# Chapter 13

## Summarizing discussion - Future perspectives on treatment of patients with metastatic castrate-resistant prostate cancer

Prostate cancer has the highest incidence of all cancers in men in the Netherlands.[1] Its incidence rises with age. Virtually all mortality caused by prostate cancer occurs after the tumor has become metastatic and castrate-resistant (mCRPC). At this stage, prostate cancer treatment is strictly palliative. However, only 10-20% of all prostate cancer patients reach this disease stage within five years after diagnosis.[2] Therefore, a majority of patients dies with prostate cancer, but not because of the disease. Nevertheless, due to its high prevalence in the population, prostate cancer is still the second deadliest cancer in men, being only surpassed by lung cancer.[3, 4] In this thesis, multiple approaches to improve mCRPC outcome were studied, focusing on novel chemotherapies for mCRPC patients.

Until 2004, mCRPC patients were treated with mitoxantrone, which improves quality of life but has not shown to extend overall survival.[5] That year docetaxel was introduced as first-line therapy for mCRPC patients. This taxane extended median overall survival by 2.4 months compared to mitoxantrone treatment in the TAX-327 trial.[6] Docetaxel targets cells by stabilizing microtubules. Its mechanism of action results in a variety of antitumor effects in the cell, such as mitotic arrest, inhibition of transnuclear localization of the androgen receptor, and p53 induced apoptosis.[7, 8] However, docetaxel is not specific for cancer cells, and as microtubules play essential roles in healthy cells too, docetaxel treatment may result in severe adverse events, such as polyneuropathy and bone marrow suppression. Furthermore, only about half of mCRPC patients respond to docetaxel treatment, and eventually all tumors become docetaxel resistant.[6] Therefore, additional research aims to further improve survival and quality of life of mCRPC patients.

Over the past few years, various novel second-line mCRPC therapies have been introduced into the clinic, such as cabazitaxel (a second-generation taxane),[9] abiraterone acetate (an inhibitor of the enzyme cytochrome P450 c17 (CYP17)),[10, 11] and enzalutamide (an androgen receptor antagonist)[12]. These agents have been studied or are being studied as first-line therapy as well; however, currently known results from studies such as COU-AA-302, a phase III study assessing abiraterone in asymptomatic or minimally symptomatic mCRPC patients, have not resulted in a change in policy regarding first-line mCRPC therapy in the Netherlands, one reason being that the control-arm received prednisone, which is not standard of care in the Netherlands.[13]

As multiple second-line mCRPC therapies have received approval, it has become unclear what therapy sequence is preferred after docetaxel therapy. In **Chapter 4**, we studied therapy outcome in mCRPC patients receiving cabazitaxel and abiraterone after docetaxel therapy. Differences in survival were minimal and non-conclusive, also as patient characteristics were not completely equal between groups due to the retrospective nature of the study. Cabazitaxel had a lower antitumor efficacy in mCRPC patients who had previously been

treated with abiraterone than in patients who received cabazitaxel before abiraterone; nevertheless, some patients still had clinical benefit by treatment with cabazitaxel. In patients treated with abiraterone after cabazitaxel, abiraterone was only slightly less effective than in patients receiving abiraterone prior to cabazitaxel. Future studies are needed to evaluate the cause of this decreased antitumor efficacy in more advanced mCRPC patients. As a final note, the CAST-study confirmed that toxicity caused by cabazitaxel, some life-threatening, remains a considerable issue with cabazitaxel therapy.

In the CAST-study, abiraterone and particularly cabazitaxel had a decreased antitumor efficacy when administered in the third-line compared to second-line therapy. Similar results were reported in patients receiving enzalutamide after docetaxel and abiraterone, abiraterone after docetaxel and enzalutamide, and docetaxel after abiraterone.[14-18] These findings may simply be a consequence of the patients having more advanced disease. Alternatively, cross-resistance between various mCRPC therapies may occur, e.g. as all therapies interact in some way with the androgen receptor pathway. Early preclinical studies indicated cross-resistance between taxanes and abiraterone.[19] However, this theory currently lacks a thorough scientific basis: aforementioned results need to be confirmed and mechanisms for cross-resistance would need to be identified. Thirdly, these therapies may only target a subgroup of prostate cancer cells, while other critical cancer cells are not inhibited by the therapy. E.g., stem cell-like prostate cancer cells may not be targeted by these therapies, resulting in proliferation and differentiation of these cells and acquired resistance.[20] It has also been suggested that epithelial-to-mesenchymal transition (EMT) may result in docetaxel resistance through reduced expression of miR-200C and miR-205, while such mesenchymal cells are prone to metastasize.[21]

