-Mulero M et al. Cancer Res 2012;72:4611-4615 (with permission)
Proposed model of taxane mechanism of action in prostate cancer. AR associates with microtubules and translocates to the nucleus via the motor protein dynein. Taxanes inhibit depolymerzation of microtubules and block microtubules dynamics. By interfering with microtubule dynamics, taxanes cause a cell cycle arrest in the G2/M phase, and inhibit AR nuclear translocation as an additional mechanism of action in mCRPC.
The mechanisms of action of enzalutamide (MDV3100) and abiraterone are also shown. Enzalutamide exerts its effect by inhibiting AR nuclear translocation, DNA-binding and co-activator recruitment. Abiraterone inhibits androgen biosynthesis by irreversibly blocking CYP17A1, a crucial enzyme in steroidogenesis.

Following phase I/II studies yielding PSA responses, pain responses, and objective tumor responses for docetaxel [9, 10], two large phase III trials TAX327 and SWOG 99-16 were initiated [11, 12]. TAX327 was conducted in 1006 men with mCRPC who were randomized to receive 3-weekly docetaxel (75 mg/m^2), weekly docetaxel (30 mg/m^2) or 3-weekly mitoxantrone (12 mg/m^2), each with prednisone [11]. OS of patients who were treated with docetaxel in the 3-weekly regime was superior as compared to mitoxantrone with an OS of 19.2 vs 16.3 months (HR 0.79, 95%CI 0.67-0.93) in the final analysis [13]. The docetaxel 3-weekly arm also showed better palliation, with more patients having pain (35% vs. 22%, p=0.01) and quality of life responses (22% vs 13%, p=0.009) as compared to mitoxantrone . The docetaxel weekly schedule showed a trend towards improved OS, but did not reach statistical significance. The TAX327 updated survival analysis also contained a post-hoc analysis which demonstrated that the trends in OS were consistent among several subgroups of patients based on age (<68 vs. ≥68 years), pain vs. no pain at baseline, and baseline PSA <115 vs. ≥115 ng/ml.

Neutropenia was the most common observed grade 3 / 4 toxicity and occurred more frequently in patients receiving 3-weekly docetaxel (32%). Despite the high incidence of neutropenia, febrile neutropenia was rare (3%) and other grade 3 / 4 toxicities all occurred in less than 5%.

A second trial, SWOG 99-16 was designed on the assumption that the combination of docetaxel and estramustine had the greatest therapeutic potential. Seven-hundred seventy patients were randomized to receive 280 mg estramustine three times daily on days 1-5, plus docetaxel 60 mg/m^2 on day 2, preceded by 60 mg of dexamethasone divided in three doses, or mitoxantrone 12 mg/m^2 on day 1 plus 5 mg of prednisone twice daily [12]. Both were given in a 21-day cycle, and dose escalation to docetaxel 70 mg/m^2 or mitoxantrone 14 mg/m^2 was allowed in cycle 2 if no grade 3/4 toxicities were observed during the first cycle. Median OS was superior in the group receiving docetaxel as compared to mitoxantrone (17.5 vs. 15.6 months respectively), with an HR of 0.80 (95% CI 0.67-0.97). The group treated with docetaxel and estramustine had significantly higher rates of grade 3 and 4 neutropenic fever (5 percent vs. 2 percent), cardiovascular events (15 percent vs. 7 percent), and nausea and vomiting (20 percent vs. 5 percent), as compared with the group treated with mitoxantrone and prednisone.

Taken together, the results of these two phase III studies showed that docetaxel in a 3-weekly regimen improved OS, which was the primary end point of both trials. Weekly docetaxel did not appear to be better tolerated than the 3-weekly regimen, and showed only a trend towards better efficacy. The SWOG study did not reveal greater benefit by the addition of estramustine. Because of the lack of superior activity and greater toxicity by the addition of estramustine, docetaxel every 3 weeks plus low-dose prednisone subsequently became the standard of care for patients with mCRPC [14].

In multivariate analysis of TAX327, a total of ten independent prognostic factors for survival were identified including the presence of liver metastases, number of metastatic sites, clinically significant pain, Karnofsky performance status, type of progression, pretreatment PSA doubling time, baseline PSA , tumor grade , baseline alkaline phosphatase, and baseline hemoglobin [15]. These prognostic factors have been elaborated into a nomogram (Figure 2). Such decision making tools are informative, can facilitate tailoring of therapy, and can simplify important clinical decisions such as when to start cytotoxic chemotherapy. Although the survival benefit obtained by docetaxel compared with mitoxantrone is consistent among patients with and without pain at baseline (HR 0.73 and 0.85 respectively), there is a substantial difference in OS time (14.4 months for patients with pain vs. 21.3 months for patients without pain). However, this does not necessarily imply benefit from the early use of chemotherapy, but may rather guide treatment in asymptomatic patients by defining patients at greater risk of imminent disease progression and death. These patients may be candidates for chemotherapy, even in the absence of symptoms.

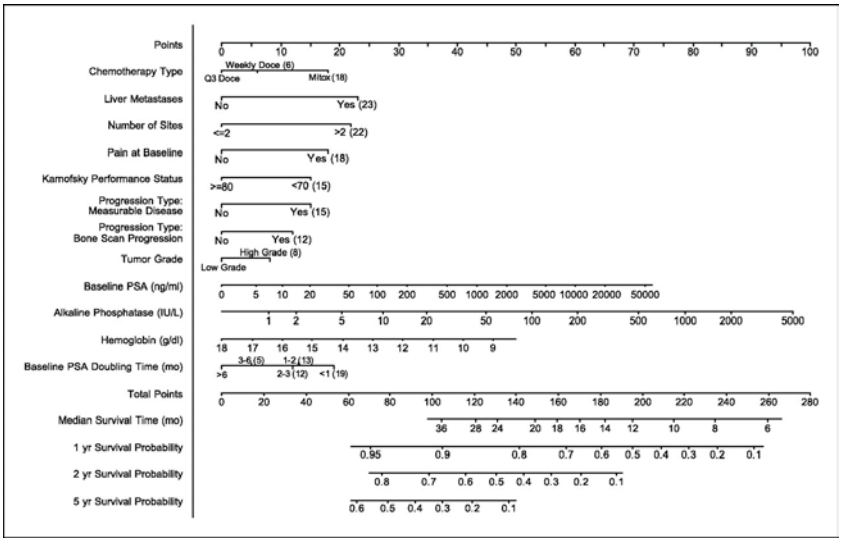

Figure 2. Adapted from Armstrong A J et al. Clin Cancer Res 2007;13:6396-6403 (with permission)
Nomogram for survival of patients with progressive mCRPC, including data derived from 686 patients and 518 mortality events. Note: a present pain intensity of ≥2 and/or an analgesic score of ≥10 were defined in the original protocol as indicative of the presence of significant pain. Instructions for physician: Locate the liver metastasis axis. Draw a straight line upward to the points axis to determine how many points toward survival the patient receives for the presence or absence of liver metastases. Repeat this process for each predictor variable and sum the points for each predictor. Locate this sum on the total points axis. Draw a straight line downward from the total points axis to identify the predicted median survival and the predicted 1-, 2-, and 5-years predicted overall survival probabilities. Instructions to patient: "Mr. X, if we had 100 men exactly like you, we would expect <nomogram prediction × 100> to be alive in 1, 2, and 5 y, respectively, and we expect 50 of those to be alive after <median survival prediction> months."

Nonetheless, in the TAX327 study a decrease in quality of life was more often observed in patients with minimal symptoms at the start of chemotherapy [16]. Therefore delaying chemotherapy may be a suitable approach in patients with minimal symptoms. Those patients who have no symptoms yet, but are more likely to develop symptoms in the near future due to bone scan progression and/or the development of anemia should be considered candidates for docetaxel chemotherapy [14].

With the recent FDA and EMA approval of the CYP17 inhibiting agent abiraterone in the pre-docetaxel setting, it has become increasingly important to identify subgroups of patients who may have greater benefit by the use of chemotherapy in order to better tailor treatment choices. Recently, Azria et al. reported a high Gleason score [8-10] at the time of diagnosis to be an independent risk factor for poor response to abiraterone [17, 18]. In addition, a retrospective analysis of patients with mCRPC enrolled in clinical trials demonstrated that patients who had a short response to prior androgen deprivation therapy (ADT) (<16 months), had poor PSA responses and PFS when treated with secondary hormonal therapies such as abiraterone, and enzalutamide [19]. In this light, a recent post-hoc analysis of the TAX327 study was conducted which revealed that the survival benefit obtained with docetaxel as compared to mitoxantrone was most pronounced in patients with high Gleason score tumors (Gleason 7-10) [20]. Furthermore, two prospective databases of patients with mCRPC demonstrated similar PSA responses and clinical benefit obtained by docetaxel, irrespective of the duration of response to ADT [21].

In an era of shifting paradigms in mCRPC with abiraterone becoming available also prior to docetaxel chemotherapy, Gleason score and prior response to ADT may serve to discriminate between patients who benefit most from docetaxel chemotherapy as first-line treatment. Docetaxel, seems to exert efficacy particularly in high Gleason score tumors irrespective of response to ADT. In contrast, in patients with better differentiated tumors and durable responses to ADT abiraterone might be a good treatment option. In the future, these observations should be prospectively validated in order to further personalize first-line treatment options for patients with mCRPC.

Mechanism of action of taxanes: emerging data on AR transport as part of their efficacy

As mentioned above, docetaxel and cabazitaxel also impair AR signaling, which in the setting of mCRPC might in fact be responsible for part of the therapeutic efficacy [6-8]. Recently, clinical and preclinical evidence is emerging about potential cross-resistance between docetaxel and abiraterone [8]. A clinical report on patients treated with docetaxel who had previously been treated with abiraterone showed an OS of only 12.5 months, which was significantly less than the 19 months predicted for this patient population [22]. Moreover, PSA declines ≥50% were observed in 26% of patients, compared to 45% in the

TAX327 study [11], and no responses to docetaxel were observed in abiraterone-refractory patients. A likely explanation is that antitumor activity of taxanes in mCRPC is partly depending on its impact on AR-signaling. When patients are treated with abiraterone first, it could very well result in impaired effectiveness of docetaxel due to annulling its effects on the AR. Hence the sequence of abiraterone followed by docetaxel upon progression could result in decreased effectiveness of the chemotherapy and thus impair the eventual clinical benefit.

Cabazitaxel however seems to retain activity in the third-line setting following docetaxel and abiraterone, with ≥50% PSA declines in 42-49% of patients [23, 24]. Prospective clinical studies should further define the implications for the optimal treatment sequence of these treatment options for patients with mCRPC.

Docetaxel retreatment

Sooner or later all patients will progress during or after treatment with docetaxel. Patients who relapse after an initial response to docetaxel may again respond to a second or even third series of docetaxel cycles [25-27]. Since the phase II data on docetaxel rechallenge have been limited to efficacy , i.e. PSA responses, pain responses and objective responses and data on survival benefit are lacking, such rechallenge has become a less likely choice following the introduction of the new agents such as cabazitaxel, abiraterone and enzalutamide , which have all demonstrated survival benefit in patients relapsing after docetaxel chemotherapy [28-30].

An alternative approach to the standard of 10-12 cycles docetaxel as used in the pivotal phase III studies, is intermittent dosing of docetaxel suspending treatment after 6 cycles or at a predefined PSA decrease, and retreatment when PSA starts to rise again. In one of the larger studies a majority of patients responded again to such retreatment [31]. These data are of particular interest because of the absence of a defined optimal duration of chemotherapy in responding cases with mCRPC [26, 32]. In a prospective phase II study, patients were enrolled who had responded to first-line docetaxel and progressed after a chemotherapy-free interval of at least 5 months. Median overall survival since enrollment was 13 months, and a 50% PSA decline was observed in 24.5% of patients [25]. Like for docetaxel retreatment, OS data for intermittent docetaxel therapy are also lacking, and a second or series of docetaxel has become questionable due to the newly available systemic treatment options.

Docetaxel-based combination therapies

In the light of improved survival and modest toxicity with docetaxel as was demonstrated in TAX 327, numerous investigators, collaborative groups and industry have investigated whether the efficacy of docetaxel could be improved by adding a second agent [33]. Here we will discuss docetaxel combination studies. An overview of the phase III combination trials with docetaxel is shown in Table 1.

PHASE III TRIALS

Immunotherapy

The GVAX platform of immunotherapies involved injection of cells derived from prostate cancer cell lines to provoke an immune response to multiple antigens expressed by the tumor cell. In addition, the cells were modified to secrete granulocyte macrophage colony-stimulating factor (GM-CSF). The VITAL-2 trial compared GVAX plus 3-weekly docetaxel with docetaxel plus prednisone and was interrupted early due to an unexpected higher death rate in the GVAX arm (67 deaths for GVAX plus docetaxel vs. 47 deaths for docetaxel plus prednisone) [34]. Another trial (VITAL-1), compared GVAX with docetaxel in patients with asymptomatic CRPC [35]. The study was prematurely terminated based on the results of a futility analysis conducted by the study's Independent Data Monitoring Committee (IDMC) which determined that the study had less than a 30% chance of meeting its predefined primary endpoint of improvement in overall survival.

Calcitriol

Calcitriol is an activated vitamin D analog that has shown to enhance antitumor activity of paclitaxel and docetaxel in vitro and in vivo [36, 37]. ASCENT-1 was a double-blind randomized phase II study that investigated weekly docetaxel plus high-dose calcitriol versus docetaxel plus placebo [38]. The primary end-point PSA response rate did not differ between the treatment groups. Although it was not the primary end-point of the trial, there was an improvement in OS for calcitriol over the placebo group. The ASCENT-2 trial was a randomized phase III trial designed to validate the observed survival benefit obtained with docetaxel plus calcitriol in the ASCENT trial [39]. In the phase III trial the control arm comprised the standard docetaxel regimen every 3 weeks. At an interim analysis, more deaths were noted in the ASCENT arm and consequently the trial was terminated early. Median OS was 17.8 months (95% CI 16.0 to 19.5) for docetaxel plus calcitriol compared to 20.2 months (95% CI 18.8 to 23.0) for docetaxel plus prednisone. Reasons for the worse OS by docetaxel plus calcitriol arm may have been attributed to the use of the weekly docetaxel schedule in the investigational arm [39].

Endothelin-A receptor antagonists

Atrasentan is an endothelin-A receptor antagonist that enhanced the effects of docetaxel against prostate cancer cells in vitro and in vivo [40, 41]. In the SWOG S0421 trial atrasentan plus docetaxel and prednisone was investigated in 991 patients with bone metastases. No difference in OS and PFS was observed for atrasentan plus docetaxel and prednisone compared with docetaxel and prednisone alone [42].

Another endothelin A receptor antagonist zibotentan was investigated in the phase III trial ENTHUSE M1C combined with standard docetaxel versus docetaxel plus placebo. Docetaxel plus zibotentan did not result in a significant improvement in OS compared with docetaxel plus placebo (HR 1.00, 95%CI 0.84 – 1.18) [43].

Angiogenesis inhibitors

The oral angiogenesis inhibitor thalidomide demonstrated additive effects to taxane chemotherapy in vitro [44]. In two randomized phase II trials, the addition of thalidomide to docetaxel resulted in an encouraging PSA decline rates. Although more tromboembolic events were observed in patients treated with thalidomide, the combination regimen was reported to be well tolerated after the administration of prophylactic low-molecular-weight heparin [45, 46]. Lenalidomide is the successor of thalidomide with greater anti-angiogenesis efficacy as well as imunomodulatory effects. The randomized phase III trial (MAINSAIL) evaluated the efficacy of lenalidomide plus docetaxel versus docetaxel and placebo as first-line treatment for mCRPC. Following an interim analysis the study was stopped due to greater toxicity in the investigational arm and possibly reduced effectiveness. This could have been due to more frequent docetaxel dose reductions in patients allocated to lenalidomide [47].

Bevacizumab is a humanized immunoglobulin G monoclonal antibody to all the isoforms of VEGF-A. The CALBG Group investigated the addition of bevacizumab to standard docetaxel and prednisone in a randomized phase III trial. Despite an improvement in PFS and objective response, the addition of bevacizumab to docetaxel and prednisone did not improve OS and was associated with greater toxicity [48].

Aflibercept, a recombinant human fusion protein that binds A and B isoforms of VEGF and placental growth factor thereby inhibiting angiogenesis, was investigated in the phase III VENICE trial. In this study 1224 men were treated with docetaxel plus prednisone and randomized to receive aflibercept or placebo. Median overall survival was 22.1 months (95.6% CI 20.3-24.1) in the aflibercept group and 21.2 months (95.6% CI 19.6-23.8) in the placebo group (stratified hazard ratio 0.94; 95.6% CI 0.82-1.08; p=0.38). The combination of aflibercept and docetaxel was associated with a higher incidence of grade 3/4 gastrointestinal disorders, hemorrhagic events, hypertension, fatigue, infections, and treatment related fatal adverse events [49].

Bone microenvironment agents

SRC-family kinases play an important role in prostate cancer growth and invasion, as well as the pathogenesis of bone metastases and the regulation of osteoclast function [50-52]. Among others, dasatinib potently inhibits the SRC family kinases (SRC, LCK, HCK, FYN, YES, FGR, BLK, LYN, and FRK [53]. In preclinical studies the tyrosine kinase inhibitor dasatinib inhibited cell duplication, migration, and invasion, and triggered apoptosis of tumoral cells. Dasatinib also acts on the tumor microenvironment, which is particularly important in the bone, where it inhibits osteoclastic activity and favors osteogenesis, exerting a bone-protecting effect [53]. These preclinical studies led to the hypothesis that combining dasatinib with docetaxel would improve treatment outcomes by targeting both the tumor and bone microenvironment. In a phase I/II study combining docetaxel with dasatinib, 18 out of 30 patients with measurable disease had a partial response and 14 patients had disappearance of lesions on bone scans [54]. However, the phase III READY trial demonstrated no survival benefit for docetaxel plus dasatinib compared to docetaxel and placebo [55].

Custirsen

Clusterin (CLU) is a stress-activated cytoprotective chaperone upregulated by a variety of anticancer therapies that lends treatment resistance when overexpressed [56]. Preclinical studies have shown that knockdown of clusterin enhances the effects of docetaxel in docetaxel-refractory cells [57]. A randomized phase II trial investigated custirsen (OGX-11), an antisense inhibitor of clusterin, in combination with docetaxel and prednisone, versus docetaxel and prednisone alone. The combination of docetaxel and prednisone with OGX-11 was associated with a longer median OS, despite similar rates of PSA and tumor response [58]. Two phase III trials of OGX-11 in first- and second line treatment of mCRPC are currently underway. Due to its unique mechanism of action, these trials are the only ongoing phase III studies with the potential of having a positive outcome in terms of survival benefit.

Table 1. Phase III trials of docetaxel-based combinations

Agent	*Result*
Docetaxel + GVAX	OS inferior in combination arm: 12.2 vs. 14.1 months
(VITAL-2)	HR 1.70 (95% CI 1.15-2.53)
Docetaxel + Calcitriol	OS inferior in combination arm: 17.8 vs. 20.2 months
(ASCENT-2)	HR 1.42 (95% CI 1.13-1.86)
Docetaxel + Atrasentan	OS not improved in combination arm: 18 vs. 17 months
(SWOG S0421)	HR 1.01 (95% CI 0.87-1.18)
Docetaxel + Zibotentan	OS not improved in combination arm: 20 vs. 19.2 months
(ENTHUSE M1C)	HR 1.00 (95% CI 0.84-1.18)
Docetaxel + Lenalidomide	OS inferior in combination arm: 17.7 vs. median not reached
(MAINSAIL)	HR 1.53 (95% CI 1.17-2.00)
Docetaxel + Bevacizumab	OS not improved in combination arm: 22.6 vs. 21.5 months
(CALBG 90401)	HR 0.91 (95% CI 0.78-1.05)
Docetaxel + Aflibercept	OS not improved in combination arm: 22.1 vs. 21.2 months
(VENICE)	HR 0.94 (95.6% CI 0.82-1.08)
Docetaxel + Dasatinib	OS not improved in combination arm: 21.5 vs. 21.2 months
(READY)	HR 0.99 (95% CI 0.87-1.13)
Docetaxel + Custirsen (SYNERGY)	Ongoing

PHASE II TRIALS

The addition of bcl-2 inhibitor AT-101 in combination with docetaxel was evaluated in a phase II trial with OS as the primary endpoint. The addition of AT-101 did not extend OS, PFS or PSA response as compared with docetaxel and prednisone [59]. The bcl-2 antisense oligonucleotide oblimersen was combined with docetaxel in an EORTC phase II trial. Primary endpoints including a rate of confirmed PSA response >30% and a major toxic event rate <45% were not reached [60].

In a randomized phase II trial of docetaxel and vandetanib, an oral inhibitor of vascular endotherlial growth factor receptor (VEGFR) and epidermal growth factor receptor (EGFR), no benefit was reported for the combination compared to docetaxel and placebo [61]. A single arm phase I/II trial of docetaxel plus sunitinib, an inhibitor of VEGFR and platelet derived growth factor (PDGFR) demonstrated PSA responses in 56.4% of patients [62]. In another single-arm phase II trial evaluating sorafenib and docetaxel PSA responses were observed in 46% of patients [63].

The PDGFR inhibitor imatinib was also investigated in combination with weekly docetaxel in a phase II trial. Increased adverse gastrointestinal events were observed in the experimental arm. These events coupled with a futility analysis which indicated that a

significant treatment difference would be unlikely for the planned accrual of 144 patients, led to early termination of the study [64].

None of these agents is currently under investigation in a phase III clinical trial.

In summary, docetaxel plus prednisone remains the gold standard of chemotherapy. None of the eight phase III docetaxel based combination trials have demonstrated a survival benefit when compared with the standard docetaxel regimen. A critical assessment by Antonarakis and Eisenberger of the phase II trials that led to the initiation of these studies showed that the results might not have been sufficient for the conduction of large phase III studies. Either no phase II data were available, or the metric for success that would prompt phase III development was not defined or reached [65].

CABAZITAXEL

Cabazitaxel was selected from 450 taxane derivatives, based on its antitumor activity in docetaxel-resistant tumor models [5]. Unlike the other taxanes (paclitaxel and docetaxel), cabazitaxel has poor affinity for the drug transporter p-glycoprotein (P-gp, ABCB1) [66, 67]. An additional characteristic of cabazitaxel is its ability to penetrate the blood-brain barrier in vivo, which is limited with other taxanes [68]. Recently it has been demonstrated that cabazitaxel also inhibits AR nuclear translocation, which could be an additional mechanism of taxane anti-tumor activity in mCRPC [8].

A phase I trial in patients with solid tumors determined that cabazitaxel had linear pharmacokinetics similar to docetaxel, but probably better tolerability [66]. The principal dose-limiting toxicity was neutropenia, with one patient experiencing febrile neutropenia and two others showing prolonged grade 4 neutropenia at the 25 mg/m^2 dose level. Non-hematologic toxicities included nausea, vomiting, diarrhea, neurotoxicity, and fatigue, and were generally mild to moderate. Objective antitumor activity was observed in two patients with partial responses including one patient with docetaxel-refractory mCRPC. One patient had an unconfirmed partial response, and two patients had minor responses. Subsequently, two proof of principle trials were conducted which demonstrated responses in patients with taxane resistant metastatic breast cancer [67, 69]. The Phase III TROPIC trial was a randomized, open-label, multicenter trial, conducted in 755 men with mCRPC who progressed during or after docetaxel chemotherapy [29]. Patients were randomized to receive either cabazitaxel 25 mg/m^2 or mitoxantrone 12 mg/m^2 in a 3 weekly regimen, each with 10 mg prednisone daily. Median OS was 15.1 months for the cabazitaxel arm versus 12.7 months in the mitoxantrone arm, with a hazard ratio (HR) for death of 0.70 (95% CI 0.59-0.83, $p<0.0001$). Secondary endpoints including progression free survival, PSA response, objective tumor response according to RECIST criteria, time to PSA progression and median time to tumor progression were all significantly improved

in the cabazitaxel arm. Pain response rates were similar between the two treatment arms. About 70% of patients had progressive disease during or within 3 months after docetaxel treatment, including about 30% of patients who had disease progression during docetaxel treatment. The benefit of cabazitaxel as compared to mitoxantrone was consistent among subgroups of patients defined by prognostic factors including patients with disease progression during docetaxel treatment and in those who received high cumulative doses of docetaxel.

In concordance with the TAX327 trial, a post-hoc analysis of the TROPIC trial linked a significant OS benefit for cabazitaxel versus mitoxantrone to patients with poorly differentiated tumors evaluated by WHO grade (median OS 15.2 months vs 12.7 months, $p<0.0001$), whereas for patients with well or moderately differentiated tumors this benefit was less robust, with a median OS of 15.5 months for cabazitaxel and 13.3 months for mitoxantrone ($p=0.56$) [70]. In this post-hoc analysis, the OS benefit obtained by cabazitaxel was independent of the duration of ADT. In contrast, a high Gleason score (Gleason 8-10) and a short response to prior ADT (≤16 months) may be predictive of a poor PSA response and PFS in patients treated with abriraterone [17, 19]. These easily available parameters could be of value in determining which treatment, cabazitaxel , or an agent like abiraterone has the greatest therapeutic potential in an individual patient as second-line treatment for mCRPC.

Patients received a median of 6 cycles for cabazitaxel, and 4 cycles for mitoxantrone. The most frequent hematological AE's were hematologic. Grade ≥3 neutropenia was more common in patients who received cabazitaxel (82%) than in patients who received mitoxantrone (58%), with febrile neutropenia rates of 8% and 1% respectively. The most frequent non-hematologic AE was diarrhea, occurring in 47% (grade ≥3, 6%) of patients treated with cabazitaxel, compared to 11% (grade ≥3, <1%) of patients treated with mitoxantrone.

A total of 18 patients (4.9%) who were treated with cabazitaxel died from causes other than disease progression within 30 days of receiving their last dose of cabazitaxel. This compares with 3 drug-related patient deaths (0.9%) in the mitoxantrone group. The most common cause of death in patients who were treated with cabazitaxel was neutropenia and its clinical consequences. However, no further deaths due to neutropenic complications occurred in the cabazitaxel group following the IDMC communication to the TROPIC investigators about the need to strictly adhere to the study protocol regarding dose delays and modifications and to manage neutropenia with granulocyte colony-stimulating factor (G-CSF) according to American Society for Clinical Oncology (ASCO) guidelines. The frequency of hematological adverse events and related deaths demonstrates that cabazitaxel treatment requires careful monitoring and management of emerging symptoms. Dose reductions as well as the administration of granulocyte

colony-stimulating factor (G-CSF) according to ASCO guidelines are strategies that should be considered in patients with high risk clinical features (age ≥65 years, poor performance status, previous episodes of febrile neutropenia, extensive prior radiation ports, poor nutritional status, or other serious comorbidities) to manage side effects of treatment with cabazitaxel. In an attempt to reduce cabazitaxel induced toxicity, an open-label randomized phase II study is currently testing whether the addition of the oral poorly resorbable steroid budesonide reduces or protects against cabazitaxel induced diarrhea.

FIRSTANA is a randomized phase III trial with OS as the primary endpoint comparing cabazitaxel 25mg/m^2 and cabazitaxel 20mg/m^2 both with prednisone, to docetaxel 75 mg/m^2 plus prednisone as first-line treatment for mCRPC. PROSELICA is an ongoing trial with a non-inferiority design comparing cabazitaxel 25mg/m^2 to cabazitaxel 20mg/m^2 both with prednisone. These studies will answer the questions whether a reduced dose of cabazitaxel may provide similar OS with the benefit of reduced toxicity, and whether cabazitaxel has greater therapeutic potential compared to docetaxel as first-line treatment for mCRPC.

At the present time, cabazitaxel has demonstrated survival benefit in patients progressing during or after treatment with docetaxel. In the Phase III TROPIC trial, the OS benefit obtained was consistent among the two thirds of patients enrolled who had either disease progression during docetaxel (29%) or within 3 months after the last docetaxel cycle (45%). Cabazitaxel has thus a different mode of action and is an important contribution to the management of patients with mCRPC who have failed docetaxel chemotherapy.

