Metastatic Prostate Cancer

Perspective on Treatment Selection and Pharmacology

Bodine P.S.I. Belderbos

Colofon

ISBN: 9789463239240
Lay-out and cover design: Wouter Aalberts, Mircea Nikkels
Printed by: Gildeprint

The printing of the thesis was financially supported by: Astellas, Stichting Wetenschappelijk Onderzoek Assistenten Heelkundige Specialismen (SWOAHS).

Metastatic Prostate Cancer

perspective on treatment selection and pharmacology

Gemetastaseerd prostaatkanker

een visie op de keuze van behandeling en farmacologie

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof. dr. R.C.M.E. Engels

en volgens het besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

Woensdag 4 December 2019 om 11.30 uur

Bodine Pauline Sophie Ida Belderbos
geboren op 19 november 1988
te Breda

Erasmus University Rotterdam

PROMOTIECOMMISSIE

Promotoren	Prof. dr. R. de Wit
	Prof. dr. A.H.J. Mathijssen
Overige leden	Prof. dr. L. Incrocci
	Prof. dr. C.H. Bangma
	Prof. dr. R.J.A. van Moorselaar
Copromotoren	Dr. M.P.J.K. Lolkema
	Dr. R.J. van Soest

The journey ♫

Tom Misch

CONTENTS

Chapter 1

General introduction

Prostate cancer is the second most commonly diagnosed non-cutaneous malignancy and the fifth leading cause of cancer-related mortality in men worldwide.[1] The prognosis depends on the extend of the disease, ranging from localized to metastatic disease. For each stage different therapeutic interventions are available. The vast majority of prostate cancer is diagnosed as localized disease, which can be monitored by active surveillance when low-risk, or treated with curative intend by radical prostatectomy or prostate radiotherapy. However, about 10-20% of the patients have loco-regional or distant metastatic disease at diagnosis.[1] Metastatic prostate cancer can be roughly divided into metastatic hormone-sensitive prostate cancer (mHSPC) and metastatic castration-resistant prostate cancer (mCRPC). The first setting is the hormone-sensitive disease phase, or equally referred to as castration-naïve, which is characterized by its sensitivity to androgen deprivation (e.g. castration).[2] The effect of castration can suppress the disease for approximately 20 months,[3-5] hereafter the disease becomes castration-resistant. For years, androgen-deprivation therapy (ADT) has been the cornerstone of treatment in metastatic prostate cancer.[6] In the past decades the treatment options for metastatic prostate cancer have drastically expanded, changing clinical practice. Because prostate cancer cells remain dependent of the androgen receptor (AR) for survival and growth, treatment for metastatic prostate cancer mainly targets this receptor axis.[7] Since 2004, large phase III trials have reported considerable survival benefit with taxane chemotherapy, androgen receptor targeted therapies (ART), and radio-active therapy in the castration-resistant phase.[8-14] Only recently, some of these treatment options have shifted from mCRPC to the mHSPC setting, allowing for more aggressive therapy in an earlier phase of the disease, resulting in an even larger survival benefit.[2-4] **Figure 1** shows the treatment options, as included in the current European (ESMO, EAU, NCCN) guidelines, and upcoming treatment options in different disease stages of metastatic prostate cancer. The constant development of new therapy options and the introduction in an earlier disease phase have brought challenges for clinicians to individualize treatment selection. Thereby, the treatment of metastatic prostate cancer patients is and will continue to be a multidisciplinary team effort.

Unmet needs in the treatment selection process for metastatic prostate cancer patients include understanding prognosis to determine when intervention is needed (prognostic factors), indicators to reduce toxicity of systemic treatments, identification of factors predictive of treatment response to select an effective treatment for a patient (predictive factors), and early response markers to ascertain a treatment is working. In this thesis we aim to increase our insight on several of these factors. Moreover, we intent to widen the understanding on pharmacological effects of anti-cancer medication to improve and maintain its safe administration.

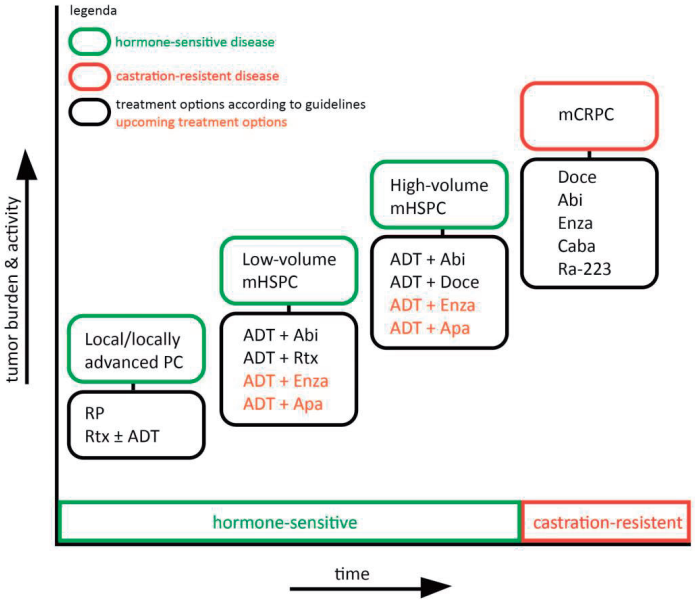


Figure 1. Treatment options in metastatic prostate cancer

PERSPECTIVE ON PHARMACOLOGY

In addition to the shift of available treatment options to an earlier disease phase, the development of new therapies is ongoing and combinations of available treatment options are subject of promising ongoing investigations.[15] The rationale for combining these therapies lies in the different working mechanisms and in previous (pre-)clinical work showing that the activity of taxanes and ART is strongly affected by hormonal manipulations.[16-18] Currently, phase I-II studies investigate the clinical effect of combination therapies in humans.[15,19] Studies combining treatment options, though, warrant thorough pharmacokinetic investigation to test for clinical relevant drug-drug interactions.
Taxanes, i.e. docetaxel and cabazitaxel, have an extensive hepatic metabolism and biliary excretion.[20] Their metabolism is mainly mediated by CYP3A iso-enzymes in the liver (80-90%), and for a small part by CYP2C8.[21] Therefore, liver (dys-)function is a major factor in taxane dose adaptations.[22] Moreover, the use of co-medication and herbal supplements, especially (strong) inducers/inhibitors of the CYP3A-system, influence pharmacokinetics of all taxanes.[23-26] Therefore, in **Chapter 2 and 3** we investigate the effects of enzalutamide and prednisone, as strong to moderate CYP3A4 inducers, on the pharmacokinetics of cabazitaxel and docetaxel, respectively.

One of the newly developed therapies for non-metastatic CRPC and potentially mHSPC is apalutamide.[27,28] Apalutamide is a new ART, binding to the ligand-binding domain of the AR, inhibiting AR nuclear translocation and impeding AR mediated transcription.[29] Before introduction in clinical practice it is important to have complete knowledge on pharmacodynamic effects of the agent. The most important and extensively investigated pharmacodynamic effects involve cardiac impairment. Medication has the ability to extent the ventricular repolarization of the heart, which could potentially result in cardiac arrhythmias and cardiac arrest. These side effects should be listed in the leaflet of the medication. Caution is needed with combinations of medication known to have an significant effect on ventricular repolarization, as an additive effect cannot be excluded. Therefore, in **Chapter 4**, the effect of apalutamide on ventricular repolarization is investigated in a thorough, international, multicenter trial involving 45 CRPC patients.

PERSPECTIVE ON TREATMENT SELECTION

Treatment options for metastatic prostate cancer, both castration-resistant and hormone-sensitive, have notably evolved in the past few years. However, the optimal individual treatment selection in mCRPC or mHSPC patients remains to be elucidated, which makes treatment decisions extremely challenging.[30,31] Prognostic factors, to estimate the probability of a specific clinical outcome such as survival, may help to make a risk assessment at that moment and to identify the best timing of treatment start. Predictive factors are biomarkers to foresee treatment response to a specific therapy. Additionally, treatment response markers are needed to determine the effect of the treatment on the tumor preferably as soon as possible after treatment start. In the absence of clinically available predictive biomarkers in the current practice, treatment choice is mainly based on clinical symptoms, metastatic burden, comorbidities, patients' preference, physicians' experience and toxicity.

mCRPC – prognostic factors

The identification of prognostic factors is usually based on cohort studies, along with data of post-hoc analysis of large phase III trials in which patients are treated with the same therapy. Trials with a long follow-up and extensive baseline parameters can most accurately identify factors which predict overall survival or toxicity. Previously, in mCRPC patients receiving first-line chemotherapy (docetaxel) prognostic models and nomograms have been developed based on the TAX327 trial,[7] which identified parameters such as performance status, time since diagnosis, metastatic extent, the presence of pain, duration of ADT, and laboratory results to predict for survival.[32-34] For second-line chemotherapy

(cabazitaxel), similar prognostic factors have been identified based on the TROPIC trial.[8,35] In **Chapter 5** we aim to confirm some of these findings and identify new factors in a different cohort of mCRPC patients treated with cabazitaxel. We used the large prospectively collected dataset of the CABARESC trial, which originally investigated the influence of budesonide on cabazitaxel-induced diarrhea in 246 mCRPC patients.[36] The CABARESC trial originated from 2011, which ensures sufficient follow-up period for this post-hoc analysis performed in 2016.