Apart from aforementioned considerations regarding treatment sequence, the optimal time to initiate and end treatment needs more investigation. Another limitation of the COU-AA-302 study is that it is unclear whether treatment at this early stage has an advantage over treating patients once they have become symptomatic. As all therapies are palliative and have adverse events, it would be best to postpone treatment as long as possible, unless clinical outcome would be worse. Until this has been studied more extensively, it seems rational to avoid treatment in non-symptomatic patients.
Treatment is currently discontinued once patients start progressing on their tumor. Furthermore, taxane treatment is generally discontinued after ten cycles. However, it is unknown whether this is the best moment to discontinue treatment. Increasing treatment duration may invoke an extra delay for therapies with improved antitumor efficacy, particularly now that multiple therapy options are available for mCRPC patients. On the other hand, even when patients have minimal progressive disease, e.g. slowly increasing PSA levels while no progression is observed during radiographic assessments, patients may

still be benefitting from this therapy.

Currently, mCRPC patients are being viewed as a homogenous population when making therapy decisions, while cancer is a heterogeneous disease. As discussed in **chapter 2**, race may be one factor in tumor heterogeneity in prostate cancer patients. Since prostate cancer prevalence and mortality differs across races, the importance of recruiting a patient population in clinical trials whose racial demographics adequately represent the average patient population in clinic is stressed in this chapter. Furthermore, markers that identify mCRPC patient populations that will respond better to therapy need to be identified. This has become more crucial now that multiple therapies are available: by treating patients with ineffective therapies, other, potentially effective therapies are being delayed, while toxicity of the ineffective therapy may be severe. Results from the Dutch compassionate use program with cabazitaxel **(chapter 3)** and the CAST-study **(chapter 4)** suggest that patients who respond well to docetaxel, i.e. completed at least 10 cycles of docetaxel, have a better response to cabazitaxel therapy than patients who had receive less than 10 cycles of docetaxel. Others have found that Gleason-score predicts docetaxel response.[22] In **chapter 9**, we report that nuclear Eg5-expression may be a biomarker predicting docetaxel response as well. Additional research is needed to identify predictive markers for therapy response, as well as markers that are able to follow therapy response more accurately during treatment. Such markers would prevent overtreatment and may aid in determining what therapy to initiate in an individual patient.

Nuclear Eg5-expression may not only be a marker for docetaxel response, but our results indicated that nuclear Eg5-expression may predict tumor aggressiveness too. While Gleason-score is now most frequently used to assess tumor aggressiveness, this assessment is limited: patients with a Gleason 6 or higher may have highly indolent disease, while others with a Gleason 6 progress rapidly into highly aggressive mCRPC. Results from multivariate analyses in our study suggested that nuclear Eg5-expression is a biomarker of tumor aggressiveness, independent of Gleason-score. If our results can be confirmed in an independent larger study population, determination of Eg5-expression in biopsies taken from patients may aid in determining whether a patient can be treated more conservatively or needs aggressive treatment, such as treatment with taxanes at an earlier disease stage.