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INTRODUCTION TO THE THESIS

CHAPTER 2

INTRODUCTION TO THE THESIS

Prostate cancer is the most commonly diagnosed cancer and the sixth leading cause of cancer-related death among men in the Western world [1]. This disease is characterized by a heterogeneous natural history, with a considerable number of patients who will never develop symptoms, and will not die from prostate cancer. When prostate cancer is still organ confined, treatment options include radical surgery, radiotherapy with or without hormonal therapy, brachytherapy, and active surveillance. Despite an abundance of local therapies, a significant proportion of patients will eventually develop metastases from prostate cancer. Patients with metastatic prostate cancer cannot be cured and have a high likelihood of ultimately dying from their disease.

Androgen receptor (AR)-targeted therapy has been the mainstay of treatment for metastatic prostate cancer since the first description of the hormonal dependence of this cancer in 1941 [2]. Since prostate cancer cells are generally dependent on AR signaling for growth and survival, patients with metastatic prostate cancer initially respond well to either medical or surgical castration, with or without the addition of anti-androgens. However, eventually all patients will progress and develop castration-resistant prostate cancer (CRPC). Castration-resistant prostate cancer is defined by either PSA or radiological progression, despite castrate levels of testosterone (<50 ng/dl) [3].

Taxanes (i.e. docetaxel and cabazitaxel) inhibit microtubule dynamics, thereby inducing cell death and apoptosis [4, 5]. For almost a decade, docetaxel chemotherapy has been the standard of care in the management of metastatic castration-resistant prostate cancer (mCRPC), following reports of improvement in survival and quality of life in the pivotal TAX327 and SWOG 99-16 studies [6, 7] (**Chapter 1**). For regulatory purposes, after the approval of docetaxel in 2004 all new drugs for mCRPC were investigated in either the pre- or post-docetaxel space. In 2010, the novel taxane cabazitaxel became approved for the treatment of mCRPC in patients who progressed during or after docetaxel. This was based on a survival benefit of cabazitaxel plus prednisone as compared with mitoxantrone plus prednisone observed in men with disease progression during or after docetaxel in the TROPIC trial [8].

In recent years, it has become clear from the biology of mCRPC that the AR remains an important driver of mCRPC. This was evidenced by the survival benefits and subsequent regulatory approval of abiraterone and enzalutamide in the post-docetaxel setting [9, 10]. Abiraterone targets the AR indirectly by inhibiting CYP17A1, a crucial enzyme in steroidogenesis, and enzalutamide directly binds to the AR ligand binding domain, inhibiting multiple steps in the AR signaling pathway [11, 12]. Recently, the treatment paradigm has shifted with evidence that these novel AR targeted agents are also effective when administered to men with mCRPC before chemotherapy [13, 14]. With these novel AR targeting therapies now also available in the pre-chemotherapy setting, treatment choices

and drug sequencing for patients with mCRPC has become increasingly challenging.
First, the optimal drug treatment sequence for patients is unknown. Emerging retrospective evidence has suggested that some of the currently available therapies might be cross-resistant [15, 16], a condition in which sensitivity to one drug is impaired by previous treatment with another drug having a similar or overlapping mechanism of action. Therefore, defining mechanisms of cross-resistance between the currently available treatment options in mCRPC is of utmost importance to unravel the optimal treatment sequence for our patients.

Second, predictive biomarkers defining which patients will benefit from the available therapies are lacking. Such markers are urgently needed by clinicians to guide treatment choices for an individual patient, in order to better tailor therapy. This will ultimately define which patients will benefit from which treatment, and could help to avoid unnecessary treatment, and improve quality of life by limiting drug-related toxicity.
In this thesis, we investigated mechanisms of cross-resistance between taxanes and new hormonal agents abiraterone and enzalutamide. Furthermore, we aimed to identify both predictive and prognostic factors for patients with mCRPC receiving docetaxel chemotherapy.

In **chapter 3** we used patient-derived CRPC cell lines to identify and give insight in mechanisms of cross-resistance between docetaxel, cabazitaxel, abiraterone and enzalutamide, all four drugs registered for the treatment of mCRPC. AR-nuclear translocation was studied as an overlapping working mechanism between these drugs, and thus a potential mechanism of cross-resistance.

In **chapter 4** we confirmed our previous in vitro findings of cross-resistance between docetaxel and enzalutamide in an in vivo model of CRPC. Mechanisms of cross-resistance were further elucidated by exploring the role of the AR pathway in enzalutamide-resistant versus enzalutamide-naïve xenografts, treated with docetaxel and cabazitaxel. The hypothesized superior efficacy of cabazitaxel over docetaxel was also evaluated in this in vivo model of CRPC.

In **chapter 5** we aimed to clinically confirm our findings from the in vivo study from chapter 4. For this purpose, we investigated whether the efficacy of cabazitaxel in mCRPC patients was affected by prior treatment with abiraterone and enzalutamide. We used data from a prospective, multicenter, randomized phase II trial (CABARESC) to compare clinical outcome of patients treated with cabazitaxel with and without prior treatment with abiraterone and/or enzalutamide.

In **chapter 6** we aimed to identify subgroups of patients who benefit the most from docetaxel chemotherapy. Since it had been hypothesized that taxane chemotherapy is particularly effective in rapidly proliferating, undifferentiated tumors [17-19], we performed a post-hoc analysis of the pivotal TAX327 study to investigate the benefit

obtained by docetaxel according to the initial biopsy Gleason score.

An emerging biomarker across different tumor types and disease settings is the neutrophil to lymphocyte ratio (NLR). An elevated NLR, a marker for host inflammation, was found to be an independent marker of adverse outcomes for several solid tumors including mCRPC [20]. In men with mCRPC treated with abiraterone, NLR ≥5 was associated with lower PSA response rates and shorter survival [21]. In **chapter 7** we explored the prognostic and predictive role of the neutrophil to lymphocyte ratio (NLR) in two multinational randomized phase III trials of mCRPC patients receiving first-line chemotherapy.

Finally, the results of this thesis are discussed and suggestions for further research are mentioned (**chapter 8**). In summary, this thesis investigated mechanisms of cross-resistance between the currently available treatment options for mCRPC in order to define the optimal treatment sequence. Furthermore, several predictive and prognostic factors in patients receiving first-line chemotherapy were explored. With this studies, we hope to ultimately contribute to unraveling the drug treatment sequence with the most benefit for our patients.

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CROSS-RESISTANCE BETWEEN TAXANES AND NEW HORMONAL AGENTS ABIRATERONE AND ENZALUTAMIDE MAY AFFECT DRUG SEQUENCE CHOICES IN METASTATIC CASTRATION-RESISTANT PROSTATE CANCER

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CHAPTER 3

ABSTRACT

Introduction: Treatment options for patients with metastatic castration-resistant prostate cancer (mCRPC) have expanded in recent years with the introduction of cabazitaxel, abiraterone, and enzalutamide. With new systemic therapies available, the optimal treatment sequence of these drugs in mCRPC becomes increasingly important. As shown recently, patients who had previously been treated with abiraterone showed impaired responses to docetaxel, suggesting clinical cross-resistance.[1] In the present study, we aimed to identify cross-resistance between taxanes (docetaxel and cabazitaxel) and the new hormonal agents abiraterone and enzalutamide. As a potential mechanism for cross-resistance, we investigated the effects on androgen receptor (AR) nuclear translocation of these compounds.

Methods: To identify cross-resistance, we determined the effects of docetaxel, cabazitaxel, abiraterone and enzalutamide on cell viability in prostate cancer cell lines with acquired resistance to abiraterone and enzalutamide. Time-lapse confocal microscopy was used to study the dynamics of AR nuclear translocation.

Results: We observed impaired efficacy of docetaxel, cabazitaxel, and enzalutamide in the abiraterone-resistant cell line, compared to the non-resistant cell line, providing evidence for in vitro cross-resistance. Impaired efficacy of docetaxel, cabazitaxel and abiraterone was observed in the enzalutamide-resistant cell line. Furthermore, docetaxel and cabazitaxel inhibited AR nuclear translocation, which was also observed for abiraterone and enzalutamide.

Conclusions: In conclusion we found substantial preclinical evidence for cross-resistance between the taxanes docetaxel and cabazitaxel, and AR targeting agents abiraterone and enzalutamide. Since these compounds all interfere with AR signaling, this strongly suggests a common mechanism of action, and thus a potential mechanism for cross-resistance in mCRPC.

INTRODUCTION

Prostate cancer cells are dependent on androgen receptor (AR) signaling for growth and survival. Therefore, patients with metastatic prostate cancer initially respond well to luteinizing hormone releasing hormone (LHRH) analogues or surgical castration, with or without anti-androgens. However, eventually all patients develop castration-resistant prostate cancer. Docetaxel is the standard first line chemotherapy for metastatic castration-resistant prostate cancer (mCRPC) and has shown survival benefit as well as palliative benefit in phase III clinical trials [2, 3]. For patients who progress after docetaxel chemotherapy several new treatment options have become available recently. Cabazitaxel and AR targeting agents abiraterone and enzalutamide all demonstrated improved overall survival (OS) in patients with mCRPC who progressed after docetaxel-based chemotherapy [4-6]. Taxanes (i.e. paclitaxel, docetaxel, and cabazitaxel) act through microtubule interaction and polymerization inducing mitotic arrest and apoptosis. Recent reports demonstrated that paclitaxel and docetaxel also impair AR-signaling, which in the setting of mCRPC might in fact be responsible for part of the therapeutic efficacy [7, 8]. AR-signaling remains an important target for therapy in mCRPC, which has been demonstrated by the survival benefit obtained by abiraterone and enzalutamide. Enzalutamide exerts its effect by inhibiting AR nuclear translocation, DNA-binding and co-activator recruitment [9]. Abiraterone inhibits androgen biosynthesis by irreversibly blocking CYP17A1, a crucial enzyme in steroidogenesis [10, 11].

Recently abiraterone has shown improved radiographic progression-free survival (PFS) and a trend towards improved OS in chemotherapy-naive patients [12]. Based on this trial, the US Federal Food and Drug Administration (FDA) and the European Medicines Agency (EMA) lent approval to the use of abiraterone in patients with mCRPC prior to docetaxel chemotherapy. With new therapies available in the pre-docetaxel setting, the challenge has become to determine the treatment sequence which yields the greatest survival benefit for patients with mCRPC. In this light, it was reported that the activity of docetaxel post-abiraterone appeared lower than anticipated, with a median OS of only 12.5 months, which was less than the 19 months observed in the TAX327 trial [1, 2]. Moreover, fewer patients had a ≥ 50% PSA response (26%) as compared to a similar abiraterone-naïve patient cohort (54%), and compared to TAX327 (48%). No PSA responses to docetaxel were observed in patients who did not have a PSA response on abiraterone either.

Likewise, the activity of abiraterone appears to be higher when used before chemotherapy than in patients who have been previously exposed to docetaxel. In two phase II trials with abiraterone, a ≥50% PSA decline was observed in 67% and 79% of chemotherapy-naive patients, respectively, compared to 29% in the post-chemotherapy COU-AA-301 phase III trial [6, 13, 14]. In addition, a ≥50% PSA decline was observed in 62%

of patients in the randomized phase III trial of abiraterone pre-chemotherapy [12].

Taken together, these data may be explained by cross-resistance, a condition in which sensitivity to one compound is impaired by another compound with a similar or overlapping mechanism of action. In this report, we describe preclinical evidence for cross-resistance between the taxanes docetaxel and cabazitaxel and new hormonal agents abiraterone and enzalutamide, all four drugs currently registered for the use in mCRPC. Furthermore, as a potential mechanism for cross-resistance, we investigated the effects of these compounds on AR nuclear translocation.

MATERIALS AND METHODS

Cell lines

The PC346C human prostate cancer cell line was derived and maintained as described previously [15-17]. Briefly, cells were cultured in special Prostate Growth Medium (PGM) based on DMEM-F12 medium with several prostate cancer growth factors [15], 100 U/ml Penicillin, and 100 µg/ml Streptomycin (Cambrex BioWhittaker, Verviers, Belgium), supplemented with 2% FCS (PAN Biotech, Aidenbach, Germany) and 0.1 nM of the synthetic androgen R1881 (NEN, Boston, MA). The PC346Abi101 and PC346Enza cell lines were generated by continuous culturing of PC346C cells in PGM medium supplemented with 2% dextran-coated charcoal stripped serum (DCC), with the addition of 1µM abiraterone for PC346Abi101 and 1µM enzalutamide for PC346Enza. After initial cell death, resistant cells started to grow out under the selection conditions used. PC346C cells stably expressing GFP labeled AR (GFP-AR) were generated using lentiviral transduction. For the experiments, cells were cultured in the same DCC-containing PGM medium [15].

The Hep3B cell lines stably expressing GFP-AR and YFP-b-tubulin were generated and maintained as described previously [18, 19]. The YFP-b-tubulin expression construct was kindly provided by Dr. Galjart (Erasmus University Medical Center).

Confocal microscopy

For confocal microscopy, Hep3B GFP-AR, Hep3B YFP-b-tubulin, and PC346C GFP-AR cells were seeded on a glass cover slip and cultured in DCC-containing medium. After overnight attachment, cells were treated with docetaxel (1 µM) [8, 20], cabazitaxel (1 µM), mitoxantrone (100 nM), abiraterone (6 µM) [21, 22]_ENREF_21, and enzalutamide (1 µM). Incubation times were 48 hours and 4 hours for Hep3B GFP-AR and PC346C GFP-AR cells respectively. Docetaxel and cabazitaxel were kindly provided by Sanofi (Paris, France). Abiraterone and enzalutamide were obtained from Sequoia Research Products (Pangbourne, UK). Mitoxantrone was obtained from EMD Serono (Rockland, MA). Confocal

microscopy was performed on a Zeiss LSM510 microscope (Carl Zeiss, Jena, Germany) equipped with a 63×/1.3 NA oil immersion objective using the 488 nm (GFP-AR) and 514 nm (YFP-b-tubulin) laser line of a 200 mW Ar laser. Cells were transferred to a live-cell chamber and maintained at 37°C and 5% CO2. For time-lapse imaging, images of Hep3B GFP-AR cells were acquired every 5 minutes during 130 minutes at multiple locations of the same sample. After 10 minutes of imaging, 1 nM of the synthetic androgen R1881 was added to the medium to induce AR nuclear translocation. Average fluorescence intensities in the nucleus and cytoplasm were measured at each time point using Image J software (RSB, NIH, Bethesda, MD). The percentage of AR nuclear localization was expressed as: nuclear signal intensity / (nuclear signal intensity + cytoplasmatic signal intensity) x 100, after background subtraction. The mean percentage of AR nuclear localization of 18-28 cells in three independent experiments ± SEM was plotted for the treatment conditions at every time point.

MTT proliferation assays

To determine the effects of docetaxel, cabazitaxel, abiraterone, enzalutamide and mitoxantrone on cell viability, we used an assay based on the enzymatic reduction of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St.Louis, MO) by metabolically active cells as described previously [23]. Briefly, cells were seeded in 96-well dishes at 5,000 cells per well in DCC medium. After overnight attachment, PC346C, PC346Abi101 and PC346Enza cells were incubated for 10 days with docetaxel, cabazitaxel, abiraterone, enzalutamide, mitoxantrone, or vehicle at indicated concentrations, with the addition of 0.1 nM R1881. Hep3B GFP-AR cells were incubated for 48 hours with the same compounds. Four replicates per condition were used. Data are expressed mean ± SEM of 3 independent experiments. IC50 values were calculated in Prism GraphPad 5.0 using the following formula: Y=100/(1+10^(X-LogIC50)). To statistically test differences in IC50 values between cell lines we used the extra sum-of-squares F test with a boundary for significance of $p<0.01$.

RESULTS

Docetaxel and cabazitaxel efficacy is impaired in PC346Abi101and PC346Enza cells

To identify cross-resistance between docetaxel and cabazitaxel, and the hormonal agents abiraterone and enzalutamide, we investigated the effects of docetaxel and cabazitaxel on cell viability in PC346Abi101 and PC346Enza cells, in which acquired resistance to abiraterone (PC346Abi101) and enzalutamide (PC346Enza) was developed in vitro (Fig. 1 A and Fig. 2 A). Protein expression of AR and PSA for PC346C, PC346Abi101, and PC346Enza was

determined using Western blotting and is shown in Supplementary Fig. S1.

We observed that docetaxel and cabazitaxel efficacy was significantly impaired in both PC346Abi101 and PC346Enza cells, as compared to the parental PC346C cells (Fig. 1 B, C and Fig. 2 B, C), suggesting cross-resistance between both taxanes and abiraterone, as well as both taxanes and enzalutamide. To determine whether the observed cross-resistance was specific for the microtubule-targeting agents docetaxel and cabazitaxel, we used mitoxantrone as a control cytotoxic agent that does not target microtubules. Mitoxantrone efficacy was not significantly impaired in PC346Abi101 and PC346Enza cells, showing similar efficacy as in PC346C cells (Fig. 1 D and Fig. 2 D). IC50 values for the various compounds in PC346Abi101 and PC346Enza versus PC346C are shown in Table 1.

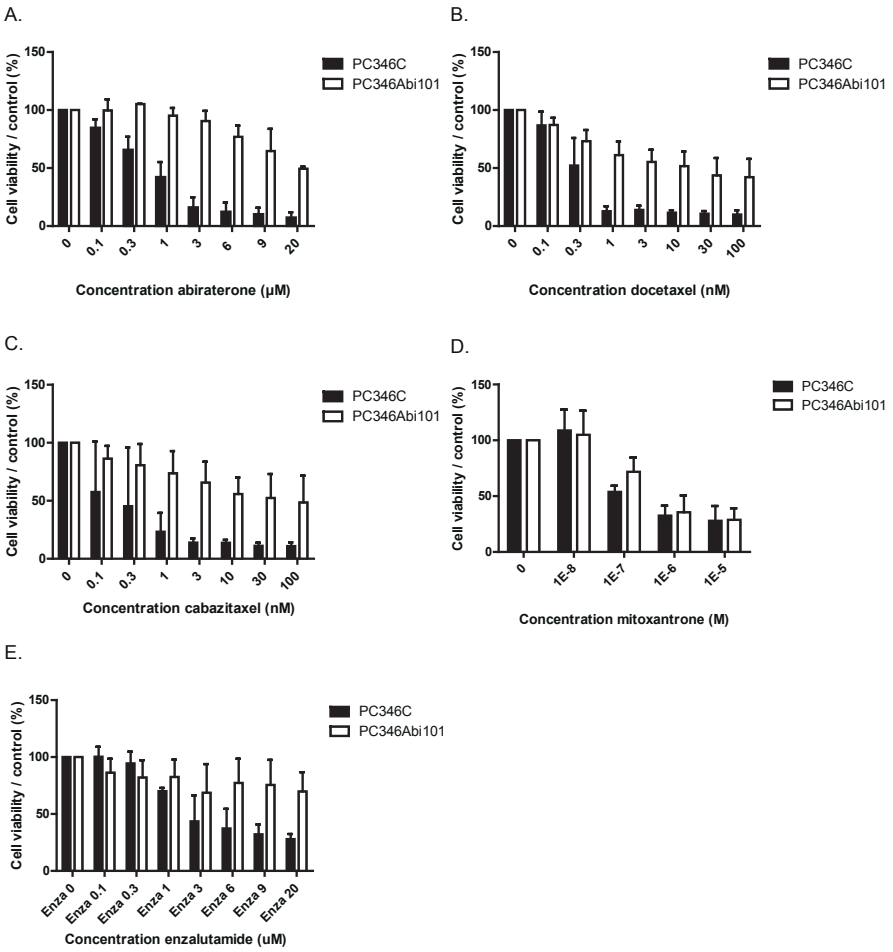

Figure 1. Docetaxel, cabazitaxel, and enzalutamide efficacy is impaired in PC346Abi101 cells, in which resistance to abiraterone was acquired by continuous culturing in the presence of 1 µM abiraterone. Cell viability of PC346Abi101 and PC346C cells is shown after 10 days of incubation with abiraterone (A), docetaxel (B), cabazitaxel (C), mitoxantrone (D), and enzalutamide (E) at indicated concentrations. Cell viability was assessed by MTT-assay. Four replicates per condition were used. Data are expressed as mean ± SEM of 3 independent experiments.

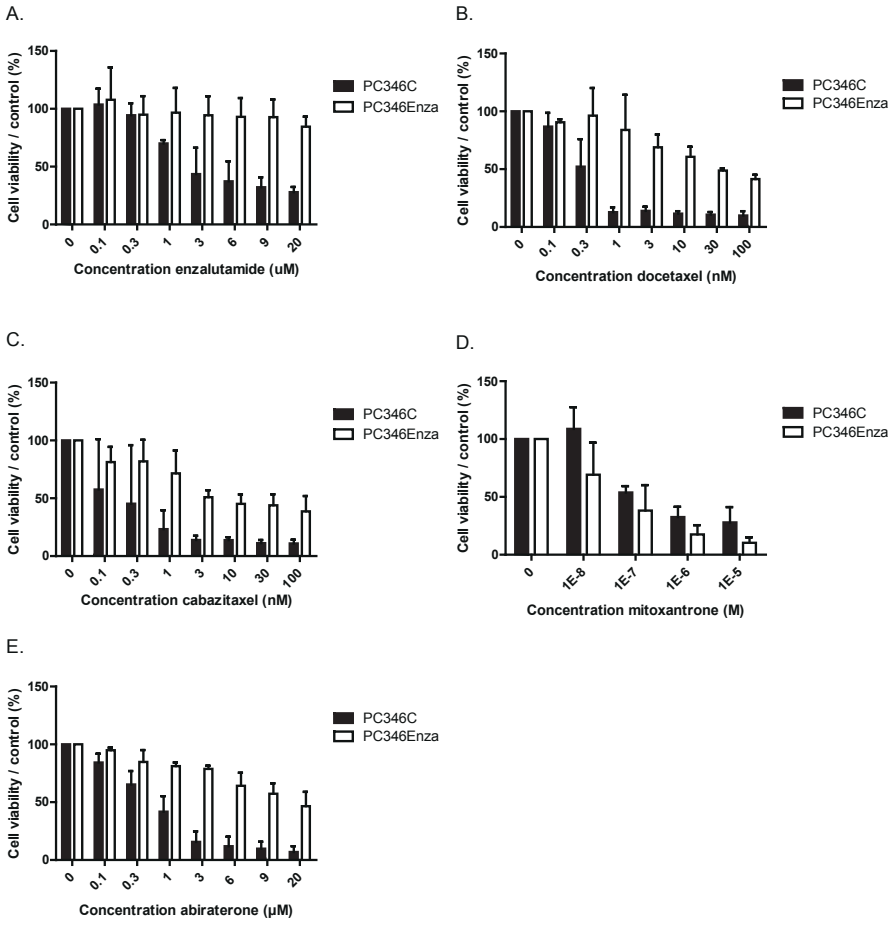

Figure 2. Docetaxel, cabazitaxel, and abiraterone efficacy is impaired in PC346Enza cells, in which resistance to enzalutamide was acquired by continuous culturing in the presence of 1 µM enzalutamide. Cell viability of PC346Enza and PC346C cells is shown after 10 days of incubation with enzalutamide (A), docetaxel (B), cabazitaxel (C), mitoxantrone (D), and abiraterone (E) at indicated concentrations. Cell viability was assessed by MTT-assay. Four replicates per condition were used. Data are expressed as mean ± SEM of 3 independent experiments.

Table 1. IC50 values for abiraterone, docetaxel, cabazitaxel, mitoxantrone and enzalutamide in PC346C versus PC346Abi101 and PC346Enza

	Cell line		
Compound:	**PC346C**	**PC346Abi101**	**PC346Enza**
	IC50 (95% CI)	**IC50 (95% CI)**	**IC50 (95% CI)**
Abiraterone (μM)	0.6 (0.5-0.8)	19.3 (15.1-24.8)*	12.2 (9.4-15.9)*
Docetaxel (nM)	0.3 (0.2-0.5)	9.3 (4.5-19.4)*	23.9 (13.6-42.0)*
Cabazitaxel (nM)	0.2 (0.1-0.5)	24.1 (11.4-51.0)*	7.2 (3.6-14.4)*
Mitoxantrone (μM)	0.2 (0.1-0.5)	0.5 (0.2-1.1)	0.05 (0.02-0.1)
Enzalutamide (μM)	3.3 (2.4-4.6)	26.6 (12.3-57.4)*	102.7 (35.7-295.8)*

*IC50 Significantly higher as compared to IC50 PC346C ($p<0.01$)

Abiraterone and enzalutamide efficacy is impaired in PC346Enza and PC346Abi101 cells

To investigate cross-resistance between the AR targeting agents abiraterone and enzalutamide, we determined the efficacy of abiraterone in the enzalutamide-resistant cell line PC346Enza, and the efficacy of enzalutamide in the abiraterone-resistant cell line PC346Abi101. We observed strongly impaired efficacy of enzalutamide in PC346Abi101 cells as compared to PC346C (Fig. 1 E). Likewise, the efficacy of abiraterone was diminished in PC346Enza as compared to PC346C, which suggests cross-resistance between these two hormonal agents (Fig. 2 E).

Docetaxel, cabazitaxel, abiraterone and enzalutamide inhibit R1881 induced AR nuclear translocation.

To investigate the dynamics of AR nuclear translocation, time-lapse microscopy was used to determine AR nuclear localization at regular time intervals after addition of R1881 in Hep3B GFP-AR cells pretreated for 48 hours with docetaxel, cabazitaxel, abiraterone, enzalutamide, and mitoxantrone (Fig. 3 A and B). Pretreatment of cells with docetaxel and cabazitaxel inhibited AR nuclear translocation with 21% and 34% respectively compared to vehicle control. We investigated mitoxantrone as a control to determine whether this effect could be linked to the interference of microtubule dynamics by docetaxel and cabazitaxel. As expected, mitoxantrone pretreatment did not cause an impairment of AR-translocation to the nucleus. Together with the observation that docetaxel and cabazitaxel clearly affected microtubules after 48 hours of treatment this strengthens the hypothesis

that microtubules may at least partly facilitate AR transport (Fig. 3 C). The reduced AR translocation after pretreatment with docetaxel and cabazitaxel for 48 hours could not be explained by cytotoxic effects, since neither of these compounds showed evidence of cellular toxicity under these conditions (Supplementary Fig. S2 A) and overall cell viability had even increased compared to day 0 (Supplementary Fig. S2 B).

Pretreatment of Hep3B GFP-AR cells with abiraterone inhibited AR nuclear translocation with 58% as compared to control. This observation demonstrates that besides inhibiting CYP17A1, abiraterone can act as an anti-androgen in the presence of R1881. As expected, no AR nuclear import was observed in enzalutamide treated cells after the addition of R1881. Cell viability of the cells was not affected by abiraterone and enzalutamide (Supplementary Fig. S2 C and D).

We confirmed our observations from the Hep3B GFP-AR cells in a prostate cancer specific model using PC346C cells stably expressing GFP-AR. Fig. 4 demonstrates that docetaxel and cabazitaxel, as well as abiraterone and enzalutamide inhibited R1881 induced AR nuclear transport in these cells as compared to vehicle control and mitoxantrone.