Prognostic factors can indicate overall survival, but can also predict for the occurrence of adverse events. In prostate cancer patients treatment with taxanes is often accompanied by considerable toxicity. Neutropenia has been the primary dose limiting toxicity of the performed phase I trials in patients with solid tumors treated with cabazitaxel.[37,38] This adverse event occurs frequently in cabazitaxel treated patients (80%) and can result in the life-threatening complication of febrile neutropenia (8%).[8] Other frequent adverse events were diarrhea, leukopenia, thrombocytopenia, and anemia. Pharmacogenetics study the differences in patients genetics, resulting in differences in proteins involved in metabolism or transportation of medication. Genetic differences could result in lower or higher levels of medication in the circulation of a patient, consequently affecting toxicity and survival. For instance, treatment with fluoropyrimidines can result in severe, even life-threatening, toxicity. This toxicity seems to be strongly affected by activity of Dihydropyrimidine Dehydrogenase (DPD), which is the main metabolizing enzyme of fluoropyrimidines. Therefore, nowadays the individual DPD activity is measured by genetic profiling of the patient before treatment is started.[39] Patient-related factors that possibly identify patients with increased risk of toxicity, or decreased risk of survival are useful to personalize treatment. Therefore, in **Chapter 6** we investigate the influence of genetic differences on cabazitaxel pharmacokinetics and treatment outcomes in 128 mCRPC patients. Single-nucleotide polymorphisms (SNPs) are the most prevalent genetic variants in humans and are defined as one single nucleotide difference in a human gene, which occurs in at least 1% of the population. SNPs in the genes encoding for transporters and enzymes involved in cabazitaxel metabolism and working mechanism are most likely to affect clinical outcomes. Transmembrane transporters P-glycoprotein (P-gp; *ABCB1*), organic anion transporters (OATP1B1; *SLCO1B1* and OATP1B3; *SLCO1B3*) and cytochrome P450 iso-enzymes (CYP3A4 and CYP3A5) are important in taxane metabolism.[40-43] Moreover, the binding site of taxanes in the cell nucleus on the microtubules, β-tubulin isoform (*TUBB*), may play a role in taxane resistance, and possibly survival.[42,44] Therefore, we assessed the association of SNPs in the following genes, *SLCO1B1* (OATP1B1), *SLCO1B3* (OATP1B3), *CYP3A4, CYP3A5, ABCB1* and *TUBB1,* with survival, toxicity, and pharmacokinetics in cabazitaxel treated mCRPC patients. Better understanding of mechanisms underlying inter-patient variability can help to personalize treatment options in these patients.

mCRPC – predictive markers

The field of metastatic prostate cancer would benefit from identification of predictive tumor biomarkers to individualize treatment. Up to now no predictive biomarkers are clinically available; however, liquid biopsies quickly gain ground as potential predictive biomarker. Liquid biopsies are simple blood collections in which valuable tumor- and patient-related information is hidden. Liquid biopsies include for instance identification and characterization of circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), cell-free DNA (cfDNA) and exosomes. Molecular characterization of the CTCs has been shown to provide disease information. The question remains how to interpret this information, and which clinical consequences it has. Currently, the most extensively investigated factor in patients with mCRPC is the androgen-receptor splice variant 7 (*AR-V7*) expression in CTCs. This mRNA splice variant of the AR lacks the ligand binding domain of the AR, resulting in a constitutively active receptor.[45] Therapies specifically targeting the AR, by ligand binding competition (enzalutamide) or reduced production of ligands (abiraterone), have been associated with decreased treatment response in *AR-V7* positive patients.[45,46] Based on a different working mechanism taxanes have been shown to still be effective in *AR-V7* positive patients.[47,48] In an ongoing trial (CABA-V7), *AR-V7* expression in CTCs is being further explored as a potential predictive biomarker, as it is prospectively investigated whether *AR-V7* positive patients will respond to cabazitaxel therapy (NCT03050866). Meanwhile, *AR-V7* positivity has also been related to unfavorable baseline characteristics and may therefore merely reflect a higher tumor burden.[49,50] To improve our understanding of the clinical value of *AR-V7*, we investigated the association of *AR-V7* to CTC count and OS (**Chapter 7**). In a post-hoc analysis of three prospectively performed clinical trials, 127 patient samples were evaluated for CTC enumeration and *AR-V7* expression to determine their associations and relate it to OS.

mCRPC – treatment response markers

Prostate-specific antigen (PSA) is the most widely used biomarker to detect and monitor treatment of men with prostate cancer. However, PSA production is androgen-dependent and as some drugs modulate androgen concentrations, PSA levels may decrease without clinical or radiological responses.[51] In combination with its low specificity, PSA is a debatable response biomarker. Usually, radiologic responses are defined by conventional imaging modalities, like PET CT/bone scan. Despite their limitations, these scans are standard in the current patient care and clinical trials to determine treatment response. Although, new, more sensitive imaging modalities are upcoming, the introduction and embedding of these modalities in clinical care awaits sufficient results of clinical trials showing their benefit over conventional imaging. Nowadays, liquid biopsies are

increasingly investigated also as potential treatment response marker. Since 2004 it is known that the enumeration of CTCs has prognostic significance in several tumor types.[52] A baseline CTC count ≥5 CTCs/7.5mL blood has a worse prognosis compared to patients with a CTC count of less than 5 CTC/7.5mL blood.[53] Therefore, a CTC count of >5 CTC/7.5mL blood is referred to as 'unfavorable' and <5 CTCs/ 7.5mL blood as 'favorable' in several tumor types.[52] Moreover, CTC conversion from unfavorable to favorable during treatment has been shown to be a stronger prognostic factor than a >50% decline in PSA and is suggested as surrogate marker for OS in clinical trials.[53,54] However, it needs further investigation before individual treatment decisions can be made based on CTC conversion or percentage change in CTC count.[55] To improve our knowledge on the clinical value of CTC responses, we performed an (unpublished) interim-analysis of the first twenty patients of the CABA-V7 trial. Herein, we evaluated the response rates in *AR-V7* positive mCRPC patients during treatment with cabazitaxel (**Chapter 8**)**.** The final results are expected in the complete analysis of the CABA-V7 trial.

mHSPC

The field of mHSPC is rapidly evolving, with treatment options shifting from the castration-resistant setting to the hormone-sensitive setting of prostate cancer. Since 2015, the addition of docetaxel/abiraterone/prostate radiotherapy to ADT in the hormone-sensitive phase has been demonstrated to improve OS.[3,4,56-58] It remains to be determined which patient may benefit most from either early concomitant docetaxel, abiraterone, or prostate radiotherapy since molecular biomarkers for early therapy response and risk stratification are currently lacking, similar to the castration-resistant setting. Based on the available trial data it has been suggested that the extent of metastatic disease may become one of the most important factors to determine treatment choice. Most physicians contemplate chemo-hormonal therapy (ADT + docetaxel) only in patients who are considered 'high-volume' metastatic by clinical parameters and conventional imaging. Abiraterone has been proven to be effective in high- and low-volume patients. And low-volume patients might also benefit from additional prostate radiotherapy. Most recently the addition of apalutamide and enzalutamide to ADT in hormone-sensitive setting has shown positive results. In **Chapter 9** we comprehensively review the available treatment options for mHSPC patients, and more importantly define tools to guide treatment selection by summarizing available trial data.