Due to the success of taxanes, researchers hypothesized that inhibitors of proteins that play essential roles during mitosis, commonly overexpressed in cancer, may be effective while causing fewer side effects. This rationale led to the development of amongst others Eg5-, polo-like kinase 1 (Plk1-) and aurora kinase-inhibitors (**Chapter 5 section A**). Early clinical studies with these inhibitors had disappointing results, amongst others as toxicities such as bone marrow suppression prevented treatment with effective dosages. For some

researchers, this casts doubt whether such therapies may be effective at all.[23] In short, it was argued that mitotic inhibitors may have been unsuccessful as tumor cells in patients divide at a slower pace than tumor cells *in vitro* and *in vivo*. Due to the differences between preclinical models and tumors in patients, it was expected that mitotic inhibitors will have limited antitumor efficacy in humans. In **Chapter 5 section B**, we explain that such statements are too simplified. We argue that while the proteins have originally been identified due to their role in mitosis, they seem to play a more extensive role in tumor progression, such as regulation of the androgen receptor pathway.[24-26] Considering the wide overexpression of these proteins in (prostate) cancer compared to healthy tissue,[27-30] we think their roles as oncogene need to be investigated in more detail, and their clinical potential more thoroughly explored. The development of mitotic inhibitors is currently in full swing for a wide variety of cancers, among others prostate cancer.

Improvement of the antitumor effect of mitotic inhibitors in prostate cancer patients can be achieved by selecting patients based on the expression of the targeted protein in the tumor. For example, Eg5 and Plk1 are overexpressed in about half of prostate cancer patients; therefore, *a priori* selection of these patients would prevent overtreatment in about half of patients.[27, 28] Since all mCRPC patients have had prostate biopsies taken and/or underwent prostate surgery, tumor tissue is readily available, hence this assessment would be non-invasive. Increased specificity of antimitotic agents may decrease toxicities. Intracellular levels in the tumor may be improved by developing agents that have low affinity to pumps such as P-glycoprotein, which transports docetaxel and other agents out of the tumor cell, limiting their efficacy.[31] Combination therapy may enhance antitumor efficacy.

Similar to breast cancer, colorectal cancer and other cancer types, it is expected that in the future treatment of mCRPC will not consist of monotherapy but a combination of antitumor agents, due to the heterogeneity of the disease and as (stem cell-like) cancer cells have the ability to bypass pathways in order to survive. To speed up the clinical development of such combination therapies and to prevent unnecessary phase II studies with combination therapies that will not have an enhanced antitumor effect, we developed and tested Analysis of Functional Annotation (AFA) as an objective strategy to create a rationale for combination therapies (**Chapter 6**). Based on our AFA results with histone deacetylase inhibitors (HDACIs), we hypothesized that combinations of mitotic inhibitors with HDACIs would have an enhanced antitumor effect. In **Chapter 7** and **Chapter 8** we show that combination therapies of HDACIs with Plk1- and aurora kinase-inhibitors, respectively, indeed have synergistic antitumor effects in preclinical studies. This combination therapy could potentially be tested in humans, and may reduce bone marrow suppression and other adverse events, as lower concentrations can be used to accomplish similar antitumor effects.

Research is ongoing to identify additional targets for prostate cancer therapy. As most morbidity and mortality from prostate cancer is caused by metastases, patients may benefit from therapies that exclusively target metastases. Recently it has been reported that radium-223 extends the median overall survival of mCRPC patients by solely targeting bone metastases, underlining the importance of metastases-targeting agents **(Chapter 10)**.[32] Two targets to prevent or treat metastases are NDRG1, a metastasis-suppressor gene in prostate cancer **(Chapter 11 section a)**, and CDK5, an enzyme necessary for prostate cancer metastases.[33, 34] In **Chapter 11 section b**, we identify irinotecan and cetrimonium bromide as agents that selectively target NDRG1-deficient prostate cancer cells; tilorone selectively targets CDK5-deficient prostate cancer cells **(Chapter 12)**. Furthermore, other targeted therapies may improve antitumor efficacy too, such as therapies that prevent epithelial-mesenchymal transition (EMT) or stimulate mesenchymal-epithelial transition (MET), therapies that target the microenvironment of the tumor, and therapies that prevent/target angiogenesis so that the tumor is deprived of its necessary oxygen and nutrients to grow and survive. Therapies that specifically target stem cell-like prostate cancer cells may be crucial, as these cells are resistant to most of the currently available therapies, while recent research suggested that such cells (the castration-resistant cells expressing the NK3 homeobox 1 (Nkx3-1) (CARNs)) may initiate mCRPC after castration.[35, 36] Future research will need to identify and establish the clinical benefit of therapy aimed at these specific molecular/cellular targets.