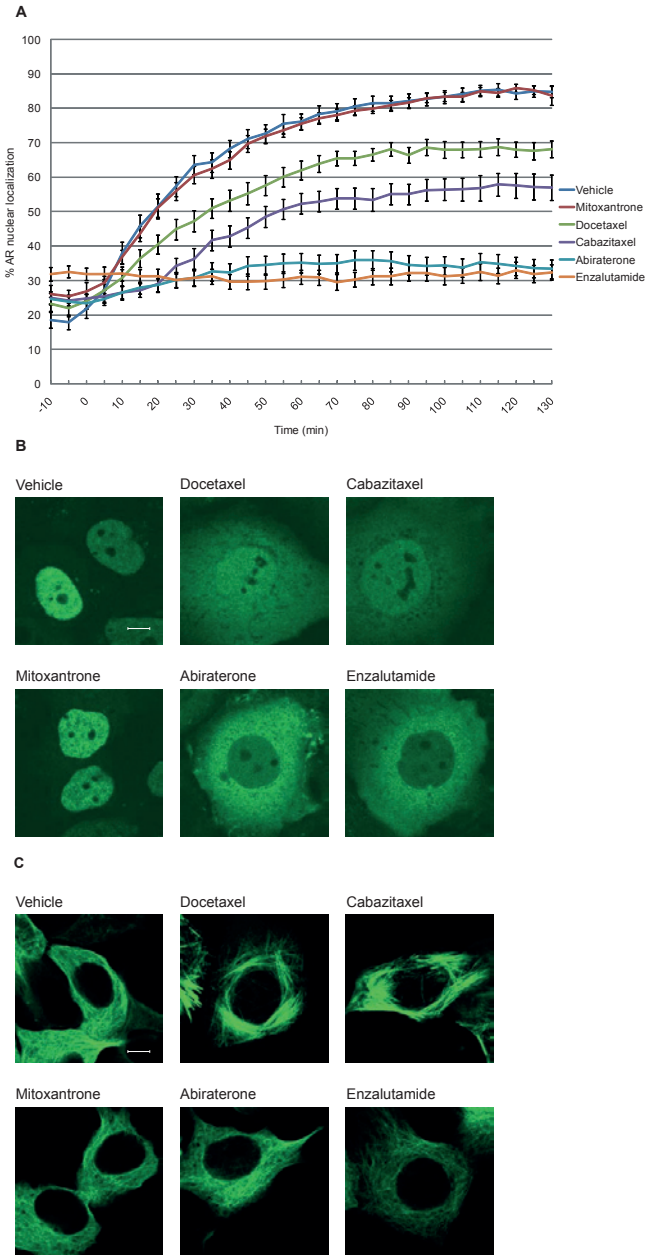

Figure 3. Docetaxel, cabazitaxel, abiraterone and enzalutamide inhibit AR nuclear import. Hep3B cells expressing GFP-AR were pre-treated with docetaxel (1 μM), cabazitaxel (1 μM), mitoxantrone (100 nM), abiraterone (6 μM), enzalutamide (1 μM), or vehicle control for 48 hours. Subsequently the synthetic androgen R1881 (1 nM) was added at t=0 to induce AR nuclear translocation. Time-lapse images were acquired every 5 minutes at multiple locations per sample. A, Dynamics and quantification of nuclear AR nuclear localization. B, Representative high resolution confocal images of AR localization were acquired after 130 minutes of incubation with R1881. Bar represents 10 μm. C, Docetaxel and cabazitaxel cause microtubule rearrangement. High resolution confocal images of Hep3B cells expressing YFP-tubulin were acquired after treatment with docetaxel (1 μM), cabazitaxel (1 μM), mitoxantrone (100 nM), abiraterone (6 μM), enzalutamide (1 μM), and vehicle control for 48 hours. Bar represents 10 μm.

DISCUSSION

In this study we present in vitro evidence for cross-resistance between taxanes (docetaxel and cabazitaxel) and AR targeting compounds abiraterone and enzalutamide in mCRPC. Furthermore, our data demonstrate that docetaxel, cabazitaxel, abiraterone and enzalutamide all act on AR nuclear transport, which is a crucial step in AR signaling, and provide a mechanistical explanation for potential cross-resistance between the two taxanes that are currently registered for treatment in mCRPC and the novel AR targeting agents abiraterone and enzalutamide. The observation that mitoxantrone did not affect AR transport and did not show impaired efficacy in the abiraterone- and enzalutamide-resistant cells, strengthened our hypothesis that cross-resistance between both taxanes and the hormonal agents might be caused by the effects on AR nuclear import of these compounds.

Darshan et al. recently reported that paclitaxel inhibits AR nuclear import [20]. Paclitaxel however, is not approved for use in mCRPC. Zhu et al. showed that also docetaxel impairs AR signaling [8]. Thus far no data have been reported on cabazitaxel, which was approved in 2010 by the US Food and Drug Administration (FDA) and in 2011 by the European Medicines Agency (EMA) for the use in mCRPC after prior treatment with docetaxel. To our knowledge, we are the first to describe preclinical evidence for cross-resistance and the effects on AR translocation dynamics by docetaxel, cabazitaxel, and abiraterone, drugs that are all three approved for the treatment of mCRPC.

Interestingly abiraterone is able to block AR nuclear import in the presence of R1881. Like testosterone or dihydrotestosterone, R1881 does not require steroidogenic conversion to bind and activate the AR. Consequently, our observed inhibition of AR nuclear transport cannot be related to CYP17A1 inhibition, a therefore must be an effect of abiraterone directly acting on the AR. This finding is supported by Richards et al, who found that abiraterone binds and inhibits AR at high but clinically relevant concentrations (≥5 μM) [21, 22]. Our observations of abiraterone and enzalutamide both directly inhibiting AR nuclear translocation, and cross-resistance between these compounds in vitro are concordant with recent clinical observations demonstrating modest efficacy of abiraterone in patients with mCRPC progressing after enzalutamide [24, 25], as well as modest efficacy of enzalutamide in patients progressing after abiraterone [26].

The inhibiting effects on AR nuclear import by abiraterone and docetaxel strongly suggest a common mechanism of action in mCRPC. Such an interaction is further augmented by our observed cross-resistance between these compounds in vitro. Although the exact mechanism needs to be further elucidated, this data may explain recent clinical observations of cross-resistance between abiraterone and docetaxel in mCRPC reported by Mezynski et al [1].

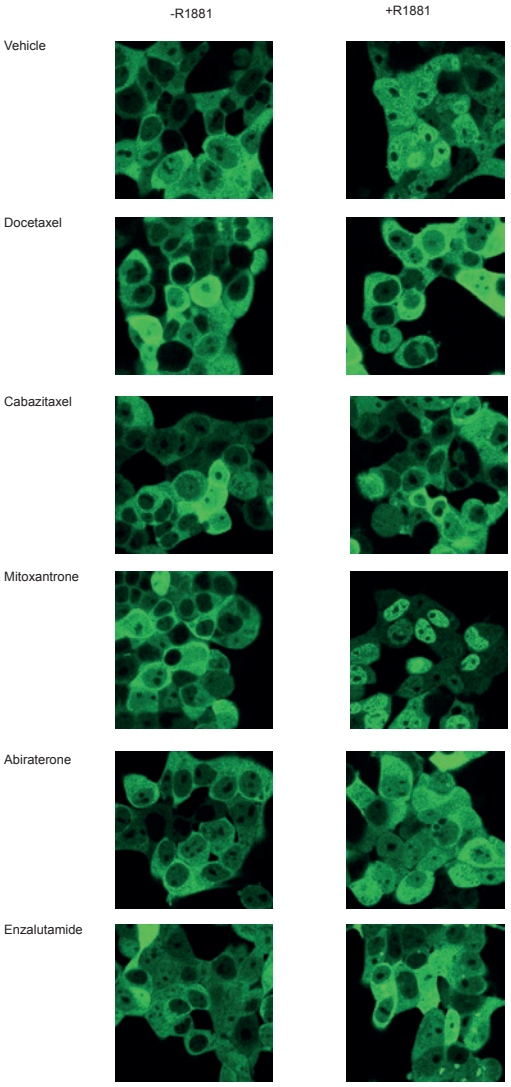

Figure 4. Docetaxel, cabazitaxel, abiraterone and enzalutamide inhibit AR nuclear import in prostate cancer cells. PC346C cells stably expressing GFP-AR were pre-treated with docetaxel (1 μM), cabazitaxel (1 μM), mitoxantrone (100 nM), abiraterone (6 μM), enzalutamide (1 μM), or vehicle control for 4 hours. High resolution confocal images were acquired after 2 hours of incubation with 1 nM R1881 to induce AR nuclear translocation.

The effects of docetaxel and cabazitaxel on AR transport could be explained by a mechanism proposed by Thadani-Mulero et al. in which AR transport is facilitated by microtubules and the motor protein dynein [7]. This model could help to better understand the effect of taxanes on AR and the molecular basis of taxane resistance._ENREF_7 In our study the effects of the taxanes on AR transport were more pronounced in PC346C as compared to the Hep3B model system, suggesting that the ability of taxanes to suppress microtubule dynamics may be cell specific, exerting optimal effects on prostate cancer cells.

With new compounds for the treatment of mCRPC becoming available for clinical use, it is warranted, especially in light of our current findings, to determine the optimal treatment sequence of these compounds in the management of patients with mCRPC. Following the recent approval by FDA and EMA of abiraterone for the use prior to docetaxel chemotherapy, prospective clinical research aiming to define the treatment sequence that provides the maximum survival benefit has become of paramount importance. The ultimate proof of clinical cross-resistance between these compounds and the magnitude of the impact of drug sequencing can only be answered in a prospective clinical trial of abiraterone or enzalutamide followed by taxane chemotherapy, versus chemotherapy followed by abiraterone or enzalutamide.

In conclusion we found substantial evidence for cross-resistance between the taxanes docetaxel and cabazitaxel, and the new hormonal agents abiraterone and enzalutamide in vitro. These preclinical observations are concordant with clinical reports of cross-resistance between docetaxel and abiraterone, as well as abiraterone and enzalutamide in mCRPC [1, 24-26]. Since docetaxel, cabazitaxel, abiraterone and enzalutamide all interfere with AR signaling, this strongly suggests a common mechanism of action, and thus a potential mechanism for cross-resistance in mCRPC. Prospective clinical studies should further define if this cross-resistance impacts the treatment sequence of these treatment options in patients with mCRPC. Survival benefit of abiraterone has been shown post-docetaxel, but since the efficacy of the taxanes may be impaired in this setting, it is critically important to demonstrate overall survival benefit when testing these agents prior to chemotherapy.

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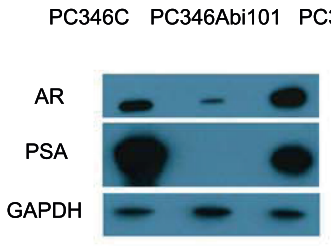
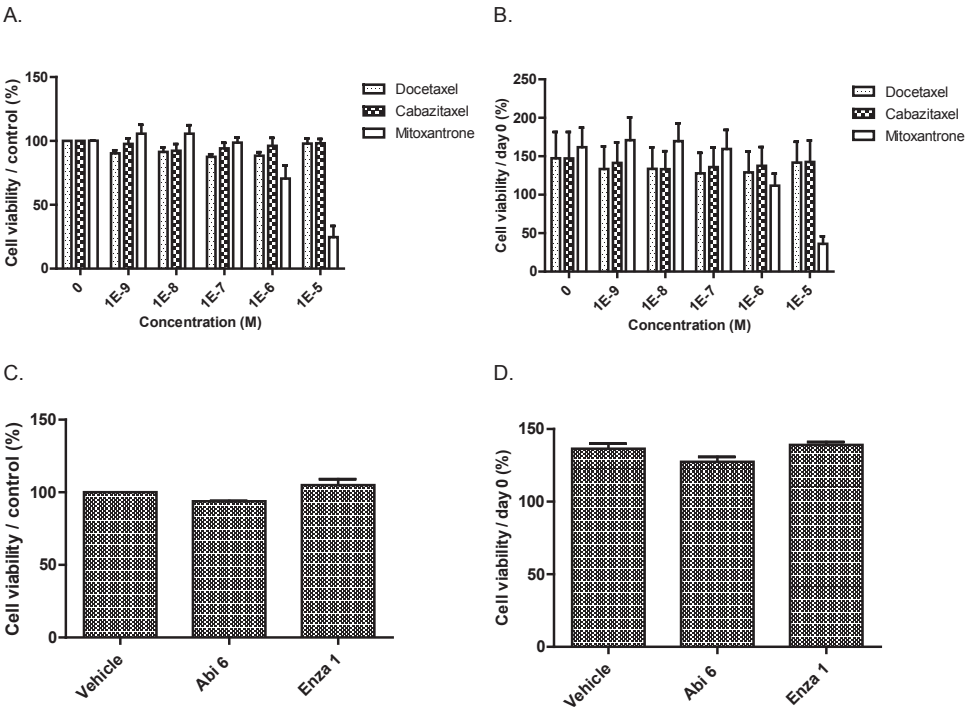
SUPPLEMENTARY FIGURES



Supplementary Figure S1. Protein expression of AR, PSA and GAPDH control for PC346C, PC346Abi101 and PC346Enza. Total proteins were extracted from PC346C, PC346Abi101 and PC346Enza cells and AR, PSA and GAPDH (loading control) were assessed by western blot.



Supplementary Figure S2. Docetaxel (1 µM), cabazitaxel (1 µM), mitoxantrone (100 nM), abiraterone (6 µM), and enzalutamide (1 µM), used in similar concentrations as for AR nuclear translocation studies, did not inhibit cell viability of Hep3B GFP-AR cells after 48 hours of treatment (A and C), showing improved overall cell viability compared to day 0 (B and D). Cell viability was assessed using MTT-assay with 48 hours of incubation. Four replicates per condition were used. Data are expressed as mean cell viability compared to control ± SEM, and mean cell viability compared to day 0 ± SEM of 3 independent experiments.

TARGETING THE ANDROGEN RECEPTOR CONFERS IN VIVO CROSS-RESISTANCE BETWEEN ENZALUTAMIDE AND DOCETAXEL, BUT NOT CABAZITAXEL, IN CASTRATION-RESISTANT PROSTATE CANCER

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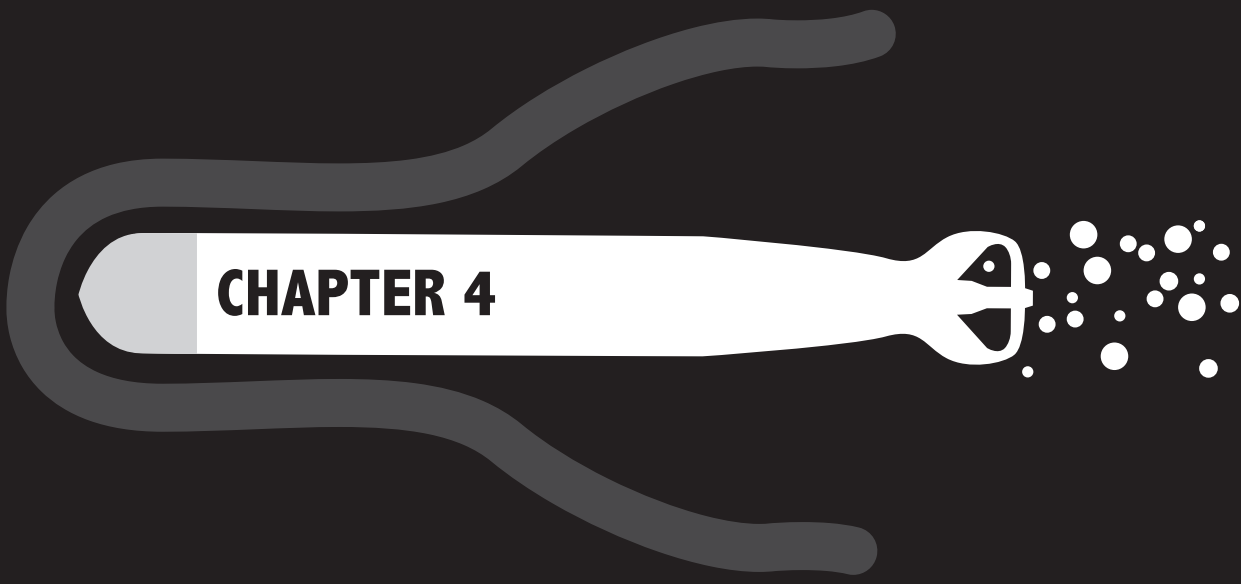

CHAPTER 4

ABSTRACT

Treatment options for metastatic castration-resistant prostate cancer (CRPC) have evolved with the established benefit of novel androgen receptor (AR)-targeted agents abiraterone and enzalutamide in the pre-chemotherapy setting. At the same time, concerns of cross-resistance between the taxanes (i.e. docetaxel and cabazitaxel) and these AR-targeted agents have risen, and the optimal drug treatment sequence is unknown. Here, we investigated the in vivo efficacy of docetaxel and cabazitaxel in enzalutamide-resistant CRPC, and mechanisms of cross-resistance between these agents. Castrated mice harboring enzalutamide-resistant tumors and enzalutamide-naïve tumors were treated with docetaxel and cabazitaxel. Tumor growth kinetics, AR nuclear localization, AR regulated gene expression, Ki67 expression, and serum levels of PSA, docetaxel, and cabazitaxel were analyzed. Docetaxel inhibited tumor growth, AR nuclear localization, and AR regulated gene expression in enzalutamide-naive tumors, but did not in enzalutamide-resistant tumors, demonstrating in vivo cross-resistance. In contrast, cabazitaxel remained highly effective in enzalutamide-resistant tumors and demonstrated superior anti-tumor activity as compared to docetaxel, independent of the AR pathway. These findings demonstrate that the AR pathway is able to confer in vivo cross-resistance between enzalutamide and docetaxel, but not cabazitaxel, in CRPC.

Patient summary: We found reduced efficacy of docetaxel, but not cabazitaxel, in enzalutamide-resistant prostate cancer.

INTRODUCTION

For almost a decade docetaxel has been the standard first-line chemotherapy for metastatic castration-resistant prostate cancer (mCRPC). In recent years, treatment options for mCRPC have evolved with the introduction of cabazitaxel, abiraterone, and enzalutamide, that all prolonged survival in the post-docetaxel setting [1]. Recently, the treatment paradigm has changed with evidence that novel AR targeted therapies abiraterone and enzalutamide are effective when administered to men with mCRPC also before chemotherapy [2, 3]. With these novel AR targeting therapies also available in the pre-chemotherapy setting, treatment sequencing has become increasingly challenging, especially since concerns have been raised regarding the efficacy of docetaxel when used after abiraterone [4, 5]. Clinical cross-resistance has been suggested in retrospective studies that demonstrated reduced efficacy of docetaxel in men with mCRPC who had previously been treated with abiraterone [4, 5]. Moreover, a preclinical study by our group identified inhibition of AR nuclear translocation as an overlapping working mechanism that potentially confers cross-resistance between taxanes and AR targeted agents abiraterone and enzalutamide [6]. Interestingly, retrospective clinical data suggested that cabazitaxel, in contrast to docetaxel, remains effective in men with mCRPC after prior abiraterone [7, 8]. The efficacy of docetaxel and cabazitaxel after first-line enzalutamide is yet unknown.

With the availability of novel hormonal agents before chemotherapy, there is an urgent need to investigate the optimal treatment sequence, and potential mechanisms of cross-resistance between the current treatment options. Here, we investigated the in vivo efficacy of docetaxel and cabazitaxel in castration-resistant prostate cancer (CRPC) with acquired resistance to enzalutamide, and mechanisms of cross-resistance between these agents.

MATERIALS AND METHODS

Cell lines and xenografts

The PC346C patient-derived prostate cancer xenograft and cell line were developed and maintained as described previously [9, 10]. The enzalutamide-resistant PC346Enza cell line was generated from the parental PC346C by long time culturing in the presence of enzalutamide(1 uM) [6]. Both PC346C and PC346Enza cells harbor wildtype AR.

In vivo experiments

PC346Enza and parental PC346C cells were subcutaneously inoculated in immunodeficient male (NMRI) mice. Mice were castrated when tumors reached a volume between 150 and 200 mm^3. After castration, mice were randomized to treatment with a

single intraperitoneal dose of docetaxel (33 mg/kg), cabazitaxel (33 mg/kg), or placebo when a tumor volume of 300 mm^3 was reached. Mice bearing the enzalutamide-resistant PC346Enza xenografts were kept under selection pressure with enzalutamide until they received their assigned treatment. To confirm enzalutamide resistance, castrate mice bearing PC346C and PC346Enza tumors were randomized to receive placebo or oral enzalutamide once daily (Axon Medchem, Groningen, the Netherlands) at a dose of 60 mg/kg, which is in line with the optimal biological dose in mice of 30-100 mg/kg as reported by Clegg et al [11]. All placebo treated PC346C xenografts were pooled for analyses. Tumor volumes were measured twice a week, and blood samples were taken every 2 weeks and analyzed for serum PSA levels. Tumor volumes were analyzed after a follow-up of 77 days after the start of treatment. Mice were euthanized before day 77 if a tumor volume of >1500-2000 mm^3 was reached. Available serum PSA samples taken at baseline (at least 2 weeks after castration), were compared with PSA levels after approximately 77 days or end of treatment (whichever came first). All animal experiments were approved by the Animal Experiments Committee under the Dutch Experiments on Animals Act. Experiments were analyzed using Graphpad 5.0. An unpaired t-test was used for statistical evaluation.

Pharmacokinetics

To determine whether enzalutamide affected the pharmacokinetics of docetaxel and cabazitaxel, a separate experiment was conducted in mice that were pretreated with enzalutamide (60 mg/kg) for at least 2 weeks and subsequently received an intraperitoneal injection of docetaxel or cabazitaxel (both 33 mg/kg). Non enzalutamide-pretreated mice also received an injection with docetaxel or cabazitaxel. Three hours after administration, blood samples were taken to determine the plasma concentration of both taxanes using a validated LC/MS/MS based assay as described previously [12, 13].

RNA isolation and real-time PCR (RT-PCR)

Total RNA from the xenografts was isolated using RNA-Bee (Tel-Test, Inc, Friendwood, TX). RT-PCR was performed using a 7500 Fast Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific Inc., Waltham, MA), as described previously [14]. Gene expression was normalized against the average of two housekeeping genes (GAPDH and PBGD) using the delta Ct method. RT-PCR experiments were carried out in duplo.

Immunohistochemistry

Immunohistochemistry to determine AR nuclear localization and Ki67 expression of the xenografts was performed using formalin-fixed paraffin embedded tissue sections. AR nuclear localization was determined after incubation with anti-AR (SP107, Dako), treatment with anti-rabbit (Ultramap) and visualization with DAB/H_2O_2. Ki67 was used

as a biotinylated anti mouse complex, detected with streptavidine-HRP, and visualized using DAB/H_20_2. AR nuclear localization scores were composed of the sum of the nuclear AR score (0 for no stain, 1 for weak stain, and 2 for intense stain), each multiplied by the corresponding percentage of cells [15]. Ki67 score was calculated by estimating the percentage of positive cells in the whole tumor section. Tissue sections that were not evaluable due to necrosis or insufficient cancer cells were excluded from analysis. Immunohistochemistry slides were scored by two readers (RJvS and CFK), blinded for treatment group and tumortype. The final blinded scored was made by consensus.

RESULTS

We first confirmed that the PC346Enza xenograft was resistant to enzalutamide in vivo (Fig.1A-B). Docetaxel showed good tumor responses as compared with placebo in castrate male mice bearing enzalutamide-naïve PC346C tumors (-78% mean tumor volume change from baseline (TVC), SEM +/- 7%), whereas its efficacy was impaired in mice bearing enzalutamide-resistant PC346Enza tumors (+364%TVC, SEM +/- 69%) demonstrating cross-resistance between docetaxel and enzalutamide in vivo ($P<0.01$) (Fig.1C-D). Progression-free survival and tumor growth curves over time are shown in Fig.1E-F, and Supplementary figure 1. Concordant with the observed tumor responses, docetaxel reduced serum PSA levels as compared to placebo in castrate mice bearing PC346C, while it did not in mice bearing PC346Enza tumors (Fig. 2A-B). Thus cross-resistance between docetaxel and enzalutamide was not only observed at the level of tumor growth, but also in terms of clinically relevant serum PSA response, which is directly related to tumor volume.

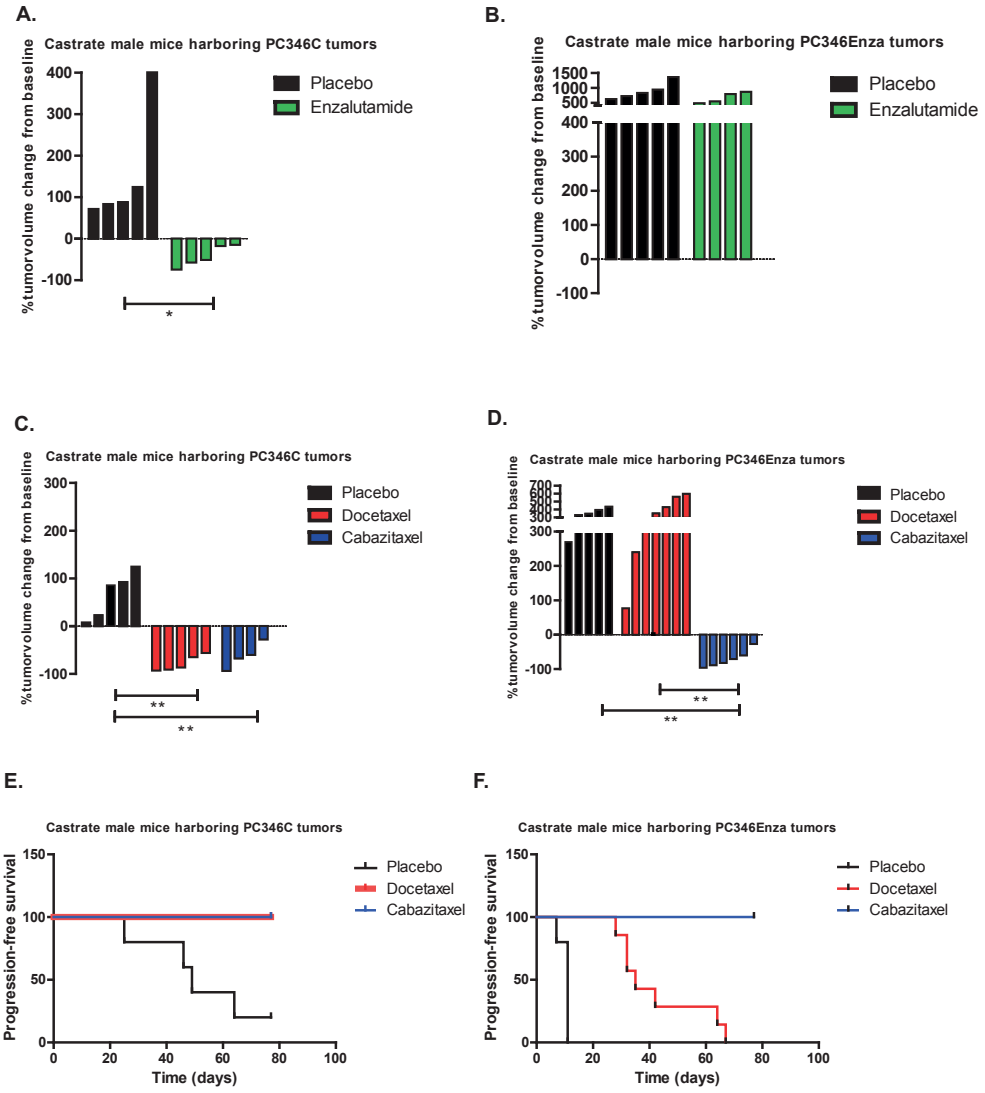

Figure 1. (A and B) Castrate male mice harboring enzalutamide-resistant PC346Enza tumors and the parental enzalutamide-naïve PC346C tumors were treated with daily oral enzalutamide (60 mg/kg) and placebo. (**C and D**) Castrate mice bearing PC346Enza and PC346C tumors were treated with docetaxel (33 mg/kg) and cabazitaxel (33 mg/kg) using a single intraperitoneal injection, or placebo. The percentage of tumor volume change form baseline was calculated after a cut-off of 77 days. Differences between groups were evaluated using an unpaired t-test. (*) represents $p<0.05$, (**) represents $p<0.01$. The exact p-values are quoted for comparisons with borderline significance ($0.05<p<0.10$), and the absence of a star indicates $p>0.10$. Bars represent individual mice. (**E and F**) Progression-free survival during 77 days was plotted with progression defined as a ≥50% increase in tumor volume.

Tumor responses for cabazitaxel were similar in PC346Enza and PC346C tumors, demonstrating that there was no cross-resistance between enzalutamide and cabazitaxel (Fig.1C-D). While docetaxel efficacy was impaired in mice bearing PC346enza tumors (+364%TVC, SEM +/- 69%), cabazitaxel remained very effective (-70%TVC, SEM +/- 10%) and demonstrated greater anti-tumor activity (P<0.01) and serum PSA declines (Fig. 2A-B) as compared to docetaxel.