REFERENCES

1. Organization WH. Cancer Today / International agency for research on cancer. https://gco.iarc.fr/today/home. . Accessed 5 may 2019, 2019.
2. Gravis G, Fizazi K, Joly F, et al. Androgen-deprivation therapy alone or with docetaxel in non-castrate metastatic prostate cancer (GETUG-AFU 15): a randomised, open-label, phase 3 trial. Lancet Oncol. 2013;14(2):149-158.
3. James ND, Sydes MR, Clarke NW, et al. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. Lancet. 2016;387(10024):1163-1177.
4. Sweeney CJ, Chen YH, Carducci M, et al. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. N Engl J Med. 2015;373(8):737-746.
5. Lonergan PE, Tindall DJ. Androgen receptor signaling in prostate cancer development and progression. J Carcinog. 2011;10:20.
6. Sartor O, de Bono JS. Metastatic Prostate Cancer. N Engl J Med. 2018;378(7):645-657.
7. Tan MH, Li J, Xu HE, Melcher K, Yong EL. Androgen receptor: structure, role in prostate cancer and drug discovery. Acta Pharmacol Sin. 2015;36(1):3-23.
8. 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.
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. 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.
12. Ryan CJ, Smith MR, Fizazi K, et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol. 2015;16(2):152-160.
13. 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.
14. Beer TM, Armstrong AJ, Rathkopf DE, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med. 2014;371(5):424-433.
15. Sternberg CN. Improving Survival for Metastatic Castrate-resistant Prostate Cancer: Will Combination Therapy Help Us To Move Forward? Eur Urol. 2016;70(5):722-723.
16. Mout L, de Wit R, Stuurman D, et al. Testosterone Diminishes Cabazitaxel Efficacy and Intratumoral Accumulation in a Prostate Cancer Xenograft Model. EBioMedicine. 2018;27:182-186.
17. Shiota M, Kashiwagi E, Murakami T, et al. Serum testosterone level as possible predictive marker in androgen receptor axis-targeting agents and taxane chemotherapies for castration-resistant prostate cancer. Urol Oncol. 2019;37(3):180 e119-180 e124.
18. von Klot CA, Kuczyk MA, Boeker A, et al. Role of free testosterone levels in patients with metastatic castration-resistant prostate cancer receiving second-line therapy. Oncol Lett. 2017;13(1):22-28.
19. Corn PG, Agarwal N, Araujo JC, Sonpavde G. Taxane-based Combination Therapies for Metastatic Prostate Cancer. Eur Urol Focus. 2017.

20. Ridoux L, Semiond DR, Vincent C, et al. A phase I open-label study investigating the disposition of [14C]-cabazitaxel in patients with advanced solid tumors. Anticancer Drugs. 2015;26(3):350-358.
21. Genzyme S. SmPC Jevtana. 2017; https://www.medicines.org.uk/emc/product/4541/smpc. Accessed 18-04-2019, 2019.
22. Joerger M. Metabolism of the taxanes including nab-paclitaxel. Expert Opin Drug Metab Toxicol. 2015;11(5):691-702.
23. Nieuweboer AJ, de Morree ES, de Graan AJ, Sparreboom A, de Wit R, Mathijssen RH. Inter-patient variability in docetaxel pharmacokinetics: A review. Cancer Treat Rev. 2015;41(7):605-613.
24. Mathijssen RH, Sparreboom A, Verweij J. Determining the optimal dose in the development of anticancer agents. Nat Rev Clin Oncol. 2014;11(5):272-281.
25. Janssen A, Verkleij CPM, van der Vlist A, Mathijssen RHJ, Bloemendal HJ, Ter Heine R. Towards better dose individualisation: metabolic phenotyping to predict cabazitaxel pharmacokinetics in men with prostate cancer. Br J Cancer. 2017;116(10):1312-1317.
26. Sarantopoulos J, Mita AC, Wade JL, et al. Phase I study of cabazitaxel plus cisplatin in patients with advanced solid tumors: study to evaluate the impact of cytochrome P450 3A inhibitors (aprepitant, ketoconazole) or inducers (rifampin) on the pharmacokinetics of cabazitaxel. Cancer Chemother Pharmacol. 2014;74(6):1113-1124.
27. Chi KN, Agarwal N, Bjartell A, et al. Apalutamide for Metastatic, Castration-Sensitive Prostate Cancer. N Engl J Med. 2019.
28. Smith MR, Saad F, Chowdhury S, et al. Apalutamide Treatment and Metastasis-free Survival in Prostate Cancer. N Engl J Med. 2018;378(15):1408-1418.
29. Clegg NJ, Wongvipat J, Joseph JD, et al. ARN-509: a novel antiandrogen for prostate cancer treatment. Cancer Res. 2012;72(6):1494-1503.
30. Lorente D, Fizazi K, Sweeney C, de Bono JS. Optimal Treatment Sequence for Metastatic Castration-resistant Prostate Cancer. Eur Urol Focus. 2016;2(5):488-498.
31. Akaza H, Procopio G, Pripatnanont C, et al. Metastatic Castration-Resistant Prostate Cancer Previously Treated With Docetaxel-Based Chemotherapy: Treatment Patterns From the PROXIMA Prospective Registry. J Glob Oncol. 2018;4:1-12.
32. Loriot Y, Eymard JC, Patrikidou A, et al. Prior long response to androgen deprivation predicts response to next-generation androgen receptor axis targeted drugs in castration resistant prostate cancer. Eur J Cancer. 2015;51(14):1946-1952.
33. Armstrong AJ, Garrett-Mayer E, de Wit R, Tannock I, Eisenberger M. Prediction of survival following first-line chemotherapy in men with castration-resistant metastatic prostate cancer. Clin Cancer Res. 2010;16(1):203-211.
34. Halabi S, Lin CY, Kelly WK, et al. Updated prognostic model for predicting overall survival in first-line chemotherapy for patients with metastatic castration-resistant prostate cancer. J Clin Oncol. 2014; 32(7):671-677.
35. Halabi S, Lin CY, Small EJ, et al. Prognostic model predicting metastatic castration-resistant prostate cancer survival in men treated with second-line chemotherapy. J Natl Cancer Inst. 2013;105(22): 1729-1737.
36. Nieuweboer AJ, de Graan AM, Hamberg P, et al. Effects of Budesonide on Cabazitaxel Pharmacokinetics and Cabazitaxel-Induced Diarrhea: A Randomized, Open-Label Multicenter Phase II Study. Clin Cancer Res. 2017;23(7):1679-1683.
37. Mita AC, Denis LJ, Rowinsky EK, et al. Phase I and pharmacokinetic study of XRP6258 (RPR 116258A), a novel taxane, administered as a 1-hour infusion every 3 weeks in patients with advanced solid tumors. Clin Cancer Res. 2009;15(2):723-730.

38. Pivot X, Koralewski P, Hidalgo JL, et al. A multicenter phase II study of XRP6258 administered as a 1-h i.v. infusion every 3 weeks in taxane-resistant metastatic breast cancer patients. Ann Oncol. 2008; 19(9):1547-1552.
39. Henricks LM, Lunenburg C, de Man FM, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. Lancet Oncol. 2018; 19(11):1459-1467.
40. Pean E, Demolis P, Moreau A, et al. The European Medicines Agency review of cabazitaxel (Jevtana(R)) for the treatment of hormone-refractory metastatic prostate cancer: summary of the scientific assessment of the committee for medicinal products for human use. Oncologist. 2012;17(4):543-549.
41. Nieuweboer AJ, Hu S, Gui C, et al. Influence of drug formulation on OATP1B-mediated transport of paclitaxel. Cancer Res. 2014;74(11):3137-3145.
42. Marsh S. Taxane pharmacogenetics. Per Med. 2006;3(1):33-43.
43. de Morree ES, Bottcher R, van Soest RJ, et al. Loss of SLCO1B3 drives taxane resistance in prostate cancer. Br J Cancer. 2016;115(6):674-681.
44. Duran I, Hagen C, Arranz JA, et al. SNPs associated with activity and toxicity of cabazitaxel in patients with advanced urothelial cell carcinoma. Pharmacogenomics. 2016;17(5):463-471.
45. Antonarakis ES, Lu C, Wang H, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med. 2014;371(11):1028-1038.
46. Armstrong AJ, Halabi S, Luo J, et al. Prospective Multicenter Validation of Androgen Receptor Splice Variant 7 and Hormone Therapy Resistance in High-Risk Castration-Resistant Prostate Cancer: The PROPHECY Study. J Clin Oncol. 2019;37(13):1120-1129.
47. Onstenk W, Sieuwerts AM, Kraan J, et al. Efficacy of Cabazitaxel in Castration-resistant Prostate Cancer Is Independent of the Presence of AR-V7 in Circulating Tumor Cells. Eur Urol. 2015;68(6):939-945.
48. Antonarakis ES, Lu C, Luber B, et al. Androgen Receptor Splice Variant 7 and Efficacy of Taxane Chemotherapy in Patients With Metastatic Castration-Resistant Prostate Cancer. JAMA Oncol. 2015; 1(5):582-591.
49. Tagawa ST, Antonarakis ES, Gjyrezi A, et al. Expression of AR-V7 and ARv567es in circulating tumor cells correlates with outcomes to taxane therapy in men with metastatic prostate cancer treated in TAXYNERGY. Clin Cancer Res. 2018.
50. Plymate SR, Sharp A, de Bono JS. Nuclear Circulating Tumor Cell Androgen Receptor Variant 7 in Castration-Resistant Prostate Cancer: The Devil Is in the Detail. JAMA Oncol. 2018;4(9):1187-1188.
51. Pantel K, Hille C, Scher HI. Circulating Tumor Cells in Prostate Cancer: From Discovery to Clinical Utility. Clin Chem. 2019;65(1):87-99.
52. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med. 2004;351(8):781-791.
53. de Bono JS, Scher HI, Montgomery RB, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res. 2008;14(19):6302-6309.
54. Scher HI, Jia X, de Bono JS, et al. Circulating tumour cells as prognostic markers in progressive, castration-resistant prostate cancer: a reanalysis of IMMC38 trial data. Lancet Oncol. 2009;10(3): 233-239.
55. Morris MJ, Autio KA, Basch EM, Danila DC, Larson S, Scher HI. Monitoring the clinical outcomes in advanced prostate cancer: what imaging modalities and other markers are reliable? Semin Oncol. 2013;40(3):375-392.
56. James ND, de Bono JS, Spears MR, et al. Abiraterone for Prostate Cancer Not Previously Treated with Hormone Therapy. N Engl J Med. 2017;377(4):338-351.