Over the past years, significant progress has been made regarding the treatment of mCRPC patients. Multiple novel therapies have been introduced into clinical practice: cabazitaxel, abiraterone, enzalutamide and radium-223. Other therapies are at advanced stages of clinical development, such as ipilimumab, tasquinimod, custirsen and cabozantinib. Additional molecular targets and its targeted therapies are slowly but steadily identified in preclinical studies. Although the development of new therapies with increased efficacy and/or decreased toxicity remains of high importance, due to this progress it has become crucial that studies optimize the use of existing therapies, so that maximum clinical benefit can be accomplished in mCRPC patients with existing therapies. Therefore, research assessing the optimal treatment strategy with available therapies and the development of improved biomarkers, both for therapy response and tumor aggressiveness, has become of high importance too. Such research may further improve survival and the quality of life of mCRPC patients.

## References


1. http://cijfersoverkanker.nl/p=5124a6e31b232[Internet]. Incidence rate of cancers in men in the Netherlands. [in Dutch]. Nederlandse Kankerregistratie (NKR), integraal kankercentrum Nederland (IKNL). Utrecht, NL. 2013.
2. Kirby M, Hirst C, Crawford ED. Characterising the castration-resistant prostate cancer population: a systematic review. Int J Clin Pract 2011;65(11):1180-1192.
3. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014;64(1):9-29.
4. http://cijfersoverkanker.nl/selecties/Dataset_1/img5102c95ec2307[Internet]. Mortality rate of cancers in men in the Netherlands. [in Dutch]. Nederlandse Kankerregistratie (NKR), integraal kankercentrum Nederland (IKNL). Utrecht, NL. 2013.
5. Tannock IF, Osoba D, Stockler MR et al. Chemotherapy with mitoxantrone plus prednisone or prednisone alone for symptomatic hormone-resistant prostate cancer: a Canadian randomized trial with palliative end points. J Clin Oncol 1996;14(6):1756-1764.
6. Tannock IF, de Wit R, Berry WR et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med 2004;351(15):1502-1512.
7. Darshan MS, Loftus MS, Thadani-Mulero M et al. Taxane-induced blockade to nuclear accumulation of the androgen receptor predicts clinical responses in metastatic prostate cancer. Cancer Res 2011;71(18):6019-6029.
8. Giannakakou P, Nakano M, Nicolaou KC et al. Enhanced microtubule-dependent trafficking and p53 nuclear accumulation by suppression of microtubule dynamics. Proc Natl Acad Sci U S A 2002;99(16):10855-10860.
9. de Bono JS, Oudard S, Ozguroglu M et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. Lancet 2010;376(9747):1147-1154.
10. de Bono JS, Logothetis CJ, Molina A et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364(21):1995-2005.
11. Fizazi K, Scher HI, Molina A et al. Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol 2012;13(10):983-992.
12. Scher HI, Fizazi K, Saad F et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 2012;367(13):1187-1197.
13. Ryan CJ, Smith MR, de Bono JS et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med 2013;368(2):138-148.
14. Mezynski J, Pezaro C, Bianchini D et al. Antitumour activity of docetaxel following treatment with the CYP17A1 inhibitor abiraterone: clinical evidence for cross-resistance? Ann Oncol 2012.
15. Schrader AJ, Boegemann M, Ohlmann CH et al. Enzalutamide in Castration-resistant Prostate Cancer Patients Progressing After Docetaxel and Abiraterone. Eur Urol 2013.
16. Bianchini D, Lorente D, Rodriguez-Vida A et al. Antitumour activity of enzalutamide (MDV3100) in patients with metastatic castration-resistant prostate cancer (CRPC) pre-treated with docetaxel and abiraterone. Eur J Cancer 2013.
17. Loriot Y, Bianchini D, Ileana E et al. Antitumour activity of abiraterone acetate against metastatic castration-resistant prostate cancer progressing after docetaxel and enzalutamide (MDV3100). Ann Oncol 2013.