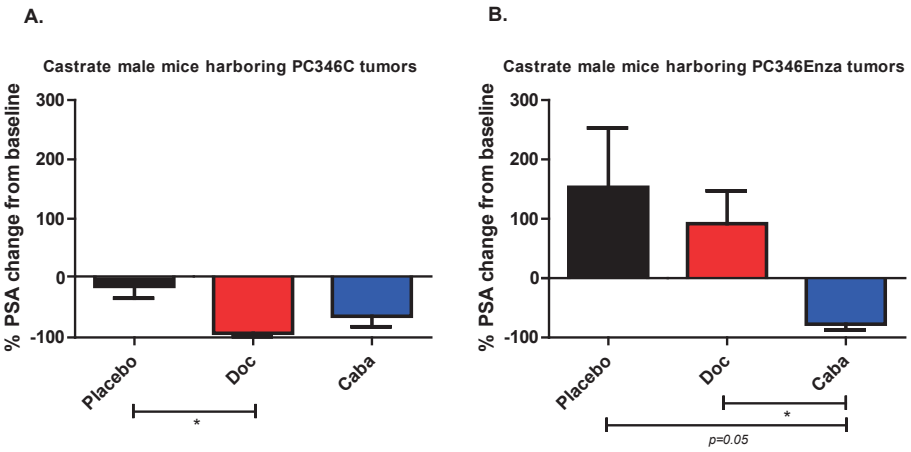

Figure 2. (A and B) Blood samples of mice harboring enzalutamide-resistant PC346Enza tumors and the parental PC346C tumors were taken every 2 weeks to determine serum PSA levels. Baseline serum PSA samples taken at least 2 weeks after castration were compared with PSA levels after approximately 77 days or end of treatment (whichever came first). The mean percentage of PSA change from baseline +/- SEM was plotted. Differences between groups were evaluated using an unpaired t-test. (*) represents p<0.05, (**) represents p<0.01. The exact p-values are quoted for comparisons with borderline significance (0.05<p<0.10), and the absence of a star indicates p>0.10.

Plasma concentrations of docetaxel and cabazitaxel were similar in enzalutamide pretreated versus non-pretreated control mice, indicating no effect of enzalutamide on the pharmacokinetics of both taxanes (Fig. 3, Table 1). Furthermore, plasma concentrations of docetaxel and cabazitaxel in mice were similar as compared to those reported in patients (Table 1) indicating that our observed cross-resistance occurs at clinical relevant concentrations.

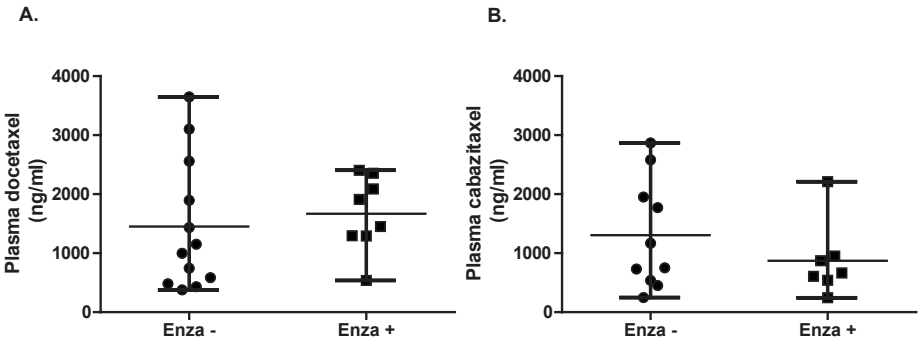

Figure 3. (A and B) Plasma concentrations 3 hours after intraperitoneal injection of docetaxel (33mg/kg) and cabazitaxel (33mg/kg) were measured in mice that were pre-treated with enzalutamide (60 mg/kg) and compared to mice that were non-pretreated. Plasma concentrations were measured using LC/MS/MS. Scatter plots including mean and range were used to represent the values. Differences between groups were evaluated using an unpaired t-test. Exact p-values are quoted in supplementary table 1. The absence of a star indicates that no statistical significant differences were observed.

Table 1. Plasma concentrations of docetaxel and cabazitaxel in mice.

	Enza-pretreated	*Non-pretreated*	*P-value (t-test)*	*Patient plasma levels [16, 17]**	*P-value as compared with mice*
Plasma docetaxel, Mean (SD) (ng/ml)	1667 (639)	1451 (1113)	0.63	2180 (170)	0.07
Plasma cabazitaxel, Mean (SD) (ng/ml)	871 (634)	1306 (931)	0.30	535 (305)	0.14

*Patient plasma levels were derived from corresponding phase I studies [16, 17]

While the expression of AR was similar among treatment groups (Fig. 4A-B), docetaxel was able to affect the downstream AR pathway by inhibiting intratumoral AR nuclear localization (Fig. 5A,C) and the AR target gene PSA (Fig. 4C) in PC346C tumors. In contrast, while expressing lower baseline levels, docetaxel did not inhibit AR nuclear localization and PSA expression as compared to placebo in PC346Enza tumors (Fig. 5A,C, and 4D), indicating a reduced anti-tumor activity via the AR pathway in these tumors. This impaired anti-AR effect in PC346Enza tumors was also observed for cabazitaxel (Fig. 5A and 4C-D) and enzalutamide (Fig. 4G-H). Although the effects of cabazitaxel via the AR were impaired in enzalutamide-resistant tumors, it demonstrated stronger antiproliferative properties compared to docetaxel as depicted in Ki67 staining (Fig. 5B-C), independent of the AR pathway.

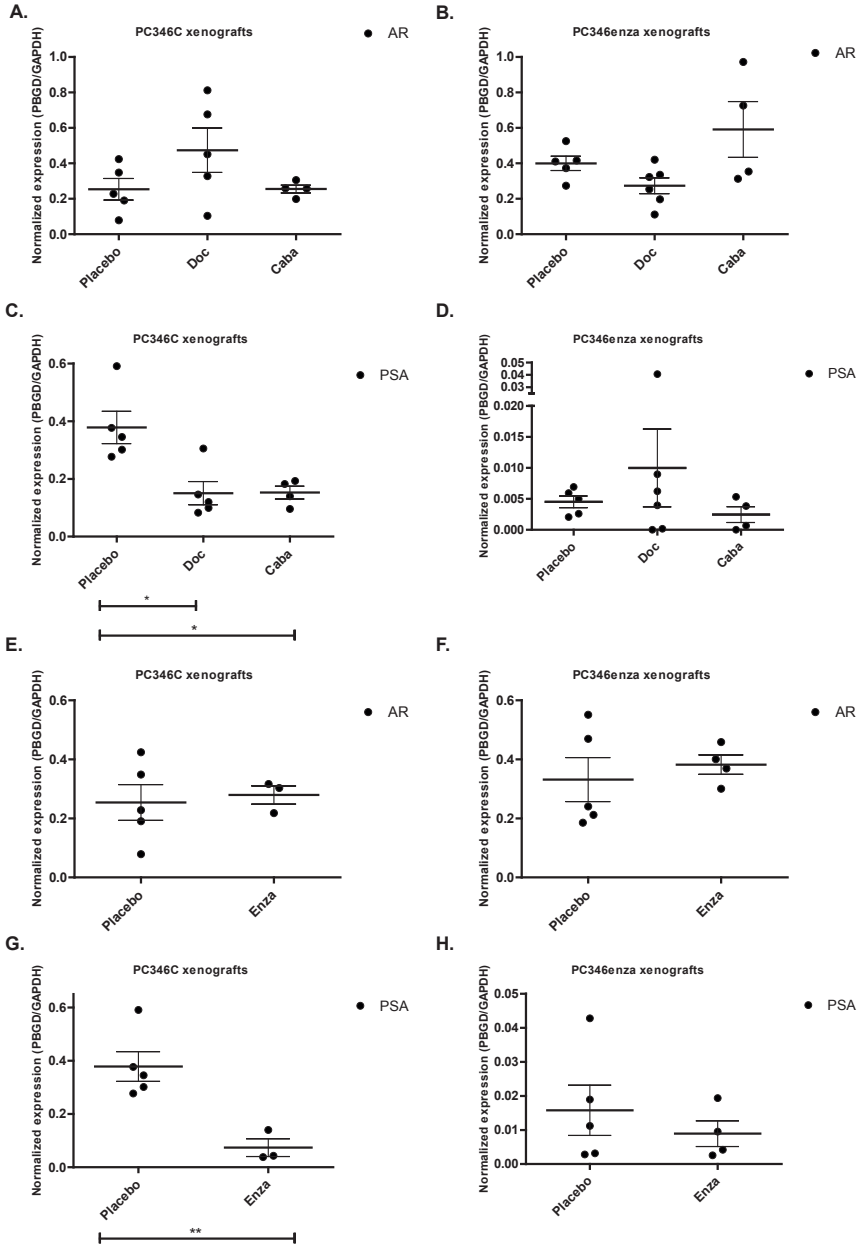

Figure 4. (A and B). Androgen receptor (AR) expression of enzalutamide-resistant PC346Enza tumors versus enzalutamide-naive PC346C tumors treated with docetaxel, cabazitaxel and placebo. **(C and D)** Expression of the downstream AR target gene PSA in PC346Enza versus PC346C tumors treated with docetaxel, cabazitaxel and placebo. (**E and F**) AR expression in PC346Enza versus PC346C tumors treated with enzalutamide and placebo. (**F and G**) Expression of the downstream AR target gene PSA in PC346Enza versus PC346C tumors treated with enzalutamide and placebo. RNA from the tumors was isolated and RT-PCR was performed as described in the supplementary methods. Gene expression was normalized against the average of two housekeeping genes (GAPDH and PBGD). Differences in gene expression were displayed using scatterplots including mean (+/- SEM) and tested using an unpaired t-test. (*) represents $p<0.05$, (**) represents $p<0.01$. The exact p-values are quoted for comparisons with borderline significance ($0.05<p<0.10$) and the absence of a star indicates $p>0.10$.

DISCUSSION

In this study, we present the first evidence for in vivo cross-resistance between docetaxel and enzalutamide in CRPC. We showed that docetaxel efficiently impaired AR nuclear localization and consequently AR signaling in enzalutamide-naïve tumors, while it did not in enzalutamide-resistant tumors. These results indicate that the inhibiting effects of docetaxel on the AR represent part of its antitumor activity, which is impaired by previous AR targeted therapy such as enzalutamide. In this light, it could also explain the reduced efficacy of docetaxel when used after abiraterone that was observed in retrospective clinical studies [4, 5]. Our findings are especially of interest with the increasing use of enzalutamide and abiraterone pre-chemotherapy.

In contrast to docetaxel, cabazitaxel demonstrated robust tumor and PSA responses in enzalutamide-resistant tumors, while the effects on AR signaling were reduced as compared to those in enzalutamide-naïve tumors. These observations indicate that cabazitaxel is less dependent on its inhibitory effects on the AR pathway, and exerts greater anti-tumor activity via AR independent mechanisms as compared to docetaxel. This is concordant with clinical observations [7, 8], and is probably caused by a higher potency of cabazitaxel to suppress microtubule dynamics as compared to docetaxel, with faster drug uptake and better intracellular retention[18]. This is further augmented by our observed lower Ki67 expression in enzalutamide-resistant tumors treated with cabazitaxel as compared to docetaxel, indicating stronger antiproliferative properties. The greater potency of cabazitaxel after AR-targeted treatment might have clinical implications, as currently docetaxel is the standard first-line chemotherapy for men with mCRPC. Considering the superior efficacy of cabazitaxel over docetaxel in enzalutamide-resistant tumors, our results provide a rationale for clinical studies comparing cabazitaxel with docetaxel in men with mCRPC who progressed on first-line enzalutamide or abiraterone.

In summary, we demonstrated that a reduced inhibition of the AR pathway by docetaxel in enzalutamide-resistant CRPC confers cross-resistance between these drugs in vivo. Cabazitaxel remained highly effective in enzalutamide-resistant tumors, demonstrating greater antiproliferative properties independent of the AR pathway. This merits further clinical evaluation of cross-resistance and the optimal treatment sequence for patients with mCRPC.

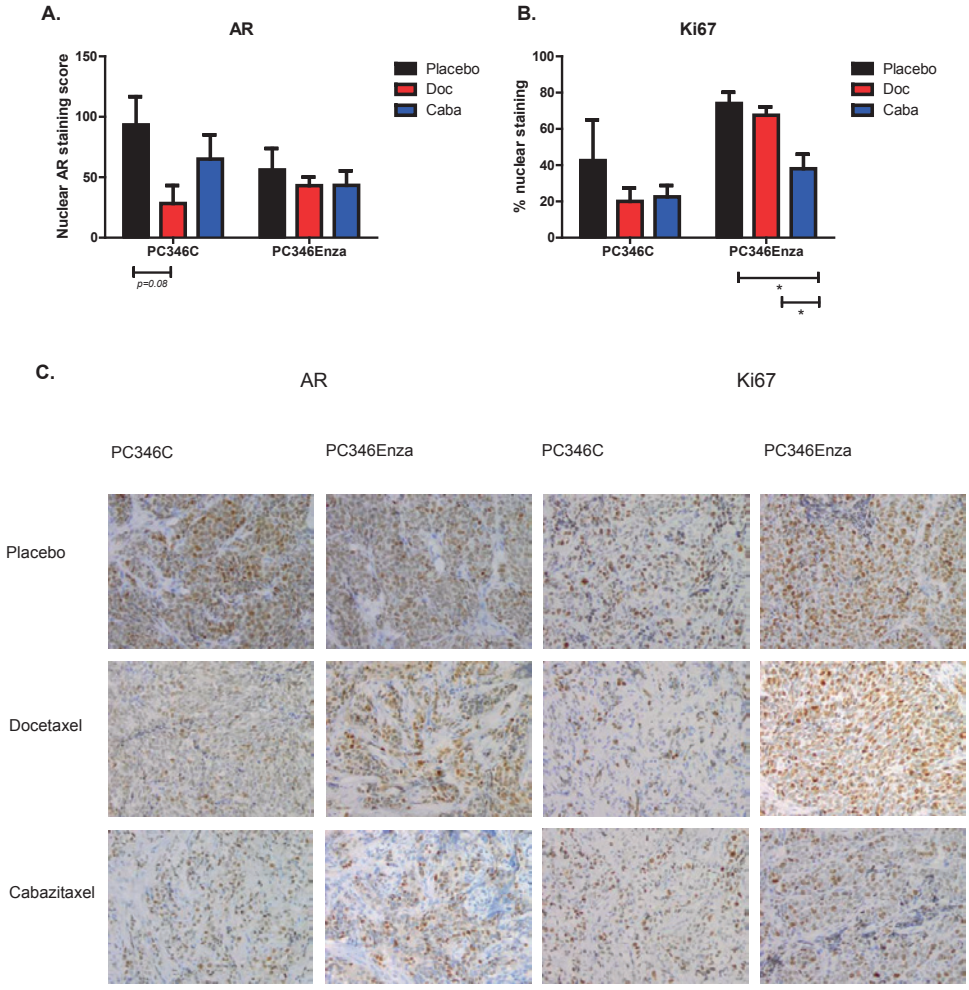

Figure 5. (**A and B**) AR nuclear localization and Ki-67 staining of enzalutamide-resistant PC346Enza tumors and the parental enzalutamide-naïve PC346C tumors. Immunostainings were scored by two readers, blinded for treatment and type of tumor. The score was composed using a sum of the nuclear AR score (0 for no stain, 1 for weak stain, and 2 for intense stain), each multiplied by the corresponding percentage of cells . Ki-67 score was calculated by estimating the percentage of positive cells in the whole tumor section. Differences in AR nuclear localization and Ki67 expression were tested using an unpaired t-test. (*) represents $p<0.05$, (**) represents $p<0.01$. The exact p-values are quoted for values with borderline significance ($0.05<p<0.10$), and the absence of a star indicates $p>0.10$.

(**C**) Representative pictures of AR nuclear localization and Ki67 staining in PC346Enza and PC346C tumors treated with docetaxel, cabazitaxel and placebo.

ACKNOWLEDGEMENTS

This work has been funded by a research grant from Sanofi. We thank Agnes Boer, Sander Hoeben and Debra Stuurman for their work and contributions to the in vivo experiments, Peter de Bruijn for the pharmacokinetic measurements, and Hans Stoop for optimizing the immunohistochemistry.

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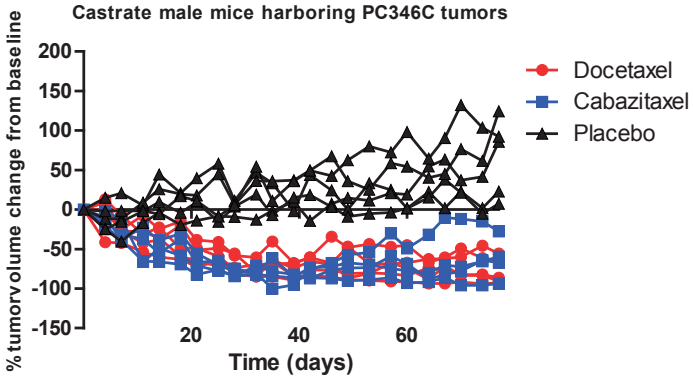
SUPPLEMENTARY FIGURES

A.



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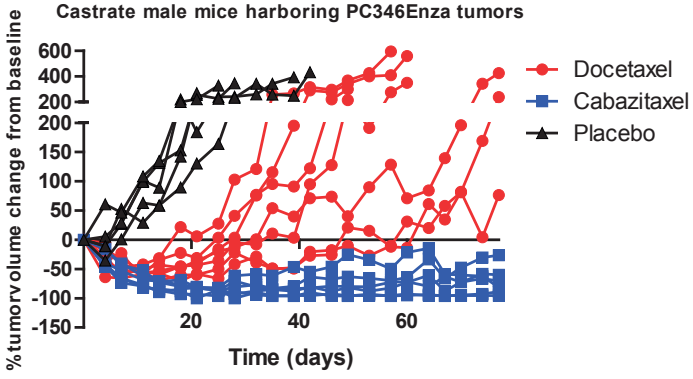

Supplementary figure 1. (A and B) Individual tumor growth curves over time in castrate mice bearing PC346C and PC346Enza tumors. Mice were treated with docetaxel (33 mg/kg), cabazitaxel (33 mg/kg) using a single intraperitoneal injection, or placebo.

THE INFLUENCE OF PRIOR NOVEL ANDROGEN RECEPTOR TARGETED THERAPY ON THE EFFICACY OF CABAZITAXEL IN MEN WITH METASTATIC CASTRATION-RESISTANT PROSTATE CANCER: DATA FROM A MULTICENTER RANDOMIZED PHASE II TRIAL

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*The first two authors contributed equally to this article

Manuscript submitted

CHAPTER 5

ABSTRACT

Background: The treatment armamentarium for metastatic castration-resistant prostate cancer (mCRPC) has expanded with the introduction of several new therapies. In this treatment continuum, it is unclear whether the efficacy of cabazitaxel is affected by prior novel androgen receptor targeted therapies (ART) abiraterone and enzalutamide.

Objective: We aimed to investigate the influence of prior ART on the efficacy of cabazitaxel in men with mCRPC.

Design, setting and participants: Data from an ongoing prospective, multicenter, randomized phase II trial (CABARESC) were used comprising 114 men with mCRPC treated with cabazitaxel (25 mg/m^2 every 3-weeks) plus prednisone in the post-docetaxel setting.

Outcome measurements and statistical analysis: The primary endpoints of this unplanned analysis were PSA response (≥50%), and overall survival (OS). Univariate and multivariable analyses were conducted to investigate the influence of prior ART on the efficacy of cabazitaxel as defined by OS and PSA response rates.

Results and limitations: From the 114 patients included in this analysis, 44 men received prior ART and 70 men did not receive prior ART before treatment with cabazitaxel. PSA response rates (≥50%) while on cabazitaxel treatment were similar in patients with and without prior ART (34% versus 40%, respectively, P=0.53). Likewise, median OS was not significantly different between men with and without prior ART (9.8 months versus 10.6 months, respectively, logrank P=0.65). In multivariable analysis, the only variables significantly associated with OS were performance score, alkaline phosphatase and albumin at baseline. This study is limited by its modest sample size.

Conclusion: Our study showed that prior treatment with ART did not influence the efficacy of cabazitaxel in men with mCRPC. With emerging evidence of cross-resistance between the currently available therapies in mCRPC, cabazitaxel provides a good treatment option irrespective of prior treatment.

Patient summary: Cabazitaxel efficacy seems not affected by prior treatment with abiraterone or enzalutamide.

INTRODUCTION

The treatment armamentarium for metastatic castration-resistant prostate cancer (mCRPC) has changed considerably over the past few years, with the introduction of several new drugs that provide substantial survival benefits [1-5]. Cabazitaxel, abiraterone and enzalutamide all demonstrated survival benefit in the post-docetaxel setting and subsequently became approved for the treatment of this disease [1, 2, 5]. Moreover, the novel androgen receptor (AR)-targeted therapies abiraterone and enzalutamide have shown survival improvement when used in the pre-docetaxel setting [1, 2, 5-7]. These advances also come with new challenges. At the current time, no established predictive biomarkers for the available treatments exist, and the optimal drug treatment sequence is still undetermined.

Retrospective series studies suggested that the overall survival benefits obtained by the new therapies cannot be simply added up, as cross-resistance between docetaxel and AR-targeted agents has been observed [8-10]. Reduced efficacy of docetaxel was observed in men with mCRPC who had previously been treated with abiraterone, suggesting clinical cross-resistance [8-10]. In addition, preclinical studies revealed that the androgen receptor (AR) is able to confer cross-resistance between enzalutamide and docetaxel in vivo, which is induced by an overlapping working mechanism on AR nuclear translocation [11, 12]. These findings raise concern whether prior treatment with abiraterone or enzalutamide may affect the efficacy of subsequent cabazitaxel treatment. Emerging preclinical and retrospective clinical data suggested that cabazitaxel, in contrast to docetaxel, has sustained efficacy in men with mCRPC after prior abiraterone treatment [13, 14]. In two retrospective studies, cabazitaxel efficacy after abiraterone treatment was investigated and compared to the TROPIC trial of cabazitaxel in abiraterone-naïve patients as an historical control group [2, 13, 14]. These studies suggested retained efficacy of cabazitaxel after prior abiraterone, as the observed PSA response rates were similar when compared to the TROPIC trial. However, to date, the efficacy of cabazitaxel has never been directly compared between patients with and without prior abiraterone or enzalutamide within the same study population, which hampers clinically meaningful conclusions. Here, we used data from a prospective, randomized, multicenter phase II study, to investigate the influence of prior AR-targeted therapies (ART) abiraterone and enzalutamide on the efficacy of cabazitaxel.

PATIENTS AND METHODS

Study population and data collection

CABARESC (Dutch Trial Registry number: NTR 2991, EudraCT number: 2011-003346-40) is an ongoing randomized, open-label, multicenter, phase II trial that was designed to investigate the effects of budesonide on cabazitaxel induced diarrhea. The primary endpoint of the original study was the incidence of grade 2-4 diarrhea. Eligible men were randomized to either cabazitaxel (25 mg/m^2) and prednisone (10 mg daily) plus oral budesonide (9 mg daily during 44 days), or standard cabazitaxel 25 mg/m^2 plus prednisone (10 mg daily). It has been shown previously that budesonide does not affect the pharmacokinectics of cabazitaxel [15].

Full inclusion and exclusion criteria of the CABARESC trial are shown in the Supplementary materials and methods. In brief, patients were eligible if they had mCRPC with documented disease progression during or after treatment with docetaxel, as defined by rising PSA levels, appearance of new lesions or documented disease progression based on CT scan or bone scan. Cabazitaxel treatment was continued until disease progression, unacceptable toxicity, or until 10 cycles have been administered. Patients were randomly assigned to the treatment groups through a centralized stratified randomization process using the following stratification factors: center, age (≥65 versus <65 years) and previous radiotherapy (yes versus no). In this study, data were prospectively collected at baseline and for every cycle including: hematology and biochemistry laboratory values, performance status, age, prior treatment with ART, duration of treatment with ART, PSA values, and survival status.

For the current unplanned analysis, we included patients from the ongoing CABARESC trial who were randomized before May 15 2014 and had a completed off-protocol form. As the CABARESC study is still recruiting, the primary endpoint of the original study (incidence of grade 3/4 diarrea) was not reported, and no data per arm were analyzed. The CABARESC study was approved by the institutional review board at each participating center. Written informed consent was obtained from all participants.

Data collection and definitions

The primary objective of the current analysis was to explore the influence of prior ART on the efficacy of cabazitaxel in men with mCRPC. Primary endpoints of this analysis were the proportion of patients with a ≥50% PSA response, and OS. As a secondary endpoint, we investigated PSA progression-free survival (PSA-PFS). For the definition of PSA response and PSA-PFS, Prostate Cancer Working Group 2 (PCWG2) criteria were used [16]. As recommended by the PCWG2, PSA response was defined as ≥50% decline from baseline, and PSA progression as a 25% increase (and a minimum of 2 ng/ml) from baseline or nadir.

In most cases this was confirmed by a second measurement; however confirmation was not routinely performed for all patients. OS was defined by time from randomization to death from any cause. Since bone scans and CT-scans were not performed according to regular intervals in the study protocol, we did not consider radiological PFS for our analyses.

Descriptive statistics were used to compare baseline characteristics in the ART pretreated versus non-pretreated patients, with statistical evaluation using Fisher's exact test for categorical variables and Kruskal Wallis test for continuous variables. OS and PSA-PFS were calculated using the Kaplan-Meier method with statistical evaluation by the logrank test.

Model building and statistical considerations

We conducted univariate and multivariable Cox regression analyses including prior treatment with ART (yes/no), and the duration of prior ART to investigate its effect on PSA response and OS of men treated with cabazitaxel. Cox proportional hazards models were constructed to adjust for known prognostic factors from the Halabi nomogram [17] including: baseline serum PSA, LDH, albumin, alkaline phosphatase, hemoglobin, and performance score. The multivariable model was constructed using backward elimination at the 5% level. A log transformation was applied to variables with a non-normal distribution.

RESULTS

Baseline characteristics

In the CABARESC trial, 141 patients who had a completed off-study form were randomized before May 15, 2014. Of these 141 patients, 27 men were excluded from analysis for the following reasons: 5 patients had missing PSA values at baseline, 9 patients were randomized but never received cabazitaxel treatment due to rapid worsening of performance status or death, and 13 patients had received previous study treatment with orteronel (Figure 1). Patients who received prior orteronel were excluded from this analysis since this is not a clinically approved regimen in the treatment of mCRPC. All patients had received prior docetaxel chemotherapy. Forty-four out of 114 patients (39%) had received prior ART in the post-docetaxel setting, of whom 41 had received abiraterone, 5 had received enzalutamide, and 2 had received both. The remaining 70 patients had received no prior ART before study treatment with cabazitaxel.

Baseline characteristics of the men with and without prior ART are shown in table 1. Known prognostic variables were evenly distributed among subgroups, except for a

significantly lower albumin level in men with prior ART (table 1). The median number of cabazitaxel cycles received was 6 in the ART group, and 5 for men without prior ART.

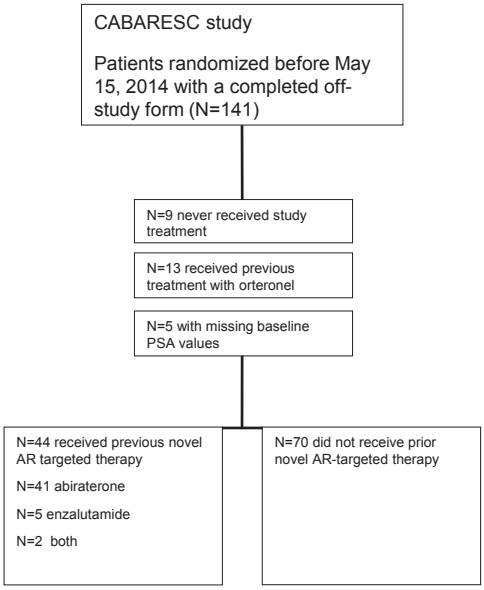


Figure 1. CONSORT diagram

The influence of prior novel AR-targeted therapy on the efficacy of cabazitaxe/

PSA response rates (≥50%) while on cabazitaxel treatment were similar in patients with and without prior ART (34% versus 40% respectively, P=0.53). Waterfall plots of the maximum PSA change while on cabazitaxel treatment for men with and without prior ART are shown in Fig. 2. Likewise, median PSA-PFS was not significantly different between the two groups. Men who received prior ART had a median PSA-PFS of 4.8 months, versus 6.5 months for men without prior ART (logrank P=0.32) (Fig. 3A). Median OS was similar for patients previously treated with ART versus patients who were not previously treated with ART, with a median OS of 9.8 versus 10.6 months respectively (logrank P=0.65) (Fig. 3B).

Univariable and multivariable analyses for OS and PSA response.