57. Fizazi K, Tran N, Fein L, et al. Abiraterone acetate plus prednisone in patients with newly diagnosed high-risk metastatic castration-sensitive prostate cancer (LATITUDE): final overall survival analysis of a randomised, double-blind, phase 3 trial. Lancet Oncol. 2019;20(5):686-700.
58. Parker CC, James ND, Brawley CD, et al. Radiotherapy to the primary tumour for newly diagnosed, metastatic prostate cancer (STAMPEDE): a randomised controlled phase 3 trial. Lancet. 2018; 392(10162):2353-2366.

Chapter 2

Influence of enzalutamide on cabazitaxel pharmacokinetics; a drug-drug interaction study in metastatic castration-resistant prostate cancer (mCRPC) patients

Bodine P.S. Belderbos, Sander Bins, Roelof W.F. van Leeuwen, Esther Oomen-de Hoop, Nelly van der Meer, Peter de Bruijn, Paul Hamberg, Esther N.M. Overkleeft, Wendy M. van der Deure, Martijn P. Lolkema, Ronald de Wit, and Ron H.J. Mathijssen

Clin Cancer Res. 2018 Feb 1; 541-546

ABSTRACT

Purpose: In ongoing clinical research on metastatic castration-resistant prostate cancer (mCRPC) treatment, the potential enhanced efficacy of the combination of taxanes with AR-targeted agents, i.e. enzalutamide and abiraterone, is currently being explored. Since enzalutamide induces the CYP3A4 enzyme and taxanes are metabolized by this enzyme, a potential drug-drug interaction needs to be investigated.

Design: Therefore, we performed a pharmacokinetic cross-over study in mCRPC patients who were scheduled for treatment with cabazitaxel Q3W (25 mg/m^2). Patients were studied for three consecutive cabazitaxel cycles. Enzalutamide (160 mg QD) was administered concomitantly after the first cabazitaxel cycle, during 6 weeks. Primary endpoint was the difference in mean area under the curve (AUC) between the first (cabazitaxel monotherapy) and third cabazitaxel cycle, when enzalutamide was added.

Results: A potential clinically relevant 22% (95%CI: 9–34%, p=0.005) reduction in cabazitaxel exposure was found with concomitant enzalutamide use. The geometric mean $AUC_{0\text{-}24h}$ of cabazitaxel was 181 ng*h/mL (95%CI 150-219 ng*h/mL) in cycle 3 and 234 ng*h/mL (95%CI 209-261 ng*h/mL) in cycle 1. This combination did not result in excessive toxicity, while PSA response was promising.

Conclusions: We found a significant decrease in cabazitaxel exposure when combined with enzalutamide. In an era of clinical trials on combination strategies for mCRPC, it is important to be aware of clinically relevant drug-drug interactions. Since recent study results support the use of a lower standard cabazitaxel dose of 20 mg/m^2, the clinical relevance of this interaction may be substantial, since the addition of enzalutamide may result in sub-therapeutic cabazitaxel exposure.

TRANSLATIONAL RELEVANCE

Preclinical data suggest enhanced efficacy of cabazitaxel when combined with hormonal therapies for metastatic castration-resistant prostate cancer (mCRPC). Clinical studies on combining taxanes, i.e. docetaxel and cabazitaxel, and AR-targeted agents, i.e. enzalutamide and abiraterone, are ongoing. It is important to be aware of potential drug-drug interactions between these agents, especially since enzalutamide is known to be a strong CYP3A4 inducer and taxanes are metabolized by this enzyme. We performed a pharmacokinetic study to identify the influence of enzalutamide on cabazitaxel concentrations in mCRPC patients. We found a potential clinically relevant reduction in cabazitaxel concentration (>20%) when combined with enzalutamide. Nonetheless, the combination was safe and well-tolerated. Moreover, PSA response levels were higher than expected in this heavily pretreated patient population. Studies with this drug combination are warranted, but investigators need to be aware of the current findings.

INTRODUCTION

The current treatment paradigm in mCRPC comprises monotherapy options with chemotherapy, i.e. docetaxel or cabazitaxel, or with novel hormonal therapies, including enzalutamide and abiraterone.[1] Clinical studies with various combinations of therapies, e.g. taxanes with novel hormonal therapies, with various designs and aims, are in progress.[2-5] A summary of ongoing clinical trials on these combinations was recently reviewed by Sternberg, and showed the rationale for combination therapies.[6] In addition, preclinical *in vivo* work has shown that the activity of cabazitaxel is strongly affected by hormonal manipulations, e.g. either by castration or testosterone supplementation.[7] Clinical studies with combined treatments, though, warrant thorough pharmacokinetic investigation to test for clinical relevant drug-drug interactions.

Taxanes, i.e. docetaxel and cabazitaxel, have an extensive hepatic metabolism (63-77%) and biliary excretion.[8] Their metabolism is mediated by CYP3A iso-enzymes in the liver, and for a small part by CYP2C8. Therefore, liver (dys-)function is a major factor in taxane dose adaptations.[9] Pharmacokinetics of docetaxel show a considerable interpatient variability (30-50%), depending on patient characteristics, like gender and age. The interpatient variability of cabazitaxel is moderate (~24%) and cabazitaxel pharmacokinetics are less susceptible for patient characteristics.[10] However, the use of co-medication and herbal supplements, especially (strong) inducers/inhibitors of the CYP3A-system influence the pharmacokinetics of all taxanes.[11-13] The influence of CYP3A-inducers on the pharmacokinetics of cabazitaxel have been extensively investigated by Sarantopoulos *et al.*, showing that cabazitaxel is susceptible for CYP3A induction or inhibition. Repeated administration of a strong CYP3A inducer, rifampin, resulted in an 21% increase in cabazitaxel clearance and a 17% decrease in cabazitaxel concentration.[14] Additionally, the pharmacokinetics of taxanes can potentially be influenced by changes in drug-transporters, like ABCB1 (P-glycoprotein).[11,15] Although cabazitaxel has less propensity for ABCB1 mediated drug resistance, the upregulation of the efflux pump can potentially impact the pharmacokinetics of cabazitaxel.[16]

Enzalutamide is known to be a moderate inducer of CYP2C9 and CYP2C19 and a strong inducer of CYP3A4. Maximal CYP3A4 induction by enzalutamide may not appear until one month after treatment start, when steady state levels of enzalutamide are reached.[17] Since enzalutamide induces CYP3A4, and since taxanes are predominantly metabolized via that enzyme, a drug-drug interaction between these agents is expected.[12,15]

Recently, Morris *et al.* published a pharmacokinetic study in patients on the combination of enzalutamide and docetaxel.[18] They found a 12% decrease in docetaxel concentrations

during concomitant enzalutamide use compared with docetaxel alone, which they regarded as clinically irrelevant. However, given the short combination period of only 3 weeks, during which steady state exposure of enzalutamide may not have been reached yet, the true drug-drug interaction might be larger than reported. The half-life and steady state level of orally administrated enzalutamide (160 mg once daily) are relatively long: 6 days and 4 weeks, respectively. So, to identify the true inductive effects of enzalutamide on cabazitaxel exposure, we aimed to study the combination for at least the time-period that steady state of enzalutamide (i.e. after 4 weeks) is reached.[17] Therefore, we studied 3 consecutive cabazitaxel cycles in our trial, where enzalutamide is used for 6 weeks by men with mCRPC.

PATIENTS AND METHODS

Between April 2015 and August 2016 this prospective, non-randomized, non-blinded, cross-over, pharmacokinetic trial was undertaken in the Erasmus University Medical Center in Rotterdam, the Netherlands. The Ethical board of the Erasmus University Medical Center approved the study protocol and written informed consent was obtained from all participants. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki. The study was registered at the Dutch Trial Register (www.trialregister.nl) by number NL51749.078.14.