18. Noonan KL, North S, Bitting RL, Armstrong AJ, Ellard SL, Chi KN. Clinical activity of abiraterone acetate in patients with metastatic castration-resistant prostate cancer progressing after enzalutamide. Ann Oncol 2013;24(7):1802-1807.
19. van Soest RJ, de Morree ES, Teubel W et al. Pre-clinical evidence for cross-resistance between taxanes and abiraterone in castration-resistant prostate cancer (mCRPC). [abstract]. European Cancer Congress 2013. 2013 Sep 27 - Oct 1; Amsterdam, the Netherlands. Abstract 2929.
20. McCubrey JA, Chappell WH, Abrams SL et al. Targeting the cancer initiating cell: the Achilles' heel of cancer. Adv Enzyme Regul 2011;51(1):152-162.
21. Puhr M, Hoefer J, Schafer G et al. Epithelial-to-mesenchymal transition leads to docetaxel resistance in prostate cancer and is mediated by reduced expression of miR-200c and miR-205. Am J Pathol 2012;181(6):2188-2201.
22. van Soest RJ, de Morree ES, Shen L, Tannock IF, Eisenberger MA, de Wit R. 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. Eur Urol 2013.
23. Komlodi-Pasztor E, Sackett DL, Fojo AT. Inhibitors targeting mitosis: tales of how great drugs against a promising target were brought down by a flawed rationale. Clin Cancer Res 2012;18(1):51-63.
24. Hou X, Li Z, Huang W et al. Plk1-dependent microtubule dynamics promotes androgen receptor signaling in prostate cancer. Prostate 2013;73(12):1352-1363.
25. Lorenzo C, Liao Q, Hardwicke MA, Ducommun B. Pharmacological inhibition of aurora-A but not aurora-B impairs interphase microtubule dynamics. Cell Cycle 2009;8(11):1733-1737.
26. Liu XS, Song B, Liu X. The substrates of Plk1, beyond the functions in mitosis. Protein Cell 2010;1(11):999-1010.
27. Xing ND, Ding ST, Saito R et al. A potent chemotherapeutic strategy in prostate cancer: S-(methoxytrityl)-L-cysteine, a novel Eg5 inhibitor. Asian J Androl 2011;13(2):236-241.
28. Weichert W, Schmidt M, Gekeler V et al. Polo-like kinase 1 is overexpressed in prostate cancer and linked to higher tumor grades. Prostate 2004;60(3):240-245.
29. Buschhorn HM, Klein RR, Chambers SM et al. Aurora-A over-expression in high-grade PIN lesions and prostate cancer. Prostate 2005;64(4):341-346.
30. Chieffi P, Cozzolino L, Kisslinger A et al. Aurora B expression directly correlates with prostate cancer malignancy and influence prostate cell proliferation. Prostate 2006;66(3):326-333.
31. Payton M, Bush TL, Chung G et al. Preclinical evaluation of AMG 900, a novel potent and highly selective pan-aurora kinase inhibitor with activity in taxane-resistant tumor cell lines. Cancer Res 2010;70(23):9846-9854.
32. Parker C, Nilsson S, Heinrich D et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. N Engl J Med 2013;369(3):213-223.
33. Feldmann G, Mishra A, Hong SM et al. Inhibiting the cyclin-dependent kinase CDK5 blocks pancreatic cancer formation and progression through the suppression of Ras-Ral signaling. Cancer Res 2010;70(11):4460-4469.
34. Strock CJ, Park JI, Nakakura EK et al. Cyclin-dependent kinase 5 activity controls cell motility and metastatic potential of prostate cancer cells. Cancer Res 2006;66(15):7509-7515.
35. Wang X, Kruithof-de Julio M, Economides KD et al. A luminal epithelial stem cell that is a cell of origin for prostate cancer. Nature 2009;461(7263):495-500.
36. Germann M, Wetterwald A, Guzman-Ramirez N et al. Stem-like cells with luminal progenitor phenotype survive castration in human prostate cancer. Stem Cells 2012;30(6):1076-1086.