Factors significantly associated with OS in univariate analysis are shown in table 2 and included performance score, alkaline phosphatase, albumin, hemoglobin and LDH at baseline. Prior ART and the duration of prior ART were not significantly associated with OS (HR=1.14; 95%CI: 0.66-1.97, P=0.65 and HR=1.00; 95%CI: 0.92-1.09, P=0.98 respectively). From the significant variables in univariate analysis, a multivariate model for OS was constructed (table 2). The only variables significantly associated with OS in multivariable analysis were performance score, alkaline phosphatase and albumin at baseline. Univariate

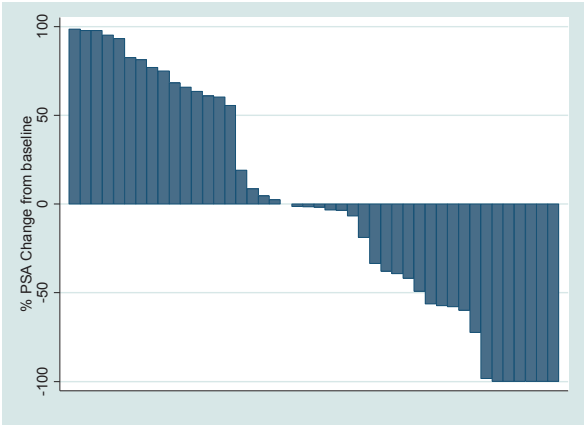
logistic regression analyses for PSA response (≥50%) are shown in Supplementary table 1. Prior ART or the duration of prior ART were not significantly associated with PSA response. Baseline hemoglobin was the only variable that was significantly associated with PSA response.

Table 1. Baseline characteristics of men with and without prior novel AR targeted therapy.

Characteristic	**Prior abiraterone or enzalutamide**	**No prior abiraterone or enzalutamide**	***P*-value**
Number of patients	44	70	
Age, years, median (range)	69 (53-83)	68 (49-82)	0.093
WHO performance score n (%)			
0	18 (41)	25 (36)	0.56
1	25 (57)	44 (63)	
Missing	1	1	
PSA, ng/ml, median (range)	210 (15-5000)	154 (12.5-4172)	0.25
LDH at baseline, median (range)	287 (90-724)	273 (38-1843)	0.83
Hemoglobin at baseline, mmol/L, median (range)	8 (6-10)	8 (5-9)	0.96
Alkaline phosphatase at baseline, IU/L, median (range)	124 (50-907)	126 (43-1023)	0.83
Albumin at baseline, g/L, median (range)	37 (26-46)	41 (25-49)	0.013
Duration of treatment with abiraterone/enzalutamide, months, median (range)	6.1 (0.9-22)	-	

PSA - prostate-specific antigen; LDH - Lactate Dehydrogenase

A.



B.

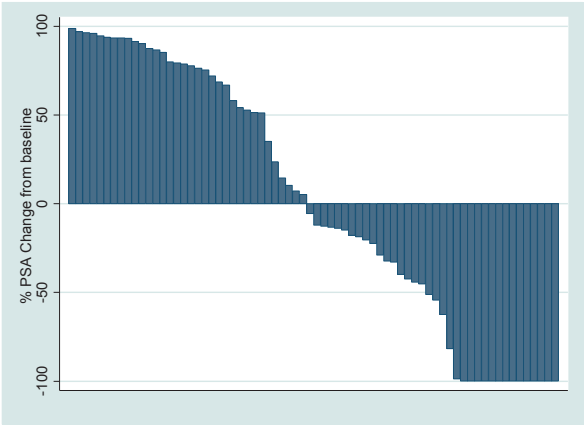


Figure 2. waterfall plots of the maximum PSA change from baseline during treatment with cabazitaxel in men with (A) and without (B) prior novel AR-targeted therapy

DISCUSSION

In this study, we demonstrated that there was no influence of prior ART on the efficacy of cabazitaxel. PSA response rates and OS were similar between patients with and without prior abiraterone or enzalutamide.

We used data from a prospective randomized phase II study to directly compare the efficacy of cabazitaxel in men who were pretreated with ART versus men who were non-pretreated. To date, only two retrospective studies have been published that investigated the response of patients treated with cabazitaxel after abiraterone, which compared their findings to historic controls [13, 14]. To our knowledge, we are the first to directly compare the efficacy of cabazitaxel between patients with and without prior ART within the same

study population, using prospective trial data.

Our observed PSA response rates in ART pretreated men (36%) are in line with those reported by Pezaro et al. and Al Nakouzi et al., which ranged from 40-45% [13, 14]. These findings are concordant with the TROPIC trial of cabazitaxel in ART-naïve men, and with the ART-naïve patients in the current analysis [2].

Table 2. Univariate and multivariable analyses for OS

Variable	Univariate Hazard Ratio (95% CI)	*P*-Value	Multivariable Hazard Ratio (95% CI)	*P*-Value
Age (≥ median)	1.01 (0.97-1.05)	0.71		
WHO performance score (1 vs. 0)	1.83 (1.01-3.32)	0.039	2.23 (1.06-4.69)	0.035
Hemoglobin at baseline	0.68 (0.53-0.88)	0.005	0.88 (0.63-1.24)	0.47
PSA at baseline	1.12 (0.92-1.37)	0.26		
Alkaline phosphatase at baseline	1.84 (1.31-2.60)	<0.001	1.65 (1.06-2.57)	0.026
LDH at baseline	1.69 (1.02-2.81)	0.049	0.74 (0.42-1.29)	0.29
Albumin at baseline	0.90 (0.86-0.95)	<0.001	0.87 (0.81-0.92)	<0.001
Prior novel AR-targeted therapy (yes/no)	1.14 (0.66-1.97)	0.65		
Duration of prior AR-targeted therapy	1.00 (0.92-1.09)	0.98		

PSA - prostate-specific antigen; LDH - Lactate Dehydrogenase; AR – Androgen receptor

Taken together, these findings strongly suggest that there is no cross-resistance between abiraterone or enzalutamide and cabazitaxel. These results are especially of interest, since an increasing number of reports have suggested an impaired efficacy of docetaxel after abiraterone, indicating cross-resistance with ART for this taxane [8-10]. These clinical findings are supported by preclinical findings from our group that showed an overlapping working mechanism on AR-nuclear translocation for both enzalutamide and docetaxel [11, 12]. In preclinical studies, this overlapping, AR-mediated, mechanism of action was able to confer cross-resistance between these drugs in vivo. Interestingly, in this preclinical model, cross-resistance was not observed for cabazitaxel, that demonstrated

sustained antitumor activity even in tumors previously treated with enzalutamide [11]. In the current analysis we clinically confirmed the findings from the reported preclinical studies, showing similar activity of cabazitaxel when delivered either before or after ART. An explanation for the lack of cross-resistance for cabazitaxel could be that cabazitaxel, in contrast to docetaxel, is less dependent on the AR for exerting its anti-tumor activity [11]. Moreover, it has been shown that cabazitaxel supresses microtubule dynamics more potently as compared with docetaxel, with higher intratumoral concentrations [18].

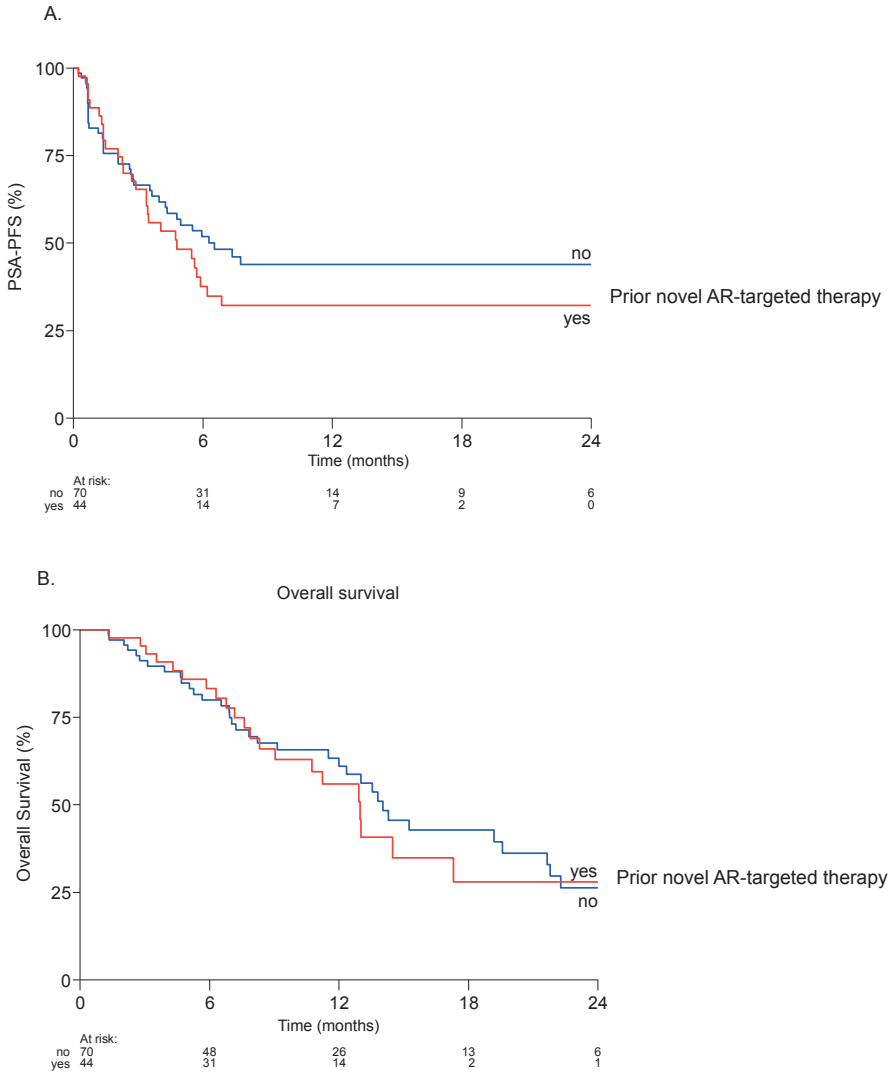


Figure 3. Kaplan-Meier estimates of PSA-PFS (A) and OS (B) in men treated with cabazitaxel with and without prior novel AR-targeted therapy

The main strength of our study is the use of prospective trial data to directly compare the efficacy of cabazitaxel in patients who did and did not receive prior ART within the same study population. Since all patients were prospectively enrolled using the same inclusion and exclusion criteria, this is a unique study enabling a direct comparison of the influence of prior ART on the efficacy of cabazitaxel. A limitation of the study is the modest sample size of 114 patients. Also, as an inherent limitation, the original CABARESC study was not designed for the aim of the current unplanned analysis and therefore had a different primary endpoint.

CONCLUSIONS

In conclusion, our study showed that prior treatment with ART did not influence the efficacy of cabazitaxel in men with mCRPC. With emerging evidence of cross-resistance between the currently available therapies in mCRPC, cabazitaxel provides a good treatment option both before and after novel AR-targeted therapies in the post-docetaxel setting.

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SUPPLEMENTARY TABLES

Supplementary table 1. Univariate logistic regression analysis for PSA response (≥50%)

	Univariate	
Variable	**Odds ratio (95% CI)**	***P*-Value**
Age	1.03 (0.98-1.09)	0.26
WHO performance score (1 vs. 0)	0.58 (0.26-1.25)	0.17
Hemoglobin at baseline	1.63 (1.06-2.50)	0.026
PSA at baseline	1.17 (0.88-1.56)	0.29
Alkaline phosphatase at baseline	0.64 (0.37-1.09)	0.11
LDH at baseline	0.68 (0.34-1.35)	0.27
Albumin at baseline	1.06 (0.99-1.14)	0.074
Prior novel AR-targeted therapy (yes/no)	0.78 (0.35-1.70)	0.53
Duration of prior AR-targeted therapy	1.00 (0.88-1.13)	0.98

PSA - prostate-specific antigen; LDH – Lactate Dehydrogenase; AR – Androgen receptor

SUPPLEMENTARY MATERIALS AND METHODS

Inclusion criteria

Metastatic castrate resistant prostate cancer (mCRPC) patients with documented disease progression, defined as:

documented rising PSA levels (at least 2 consecutive rises in PSA over a reference value taken at least 1 week apart, or a PSA rise of ≥ 2.0 µg/l), appearance of new lesions or documented disease progression based on CT scan or bone scan.

Previous treatment with a docetaxel-containing regimen

Age ≤ 18 years;

WHO performance status ≤ 1

Adequate renal function (within 21 days before randomization) defined as serum creatinin ≤ 1.5 x ULN and/or calculated creatinin clearance ≥ 50ml/min, according to MDRD formula.

Adequate hepatic functions (within 21 days before randomization) defined as: total bilirubin ≤ 1.0 x ULN; alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) ≤2.5x ULN, in case of liver metastasis < 5 ULN; alkaline phosphatase (AF) < 5x ULN) In case of bone metastasis, AF < 10x ULN is accepted;

Adequate hematological blood counts (within 21 days before randomization) defined as (absolute neutrophil count (ANC) ≥1.5 x 109/L and platelets ≥ 100 x 109/L);

Castration, either surgically or by continued LHRH agonist therapy

Written informed consent according to ICH-GCP

Exclusion criteria

Impossibility or unwillingness to take oral drugs;

Serious illness or medical unstable condition requiring treatment, brain metastases or history of psychiatric disorder that would prohibit the understanding and giving of informed consent;

Use of medications or dietary supplements known to induce or inhibit CYP3A

Known hypersensitivity to corticosteroids

Any active systemic or local bacterial, viral, fungal - or yeast infection.

Ulcerative colitis, Crohn's disease or celiac disease (active or in medical history)

Ostomy

Planned/active simultaneous yellow fever vaccine

Geographical, psychological or other non-medical conditions interfering with follow-up

THE INITIAL BIOPSY GLEASON SCORE AS A PREDICTIVE MARKER FOR SURVIVAL BENEFIT IN PATIENTS WITH CASTRATION-RESISTANT PROSTATE CANCER TREATED WITH DOCETAXEL: DATA FROM THE TAX327 STUDY

Robert J. van Soest, Ellen S. de Morrée, Liji Shen, Ian F. Tannock, Mario A. Eisenberger, and Ronald de Wit

Eur Urol 2014;66(2):330-6

CHAPTER 6

ABSTRACT

Background: Since 2004 docetaxel has been the standard first-line systemic therapy for patients with metastatic castration-resistant prostate cancer (mCRPC). With abiraterone recently becoming available in the pre-docetaxel setting, it is warranted to identify subgroups of patients who may obtain the greatest benefit from docetaxel and particularly qualify for receiving docetaxel as first-line treatment for mCRPC.

Objective: We aimed to identify factors that could characterize subgroups of patients who obtain the greatest benefit from the use of docetaxel.

Design, setting and participants: TAX327 was multinational randomized phase 3 study that was conducted from 2000 to 2002 in 1006 men with mCRPC.

Intervention: Patients were randomized to receive 3-weekly docetaxel (D3), weekly docetaxel (D1) or 3-weekly mitoxantrone (M), each with prednisone.

Outcome measurements and statistical analysis: We investigated whether patients with poorly differentiated tumors (Gleason ≥7) at diagnosis had greater benefit from D3 as compared to M, than patients with better differentiated tumors (Gleason ≤6). Using a Cox model, we compared overall survival (OS), between the treatment groups within each subgroup of Gleason score.

Results and limitations: The TAX 327 data showed that the OS benefit of D3 versus M is greater in patients with high grade tumors (median OS 18.9 vs 14.5 months, p=0.009) than in patients with low grade tumors (median OS 21.6 vs 20.7 months, p=0.674). Limitations of a retrospective analysis apply.

Conclusions: The survival benefit obtained with docetaxel is most pronounced in patients with high Gleason score tumors (Gleason ≥7). In a time of shifting paradigms in mCRPC with abiraterone becoming available prior to docetaxel chemotherapy, Gleason score may help in selecting patients who obtain the greatest benefit from docetaxel as first-line treatment for mCRPC. Prospective validation of these findings is warranted.

INTRODUCTION

Treatment options for patients with metastatic castration-resistant prostate cancer (mCRPC) have expanded in recent years with the introduction of new agents like cabazitaxel, abiraterone, and enzalutamide. The current standard first line chemotherapeutic agent docetaxel has shown survival benefit as well as palliative benefit in the TAX327 and the Southwest Oncology Group 99-16 studies [1, 2]. TAX327 was conducted in 1006 men with metastatic castration-resistant prostate cancer who were randomized to receive 3-weekly docetaxel (D3), weekly docetaxel (D1) or 3-weekly mitoxantrone (M), each with prednisone. Overall survival (OS) of patients who were treated with D3 was superior as compared to M with an OS benefit of 2.9 months in the final analysis [3]. The D3 arm also showed better palliation, with more patients having a pain and quality of life response as compared to the M arm. Neutropenia was the most commonly observed grade 3 or 4 adverse event and occurred more frequently in patients receiving D3 (32%).

Recently the COU-AA-302 trial demonstrated superior radiological progression-free survival and a trend towards improved OS for abiraterone in docetaxel-naïve mCRPC patients, as compared with prednisone alone [4]. Based on this trial, the US Federal Food and Drug Administration (FDA) and the European Medicines Agency (EMA) lent approval to the use of abiraterone in patients with metastatic mCRPC prior to docetaxel chemotherapy. With new therapies available in the pre-docetaxel setting it is warranted to identify subgroups of patients who may respond better to one of the treatment options in order to better tailor therapy.

Gleason score is one of the strongest predictors of prostate cancer mortality in men with localized disease [5, 6]. Men with poorly differentiated localized tumors (Gleason 7-10) have a high probability of dying from prostate cancer within 10-years of diagnosis when treated conservatively [5]. High biopsy Gleason scores are observed in 9% of patients diagnosed with localized prostate cancer [7]. In patients progressing to mCRPC this proportion is higher, ranging from 25 to 30% of chemotherapy-naive patients, to around 52% in men progressing after docetaxel chemotherapy [1, 8, 9]. Recently, a high Gleason score (8-10) at the time of diagnosis was reported to be an independent risk factor for poor response to abiraterone [10, 11]. For patients treated with the second-line taxane cabazitaxel poorly differentiated tumors have been associated with pronounced benefits in terms of progression-free survival (PFS) and OS [12, 13]. In this post-hoc analysis of the TAX327 study we aimed to investigate whether Gleason score at initial diagnose could characterize a subgroup of patients who obtain the greatest benefit from D3 treatment as compared to M.

PATIENTS AND METHODS

Subjects and treatment

TAX327 was a randomized, non-blinded, phase III study, involving 1006 men with mCRPC, and was conducted in 24 countries. Full details of the trial are provided in the original report [1]. Briefly, patients were eligible if they had metastatic adenocarcinoma of the prostate, castrate levels of serum testosterone (<50 nl/ml), and disease progression during hormonal therapy defined as clinically or radiographically measurable disease or by PSA (prostate-specific antigen) criteria. No prior treatment with chemotherapeutic agents other than estramustine was allowed. Participants were randomized to receive 3-weekly docetaxel (75 mg/m^2), weekly docetaxel (30 mg/m^2) or 3-weekly mitoxantrone (12.5 mg/m^2), each with prednisone 5 mg twice daily. Baseline data were obtained for D3 and M in subgroups of Gleason score ≥7 and Gleason score ≤6 at diagnosis. Baseline information collected on each individual included PSA, age, performance status, pain score, hemoglobin (Hb), alkaline phosphatase, prior treatments, time from first hormonal treatment to start of study drug, time from diagnosis to start of study drug, the presence of visceral disease, and the proportion of patients with bone metastases. Treatment was planned for 30 weeks in the absence of progression. The primary end point of the study was OS. The study was conducted from March 2000 through June 2002 and was approved by an institutional review board at every participating institution. Written informed consent was obtained from all participants.

PSA response

Serum PSA was measured at baseline and every three weeks during treatment. PSA decline was defined as a reduction of at least 30 percent or 50 percent from baseline that was maintained for at least three weeks.

Statistical analyses

The TAX327 database was used to investigate if patients with poorly differentiated tumors (Gleason ≥7) at diagnosis had greater benefit from D3 as compared to M, compared to patients with better differentiated tumors (Gleason ≤6). To test whether there were differences in baseline variables known to predict OS between D3 and M in the Gleason score subgroups, we used Pearson Chi-square test for all categorical variables, and a two sample t-test with Cochran and Cox approximation for all continued variables. OS for D3 and M in the Gleason subgroups was assessed using the Kaplan-Meier method and tested by a logrank test. To correct for baseline variables, OS between the treatment groups was further tested by a Cox regression analysis stratified by each single prognostic factor. A Cox regression without stratification was also performed.

PSA response was evaluated for D3 and M in the subgroups of Gleason score ≥7 and Gleason score ≤6. Fisher's exact test was used to compare PSA response for D3 and M within the Gleason score subgroups.

RESULTS

Baseline characteristics

Biopsy Gleason scores were available from 482 of 672 patients who received either D3 or M. We identified 349 patients with a biopsy Gleason score of ≥7, of which 185 were in the D3 arm, and 164 in the M arm. There were 133 patients with a biopsy Gleason score of ≤6, of which 62 in the D3 arm, and 71 in the M arm. Baseline characteristics of the Gleason score subgroups are listed in Table 1 and were well balanced between patient groups, except for an imbalance in the group of patients with an impaired Karnofsky performance score (PS) (14.6% for D3 vs 7.6% for M, p=0.034), and the proportion of patients with bone metastases (89.7% for D3 vs. 96.3 % for M, p=0.021).

Overall survival in the Gleason score subgroups

The analysis showed that the OS benefit of D3 versus M is greater in patients diagnosed with a biopsy Gleason score ≥7 (logrank test p=0.009, median OS 18.9 vs 14.5 months), compared to patients diagnosed with a biopsy Gleason score ≤6 (logrank test p=0.674, median OS 21.6 vs 20.7 months). Survival curves are shown in Figure 1 and details are listed in Table 2. Hazard ratios (HR) for the comparison between treatment groups were 0.69 (CI 0.52-0.91) for D3 versus M in the Gleason ≥7 subgroup and 0.90 (CI 0.56 – 1.46) for D3 versus M in the Gleason ≤6 subgroup.

To correct for baseline variables, we performed a stratified Cox regression analysis on treatment effect as assessed by OS for the Gleason subgroups, with each single stratified prognostic factor. When stratified for every baseline variable known to predict OS, p-values for treatment effect on OS in the Gleason score ≥7 subgroup remained significant (Table 3).

Table 1. Baseline characteristics for D3 and M in the Gleason score subgroups.

	Gleason 7-10		Gleason 2-6	
	D3	**M**	**D3**	**M**
Number of patients	185	164	62	71
Age, years, median (range)	67 (42-92)	68 (43-83)	67 (49-86)	69 (45-86)
p-value	0.825		0.667	
Visceral disease, n (%)	41 (22.2)	39 (23.8)	12 (19.4)	13 (18.3)
p-value	0.720		0.878	
Karnofsky performance score ≤70 n (%)	14 (7.6)	24 (14.6)	10 (16.1)	10 (14.1)
p-value	0.034		0.742	
PSA, ng/ml, median (range)	92 (0-40740)	144 (3-8022)	87 (4-2259)	113 (0-5720)
p-value	0.444		0.163	
Time from first hormonal treatment to start study drug, years, n (%)				
<2.5 years	77 (41.6)	71 (43.3)	22 (35.5)	20 (28.2)
≥2.5 years	68 (36.7)	60 (36.6)	29 (46.7)	39 (54.9)
missing	40 (21.6)	33 (20.1)	11 (17.7)	12 (16.9)
p-value	0.920		0.806	
Pain at baseline n (%)	85 (45.9)	81 (49.4)	28 (45.2)	35 (49.3)
p-value	0.451		0.697	
Hb at baseline, g/dl, median (range)	12.7 (8.9-16.2)	12.7 (8.6-16)	13 (6.4-16.2)	12.6 (9-15.7)
p-value	0.905		0.292	
Alkaline phosphatase at baseline, IU/L, median (range)	191 (26-4438)	207 (51-6075)	176 (59-9900)	172 (18-5005)
p-value	0.826		0.721	
Prior prostatectomy, n (%)	32 (17.3)	27 (16.5)	19 (30.6)	21 (29.6)
p-value	0.836		0.893	
Prior radiotherapy, n (%)	100 (54.1)	87 (53.0)	39 (62.9)	42 (59.2)
p-value	0.851		0.659	
Bone metastases, n(%)	166 (89.7)	158 (96.3)	56 (90.3)	63 (88.7)
p-value	0.017		0.766	
Time from diagnosis to start study drug, median (range)	39.0 (2.8-197.5)	36.6 (2.3-163.6)	60.3 (8.6-234.6)	62.8 (6.6-146.3)
p-value	0.716		0.394	

D3 = Docetaxel 3-weekly; M = Mitoxantrone 3-weekly; PSA = Prostate-specific antigen; Hb = Hemoglobin

PSA declines in the Gleason score subgroups

Clinical benefit of D3 versus M as assessed by the proportion of patients with a ≥ 30% PSA decline was higher in patients with Gleason score ≥7 (71.3% for D3 vs 51.3% for M, p<0.001), as compared to patients with Gleason score ≤6 (67.7% for D3 vs 56.3% for M P=0.212) (Table 4). Likewise, the effects of D3 versus M as assessed by the proportion of patients with a ≥ 50% PSA decline was also higher in the subgroup with Gleason score ≥7 (61.9% for D3 vs 41.9% for M, p<0.001), as compared to the subgroup with Gleason score ≤6 (58.1% for D3 vs 47.9% for M, p=0.297).

Table 2. Median overall survival for D3 and M in the Gleason score subgroups.

	Gleason 7-10		Gleason 2-6	
	D3	**M**	**D3**	**M**
Number of patients	185	164	62	71
Median OS, months	18.9	14.5	21.6	20.7
(95% CI)	(16.7 - 21.2)	(12.6 -16.5)	(16.8 – 23.7)	(17.1 – 23.5)
P-value (logrank test)	0.009		0.674	

D3 = Docetaxel 3-weekly; M = Mitoxantrone 3-weekly; OS = Overall survival; CI = Confidence interval

DISCUSSION

In this post-hoc analysis of the TAX327 study, we found that the survival benefit obtained with docetaxel as compared to mitoxantrone is greatest in patients with high Gleason score tumors (Gleason 7-10) at diagnosis. In addition, the PSA response rates for patients treated with docetaxel, as compared to mitoxantrone were higher in patients with high Gleason score tumors.

To our knowledge, we are the first to demonstrate the pronounced benefit of docetaxel in high Gleason score tumors. Our results are mirrored by findings from the TROPIC-trial, which was conducted in patients with mCRPC who progressed after docetaxel chemotherapy and were randomized to cabazitaxel plus prednisone (CP) or mitoxantrone plus prednisone (MP) [14]. In that study the OS benefit was 2.4 months superior for CP as compared to MP. In a recent post-hoc analysis a significant OS benefit for cabazitaxel versus mitoxantrone was also linked to patients with poorly differentiated tumors evaluated by WHO grade (median OS 15.2 months vs 12.7 months, p<0.0001), whereas for patients with well or moderately differentiated tumors this benefit was less robust, with a median OS of 15.5 months for cabazitaxel and 13.3 months for mitoxantrone (p=0.56) [12].

In the present study, more pronounced effects of docetaxel in patients diagnosed with high Gleason score tumors were demonstrated by both OS benefit and PSA response rate. Interestingly this reflects findings of an earlier report on the TAX327 trail by Armstrong

et al., which demonstrated a PSA-decline of ≥ 30% within 3 months of chemotherapy initiation to have the highest degree of surrogacy for OS [15].