Study population

Patients enrolled in this study had histologically proven adenocarcinoma of the prostate with documented progression after docetaxel treatment and were eligible to receive second line chemotherapy, i.e. cabazitaxel. As the pharmacokinetic interaction was our primary endpoint, prior cabazitaxel treatment was allowed and prior enzalutamide treatment had to be ceased at least 6 weeks before start of the study. Eligible patients were aged at least 18 years, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and adequate liver function, defined by total bilirubin ≤ 1.5 x the upper limit of normal (ULN; except for documented Gilbert's disease), ASAT and ALAT ≤ 2.5 x ULN (or ≤ 5 x ULN if liver metastases are present). Patients with a medical history of seizures or predisposition to seizures were excluded. Medication or herbal supplements which may interact with either cabazitaxel or enzalutamide, e.g. by induction or inhibition of CYP3A4, CYP2C8, CYP2C9 or CYP2C19, were prohibited during this trial.

Study design

Patients received three consecutive cycles of cabazitaxel as a 1-hour infusion of 25 mg/m^2 once every three weeks. Dose modifications of cabazitaxel were allowed to a minimal dose of 12.5 mg/m^2 (50% of the registered dose). Premedication consisted of intravenously given dexamethasone (10 mg), followed by granisetron 1 mg. Oral prednisone, at a dose of 5 mg twice daily, was taken for as long as cabazitaxel treatment continued. At day 7 after the first cabazitaxel cycle within the study, a daily dose of 160 mg enzalutamide (4 capsules of 40 mg, orally) was added until eight (plus/minus one) days after the third cabazitaxel cycle. So, enzalutamide was co-treated for a total of six weeks. Enzalutamide administration was at 10.00 AM, without permission of dose interruptions or modifications. Patient compliance was assessed through a patient diary. Using this cross-over design all patients were their own control, making enzalutamide co-medication the only structural varying factor. See **Figure 1** for a simplified scheme of the study design.

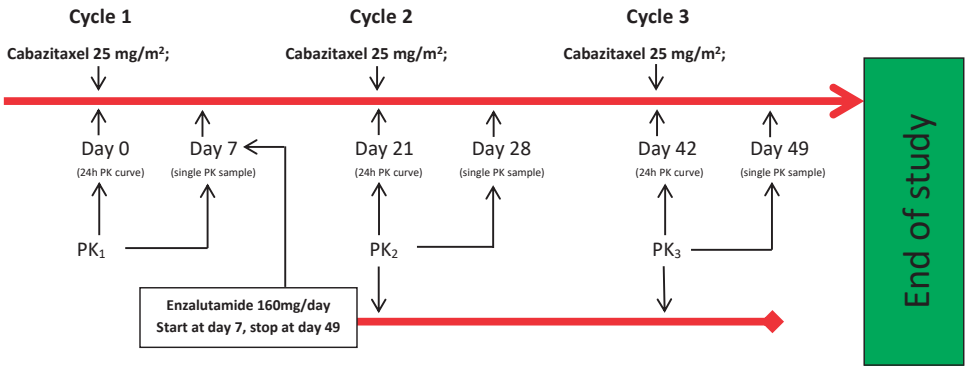


Figure 1. Study Design
The study included three consecutive courses of cabazitaxel, and one week after the first cabazitaxel cycle enzalutamide was added for a period of six weeks.Abbreviations: PK=pharmacokinetics

PK-assessments

Plasma samples for cabazitaxel pharmacokinetic (PK) assessments were obtained at cycle 1 (before initiation of enzalutamide dosing), cycle 2 and at cycle 3, when steady state levels of enzalutamide have been reached. Blood samples (4 mL) were withdrawn on the first day of each cabazitaxel cycle, at different time-points (pre-infusion and at 0.5, 0.92, 1.08, 1.25, 1.5, 2, 3, 5, 7, 11-13, 24, and 192 hours after the start of cabazitaxel). Measurement of plasma concentrations of cabazitaxel was performed using a validated liquid chromatography with tandem mass spectrometry methods (UP-MS/MS).[19] We used non-compartmental analysis (Phoenix version 6.1; Pharsight) and estimated the residual

AUC by a linear PK curve from the latest measurable PK-point. The last PK-sample was taken at 192h, which corresponds with the regular control at the outpatient clinic at 1 week after cabazitaxel infusion. PK parameters determined included cabazitaxel exposure (expressed as dose-corrected area under the plasma concentration time curves from time zero to infinity ($AUC_{0\text{-}inf}$)). When cabazitaxel concentrations were below the limit of detection at this point, we used the 24 hour sampling point as latest PK-sample, and therefore the dose corrected $AUC_{0\text{-}24h}$ as primary outcome measurement. Other PK parameters included were the maximum drug concentration (C_{max}), and its half-life ($t_{1/2}$).

PSA-assessments

Although not specified per protocol, serum PSA levels were determined at study baseline and before start of each cycle. Exploratory analyses were performed to identify proportion of patients with ≥ 50% decline of PSA from study start compared to the end of the treatment period.

Statistical Analysis

In order to take into account dose-reduced cabazitaxel cycles, all measured concentrations were dose-corrected to a dose of 25 mg/m^2 by the formula AUC*(25/dose given in that cycle). Before analyzing the dose-corrected areas under the curve (AUC) and maximum cabazitaxel concentrations (C_{max}), a natural log-transformation of these data was performed in order to take into account possible deviations from normality. The (log) dose-corrected area under the curve (AUC) of cabazitaxel without enzalutamide (AUC Cycle 1) was compared to the (log) dose-corrected AUC of cabazitaxel with enzalutamide (AUC Cycle 3), using a paired *t*-test. In addition, as a secondary endpoint, the same test was used to compare (log) dose-corrected AUC of cycle 1 to cycle 2, the (log) maximum cabazitaxel concentration (C_{max}) in cycle 1 to cycle 3, and the same for the half-life ($t_{1/2}$) of cabazitaxel. The mean differences and 95% CIs for the differences were exponentiated to provide point estimates of the ratio of geometric means and 95% CIs for these ratios, which can be interpreted as relative differences in percentages. The study required an estimated sample size of 14 evaluable patients to detect a clinical relevant difference in AUC with 80% power and a two-sided significance level of 0.05. IBM SPSS Statistics 21 was used for all analyses.

RESULTS

Patient characteristics & Pharmacokinetic parameters

Baseline characteristics are available for all 14 patients and are shown in **Table 1.** All patients were chemically castrated and used androgen-deprivation therapy throughout the whole study period. Cabazitaxel pharmacokinetic parameters are shown in **Table 2**. We aimed to identify the $AUC_{0\text{-}inf}$ of cabazitaxel by collecting PK samples up to 192 hours. However, in most cases the concentration of cabazitaxel was below the detection limit (LLQ) in the 192 hour-PK samples. As a result the residual AUC was >20% of the total AUC, which is not acceptable according to the general PK-assumptions. Therefore we had to decide to limit our AUC calculation up to the 24 hour sample ($AUC_{0\text{-}24h}$).

The geometric mean exposure was 22% (95% CI 9 – 34%, p=0.005) lower in the third cycle (cabazitaxel combined with enzalutamide: $AUC_{0\text{-}24h}$ of 181 ng*h/mL, 95%CI 150-219 ng*h/mL) compared to the first cycle (cabazitaxel monotherapy: $AUC_{0\text{-}24h}$ of 234 ng*h/mL, 95%CI 209-261 ng*h/mL). Interestingly, this decrease was already observed during

Table 1. Patient Characteristics

Characteristic	Value No. (%)
Evaluable patients (n)	14 (100)
Age (years) , mean ± SD	67.7 ± 6.1
WHO performance status	
- 0	4 (29)
- 1	10 (71)
Liver function baseline, mean ± SD	
- Bilirubine (µmol/L)	5.4 ± 2.1
- ASAT (U/L)	24.6 ± 9.9
- ALAT (U/L)	19.1 ± 8.5
Prior therapy	
- Docetaxel	14 (100)
- Abiraterone	6 (43)
- Enzalutamide	3 (21)
- Radiotherapy	3 (21)
- Experimental*	5 (36)
- Cabazitaxel	2 (14)
Type of castration	
- Chemical	14 (100)

*Abbreviations: n= number, SD= standard deviation; WHO=World Health Organization.*Experimental included participation in phase I and II clinical trial with a variety of agents: TAS-119, ARN-509, Dendritic cell therapy*

Table 2. Cabazitaxel pharmacokinetics

Cabazitaxel PK parameters	C1 (caba)	C2 (caba+enza)	C3 (caba + enza)	Difference C3/C1 % (95% CI)	P-value*
$AUC_{0\text{-}24h}$ ng*h/mL (CV%)	234 (19)	182 (26)	181 (34)	-22 (-9 till -34)	0.005
C_{max} ng/mL (CV%)	138 (39)	96 (49)	96 (20)	-30 (-42 till -16)	0.001
$t_{1/2}$ h (CV%)	22 (87)	14 (41)	18 (79)	-18 (-59 till 64)	0.552

*Abbreviations: PK = pharmacokinetics; C1/2/3 = cycle 1/2/3, caba = cabazitaxel; enza = enzalutamide; CI = Confindence Interval; $AUC_{0\text{-}24h}$= Area under the curve for 24 hours, expressed as geomean; C_{max}= max concentration, expressed as geomean; $t_{1/2}$= half-life, expressed as geomean; CV% = coefficient of variation . * = comparison between cycle 1 and cycle 3.*

cycle 2 ($AUC_{0\text{-}24h}$ of 182 ng*h/ml, 95%CI 157-211 ng*h/mL; relative difference = -22%, 95% CI -13 – -31%, p<0.001) despite the shorter period of the drug-drug combination during that cycle. In addition, the variation in pharmacokinetic effects, as expressed by the coefficient of variation (CV%), was higher in the third cycle than in cycle 1 and cycle 2 in the study, respectively 34%, 19% and 26%, see **Figure 2**. As secondary endpoints other pharmacokinetic endpoints; maximum concentration (C_{max}) of cabazitaxel and half-life ($t_{1/2}$) of cabazitaxel were measured, and compared between cycle 1 and cycle 3. The maximum concentration of cabazitaxel in the first cycle (C_{max} 138 ng/mL, 95% CI 111-171 ng/mL) was significantly higher than in the third cycle (C_{max} 96 ng/mL, 95% CI 86-108 ng/mL; relative difference = -30%, 95% CI -42 – -16%, p=0.001). There was no difference in half-life of cabazitaxel between both cycles (cycle 1: $t_{1/2}$ 22h, 95% CI 14-33h vs. cycle 3: $t_{1/2}$ 18h, 95% CI 12-27h; relative difference = -18%, 95% CI -59 – 64%, p=0.552).

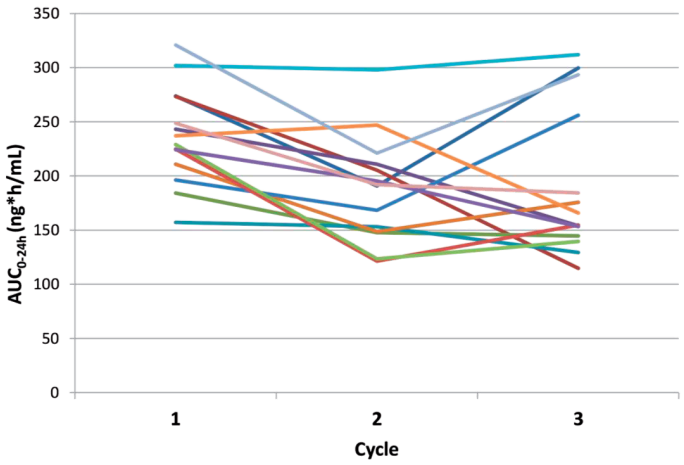


Figure 2. Individual dose-corrected cabazitaxel $AUC_{0\text{-}24h}$.
Each line refers to one patient. The dose-corrected $AUC_{0\text{-}24h}$ of cabazitaxel (Y-axis) is measured per cycle (X-axis), and AUC-values for individual patients are connected between the cycles.

Toxicity & PSA response

There were no unexpected serious adverse events (SAEs) during combined treatment with these drugs. A total of four SAEs were generally related to cabazitaxel treatment (monotherapy), including neutropenic fever (2 times), deep venous thrombosis, and hypertension. Hypertension (grade 3) occurred during the third cycle, although this patient was already known with hypertension. The deterioration of the blood pressure may be attributed to enzalutamide use as well. Four out of six (67%) grade 3-4 adverse events occurred during cabazitaxel monotherapy, while the other two adverse events happened during the combination therapy of cabazitaxel and enzalutamide, indicating an overall well-tolerated combination strategy.

Since PSA response was not specified per protocol, we analyzed response rates in an explorative way. Since baseline PSA was missing for one patient, PSA analyses were performed in 13 of 14 patients. PSA responses of 50% or more were observed in eight (62%) of the 13 patients at completion of the three consecutive cycles of cabazitaxel in the study, which we regarded as high given the extensive prior treatment in this typical patient cohort (**Figure 3**). Five of the PSA responders had prior treatment with an AR-agent, and both cabazitaxel pre-treated patients had a >50% PSA decline.

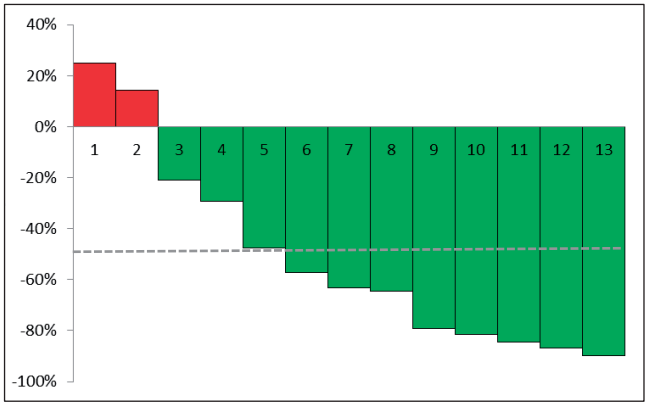


Figure 3: PSA response after 3 cycles of cabazitaxel within the study
PSA response is defined as ≥ 50% decline of PSA compared to baseline. N=13, since a baseline PSA-value was missing for one patient.

DISCUSSION

In this pharmacokinetic cross-over study in patients with mCRPC treated with cabazitaxel, we evaluated the difference in exposure ($AUC_{0\text{-}24h}$) of cabazitaxel when administered with or without enzalutamide. Our study showed a significant and potentially clinically important reduction (22%) in cabazitaxel exposure by simultaneous treatment with enzalutamide.

As mentioned, enzalutamide is classified as a strong inducer of CYP3A4 enzyme,[17] and all taxanes, including cabazitaxel, are metabolized primarily by this enzyme. Therefore, a drug-drug interaction was expected *a priori*, although the magnitude of that interaction was uncertain. In this study, the inductive effects of enzalutamide on cabazitaxel exposure is relatively high and results in a clinically relevant interaction between these agents. In contrast, Morris *et al.*[18] found a moderate 12% reduction of docetaxel concentration (AUC_{inf}) with concomitant enzalutamide use. The substantial difference between the identified percentages of enzalutamide related drug-induction in the study of Morris *et al.*[18] and our cohort may be due to the different taxanes that were used. Although our primary endpoint was to compare cabazitaxel study cycle 1 to cabazitaxel study cycle 3, we already saw comparable geomean cabazitaxel $AUC_{0\text{-}24h}$ decreases during cabazitaxel study cycle 2, following 14 days of treatment with enzalutamide. Although the steady state levels of enzalutamide are reached after 4 weeks, the inducing effect on CYP3A4 is probably complete after two weeks. This observation is consistent with prior observations that induction of CYP enzymes is complete within 9-14 days after start of treatment with strong CYP inducers.[20] Nonetheless, study cycle 3 was not similar to study cycle 2: the interpatient variability was substantially higher in the third cycle, as several patients had higher cabazitaxel concentrations in that cycle than in cycle 2. Contrarily, other patients had a further decrease in cabazitaxel exposure in the third cycle (see **Figure 2**). The interpatient variability of cabazitaxel is moderate with 24%.[10] In this study, it appears that interpatient variability (%CV) increases when cabazitaxel is combined with enzalutamide and even further in consecutive cycles. We cannot provide a clear explanation for this phenomenon. This increased variation was not dependent on occurrence of hepatotoxicity, co-medication, or number of cabazitaxel cycles preceding the start of this study. Also patient compliance to daily take oral enzalutamide did not differ during the cycles, based on notes in patient diaries. Moreover, medication or herbal supplements that could interact with enzalutamide were prohibited per protocol, so the risk of interpatient variation in pharmacokinetics of enzalutamide, with potential impact on pharmacokinetics of cabazitaxel, is brought back to the minimum. However, enzalutamide concentrations were not measured, which is a limitation of our study.

Recently, the results of the 'PROSELICA' study have become available. PROSELICA was a post-marketing study, mandated by the Food and Drug Administration (FDA), to investigate if a 20 mg/m^2 three-weekly dose of cabazitaxel is non-inferior to the standard dose of 25 mg/m^2.[21] The PROSELICA study showed a non-inferior overall survival outcome with the 20 mg/m^2 dose. As expected the lower dose was also associated with a notably favorable adverse event profile. Although to date the dose in the label has not been changed, in clinical practice the use of the 20 mg/m^2 dose is being adopted widely, both in the US and in Europe. If indeed such a lower dose of cabazitaxel is applied, an additional reduction of 22% in cabazitaxel exposure by enzalutamide may result in sub-therapeutical cabazitaxel concentrations.