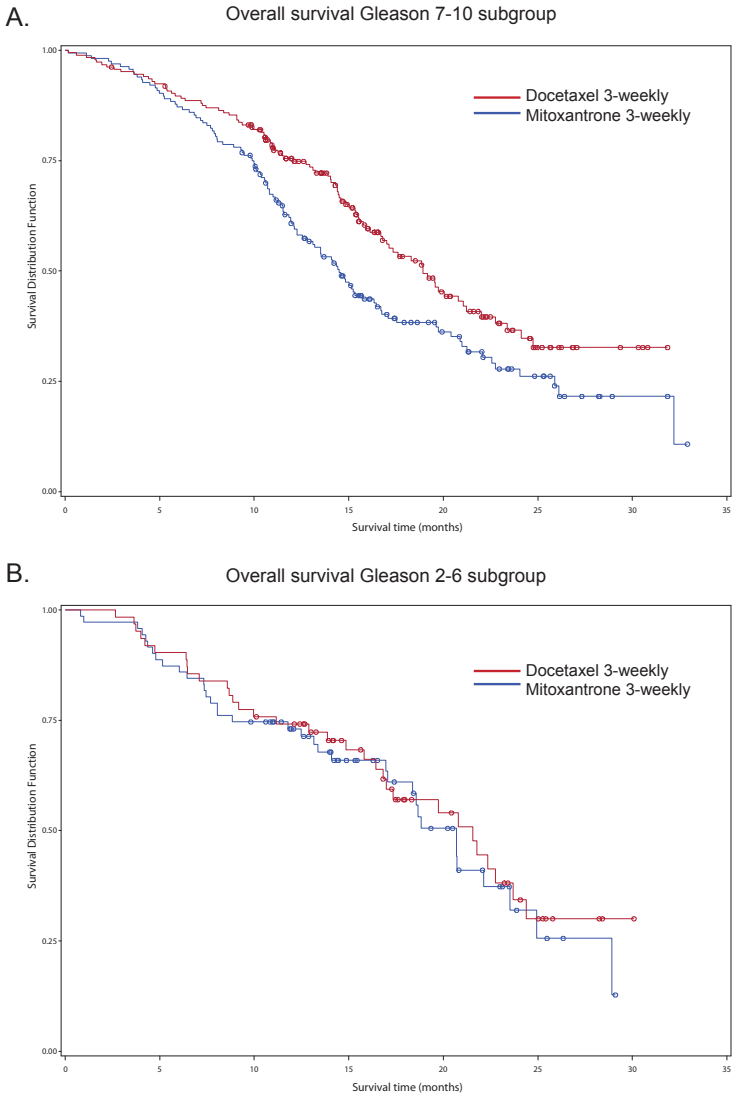

Figure 1. Kaplan-Meier estimates of overall survival for the D3 and the M arm in the subgroups of Gleason score 7-10 (A) and Gleason score 2-6 (B).

Table 3. Stratified Cox regression analysis on treatment effect assessed by OS with each single stratified prognostic factor.

	OS D3 vs M Gleason 7-10		OS D3 vs M Gleason 2-6	
Stratified baseline prognostic factors	***P*-value**	**Hazard ratio (95% CI)**	***P*-value**	**Hazard ratio (95% CI)**
Without stratification	0.009	0.69 (0.52-0.91)	0.675	0.9 (0.56-1.46)
Age	0.008	0.68 (0.51-0.91)	0.654	0.9 (0.55-1.45)
Visceral disease	0.008	0.68 (0.52-0.91)	0.508	0.85 (0.52-1.38)
Bone metastases	0.020	0.72 (0.54-0.95)	0.693	0.91 (0.56-1.47)
Karnofsky performance score	0.014	0.7 (0.52-0.93)	0.625	0.89 (0.55-1.44)
PSA	0.002	0.64 (0.48-0.85)	0.776	0.93 (0.57-1.51)
Time from first hormonal treatment to start study drug	0.006	0.64 (0.47-0.88)	0.983	0.99 (0.57-1.74)
Pain	0.015	0.7 (0.53-0.93)	0.966	1.01 (0.62-1.64)
Hb	0.009	0.69 (0.52-0.91)	0.625	0.89 (0.55-1.44)
Alkaline phosphatase	0.002	0.63 (0.48-0.85)	0.667	0.9 (0.56-1.45)

D3 = Docetaxel 3-weekly; M = Mitoxantrone 3-weekly; CI = Confidence interval; PSA = Prostate-specific antigen; Hb = Hemoglobin

The observations that taxanes have high antitumor activity in patients with high Gleason score tumors are especially of interest in the light of potential contrasting findings with the new generation hormonal agent abiraterone. In contrast with the findings for docetaxel and cabazitaxel, high Gleason score (8-10) at the time of diagnosis seems to be an independent risk factor for poor response to abiraterone (OR 0.60, 95%CI 0.39-0.85) [10, 11]. This finding points towards a modest efficacy of abiraterone in poorly differentiated tumors. With new compounds for the treatment of mCRPC becoming available for clinical use, and the recent FDA and EMA approval of abiraterone in the pre-docetaxel setting, treatment selection for individual patients becomes increasingly challenging. The observation in our study that docetaxel has the most pronounced anti-tumor activity in patients with high Gleason score tumors may provide additional guidance in treatment decisions regarding the use of docetaxel chemotherapy as first line treatment for patients with mCRPC, especially for those patients with high Gleason score tumors.

Table 4. PSA response for D3 and M in the Gleason score subgroups

	Gleason 7-10		**Gleason 2-6**	
	D3	**M**	**D3**	**M**
Number of patients	185	164	62	71
Data missing (n)	4	4	0	0
≥ 30% PSA decline, n(%)	129 (71.3)	82 (51.3)	42 (67.7)	40 (56.3)
p-value	<0.001		0.212	
≥ 50% PSA decline, n(%)	112 (61.9)	67 (41.9)	36 (58.1)	34 (47.9)
p-value	<0.001		0.297	

D3 = Docetaxel 3-weekly; M = Mitoxantrone 3-weekly; PSA = Prostate-specific antigen

A limitation of the current study is that the revision of the Gleason grading at the 2005 International Society of Urological Pathology (ISUP) consensus conference led to a grade migration or upgrading, both in needle biopsies and radical prostatectomy specimens [16, 17]. Billis et al. reported a change from Gleason 5–6 to group 7 and Gleason 5–6 to 8–10 in 15.7 and 0.6% of biopsies respectively using the revised Gleason grading compared to the Gleason grading before 2005 [18]. These data indicate that our results might not be applicable to a small proportion of patients (16.3%) with Gleason score 7-10 nowadays, who might have had a Gleason score 2-6 the TAX327 era. However although based on the Gleason grading before 2005, we demonstrate a very strong relation between clinical benefit obtained by docetaxel and tumor differentiation in a pivotal phase III trial that led

to the approval of this drug, which is the largest database available to address the current clinical question. Taking into account the retrospective nature of the analysis, our findings should be prospectively validated in the current setting to evaluate the implications for individual treatment decisions. With prospective studies to personalize treatment choices for the current treatment options in mCRPC still lacking, our findings are important to take into account when qualifying patients for treatment with docetaxel.

Greater benefit from docetaxel in patients with high-grade tumors might be caused by a higher proliferation rate of these tumors. Multiple reports have found a positive correlation between Gleason score and the proliferation marker Ki-67 [19-21]. In breast cancer, clinical benefit from docetaxel-based chemotherapy has been consistently higher in tumors with high Ki-67 expression [22-24]. Moreover, poor histological grade in locally advanced breast cancer proved to be predictive of pathological complete response to chemotherapy containing docetaxel in a neoadjuvant setting [25]. The relationship between markers such as Ki-i67 and clinical benefit from docetaxel in prostate cancer still needs to be elucidated.

CONCLUSIONS

In conclusion, in the setting of mCRPC, the survival benefit obtained with docetaxel as compared to mitoxantrone is most pronounced in patients with poorly differentiated tumors (Gleason 7-10). In an era of shifting paradigms in mCRPC with abiraterone becoming available also prior to docetaxel chemotherapy, Gleason score may serve to discriminate between patients who benefit most from docetaxel chemotherapy as first-line treatment, which seems to exert efficacy particularly in high Gleason score tumors, or patients with better differentiated tumors in whom treatment with abiraterone might be more beneficial. Prospective validation of the implications for the choice of first-line treatment in mCRPC is warranted.

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NEUTROPHIL TO LYMPHOCYTE RATIO AS A PROGNOSTIC BIOMARKER FOR MEN WITH METASTATIC CASTRATION-RESISTANT PROSTATE CANCER RECEIVING FIRST-LINE CHEMOTHERAPY: DATA FROM TWO RANDOMIZED PHASE III TRIALS

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CHAPTER 7

ABSTRACT

Background: The neutrophil to lymphocyte ratio (NLR), a marker of host inflammation, has been associated with poor outcome in several solid tumors. Here, we investigated associations of the derived NLR (dNLR) and duration of initial androgen deprivation therapy (ADT) with survival of men with metastatic castration-resistant prostate cancer (mCRPC) receiving first-line chemotherapy.

Patients and methods: Data from the multinational randomized phase III studies VENICE and TAX327 included a total of 2230 men with mCRPC randomized to receive first-line chemotherapy, and were used as training and validation sets respectively. Associations of dNLR and duration of initial ADT with OS were evaluated by multivariable Cox regression analysis in the training set stratified for performance status and treatment arm. The model was then tested in the validation set. Subsequently we investigated the treatment effect of docetaxel on OS in subgroups according to dNLR and duration of initial ADT.

Results: In the training set both dNLR ≥median (2) and duration of initial ADT <median (15 months) were associated with increased risk of death (HR=1.29;95%CI: 1.11-1.50, P<0.001 and HR=1.41; 95%CI: 1.21-1.64, P<0.001 respectively) after adjustment for age, alkaline phosphatase, hemoglobin, and pain at baseline. In the validation set, dNLR remained an independent prognostic factor for OS (HR=1.43; 95%CI: 1.20-1.70, P<0.001), whereas duration of initial ADT was not (HR=1.16; 95%CI: 0.97-1.37, P=0.10). In subgroup analyses of the TAX327 study, docetaxel improved OS irrespective of dNLR and duration of initial ADT.

Conclusion: The dNLR was prognostic for OS in men with mCRPC receiving first-line chemotherapy in two randomized phase III trials. A high dNLR (≥2) was associated with shorter survival irrespective of the received treatment. This readily available biomarker may serve for risk stratification in future clinical trials and could be incorporated into prognostic nomograms.

INTRODUCTION

Treatment options for men with metastatic castration-resistant prostate cancer (mCRPC) have expanded with the introduction of several new approved agents including abiraterone, enzalutamide, cabazitaxel and radium-223 [1]. Docetaxel with prednisone is the standard first-line chemotherapy and has shown to improve survival and its quality as compared with mitoxantrone in the TAX327 study [2]. Many trials have been conducted to investigate whether the efficacy of docetaxel could be improved by adding a targeted agent. The VENICE study was a randomized phase III trial that investigated the combination of docetaxel and aflibercept, a recombinant human fusion protein that binds A and B isoforms of vascular endothelial growth factor and placental growth factor [3]. Median overall survival (OS) was similar in the two arms of the trial.

With evidence that both abiraterone and enzalutamide are effective agents when administered to men with mCRPC either before or after chemotherapy [1,4,5], treatment selection and sequencing for an individual patient has become more challenging. Therefore, prognostic and predictive biomarkers for response to the various drugs approved for treatment of mCRPC are needed to guide clinical practice and trial design. An elevated neutrophil to lymphocyte ratio (NLR), a marker for host inflammation, was found to be an independent marker of adverse outcomes for several solid tumors including mCRPC [6-8]. In men with mCRPC treated with abiraterone, NLR ≥5 was associated with lower PSA response rates and shorter survival [9]. Since peripheral blood contains few white cells other than neutrophils or lymphocytes, a derived NLR (dNLR) calculated by the absolute neutrophil count divided by the difference between white cell and neutrophil counts, approximates NLR and has demonstrated similar prognostic value to the NLR [10]. Another potential prognostic factor in men with mCRPC, the duration of previous androgen deprivation therapy (ADT), was incorporated in a prognostic nomogram for men receiving second-line chemotherapy [11]. A longer duration of ADT was also associated with favorable outcomes in men treated with abiraterone in the post-docetaxel setting [12]. The implications of both dNLR and duration of initial ADT for men receiving first-line chemotherapy are unknown.

In this analysis, we used data from two randomized phase III studies to investigate the prognostic value of the dNLR and duration of initial ADT in men with mCRPC receiving first-line chemotherapy. We hypothesized that an elevated dNLR and shorter duration of initial ADT are associated with shorter survival. As a secondary objective, we aimed to investigate whether dNLR and duration of initial ADT were able to predict the treatment effect of docetaxel on OS and PSA response.

PATIENTS AND METHODS

Study population

For this study we used the VENICE study as a training set and the TAX327 study as an independent validation cohort [2,3]. These two large trials provided independent databases with mCRPC patients receiving first-line chemotherapy.

The VENICE study was a multinational phase III trial conducted in 1224 men with mCRPC who were treated with docetaxel (75 mg/m^2) every 3 weeks plus prednisone (10 mg daily) (D3) and were randomized to receive aflibercept (6 mg/kg) or placebo [3]. TAX327 was a multinational randomized phase III study involving 1006 men with mCRPC, who were randomized to receive D3, weekly docetaxel (30 mg/m^2) (D1) or 3-weekly mitoxantrone (12.5 mg/m^2) (M), each with prednisone. Full details of the VENICE and TAX327 trials are provided in the original reports [2,3]. Both studies were approved by an institutional review board at each participating institution. Written informed consent was obtained from all participants.

Data collection and definitions

The primary objectives of this study were to explore the prognostic role of the dNLR and the duration of initial ADT on OS, as defined by time from randomization to death from any cause. As a secondary objective we investigated treatment effects of docetaxel on OS and PSA response according to dNLR and the duration of initial ADT.

The variables that were considered for the prognostic model are shown in Supplementary table S1 and included among others; metastatic site, pain at baseline, baseline PSA, PSA doubling time, baseline testosterone, alkaline phosphatase, and hemoglobin [13]. The dNLR was calculated from peripheral blood counts as neutrophils divided by the difference of leukocytes and neutrophils. The duration of initial ADT was defined as the time from start of first-line hormonal therapy, (i.e.LHRH agonists, LHRH antagonists, surgical castration, with or without the addition of anti-androgens) to the start date of first subsequent anticancer treatment. Adjuvant hormonal therapy and anti-androgens given for short period to avoid PSA flare were not considered.

Model building and statistical considerations

We conducted univariable and multivariable Cox regression analyses in our training set (VENICE), stratified for ECOG performance status (0-1 vs 2) and treatment arm as was done in the initial study. The multivariable model was constructed using backward elimination at the 5% level. From this multivariable model, a risk score was composed for patients with 0-1,2,3,4, and 5-6 risk factors respectively. We performed sensitivity analyses using backward elimination without including pain to investigate whether there was any

impact of excluding pain data (N=197 missing in training set) on our multivariable model

The dNLR and duration of initial ADT were dichotomized according to their medians to make our results more generalizable. For supportive analyses dNLR and duration of initial ADT were studied by calculating the area under receiver operating characteristic curves (AUC) to identify optimal cutoffs with the end point dead or alive at month 24. Baseline laboratory variables were dichotomized using the median value.

Validation

The TAX327 database including all randomized men was used to externally validate and test the prognostic factors and prognostic score identified from the training set. The analysis was stratified for Karnofsky performance status (≤70 vs ≥80) and treatment arm [2]. Cutoffs were defined by the medians of the training set.

The statistical analyses for model development and validation were carried out using SAS version 9.2 (SAS Institute, Cary, NC). All statistical tests were two sided, and statistical significance was defined as $P<0.05$. Since this was an exploratory analysis, no correction for multiple testing was applied.

Treatment effect of docetaxel according to dNLR and duration of initial ADT

We used subgroup analyses to investigate whether dNLR and duration of initial ADT were able to predict the treatment effect of docetaxel on OS and PSA response. The TAX327 database was used to investigate OS benefit and PSA response in men receiving D3 as compared to the M control arm in subgroups of dNLR ≥median and <median, and in subgroups defined by duration of initial ADT ≥median and <median. The D1 arm was not included in the subgroup analyses, since D3 is the approved standard docetaxel regimen. To test whether there were differences in baseline variables known to predict OS between D3 and M in the defined subgroups, we used Fisher's exact test for all categorical variables, and the Wilcoxon Rank Sum test for all continuous variables. OS for D3 and M in the subgroups of dNLR and duration of initial ADT was assessed using the Kaplan-Meier method with statistical evaluation by the logrank test.

Confirmed PSA response, defined according to the Prostate Cancer Working Group 2 criteria was evaluated in subgroups of dNLR and duration of initial ADT in both TAX327 and VENICE databases, with statistical evaluation using Fisher's exact test. We used logistic regression with backward elimination adjusted on baseline PSA, and stratified for performance status and treatment arm to construct a model for the prediction of a confirmed PSA response (≥50%) in the VENICE database, with validation in TAX327.

RESULTS

Baseline characteristics

Baseline characteristics of the training and validation sets were generally well comparable (table 1). Median OS was 21.1 and 19.2 months for men treated with D3 in the VENICE and TAX327 studies respectively [2,3].

The median dNLR was 2.0 in the training set and 2.1 in the validation set, and the median duration of initial ADT was 15 months in both datasets. The optimal threshold for the prediction of OS by ROC-analysis in the training set was 2.05 for dNLR (AUC 0.58), which was almost identical to the median. For the duration of initial ADT the optimal threshold for the prediction of OS in the training set was 13.5 months (AUC 0.58).

Table 1. Baseline characteristics of men in the training and validation sets.

Characteristic	**VENICE**	**TAX327**
Number of patients randomized	1224	1006
Age, years, median (range)	68 (40-88)	68 (36-92)
Missing	0	0
Performance score n (%)		
ECOG 0–1	1170 (95.6%)	
2	54 (4.4%)	
Missing	0	
Karnofsky		
≥80		875 (87.1)
≤70		130 (12.9)
Missing		1
Metastatic site n (%)		
Visceral	347 (28.4)	229 (22.9)
Bone with no visceral involvement	800 (65.4)	720 (72.1)
Lymph node only	76 (6.2)	50 (5.0)
Missing	1	7

PSA, ng/ml, median (range)	86.8 (0-6138)	114 (0-40740)
Missing	1	2
Pain at baseline n (%)	366 (35.6)	458 (45.8)
Missing	197	6
Hemoglobin at baseline, g/dl, median (range)	12.7 (7.9-16.4)	12.7 (6.4-16.3)
Missing	0	0
Alkaline phosphatase at baseline, IU/L, median (range)	142 (20-6498)	205 (18-9900)
Missing	7	0
dNLR, median (range)	2.0 (0.2-19.4)	2.1 (0.3-19)
Missing	6	5
Duration of initial ADT, median, months, (range)	15.0 (2-272)	15.4 (0-226)
Missing	5	2

PSA, prostate-specific antigen; dNLR, Derived neutrophil to lymphocyte ratio; ADT, Androgen deprivation therapy

Associations of dNLR and duration of initial ADT with survival in the training set

In multivariable analysis of the training set, both dNLR ≥median and a duration of ADT <median were associated with an increased risk of death (HR=1.29; 95%CI: 1.11–1.50, *P*<0.001 and HR=1.41; 95%CI: 1.21–1.64, *P*<0.001 respectively) after adjustment for age, alkaline phosphatase, hemoglobin, and pain at baseline. The final multivariable model for the prediction of OS constructed in the training set is shown in table 2. Sensitivity analyses without including pain resulted in selection of the same variables, demonstrating that there was no impact on our multivariable model of excluding pain data (*N*=197 missing in training set). Univariable analyses of all considered variables such as baseline PSA, PSA doubling time, metastatic site, and baseline testosterone in the training set are shown in Supplementary table S1.

Table 2. Multivariable model for OS in the training and validation sets.

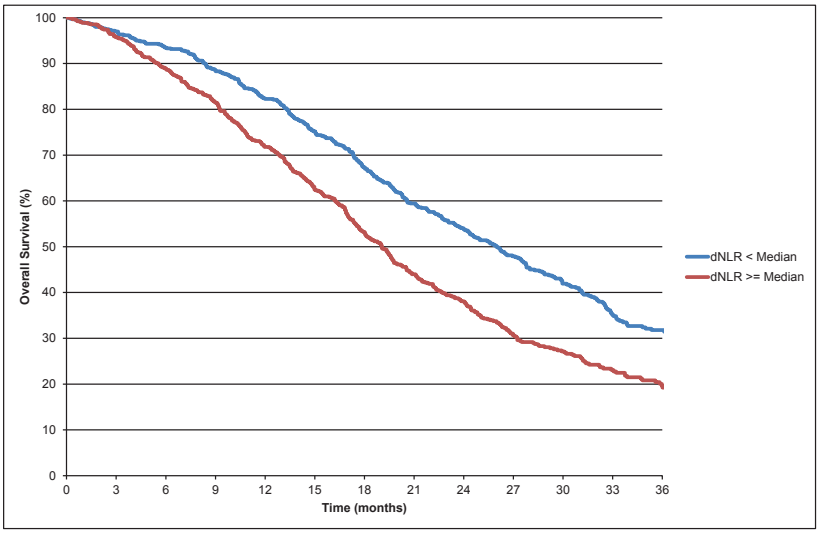
	Training (VENICE)		Validation (TAX327)	
Variable	**Hazard Ratio (95% CI)**	***P*-Value**	**Hazard Ratio (95% CI)**	***P*-Value**
Age (≥median)	1.24 (1.06-1.44)	0.006	1.02 (0.86-1.22)	0.82
Alkaline Phosphatase at baseline (≥median)	1.65 (1.41-1.93)	<0.001	1.43 (1.18-1.74)	<0.001
Duration of initial ADT (<median)	1.41 (1.21-1.64)	<0.001	1.16 (0.97-1.37)	0.10
dNLR (≥median)	1.29 (1.11-1.50)	<0.001	1.43 (1.20-1.70)	<0.001
Hemoglobin at baseline (<median)	1.45 (1.24-1.69)	<0.001	1.38 (1.16-1.65)	<0.001
Pain at baseline (PPI >=2)	1.56 (1.33-1.82)	<0.001	1.59 (1.33-1.90)	<0.001

ADT, androgen deprivation therapy; dNLR, derived neutrophil to lymphocyte ratio; PPI, Present Pain Intensity Scale from the McGill-Melzack questionnaire

Survival curves according to dNLR in the training set are shown in Figure 1 A, with an OS of 26.0 months (95%CI: 23.8–27.8) for men with a dNLR <median and 19.1 months (95%CI: 17.7–20.2) for men with a dNLR ≥median (logrank *P*<0.001). Men with a duration of initial ADT <median had an OS of 18.8 months (95%CI: 17.7–20.2) as compared to 25.0 months (95%CI: 23.6–27.2) for a duration of initial ADT ≥median (logrank *P*<0.001) (Supplementary figure S1 A).

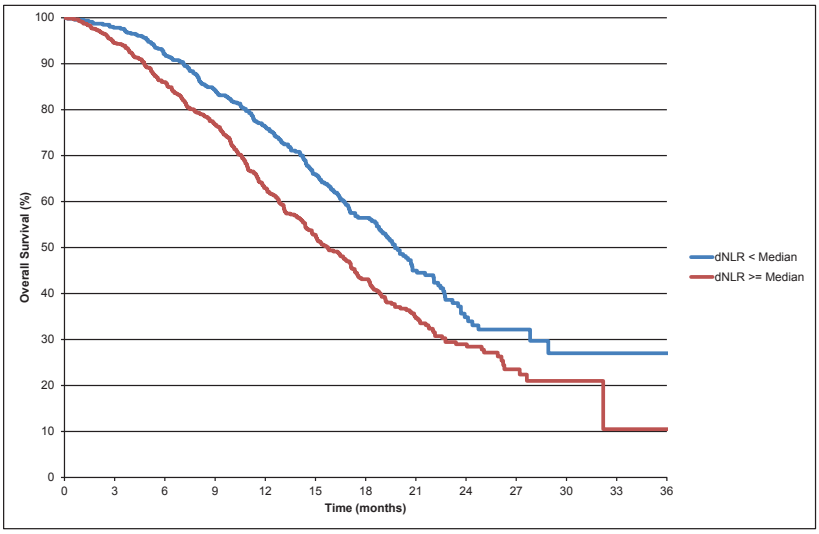
Four risk groups were composed for men with 0-1,2,3,4, and 5-6 risk factors from the multivariable model in the training set. Outcomes according to these risk groups are shown in Supplementary table S2 and Supplementary figure S2 A. The risk score was significantly and inversely associated with OS (*P*<0.001).

A.



No.at risk	0 months	6 months	12 months	18 months	24 months	30 months	36 months
dNLR <median	601	558	491	401	314	184	94
dNLR ≥median	617	545	439	325	230	111	49

B.



No. at risk	0 months	6 months	12 months	18 months	24 months	30 months	36 months
dNLR <median	457	420	303	153	41	7	0
dNLR ≥median	544	466	305	150	54	5	0

Figure 1. Kaplan-Meier estimates of overall survival in men with dNLR ≥median and <median in the training set (A) and in thevalidation set using the median as defined in the training set (B).

Validation

Testing the final model in the validation set showed that dNLR remained an independent prognostic factor for OS with a HR of 1.43 for men with a dNLR ≥2.0 (95%CI: 1.20–1.70, P<0.001), whereas duration of ADT was not independently associated with OS (HR=1.16; 95%CI: 0.97–1.37, P=0.10) (table 2).

Median OS was 19.8 months (95%CI: 18.7–21.6) versus 15.7 months (95%CI: 14.4–17.2) respectively for men with a dNLR <2.0 versus ≥2.0 in the validation set (logrank P<0.001) (Figure 1B). In men with a duration of initial ADT <15 months median OS was 16.8 months (95%CI: 15.1–17.6) as compared to 19.2 months (95%CI 17.4–20.9) for men with a duration of initial ADT ≥15 months (logrank P=0.06) (Supplementary figure S1 B). The composed risk score derived from the training set was also significantly associated with OS in the validation set (P<0.001) (Supplementary table S2 and Supplementary figure S2 B).

Treatment effect of docetaxel on OS and PSA response according to dNLR and duration of initial ADT

In the TAX327 database, baseline characteristics including known prognostic factors were similar among subgroups of dNLR (≥median and <median) and duration of initial ADT (≥median and <median) (Supplementary table S3). Comparing men treated with D3 versus M in subgroups according to dNLR and duration of initial ADT, the risk reduction of death consistently favored the D3 arm (table 3). No significant interaction of dNLR and duration of initial ADT on the treatment effect of D3 versus M on OS was observed (P=0.50 and P=0.30 respectively).

Table 3. Median overall survival for D3 versus M in subgroups according to dNLR and duration of initial ADT.

	dNLR ≥median		dNLR <median		Duration of initial ADT ≥median		Duration of initial ADT <median	
	D3	**M**	**D3**	**M**	**D3**	**M**	**D3**	**M**
Number of patients	176	155	156	181	182	164	153	172
Median OS, months (95% CI)	17.4 (15.1-19.6)	13.1 (11.5-16.3)	20.8 (18.8-23.7)	18.6 (16.5-20.5)	21.1 (16.7 - 23.7)	19.2 (14.8 -20.8)	18.3 (15.9 – 20.8)	14.8 (12.1 – 16.8)
Hazard Ratio (95% CI)	0.72 (0.54-0.95)		0.80 (0.59-1.09)		0.87 (0.64-1.17)		0.70 (0.53-0.94)	
P-value (logrank test)	0.022		0.160		0.343		0.015	

dNLR, derived neutrophil to lymphocyte ratio; ADT, androgen deprivation therapy; D3, Docetaxel 3-weekly; M, Mitoxantrone 3-weekly; OS, Overall survival; CI, Confidence interval

The overall rate of confirmed PSA response (≥50%) in men treated with D3 was higher in the subgroup with a dNLR <median as compared to ≥median in both TAX327 (70% vs 53%, P=0.001) and VENICE databases (76% vs. 67% P=0.001) (Table 4). When adjusted for other prognostic factors dNLR remained associated with PSA response in a multivariable logistic regression model constructed in the VENICE dataset (P=0.009) with validation in TAX327 (P<0.001) (Supplementary table S4). PSA response rates were similar among subgroups defined by duration of initial ADT (Table 4).

When assessing the treatment effect of D3 on PSA response, D3 improved PSA response rates over M for both dNLR ≥median (53% for D3 versus 36% for M, P=0.002) and <median (70% for D3 versus 49% for M, P<0.001). Accordingly, PSA response rates were higher for D3 versus M in men with a duration of initial ADT ≥median (60% and 45% for D3 versus M respectively, P=0.009) and <median (62% for D3 versus 39% for M, P<0.001).

Table 4. PSA response rates in men treated with D3 according to dNLR and duration of initial ADT.

	dNLR ≥median	dNLR <median	Duration of initial ADT ≥median	Duration of initial ADT <median
TAX327				
≥50% PSA decline n(%)	91 (53)	107 (70)	107 (60)	94 (62)
P-value	0.001		0.734	
VENICE				
≥50% PSA decline n(%)	402 (67)	451 (76)	444 (74)	413 (70)
P-value	0.001		0.122	

dNLR, derived neutrophil to lymphocyte ratio; ADT, androgen deprivation therapy PSA, prostate-specific antigen

DISCUSSION

In this study we identified and externally validated the prognostic role of dNLR in men with mCRPC receiving first-line chemotherapy. A high dNLR (≥2) was associated with shorter survival irrespective of the received treatment and other prognostic factors. The duration of initial ADT was prognostic for OS in the training set, but did not reach statistical significance in the validation set. Both dNLR and duration of initial ADT did not have predictive value, as docetaxel improved OS and PSA response in all defined subgroups.

To our knowledge, we are the first to identify and validate the prognostic value of dNLR in men with mCRPC receiving first-line chemotherapy, using data from two randomized phase III trials. The dNLR is a readily available biomarker, which is easy to obtain and comes with no extra costs. It may be used for risk stratification in clinical trials, could be incorporated in prognostic nomograms, and may give direct prognostic information on patients with mCRPC in daily practice.

The prognostic role of NLR has been shown in many solid tumors included in a recent meta-analysis of one hundred studies [6]. These observations suggest a broad prognostic impact of NLR across different tumor types, disease settings, and treatments. Although the exact mechanisms behind the unfavourable prognostic implications of an elevated NLR remain to be elucidated, it may relate to increased neutrophil-dependent inflammation, and reduced lymphocyte mediated tumor response [14]. Neutrophils are able to favour tumor development and inhibit the activity of lymphocytes and other immune cells, while the presence of intratumoral lymphocytes is associated with better responses to cytotoxic treatment and more favorable prognosis in cancer patients [15]. We studied the dNLR instead of the NLR as lymphocyte counts were not available in both datasets. Most leukocytes in the peripheral blood are either neutrophils or lymphocytes, so that subtraction of neutrophils from the total leukocyte count gives a good estimate of the lymphocyte count. It has been confirmed in other studies that dNLR provides similar prognostic value to the NLR [10].

As a secondary objective of our study we evaluated the treatment effect of docetaxel in subgroups according to dNLR. A high NLR has been shown previously to be associated with low PSA response rates to abiraterone (16%) and worse OS in patients with mCRPC [9]. Here we report that although patients with a high dNLR have an unfavorable prognosis, docetaxel provides a robust OS benefit of 4.3 month and ≥50% PSA declines in 53-67% of this patient group. Although our patient population and the cohort reported for abiraterone are not directly comparable, this generates the hypothesis that in patients with a high dNLR treatment with chemotherapy might be more beneficial than abiraterone. Unfortunately, there is no data to confirm whether NLR is a marker of better response to AR-targeted agents or chemotherapy.

Halabi et al. have previously demonstrated the prognostic importance of the duration of ADT in patients with mCRPC receiving second-line chemotherapy [11]. The duration of ADT was also associated with survival in a prognostic model of men treated with abiraterone in the post-docetaxel setting [12]. In our analysis, duration of initial ADT only demonstrated independent prognostic value in the training set. We investigated the duration of initial ADT as it better approximates the biologically and clinically relevant time of actual response to hormonal therapy [16]. ADT by either surgical or hormonal castration as defined in our analysis was a mandatory inclusion criterion of both studies.

Furthermore, all men included in our analysis continued LHRH agonists/antagonists during chemotherapy. As such, the total duration of ADT would not provide a good estimate of the response duration. In contrast, the use of additional anti-androgens (e.g. bicalutamide) after initial castration was not mandatory and thus highly variable between patients. Therefore, use of such drugs was not included in our definition.

The main strength of our study is the use of two large databases of men with mCRPC receiving first-line chemotherapy in randomized phase III studies, as a training set and independent validation set. An inherent limitation is its retrospective nature. Also, data were not available to allow correction for some known prognostic factors such as baseline levels of lactate dehydrogenase (LDH) and albumin [13]. However, previous data investigating NLR in men with mCRPC receiving docetaxel showed a significant independent association between NLR and OS even after adjustment for LDH and albumin [7]

In conclusion, dNLR was prognostic for OS in men with mCRPC receiving first-line chemotherapy in two randomized phase III trials. A high dNLR (≥2) was associated with shorter survival irrespective of the received treatment. This readily available biomarker may serve for risk stratification in future clinical trials and could be incorporated into prognostic nomograms. In addition, it could give direct prognostic information on patients in daily practice.

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SUPPLEMENTARY TABLES

Supplementary table S1. Univariable analyses for OS of all variables considered in the training set, stratified for performance status and treatment arm.

	Training (VENICE)	
Variable	**Hazard Ratio (95% CI)**	***P*-Value**
Age (≥median)	1.11 (0.97-1.27)	0.120
Metastatic site		
Lymph node only	Reference	0.002
Bone metastases with no visceral involvement	1.62 (1.18-2.22)	
Any visceral metastases	1.83 (1.31-2.54)	
Alkaline Phosphatase at baseline (≥median)	2.03 (1.77-2.33)	<0.001
Gleason score (8-10 vs. ≤7)	1.08 (0.94-1.23)	0.299
Duration of initial ADT (<median)	1.39 (1.21-1.59)	<0.001
Type of progression		
PSA only	Reference	0.091
Bone scan with no radiologic progression	1.17 (1.01-1.36)	
Radiologic progression	1.03 (0.86-1.24)	
Prior prostatectomy	0.94 (0.82-1.08)	0.378
Prior radiotherapy	1.05 (0.91-1.21)	0.539
dNLR (≥median)	1.49 (1.30-1.71)	<0.001
PSA at baseline (≥median)	1.48 (1.30-1.70)	<0.001
PSA doubling time (<median)	1.29 (1.13-1.49)	<0.001
Testosterone at baseline (≥median)	1.01 (0.88-1.15)	0.935
Hemoglobin at baseline (<median)	1.86 (1.63-2.13)	<0.001
Pain at baseline (PPI >=2)	1.88 (1.62-2.18)	<0.001

dNLR, Derived neutrophil to lymphocyte ratio; PPI, Present Pain Intensity Scale from the McGill-Melzack questionnaire

Supplementary table S2. Clinical outcome according to risk score in the training and validation sets

	Training set (VENICE)					Validation set (TAX327)				
Risk score	0-1	2	3	4	5-6	0-1	2	3	4	5-6
Number of patients (%)	184 (18.2)	232 (23)	258 (25.5)	202 (20)	134 (13.3)	109 (11.0)	200 (20.1)	269 (27.1)	259 (26.1)	156 (15.7)
Median OS, months	33.8	26.9	20.3	17.7	12.8	24.7	21.1	19.0	15.1	10.9
(95% CI)	(30.8-39.0)	(24.4-28.9)	(19.0-22.7)	(14.7-19.8)	(10.6-13.7)	(20.8-NR)	(18.9-23.2)	(16.7-21.2)	(13.1-17.1)	(9.5-12.3)
Hazard Ratio	Ref	1.57	2.21	3.14	5.21	Ref	1.54	1.75	2.62	3.9
(95% CI)		(1.21-2.04)	(1.72-2.84)	(2.43-4.06)	(3.95-6.89)		(1.04-2.29)	(1.20-2.55)	(1.81-3.81)	(2.66-5.74)
***P*-value**			<0.001					<0.001		

OS, Overall survival; CI, Confidence interval

Supplementary table S3. Baseline characteristics of patients according to dNLR and the duration of initial ADT in the TAX327 study.

	Duration of initial ADT ≥median		Duration of initial ADT <median		dNLR ≥ median		dNLR <median	
	D3	**M**	**D3**	**M**	**D3**	**M**	**D3**	**M**
Number of patients	182	164	153	172	176	155	156	181
Age, years, median	69	70	66	66	69	68	67	69
(range)	(49-86)	(43-86)	(42-92)	(45-86)	(46-92)	(45-86)	(42-83)	(43-86)
Missing	0	0	0	0	0	0	0	0
P-value	0.231		0.995		0.873		0.483	
Karnofsky performance score ≤70 n (%)	21 (11.5)	20 (12.2)	21 (13.7)	26 (15.1)	17 (9.7)	27 (17.4)	25 (16.0)	20 (11.0)
Missing	0	0	0	0	0	0	0	0
P-value	0.869		0.754		0.051		0.201	
Metastatic site n(%)								
Visceral	47 (26.3)	36 (22.1)	28 (18.4)	39 (22.7)	47 (27.0)	41 (26.5)	27 (17.5)	34 (18.9)
Bone with no visceral involvement	125 (69.8)	118 (72.4)	116(76.3)	123 (71.5)	124(71.3)	105 (67.7)	115 (74.7)	137 (76.1)
Lymph node only	7 (3.9)	9 (5.5)	8 (5.3)	10 (5.8)	3 (1.7)	9 (5.8)	12 (7.8)	9 (5.0)
Missing	3	1	1	0	2	0	2	1
P-value	0.574		0.601		0.157		0.578	

PSA, ng/ml, median	95	120	148	123	107	117	129	138
(range)	(0-5001)	(3-8022)	(1-40740)	(0-7852)	(0-40740)	(0-8022)	(3-3450)	(3-7852)
Missing	0	1	0	0	0	0	0	1
P-value	0.061		0.968		0.251		0.499	
Pain at baseline n (%)	80 (44.2)	73 (45.1)	72 (47.1)	81 (47.1)	85 (48.6)	83 (53.5)	65 (41.7)	71 (39.7)
Missing	1	2	0	0	1	0	0	2
P-value	0.914		1,000		0.379		0.739	
Hb at baseline, g/dl, median	12.7	12.6	12.5	12.7	12.7	12.6	12.6	12.8
(range)	(9.4-16.2)	(7.9-16.1)	(6.4-16.1)	(9.0-16.0)	(6.4-16.2)	(8.6-16.1)	(10.1-16.2)	(7.9-16.0)
Missing	0	0	0	0	0	0	0	0
P-value	0.905		0.570		0.539		0.267	
Alkaline phosphatase at baseline, IU/L, median	198	176	219	221	210	227	198	181
(range)	(51-9900)	(51-4637)	(26-4438)	(18-6075)	(40-9900)	(18-4637)	(26-4517)	(51-6075)
Missing	0	0	0	0	0	0	0	0
P-value	0.176		0.815		0.821		0.554	

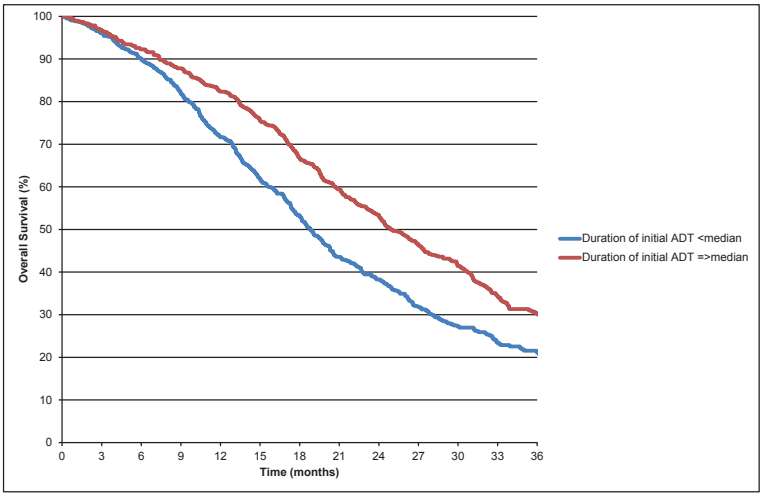
dNLR, Derived neutrophil to lymphocyte ratio; ADT, Androgen deprivation therapy; D3, Docetaxel 3-weekly; M, Mitoxantrone 3-weekly; PSA, prostate specific antigen; Hb, hemoglobin

Supplementary table S4. Multivariable logistic regression model for PSA response (≥50%) in VENICE, with validation in TAX327.

	VENICE		TAX327	
Variable	**Odds ratio (95% CI)**	***P*-Value**	**Odds ratio (95% CI)**	***P*-Value**
Alkaline Phosphatase at baseline (≥median)	0.73 (0.55; 0.99)	0.040	0.69 (0.52-0.91)	0.008
Pain at baseline (PPI >=2)	0.65 (0.48; 0.87)	0.005	0.76 (0.58-1.00)	0.046
dNLR (≥median)	0.68 (0.51-0.91)	0.009	0.56 (0.43-0.73)	<0.001

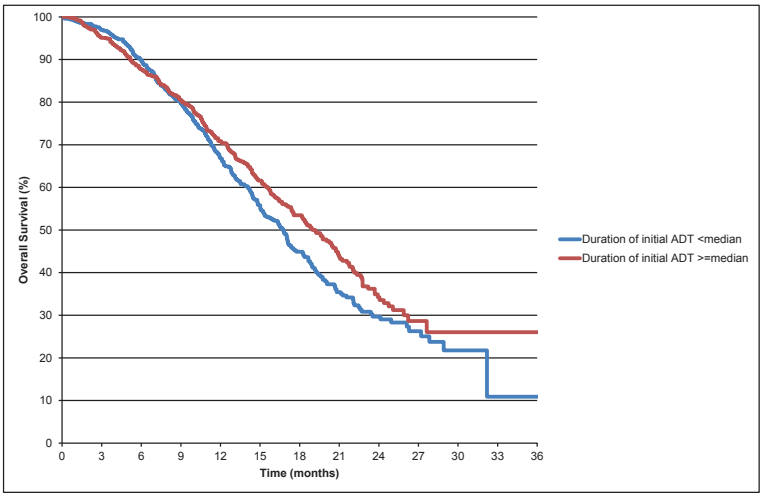
dNLR, Derived neutrophil to lymphocyte ratio; PPI, Present Pain Intensity Scale from the McGill-Melzack questionnaire

SUPPLEMENTARY FIGURES



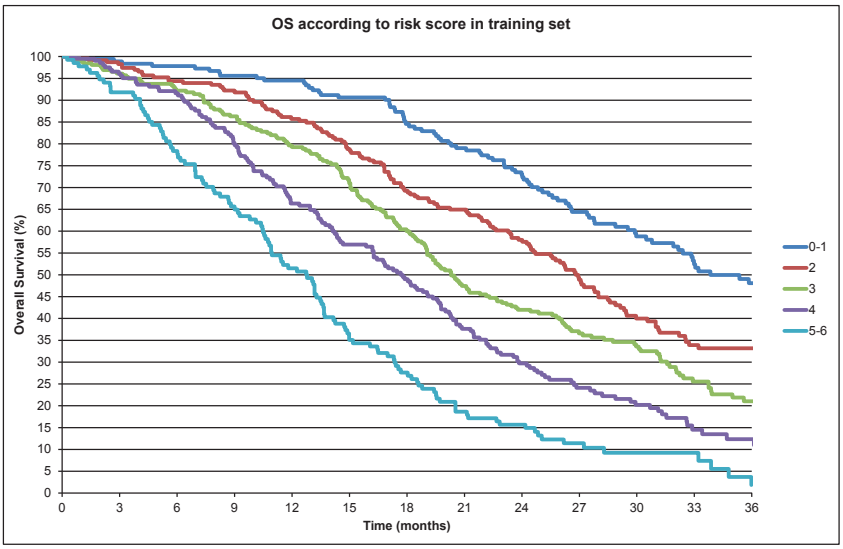
No.at risk	0 months	6 months	12 months	18 months	24 months	30 months	36 months
Duration of initial ADT <median	609	546	434	323	226	110	52
Duration of initial ADT ≥median	610	558	497	402	317	184	90

B.



No.at risk	0 months	6 months	12 months	18 months	24 months	30 months	36 months
Duration of initial ADT <median	495	444	302	145	45	5	0
Duration of initial ADT ≥median	509	445	306	158	50	7	0

Supplementary Figure S1. Kaplan-Meier estimates of overall survival in men with a duration of initial ADT ≥median and <median in the training set (A) and in the validation set using the median as defined in the training set (B).



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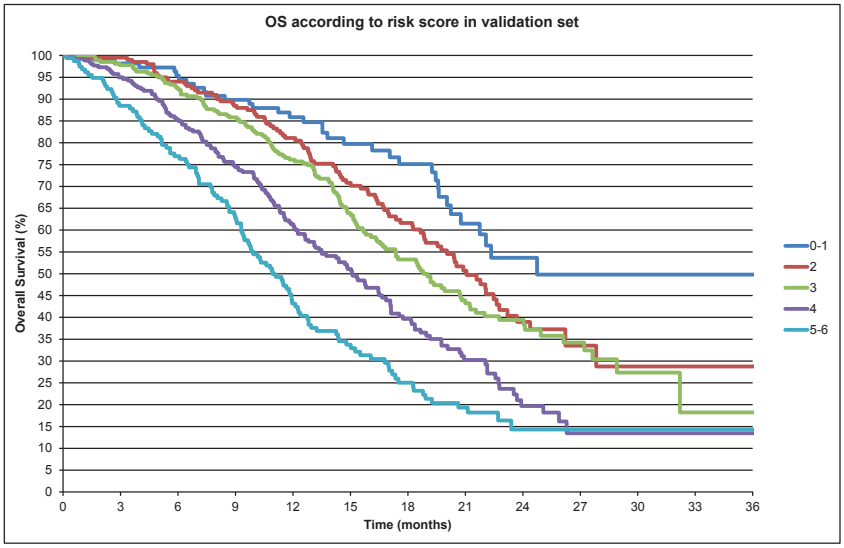

Supplementary Figure S2. Kaplan-Meier estimates of overall survival according to the number of risk factors derived from the multivariable model in the training set (A) and in the validation set (B).

GENERAL DISCUSSION

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CHAPTER 8

CROSS-RESISTANCE BETWEEN TAXANES AND NEW HORMONAL AGENTS AND POTENTIAL IMPLICATIONS FOR CLINICAL STUDIES ON TREATMENT SEQUENCE

The treatment armamentarium for mCRPC has evolved over the past few years, with the introduction of several new approved drugs including cabazitaxel, abiraterone, enzalutamide, and radium-223 [1-4]. At the current time, no predictive biomarkers for the available treatments exist, and the optimal drug treatment sequence is unknown. Treatment decisions are largely based on personal preferences, reimbursement policies, and toxicity profiles, as no level 1 evidence to tailor therapy is available. Retrospective studies suggested that the overall survival benefits obtained by the new therapies cannot be simply added up, as cross-resistance between taxanes and AR-targeted has been observed. Therefore, it is of utmost importance to identify the mechanisms of cross-resistance between the current therapies in mCRPC, in order to determine the optimal treatment sequence for individual patients.

Taxanes (i.e. docetaxel, and cabazitaxel) suppress dynamic instability of microtubules and alter microtubule polymer mass as their main mechanism of action. Recent reports demonstrated that by inhibiting microtubules, paclitaxel and docetaxel also impair AR signaling, which in the setting of mCRPC may represent part of their therapeutic efficacy [5, 6]. In this thesis we have developed CRPC cell lines with acquired resistance to abiraterone and enzalutamide to identify and give insight in mechanisms of cross-resistance between the currently available therapies: docetaxel, cabazitaxel, abiraterone and enzalutamide. We showed that the efficacy of taxanes was reduced in abiraterone- and enzalutamide-resistant cells. Of note, the efficacy of abiraterone was reduced in enzalutamide-resistant cells and vice versa. These observations are exactly concordant with retrospective clinical studies suggesting a degree of cross-resistance between these drugs, reflecting the clinical relevance of our cell line model [7-11]. Thus far, the clinical studies that have reported on cross-resistance between mCRPC treatments have all been retrospective, with the risk of potential selection bias. The major strength of our cell line model is that it enables us to study CRPC and mechanisms of cross-resistance under standardized conditions, in well-defined patient-derived cells.

Subsequently, we showed that docetaxel, cabazitaxel, abiraterone and enzalutamide all act on AR nuclear transport, which is a crucial step in AR signaling, and provide a mechanistical explanation for cross-resistance between taxanes and the novel AR targeting agents abiraterone and enzalutamide. It has been reported by other groups that AR transport is facilitated by microtubules and the motor protein dynein, which explains the inhibitory effects of taxanes on the AR [5, 6, 12, 13]. Interestingly, the inhibiting effects on the AR by abiraterone were observed in the presence of the synthetic androgen R1881, suggesting that abiraterone might be able to directly inhibit the AR, independent of CYP17A1 inhibition. This was also shown by Richards et al., who found that abiraterone

is able to bind and inhibit the AR at high but clinically relevant concentrations (≥5 uM).

As a next step, we confirmed our findings of cross-resistance between docetaxel and enzalutamide in an in vivo model of CRPC. Docetaxel efficacy was impaired in enzalutamide-resistant tumors (PC346Enza), as compared to enzalutamide-naïve tumors (PC346C), demonstrating in vivo cross-resistance between these drugs. As a mechanism for our observed cross-resistance, we showed that docetaxel efficiently impaired AR nuclear localization and consequently AR regulated gene expression in enzalutamide-naïve tumors, while it did not in enzalutamide-resistant tumors. The role of microtubules in AR transport and the resulting inhibitory effects of docetaxel on the AR have only recently been elucidated [13, 14]. In the present thesis we demonstrated that the reduced inhibition of docetaxel on the AR in enzalutamide-resistant tumors confers cross-resistance between these drugs in vivo. These results indicate that the inhibitoiry effects of docetaxel on the AR represent part of its antitumor activity, which is impaired by previous AR targeted therapy such as enzalutamide. In this light, it could also explain the reduced efficacy of docetaxel when used after prior treatment with abiraterone observed in retrospective clinical studies [9, 15, 16]. Ultimately, the proof of cross-resistance and the consequences for treatment sequencing should be investigated in prospective randomized trials. Such studies could investigate the sequence of docetaxel followed by AR-targeted therapy or vice versa, as well as AR-targeted therapy followed by AR targeted therapy versus a taxane. It will however take years to conduct these studies, and it is uncertain whether such a trial would provide definitive answers in the current treatment landscape. With an abundance of both approved and experimental therapies, subsequent treatments are likely to have a confounding effect on survival outcomes. Recently, the promising and biologically active drug orteronel (TAK-700) failed to demonstrate a survival benefit at least in part due to confounding effects of cross-over in the control arm of the study [17, 18].

In our in vivo model of enzalutamide-resistant CRPC we found superior efficacy of cabazitaxel as compared to treatment with docetaxel. Cabazitaxel demonstrated robust tumor and PSA responses in our enzalutamide-resistant xenografts, while the effects on AR signaling were reduced as compared to those in enzalutamide-naïve tumors. These observations indicate that cabazitaxel is less dependent on its inhibitory effects on the AR pathway, and exerts greater antitumor activity via AR independent mechanisms as compared to docetaxel. This sustained efficacy is concordant with clinical observations [19, 20], and is probably caused by a higher potency of cabazitaxel to suppress microtubule dynamics as compared to docetaxel, with faster drug uptake and better intracellular retention [21]. These superior properties of cabazitaxel were best reflected in our in vivo experiments as compared to the in vitro experiments, which is probably caused by the lack of a three dimensional tumor representation of an in vitro system. The greater potency of cabazitaxel after AR-targeted treatment might have clinical implications, as currently

docetaxel is the standard first-line chemotherapy for men with mCRPC. Considering the superior efficacy of cabazitaxel over docetaxel in our enzalutamide-resistant tumors, these results provide a rationale for clinical studies comparing cabazitaxel with docetaxel in men with mCRPC who progressed on first-line enzalutamide or abiraterone.

The question whether cabazitaxel is superior to docetaxel as first line-treatment for mCRPC is currently addressed in the FIRSTANA study (NCT01308567) which compares cabazitaxel 25 mg/m^2 and cabazitaxel 20 mg/m^2 both with prednisone, to docetaxel 75 mg/m^2 plus prednisone. The study has completed accrual and results are expected in 2016.

In the present thesis, we aimed to clinically confirm our preclinical findings of sustained cabazitaxel efficacy after prior treatment with novel AR-targeted therapie. Therefore, we used data from a prospective, multicenter, randomized phase II trial (CABARESC) to compare the clinical outcome of patients receiving cabazitaxel with and without prior abiraterone or enzalutamide treatment. Concordant with our preclinical findings, we demonstrated in patients that prior novel AR-targeted therapy did not affect the efficacy of cabazitaxel. These results clinically confirmed that cabazitaxel provides a good treatment option both before and after novel AR-targeted therapies in the post-docetaxel setting. It also shows that cabazitaxel has truly different properties as compared to docetaxel, which has been shown to loose part of its efficacy when used after novel AR-targeted therapy [15,16].

Future efforts in mCRPC are currently focusing on combination therapy. The efficacy of abiraterone plus enzalutamide is currently evaluated as compared with enzalutamide alone in a randomized phase III trial of chemotherapy-naïve mCRPC patients (NCT01949337). The rationale for this combination is an observed increased androgen synthesis in patients treated with enzalutamide [22]. This could theoretically result in competition between enzalutamide and testosterone at the level of the AR [23]. On the other hand, increasing AR copy numbers were observed in tumors of patients treated with abiraterone [24]. Combining the two drugs could potentially inhibit these adaptive feedback mechanisms.

In the absence of prospective studies on the treatment sequence, clinicians should be aware of potential cross-resistance between the available therapies. Novel AR targeted agents abiraterone and enzalutamide have proven to be very effective agents either when delivered before or after docetaxel chemotherapy. However, since all available studies consistently point to reduced efficacy of docetaxel when used after abiraterone, clinicians should beware that cross-resistance might occur when treating patients with hormonal agents before docetaxel. Also, sequential treatment with AR-targeted agents could be a strategy that potentially leads to cross-resistance. In this treatment continuum there might

be an important role for cabazitaxel, for which there is no evidence of cross-resistance with the other therapies in mCRPC. In this thesis we showed that although cabazitaxel is able to inhibit AR nuclear translocation, it exhibits superior antitumor activity independent of the AR pathway in both preclinical and clinical studies. Since cross-resistance might develop by subsequently targeting the AR, cabazitaxel may very well become to future taxane of choice in patients with progressive disease on either abiraterone or enzalutamide.

In summary, there has been major progress in the treatment of mCRPC over the past few years. The current challenge is how to sequence these drugs, and to deliver them to the patients that will benefit. Ultimately, biomarkers for response to the various treatment options may facilitate treatment decisions. Our findings of cross-resistance presented here provide a mechanism for reduced docetaxel efficacy in enzalutamide-resistant prostate cancer. It also explains observations of cross-resistance between abiraterone and enzalutamide when used in sequence. Rather than randomly sequencing agents based on the way of administration (oral vs. intravenous), or toxicity profiles, efforts should be taken to avoid cross-resistance that might occur when delivering docetaxel after hormonal agents or repeatedly targeting the AR pathway. Cabazitaxel might prove a valuable treatment option here without concerns for cross-resistance. Based on the results presented in this thesis and retrospective clinical data, randomized controlled trials should further evaluate the treatment sequence.

PREDICTIVE AND PROGNOSTIC FACTORS IN MCRPC PATIENTS RECEIVING DOCETAXEL

With evidence that both abiraterone acetate and enzalutamide are effective agents when administered to men with mCRPC either before or after chemotherapy [1, 3, 25, 26], treatment selection and sequencing for an individual patient has become more challenging. Since no predictive biomarkers for the available treatments exist at the current time, it is unknown which patient will benefit from which treatment. Therefore, predictive biomarkers for response to the various drugs approved for treatment of mCRPC are needed to guide clinical practice and trial design. Since prospective studies of established predictive biomarkers facilitating treatment decisions are largely lacking, we herewith provide an overview of potential predictive factors to the currently available treatment options in mCRPC.

Gleason score

In this thesis, we aimed to identify subgroups of patients who derive the greatest benefit from docetaxel chemotherapy. A post-hoc analysis of the TAX327 study was performed and showed that the overall survivial (OS) benefit obtained by docetaxel

was most pronounced in men with high Gleason score tumors (OS benefit 4.4 months versus 2.9 months in the whole patient population). At the same time, a high Gleason score (8–10) at the time of diagnosis had been reported to be an independent risk factor for poor response to abiraterone [27]. These findings suggested a potential predictive role of Gleason score. However, although the OS benefit in the COU-AA-302 study of abiraterone pre-chemotherapy was less pronounced in patients with high Gleason score tumors (7-10), the clinical benefit of abiraterone as assessed by radiographic progression-free survival demonstrated to be irrespective of Gleason score [28]. Similarly, a post-hoc analysis of the TROPIC trial investigating cabazitaxel versus mitoxantrone has shown that also cabazitaxel provided clinical benefit regardless of the tumor grading at initial diagnosis [29].Thus, Gleason score could be useful in identifying patients who derive the most benefit from docetaxel chemotherapy, but might be of less value to predict benefit from other treatments.