Interestingly, the PSA response, which was studied exploratively, was higher in this study than the response rate reported in the TROPIC trial, which was the registration trial for cabazitaxel treatment (62% versus 39%).[22,23] The higher PSA response is encouraging, since most of our patients had received prior AR-targeted therapy, several had previously been treated with cabazitaxel, and several had been on clinical phase I and II studies with a variety of drugs, rendering the 4th or even 5th line treatment. Moreover, in our cohort the cabazitaxel concentration was reduced with a mean of 22% for 2 cycles due to the drug-drug interaction with enzalutamide. This implies that the additive effect of the drug-drug combination on PSA response is promising, despite the inductive effects of enzalutamide on cabazitaxel pharmacokinetics. Still, our response rates should be interpreted with caution due to the exploratory design of the analysis, the small sample size, and the limited study period.

Furthermore, the combination treatment was very well tolerated considering the low incidence and severity of adverse events, although our results should be seen in perspective given the short treatment period and the prior cabazitaxel cycles that were allowed. In a recent study by Massard *et al.* on the combination of cabazitaxel and abiraterone, also a lower incidence of grade ≥ 3 neutropenia was reported than in the TROPIC trial (56%). This lower incidence of neutropenia did not result in less (marker) efficacy, as PSA levels dropped in 46% patients.[4]

In conclusion, our study shows a significant and clinically relevant reduction in cabazitaxel exposure when combined with enzalutamide, most probably due to CYP3A4 induction by enzalutamide. Prospective clinical studies with this promising combination are warranted, but investigators need to be aware of the observed drug-drug interaction.

REFERENCES

1. Fitzpatrick JM, Bellmunt J, Fizazi K, et al. Optimal management of metastatic castration-resistant prostate cancer: highlights from a European Expert Consensus Panel. Eur J Cancer. 2014;50(9): 1617-1627.
2. Chowdhury S, Davidson R, Coppel A, et al. The PRESIDE trial: a randomized, double-blind, placebo-controlled Phase 3 efficacy and safety study of continued enzalutamide plus docetaxel after disease progression on enzalutamide alone in patients with metastatic castration-resistant prostate cancer. 2015, Journal of Clinical Oncology 33, no. 15_suppl #TPS5083. ASCO Annual Meeting (suppl; #TPS5083).
3. Tagawa ST, Posadas EM, Bruce J, et al. Phase 1b Study of Abiraterone Acetate Plus Prednisone and Docetaxel in Patients with Metastatic Castration-resistant Prostate Cancer. Eur Urol. 2016;70(5): 718-721.
4. Massard C, Mateo J, Loriot Y, et al. Phase I/II trial of cabazitaxel plus abiraterone in patients with metastatic castration-resistant prostate cancer (mCRPC) progressing after docetaxel and abiraterone. Ann Oncol. 2017;28(1):90-95.
5. Kessler ER, Gao D, Flaig TW, et al. Phase II study of cabazitaxel with or without abiraterone acetate and prednisone in patients with metastatic castrate resistant prostate cancer after prior docetaxel and abiraterone acetate. Ann Oncol. 2017;28(3):668.
6. Sternberg CN. Improving Survival for Metastatic Castrate-resistant Prostate Cancer: Will Combination Therapy Help Us To Move Forward? Eur Urol. 2016;70(5):722-723.
7. Mout L, de Ridder C, Stuurman D, et al. Testosterone abolishes cabazitaxel efficacy in an in vivo model of prostate cancer and affects intratumoral concentrations. AACR AACR 2017 #1099.
8. Ridoux L, Semiond DR, Vincent C, et al. A phase I open-label study investigating the disposition of [14C]-cabazitaxel in patients with advanced solid tumors. Anticancer Drugs. 2015;26(3):350-358.
9. Joerger M. Metabolism of the taxanes including nab-paclitaxel. Expert Opin Drug Metab Toxicol. 2015;11(5):691-702.
10. Ferron GM, Dai Y, Semiond D. Population pharmacokinetics of cabazitaxel in patients with advanced solid tumors. Cancer Chemother Pharmacol. 2013;71(3):681-692.
11. Nieuweboer AJ, de Morree ES, de Graan AJ, Sparreboom A, de Wit R, Mathijssen RH. Inter-patient variability in docetaxel pharmacokinetics: A review. Cancer Treat Rev. 2015;41(7):605-613.
12. Mathijssen RH, Sparreboom A, Verweij J. Determining the optimal dose in the development of anticancer agents. Nat Rev Clin Oncol. 2014;11(5):272-281.
13. Janssen A, Verkleij CPM, van der Vlist A, Mathijssen RHJ, Bloemendal HJ, Ter Heine R. Towards better dose individualisation: metabolic phenotyping to predict cabazitaxel pharmacokinetics in men with prostate cancer. Br J Cancer. 2017;116(10):1312-1317.
14. Sarantopoulos J, Mita AC, Wade JL, et al. Phase I study of cabazitaxel plus cisplatin in patients with advanced solid tumors: study to evaluate the impact of cytochrome P450 3A inhibitors (aprepitant, ketoconazole) or inducers (rifampin) on the pharmacokinetics of cabazitaxel. Cancer Chemother Pharmacol. 2014;74(6):1113-1124.
15. Pean E, Demolis P, Moreau A, et al. The European Medicines Agency review of cabazitaxel (Jevtana(R)) for the treatment of hormone-refractory metastatic prostate cancer: summary of the scientific assessment of the committee for medicinal products for human use. Oncologist. 2012;17(4):543-549.
16. Bouchet BP, Galmarini CM. Cabazitaxel, a new taxane with favorable properties. Drugs Today (Barc). 2010;46(10):735-742.

17. Gibbons JA, Ouatas T, Krauwinkel W, et al. Clinical Pharmacokinetic Studies of Enzalutamide. Clin Pharmacokinet. 2015;54(10):1043-1055.
18. Morris MJ, Rathkopf DE, Novotny W, et al. Phase Ib Study of Enzalutamide in Combination with Docetaxel in Men with Metastatic Castration-Resistant Prostate Cancer. Clin Cancer Res. 2016;22(15): 3774-3781.
19. de Bruijn P, de Graan AJ, Nieuweboer A, et al. Quantification of cabazitaxel in human plasma by liquid chromatography/triple-quadrupole mass spectrometry: a practical solution for non-specific binding. J Pharm Biomed Anal. 2012;59:117-122.
20. Del Re M, Fogli S, Derosa L, et al. The role of drug-drug interactions in prostate cancer treatment: Focus on abiraterone acetate/prednisone and enzalutamide. Cancer Treat Rev. 2017;55:71-82.
21. Eisenberger M, Hardy-Bessard AC, Kim CS, et al. Phase III Study Comparing a Reduced Dose of Cabazitaxel (20 mg/m2) and the Currently Approved Dose (25 mg/m2) in Postdocetaxel Patients With Metastatic Castration-Resistant Prostate Cancer-PROSELICA. J Clin Oncol. 2017;35(28):3198-3206.
22. 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.
23. van Soest RJ, Nieuweboer AJ, de Morree ES, et al. The influence of prior novel androgen receptor targeted therapy on the efficacy of cabazitaxel in men with metastatic castration-resistant prostate cancer. Eur J Cancer. 2015;51(17):2562-2569.

Chapter 3

Effects of prednisone on docetaxel pharmacokinetics in men with metastatic prostate cancer: a randomized drug-drug interaction study

Bodine P.S. Belderbos, Koen G.A.M Hussaarts, Leonie J. van Harten, Esther Oomen-de Hoop, Peter de Bruijn, Paul Hamberg, Robbert J. van Alphen, Brigitte C.M. Haberkorn, Martijn P. Lolkema, Ronald de Wit, Robert J. van Soest, Ron H.J. Mathijssen.

Br J Clin Pharmacol. 2019 May; 986-992

ABSTRACT

Aim: Docetaxel has been approved for the treatment of metastatic prostate cancer in combination with prednisone. Since prednisone is known to induce the cytochrome P450 iso-enzyme CYP3A4, which is the main metabolizing enzyme of docetaxel in the liver, a potential drug-drug interaction (DDI) may occur. In this prospective randomized pharmacokinetic cross-over study we investigated docetaxel exposure with concomitant prednisone, compared to docetaxel monotherapy in men with metastatic prostate cancer.

Methods: Patients scheduled to receive at least 6 cycles of docetaxel (75 mg/m^2) and who lent written informed consent, were randomized to receive either the first 3 cycles, or the last 3 consecutive cycles with prednisone (BID 5mg). Pharmacokinetic blood sampling was performed during cycle 3 and cycle 6. Primary endpoint was difference in docetaxel exposure, calculated as area under the curve ($AUC_{0\text{-}inf}$) and analyzed by means of a linear mixed model. Given the cross-over design the study was powered on eighteen patients to answer the primary, pharmacokinetic, endpoint.

Results: Eighteen evaluable patients were included in the trial. Docetaxel concentration with concomitant prednisone ($AUC_{0\text{-}inf}$ 2784 ng*h/mL, 95% CI 2436-3183 ng*h/mL) was similar to the concentration of docetaxel monotherapy ($AUC_{0\text{-}inf}$ 2647 ng*h/mL, 95%CI 2377-2949 ng*h/mL). Exploratory analysis showed no toxicity differences between docetaxel monotherapy and docetaxel cycles with prednisone.