Duration of response to initial ADT

An interesting retrospective study of 108 mCRPC patients who received hormonal agents including abiraterone in clinical trials demonstrated that patients with a short response to prior androgen deprivation therapy (ADT) (<16 months) had poor PSA responses and PFS [30]. These findings suggested that patients who progress rapidly to castration-resistant disease, with short responses to prior ADT, might do worse on consecutive hormonal treatments. In contrast, we performed post-hoc analyses of 2 large phase III trials of patients with mCRPC receiving docetaxel, and showed that the treatment benefit was irrespective of duration of initial ADT. Likewise, cabazitaxel improved OS regardless of duration of initial ADT [29]. Prospective validation of these findings for the various compounds approved for the treatment of mCRPC should define whether duration of response to prior ADT could be a good parameter for further personalizing treatment.

Neutrophil to lymphocyte ratio

The neutrophil to lymphocyte ratio (NLR) is an emerging marker for systemic inflammation in cancer. NLR demonstrated independent prognostic value in several solid tumors including hepatocellular, gastric, renal cell and colorectal cancer [31-36]. In this thesis, we explored the prognostic and predictive role of the neutrophil to lymphocyte ratio (NLR) in two multinational randomized phase III trials of mCRPC patients receiving first-line chemotherapy. We identified and externally validated dNLR as an independent prognostic factor for OS. Men with an elevated dNLR (≥2) had a shorter survival irrespective of the received treatment and other prognostic factors (HR 1.29, P<0.001 in training set and 1.43, P<0.001 in validation set). Although men with an elevated dNLR had a worse prognosis, docetaxel provided a substantial OS benefit of 4.3 months and PSA response

rates of 53-67% in this patient population. Of note, a recent study exploring the role of NLR in mCRPC patients treated with abiraterone revealed that men with an elevated NLR had low PSA response rates (16%) and shorter survival. Taken together, these findings indicate that dNLR is useful as a prognostic marker in men receiving first-line chemotherapy. The dNLR is not predictive of response in men treated with docetaxel, but might be in men receiving abiraterone. Further research should define whether NLR could be a marker of better response to AR-targeted agents or chemotherapy.

This readily available biomarker may serve for risk stratification in future clinical trials and could be incorporated into prognostic nomograms. In addition, it may provide direct prognostic information on patients in daily practice. The prognostic implications of systemic inflammation and NLR also provide a rationale for the evaluation of anti-inflammatory therapy in cancer. A retrospective study in women who underwent resection of a primary breast tumor revealed that intraoperative administration of an non-steroidal anti-inflammatory drug (NSAID) provided better outcome, which was even more pronounced in women with en elevated NLR [44].

The prognostic role of NLR has been shown in many solid tumors included in a recent meta-analysis of one hundred studies [35]. These observations suggest a broad prognostic impact of NLR across different tumor types, disease settings, and treatments. Although the exact mechanisms behind the unfavourable prognostic implications of an elevated NLR remain to be elucidated, it may relate to increased neutrophil-dependent inflammation, and reduced lymphocyte mediated tumor response [37, 38]. Neutrophils are able to favour tumor development and inhibit the activity of lymphocytes and other immune cells [39, 40], while the presence of intratumoral lymphocytes is associated with better responses to cytotoxic treatment and more favorable prognosis in cancer patients [41-43].

AR splice variants: ARv7

The AR splice variant 7 lacks the ligand-binding domain, and remains constitutively active in the absence of ligand. ARv7 expression develops in the course of disease progression, and is more likely to occur in patients with more advance disease, and previous treatment with abiraterone or enzalutamide. A well designed, prospective study by Antonarakis et al. showed that men expressing ARv7 in circulating-tumor cells did not respond to treatment with either abiraterone or enzalutamide [45]. Also, clinical/radiographic progression-free survival and OS were inferior in men expressing ARv7. Although these findings require large scale validation, it is a promising first step towards a more personalized treatment approach in mCRPC, where ARv7 expression in circulating-tumor cells might be used to facilitate treatment decisions. Future research efforts should define the role of Arv7 in resistance versus sensitivity to taxane chemotherapy.

Taken together, with a rapid evolvement of the treatment landscape for mCRPC,

predictive biomarkers for the current treatment options are urgently needed. At this time, ARv7 expression in circulating-tumor cells is the most promising biomarker for future treatment selection. Other potential patient factors that might facilitate treatment decisions are derived from retrospective analyses and should therefore be interpreted with care. However, in the absence of prospectively derived biomarkers, duration of response to previous hormonal therapy and NLR are clinical parameters that are readily available, and might give some guidance when treating patients with mCRPC.

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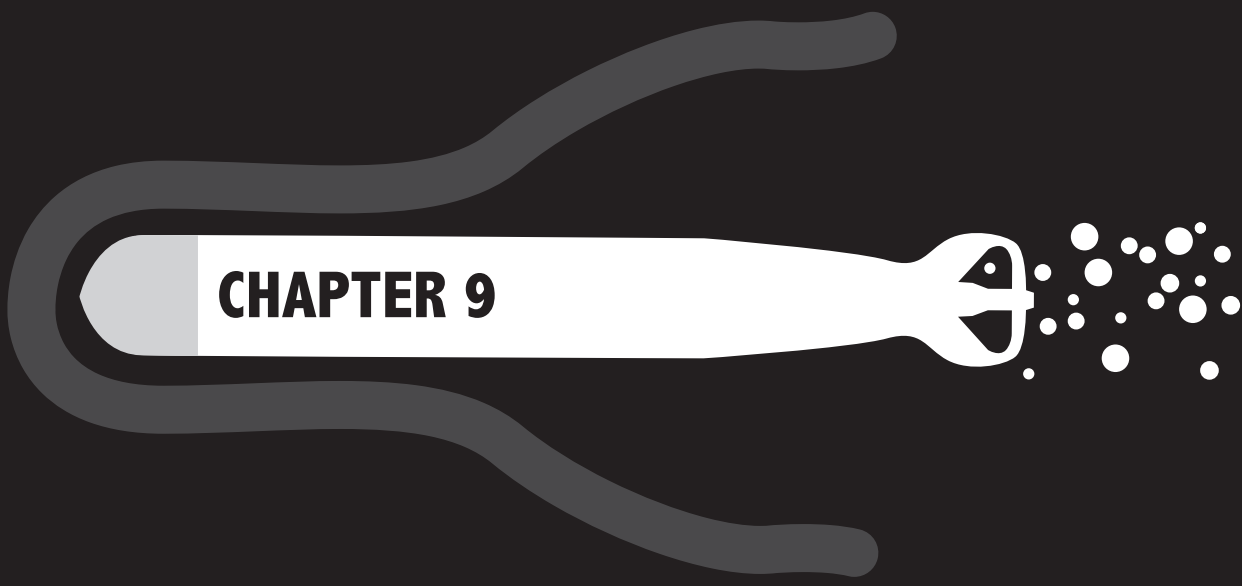
SUMMARY



CHAPTER 9

SUMMARY

The treatment landscape of metastatic castration-resistant prostate cancer (mCRPC) has changed dramatically over the past few years, with the introduction of several new approved drugs including cabazitaxel, abiraterone, enzalutamide, and radium-223 [1-5]. This considerable progress also comes with new challenges. Concerns of cross-resistance between the taxanes (i.e. docetaxel and cabazitaxel) and androgen receptor (AR)-targeted agents have arisen, and the optimal drug treatment sequence is still undetermined. There is increasing evidence for a reduced efficacy of docetaxel in men with mCRPC who had previously been treated with abiraterone, suggesting clinical cross-resistance in several retrospective studies [6-8]. Moreover, only modest efficacy have been reported in studies investigating abiraterone when used after enzalutamide and vice versa [9-12].

Taxanes act through microtubule interaction and polymerization inducing mitotic arrest and apoptosis. Recent reports demonstrated that paclitaxel and docetaxel also impair AR-nuclear translocation and consequently AR signaling, which in the setting of mCRPC might be responsible for part of their therapeutic efficacy [13, 14]. In **chapter 3**, castration-resistant prostate cancer (CRPC) cell lines with acquired resistance to abiraterone and enzalutamide were developed and used to identify and provide insight in mechanisms of cross-resistance between docetaxel, cabazitaxel, abiraterone and enzalutamide. In these cell lines, we observed cross-resistance between the taxanes and AR targeted agents abiraterone and enzalutamide. As a potential mechanism for our observed cross-resistance, we identified the inhibition of AR nuclear translocation as an overlapping working mechanism between these drugs. The role of microtubules and the motor protein dynein in AR transport have only recently been elucidated [15, 16]. In our study, we showed that taxanes are able to inhibit microtubule mediated AR nuclear translocation as an additional mechanism of action. This effect was also observed for the novel AR targeted agents abiraterone and enzalutamide, identifying an overlapping working mechanism that potentially confers cross-resistance between the taxanes and novel AR targeted agents.

In **chapter 4** we confirmed our findings of cross-resistance between docetaxel and enzalutamide in a mouse model of enzalutamide-resistant versus enzalutamide-naïve CRPC. Docetaxel efficacy was reduced in tumors with acquired resistance to enzalutamide (PC346Enza) as compared to enzalutamide-naïve tumors (PC346C). This impaired efficacy of docetaxel was caused by a reduced inhibition of AR nuclear localization and consequently AR regulated gene expression in enzalutamide-resistant tumors as compared to enzalutamide-naïve tumors. In contrast to docetaxel, cabazitaxel remained highly effective in enzalutamide-resistant xenografts, demonstrating greater antiproliferative properties independent of the AR pathway. These findings are especially of interest, since it is exactly concordant with clinically observed cross-resistance for

docetaxel, but not cabazitaxel, [6, 7, 9, 10], which can be mechanistically explained by the findings in our xenograft studies.

In **chapter 5** we clinically confirmed the findings from our preclinical in vivo studies (chapter 4). We used data from a prospective, multicenter, randomized phase II study to investigate the influence of prior novel AR-targeted therapies abiraterone and enzalutamide on the efficacy of cabazitaxel. We found that PSA response rates (≥50%) while on cabazitaxel treatment were similar in patients with and without prior novel AR-targeted therapy (34% versus 40% respectively, P=0.53). Likewise, median OS was not significantly different between men with and without prior novel AR-targeted therapy, with a median OS of 9.8 months versus 10.6 months respectively (logrank P=0.65). These findings strongly suggest that there is no cross-resistance between abiraterone or enzalutamide and cabazitaxel.

In **chapter 6** we aimed to identify subgroups of patients who derive the greatest benefit from docetaxel chemotherapy. A post-hoc analysis of the TAX327 registration study was performed and showed that the clinical benefit obtained by docetaxel was most pronounced in men with high Gleason score tumors. Median OS in men with high Gleason score tumors (7-10) was 4.4 months (logrank P=0.009), as compared to 2.9 months in the whole patient population. In a time of shifting paradigms in mCRPC with AR targeted agents available prior to docetaxel chemotherapy, Gleason score may help in selecting patients who obtain the greatest benefit from docetaxel as first-line treatment for mCRPC.

The neutrophil to lymphocyte ratio (NLR) is a marker for systemic inflammation, which is one of the emerging hallmarks of cancer [17]. In **chapter 7** we explored the prognostic and predictive role of the derived neutrophil to lymphocyte ratio (dNLR) in two multinational randomized phase III trials of mCRPC patients receiving first-line chemotherapy. In conclusion, the dNLR was prognostic for OS in men with mCRPC receiving first-line chemotherapy. We found that an elevated dNLR (≥2) was associated with shorter survival irrespective of the received treatment and other known prognostic factors. Furthermore, we composed a risk score to predict survival of men with mCRPC receiving first-line chemotherapy. Factors included in the final risk score were; age, alkaline phosphatase, hemoglobin, duration of initial androgen deprivation therapy, dNLR, and the presence of pain at baseline. The composed score and was significantly and inversely associated with survival in both databases. The dNLR is a readily available biomarker that comes with no extra costs, which can be used for risk stratification in future clinical trials and should be incorporated into new prognostic nomograms. In addition, it may provide direct prognostic information on patients in daily practice.

In **chapter 8** the results of chapters 3-7 are discussed and recommendations for future research are provided. In this thesis, we have identified mechanisms of cross-resistance between the available therapies in mCRPC. These may ultimately help in defining the

optimal drug treatment sequence. Furthermore, we have provided both predictive and prognostic factors, which can help to define which patients will benefit the most from treatment with docetaxel chemotherapy in mCRPC.

Despite the rapid therapeutic advances, mCRPC is still a lethal disease. Biomarker driven studies are key in identifying mechanisms of resistance to new drugs, and developing strategies to overcome resistance. Such translational efforts are urgently needed to change the current one-size-fits-all approach into a more personalized treatment strategy for mCRPC. Novel therapies and drug combinations targeting multiple tumorigenic pathways may ultimately turn mCRPC into a chronic rather than a lethal disease.

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SAMENVATTING

APPENDIX 1

SAMENVATTING

De behandeling van het gemetastaseerd castratie-resistent prostaatcarcinoom (mCRPC) heeft zich de laatste jaren sterk ontwikkeld met de introductie van verschillende nieuwe middelen waaronder cabazitaxel, abiraterone, enzalutamide en radium-223 [1-5]. Deze vooruitgang brengt ook nieuwe uitdagingen met zich mee. Het wordt vermoed dat er kruisresistentie zou kunnen optreden tussen taxanen (zoals docetaxel en cabazitaxel) en androgeen receptor (AR) gerichte middelen abiratrone en enzalutamide. Daarnaast is de optimale behandelingsvolgorde voor mCRPC nog onbekend.

Er is toenemend wetenschappelijk bewijs uit retrospectieve klinische studies, welke suggereren dat de effectiviteit van docetaxel verminderd zou zijn in patiënten met mCRPC die eerder behandeld zijn met abiraterone [6-8]. Daarnaast zijn er sterke aanwijzingen dat de effectiviteit van abiraterone verminderd is na eerdere behandeling met enzalutamide en vice versa. [9-12].

Taxanen werken door interactie met microtubili middels polymerisatie, leidend tot mitose arrest en apoptose. Recente studies suggereren dat paclitaxel en docetaxel ook in staat zijn om AR-translocatie en signalering te inhiberen, hetgeen in mCRPC deels verantwoordelijk zou kunnen zijn voor het therapeutisch effect. In **hoofdstuk 3** hebben we castratie-resistante prostaatkanker (CRPC) cellijnen met verworven resistentie voor abiraterone en enzalutamide gebruikt om inzicht te krijgen in mechanismen van kruisresistentie tussen docetaxel, cabazitaxel, abiraterone en enzalutamide. In deze cellijnen hebben we kruisresistentie waargenomen tussen de taxanen en nieuwe hormonale middelen abiraterone en enzalutamide. Als een potentieel mechanisme voor kruisresistentie identificeerden we AR translocatie naar de celkern, welke een overlappend werkingsmechanisme tussen deze middelen is. De rol van microtubili en het motoreiwit dyneine in AR transport is recent aan het licht gekomen in preklinische studies [15,16]. In onze studie, hebben we laten zien dat taxanen in staat zijn om AR translocatie naar de celkern te remmen als een additioneel werkingsmechanisme. Dit effect zagen we ook voor de nieuwe hormonale middelen abiraterone en enzlautamide, hetgeen een overlappend werkingsmechanisme identificeert dat potentieel zorgt voor kruisresistentie tussen taxanen en deze nieuwe hormonale middelen.

In **hoofdstuk 4** hebben we onze in vitro bevindingen van kruisresistentie tussen docetaxel en enzalutamide bevestigd in een muismodel van enzalutamide-resistent versus enzalutamide-naïef CRPC. De effectiviteit van docetaxel was verminderd in tumoren met verworven resistentie voor enzalutamide (PC346Enza) vergeleken met de enzalutamide-naïve tumoren (PC346C). Deze verminderde effectiviteit werd veroorzaakt door een verminderde inhibitie van AR translocatie naar de celkern en AR gereguleerde genexpressie in enzalutamide-resistente tumoren vergeleken met enzalutamide- naïve tumoren. In tegenstelling tot docetaxel, bleef cabazitaxel zeer effectief in enzalutamide-

resistente tumoren, hetgeen grotere anti-proliferatieve eigenschappen laat zien welke onafhankelijk zijn van de AR pathway. Deze bevindingen zijn interessant omdat ze exact overeenkomen met klinische studies welke kruisresistentie toonden voor docetaxel, maar niet voor cabazitaxel, hetgeen mechanistisch verklaard kan worden door de bevindingen in onze xenograft studies [6,7,9,10].

In **hoofdstuk 5** hebben we de bevindingen uit onze preklinsiche in vivo studies klinisch bevestigd in patiënten. Middels data uit een prospectieve, multicenter, gerandomiseerde fase 2 studie hebben we de invloed van eerdere therapie abiraterone en enzalutamide op de effectiviteit van cabazitaxel onderzocht. In deze studie vonden we dat de PSA respons onder behandeling met cabazitaxel vergelijkbaar was tussen patiënten met en zonder eerdere hormonale therapie (respectievelijk 34% versus 40%, P=0.53). Tevens was de mediane overleving niet significant verschillend tussen mannen met en zonder eerdere hormonale therapie, met een overleving van respectievelijk 9.8 maanden versus 10.6 maanden (logrank P=0.65). Deze bevindingen wijzen er sterk op dat er geen kruisresistentie is tussen abiraterone/enzalutamide en cabazitaxel.

In **hoofdstuk 6** hebben we onderzocht welke patiënten het meeste baat hebben bij chemotherapie met docetaxel. Hiertoe hebben we een post-hoc analyse uitgevoerd op de TAX327 registratie studie. Deze analyse liet zien dat de klinische baat bij docetaxel het grootst was voor patiënten met een hoge Gleason score bij diagnose. De mediane overleving in mannen met een hoge Gleason score (7-10) was 4.4 maanden (logrank P=0.009), vergeleken met 2.9 maanden in de gehele patiëntenpopulatie. Nu de nieuwe hormonale middelen abiraterone en enzalutamide ook beschikbaar zijn voor behandeling met chemotherapie kan de Gleason score helpen met het selecteren van patiënten die de meeste baat hebben bij chemotherapie met docetaxel als eerstelijns therapie voor mCRPC.

De neutrofielen-lymfocyten ratio (NLR) is een marker voor systemische inflammatie, hetgeen beschreven is als één van de specifieke kenmerken van kanker [17]. In **hoofdstuk 7** hebben we de prognostische en predictieve rol van de afgeleide NLR (dNLR) onderzocht in twee multinationale gerandomiseerde fase 3 studies van patiënten met mCRPC die eerstelijns chemotherapie ondergingen. Concluderend, was de dNLR geassocieerd met overlevingsduur in mannen met mCRPC behandeld met eerstelijns chemotherapie. Een verhoogde dNLR (≥2) was geassocieerd met een kortere overlevingsduur, onafhankelijk van de ontvangen behandeling en andere prognostische factoren. Vervolgens hebben we een risicoscore ontworpen die de overleving van mannen met mCRPC die eerstelijns chemotherapie ondergaan kan voorspellen. Factoren die meegewogen zijn in de risicoscore zijn; leeftijd, alkalisch fosfatase, hemoglobine, de duur van initiële hormonale therapie, dNLR, en de aanwezigheid van pijn. De samengestelde risicoscore was significant geassocieerd met overlevingsduur in beide databases. Dit maakt dat de dNLR is een

goedkope biomarker is, die klaar is voor klinisch gebruik en de prognose van patiënten met mCRPC kan voorspellen.

In **hoofdstuk 8** worden hoofdstukken 3-7 in perspectief geplaatst en worden aanbevelingen voor toekomstig onderzoek gedaan. In dit proefschrift hebben we mechanismen van kruisresistentie tussen de beschikbare therapieën voor mCRPC geïdentificeerd. Deze bevindingen kunnen uiteindelijk bijdragen aan het vinden van de optimale behandelingsvolgorde voor patiënten met mCRPC. Hiernaast hebben we zowel predictieve als prognostische factoren gevonden welke kunnen helpen met het identificeren van patiënten die de meeste baat hebben bij chemotherapie met docetaxel.

Ondanks de snelle vooruitgang in de behandeling is mCRPC nog steeds een dodelijke ziekte. Biomarker gedreven studies zijn de sleutel in het identificeren van resistentiemechanismen voor nieuwe middelen, en in het ontwikkelen van strategieën om resistentie te voorkomen. Zulke translationele studies zijn dringend nodig om de huidige one-size-fits-all aanpak te veranderen in een gepersonaliseerde behandeling voor mCRPC. Nieuwe therapieën en behandelcombinaties gericht op meerdere tumorpathways zouden van mCRPC uiteindelijk een chronische in plaats van een dodelijke ziekte kunnen maken.

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DANKWOORD

APPENDIX 2

DANKWOORD

Het belangrijkste hoofdstuk van dit proefschrift ligt hier voor u. Vele besprekingen, experimenten, wetenschappelijke discussies, en slapeloze nachten verder, is dan toch eindelijk dit boekje voltooid. Zonder intensieve samenwerking met vele anderen was het er nooit gekomen. Veel dank wil ik ook betuigen aan alle patiënten en hun families die hebben deelgenomen aan de klinische studies in dit proefschrift. Een aantal mensen wil ik in het speciaal bedanken.

Allereerst mijn promotor; Prof. de Wit, beste Ronald, ik ben je erg dankbaar voor de unieke kansen die je me hebt gegeven. Jouw toegewijde begeleiding en scherpe klinische visie zijn de sleutel tot dit proefschrift geweest. Dankzij je grote netwerk heb ik de mogelijkheid gehad om met veel internationale onderzoekers samen te werken. Ondanks dat ik je teleur heb moeten stellen (en geen internist-oncoloog ben geworden), hoop ik van harte dat je nog lang doorgaat, zodat we ook in de toekomst samen kunnen blijven werken.

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Graag wil ik alle nog niet genoemde co-auteurs bedanken en met name Arnoud Templeton (Kantonnspital, St. Gallen en Princess Margaret Cancer Centre, Toronto) voor onze samenwerking bij het onderzoek naar de neutrofielen-lymphocyten-ratio in mCRPC.

Mijn paranimfen Linda van Soest en Rik van Zijp, het is een eer dat jullie me op deze dag terzijde staan. Linda, lief zusje, ik ben heel trots op je en kan altijd op je rekenen. Heel knap hoe je je altijd staande heb gehouden afgelopen jaren. Rik, beste maat vanaf groep 1, met jou heb ik alles meegemaakt. Van onze eerste stappen in de Kuip tot zwemfeestjes op de plaats waar straks mijn promotieborrel is.

Mijn moeder, lieve Marja, bedankt voor je onvoorwaardelijke steun in moeilijke tijden. Je bent een ongelofelijk sterke vrouw. Papa, je hebt me gevormd tot wie ik ben, door jou ben ik geneeskunde gaan studeren. Dit zijn de moeilijkste dagen om je te missen, maar ik weet dat je er straks toch stiekem bij bent.

Lieve Maloes, je betekent alles voor me en heb me altijd gesteund. Doeset daram joenam. En mijn tweede familie: Mamijoen, Babajoen, Lisa en Robert, Lilian, Isa, bedankt voor alle liefde.

CURRICULUM VITAE

APPENDIX 3

CURRICULUM VITAE

Robert van Soest werd geboren op 3 juni 1987 te Rotterdam. Hij groeide op in Rotterdam en behaalde hier zijn gymnasium diploma in 2005. In hetzelfde jaar begon hij met de studie geneeskunde aan de Erasmus Universiteit Rotterdam, alwaar hij in 2012 zijn artsenbul kreeg uitgereikt. Tijdens de co-schappen werd zijn interesse in de urologie al snel gewekt met als gevolg een oudste co-schap en afstudeeronderzoek op de afdeling urologie van het Erasmus MC onder leiding van Prof. C.H. Bangma. Aansluitend ging hij verder met een promotietraject op de afdelingen urologie en interne-oncologie van het Erasmus MC onder supervisie van Prof. R. de Wit, Prof. G. Jenster en Dr. W. van Weerden. Na een korte stage als arts-assistent urologie in het St. Franciscus Gasthuis te Rotterdam werd hij in 2014 aangenomen voor de opleiding tot uroloog. Sinds januari 2015 is hij begonnen met de vooropleiding heelkunde in het St. Franciscus Gasthuis. In 2017 zal hij zijn opleiding urologie voortzetten in het Erasmus MC te Rotterdam.

LIST OF PUBLICATIONS

APPENDIX 4

LIST OF PUBLICATIONS

van Soest RJ, de Morrée ES, Shen L, Tannock IF, Eisenberger MA, de Wit R.
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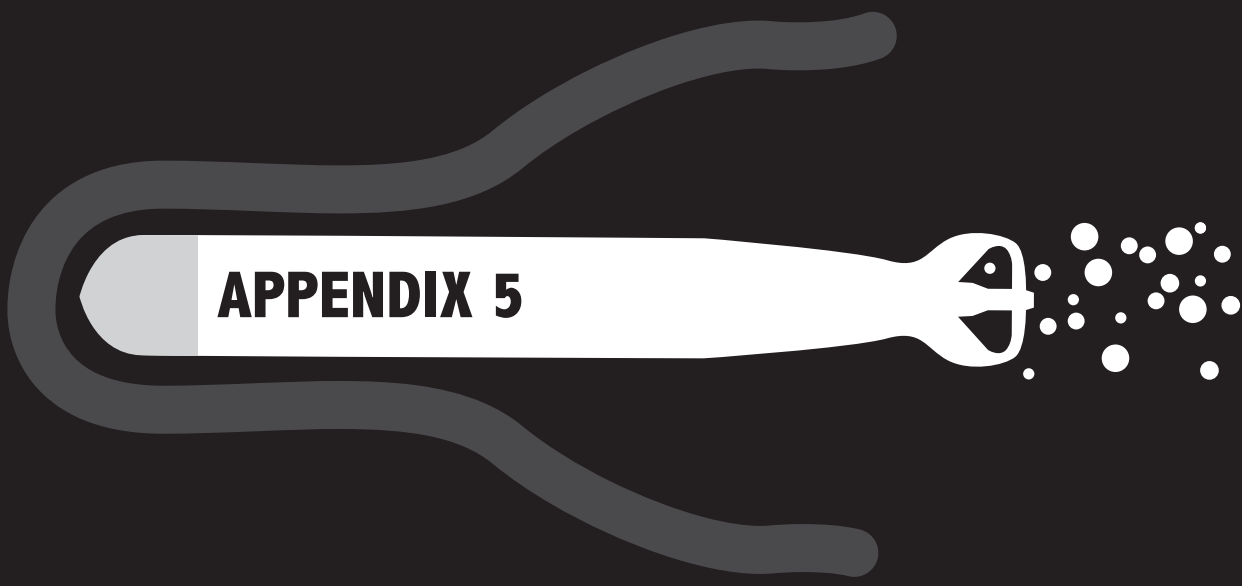
PHD PORTFOLIO



APPENDIX 5

PHD PORTFOLIO

Name PhD student:	Robert J. van Soest
Erasmus MC department:	Urology
PhD period:	2012-2014
Promotors:	Prof.dr. R. de Wit Prof.dr.ir. G. Jenster
Copromotor:	Dr.ir. W.M. van Weerden

PhD training	Year	Workload (ECTS)
General courses		
English biomedical writing and communication	2012-2013	4
Course on molecular diagnostics	2012	1
Course on microscopic image analysis	2012	0.5
Course on survival analysis	2013	0.5
Biostatistical methods and principles	2013	2
Clinical research course on Good Clinical Practice (BROK)	2013	1.5
Seminars and workshops		
Department of Urology Journal club	2012-2014	1
Department of Urology PhD meeting	2012-2014	0.5
Department of Urology 'refereeravond'	2012-2014	1
Presentations national		
NVU voorjaarsvergadering/najaarsvergadering (Vlietstraprijs)	2012-2014	1
Dutch uro-oncologic study group (DUOS)	2012-2014	1.5
IKNL netwerkdagen	2013	0.5
Tour d'Europe Rotterdam	2014	0.5
Presentations international		
ASCO Annual meeting (merit award)	2013-2014	1.5
EAU Annual Congress (poster award)	2013-2014	0.5
ASCO-GU meeting (merit award)	2013	0.5
European Cancer Conference	2013	0.5
Prostate Cancer Translational Research in Europe	2013	1
ESMO congress	2014	0.5
Interactive Genitourinary Cancer Conference (faculty member)	2014	1.5
EAU Annual Congress (faculty member)	2015	1
Lecturing		
'The androgen receptor' Medical students Erasmus MC	2012-2014	1.5
'Urological anatomy' Medical students Erasmus MC	2012-2013	1
Supervision of master thesis, students biopharmaceutical sciences, LUMC	2013-2014	4