Conclusion: No significant difference in docetaxel concentrations was observed. In addition, we found similar toxicity profiles in absence and presence of prednisone. Therefore, from a pharmacokinetic point of view, docetaxel may be administrated with or without prednisone.

INTRODUCTION

Docetaxel, a taxane chemotherapeutic agent, was approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) in 2004 as first-line chemotherapy for metastatic castration-resistant prostate cancer (mCRPC) as a result of survival benefit obtained in TAX327.[1,2] In that study, mitoxantrone plus prednisone treatment was compared to a 3-weekly docetaxel (75 mg/m^2) regimen in mCRPC patients. Prednisone (5 mg bi-daily BID) was added to docetaxel to equally compare both treatment arms, although the preceding phase 2 trials with docetaxel (36 mg/m^2, weekly) in mCRPC had been conducted without prednisone.[3,4] In the final analysis, treatment with docetaxel plus prednisone improved overall survival (OS) with 2.9 months compared to the mitoxantrone group. Subsequently, docetaxel and prednisone became first-line chemotherapy for mCRPC .

After the registration of docetaxel plus prednisone, the role of corticosteroids in the treatment of mCRPC remained controversial. In patients with symptomatic bone metastases corticosteroids may have a favorable palliative effect, and a reduction in docetaxel-induced toxicity has been suggested.[5-7] However, the effect of prednisone on OS in mCRPC patients remains unclear.[6,8] Of note, prolonged use of corticosteroids may lead to the development of multiple severe toxicities including osteoporosis, adrenal insufficiency, immune suppression, and may exacerbate comorbidities like diabetes.[9] These side-effects of long-term corticosteroid are a justifiable reason to reconsider the addition of prednisone to the docetaxel regimen.

Recently, two large clinical trials, CHAARTED and STAMPEDE, assessed the survival benefit of docetaxel combined with androgen-deprivation therapy (ADT) in metastatic hormone-sensitive prostate cancer (mHSPC).[10,11] In order to avoid long term exposure to steroids, the investigators of the CHAARTED trial decided to administer docetaxel without prednisone, whereas docetaxel was administered with prednisone in the STAMPEDE study. At the time of the initiation of our study, only the results of CHAARTED were available, showing a robust survival benefit of 13.6 months compared to androgen deprivation therapy alone. Toxicity rates were similar to previously published work on docetaxel plus prednisone in mCRPC patients , except for a higher febrile neutropenia rate in CHAARTED without prednisone, as compared to TAX327 where docetaxel was administered with prednisone.[1,12] Likewise, a retrospective trial by Kongsted *et al.* showed that the toxicity rates of febrile neutropenia and edema were significantly higher in the docetaxel monotherapy group compared to the docetaxel plus prednisone-group (for an overview of toxicity rates previously reported on docetaxel with or without prednisone, see **Table 1**).[5]

Table 1. Literature review of docetaxel toxicities with and without prednisone

Trials	Prednisone	Neutropenia (Gr3-4)	Febrile neutropenia
TAX-327	Yes	32%	3%
Venice	Yes	7%	<1%
Mainsail	Yes	16%	5%
GETUG-AFU15	No	32%	8%
CHAARTED	No	12%	6%
STAMPEDE	Yes	12%	15%
Kongsted et al.	No	-	25%
	Yes	-	10%

As a underlying mechanism, prednisone could influence docetaxel pharmacokinetics via the CYP3A4 iso-enzyme. Glucocorticoids are known as CYP3A inducers, and docetaxel is mainly metabolized in the liver by the cytochrome P450 iso-enzymes CYP3A4 and CYP3A5.[13] Consequently, this potential drug-drug interaction could lead to higher clearance of docetaxel and therefore diminished docetaxel exposure. In this study, we therefore investigated the effects of prednisone on docetaxel pharmacokinetics in patients with metastatic prostate cancer.

METHODS

This prospective, randomized, cross-over pharmacokinetic trial was carried out between September 2016 and February 2018 at the Erasmus MC Cancer Institute in Rotterdam, the Netherlands. The study protocol was approved by the Ethical board of the Erasmus MC, and the study was conducted according to the ethical guidelines of the Declaration of Helsinki. All participants signed informed consent before start of the study. The study was registered at the European Clinical Trials Database (EudraCT 2016-001269-10) and the Dutch Trial Register ('www.trailregister.nl' by NTR-number NTR6037 or acronym Doc-Pred).

Patients

We included patients with histologically confirmed metastatic prostate cancer, both hormone-sensitive or castration-resistant, who were scheduled to receive a minimum of 6 cycles of docetaxel chemotherapy. Eligible patients were 18 years and older, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Adequate organ function was required, defined by creatinine clearance >60mL/min, bilirubin levels <1x ULN, ALAT/ASAT <2.5x ULN, alkaline phosphatase (AF) < 5x ULN, absolute neutrophil count >1,5x10^9/L and platelets >100x10^9/L. Patients had to be castrated either by

continued androgen deprivation therapy (ADT) with gonadotropin releasing hormone (GnRH) analogues or by surgical orchiectomy. It was preferred ADT started four weeks prior to chemotherapy, to reach castration-levels of testosterone before treatment start. Prior hormonal treatment, like enzalutamide and abiraterone, was allowed. However, these therapies, including prednisone, had to be stopped at least 6 weeks before the start of this study. Medication or herbal supplements known to induce or inhibit CYP3A pathway were prohibited.

Study Design

Patients received 6 consecutive cycles of 3-weekly docetaxel (75 mg/m^2) and were digitally randomized to receive either the first 3 docetaxel cycles or the last 3 cycles with prednisone (cross-over). Prednisone 5 mg BID was administered during three consecutive cycles. Prednisone started at day 1 of cycle 1 or cycle 4 and was stopped after the last day of cycle 3 or cycle 6 (depending on randomization arm, A or B respectively). Prednisone dose-modifications were not allowed during the last week before pharmacokinetic sampling (cycle 3 day 1 and cycle 6 day 1) and patient compliance was assessed through a patient diary. Docetaxel dose-modifications because of hematological or non-hematological toxicities were allowed, and schedule modifications were allowed up to one week. Dexamethasone is a strong CYP3A4 inducer, its use, as premedication, was restricted to 12 and 3 hours before docetaxel-infusion to reduce its influence on docetaxel pharmacokinetics.

Pharmacokinetic sampling

To have maximum inducible effects of prednisone on the CYP-enzymes and to ensure a sufficient wash-out period after prednisone, we decided to undertake PK-samples during cycle 3 and cycle 6. Hospital admission during the first day of the 3rd and the 6th docetaxel cycle was required to obtain 24-hour pharmacokinetic-blood samples. Blood/plasma samples for determination of docetaxel pharmacokinetics were taken at predefined time points (pre-infusion and at 0.5, 0.92, 1.25, 1.5, 2, 3, 4, 6, 8 and 24 hours after the start of docetaxel). Plasma concentrations of docetaxel were measured using a validated liquid chromatography with tandem mass spectrometry method (UP-LCMS/MS).[14] Pharmacokinetic parameters were docetaxel concentration, expressed as dose-corrected area under the curve from pre-infusion time-point to infinity ($AUC_{0\text{-}inf}$), maximum drug concentration (C_{max}), docetaxel half-life ($t_{1/2}$) and docetaxel clearance. $AUC_{0\text{-}inf}$ was calculated using a linear pharmacokinetic curve to estimate the residual AUC from the latest measurable pharmacokinetic point (24h time-point).

Toxicity

Secondary endpoint was describing toxicity rates during docetaxel monotherapy cycles and docetaxel with prednisone cycles. Standard laboratory control was performed prior to each docetaxel cycle and when indicated according to the physician. Toxicities were scored using the CTCAE (v.4.0) grading. If relevant differences in toxicity rates between the treatment arms occurred, these were analyzed by means of McNemar test.

Statistical analysis

A difference in systemic exposure to docetaxel of 25% was determined to be clinically relevant and it was assumed that the within-patient standard deviation in docetaxel pharmacokinetics was 25%. Given a power of 80% and a two-sided alpha of 5%, 18 patients were required to detect a difference.[15] Since docetaxel dose-modifications were allowed, a dose-correction was applied for all docetaxel concentrations to the standard dose of 75 mg/m^2. All docetaxel cycles with prednisone were compared to all docetaxel cycles without prednisone, regardless of the randomization arm. Analyses of the $AUC_{0\text{-}inf}$ and C_{max} were performed on log-transformed values, since these parameters were assumed to follow a lognormal distribution.[16] Estimates for the mean differences in (log) $AUC_{0\text{-}inf}$, C_{max} and clearance were obtained using a linear mixed effect model with treatment, sequence, and period as fixed effects and subject within sequence as a random effect.[17] Variance components were estimated based on restricted maximum likelihood (REML) methods and the Kenward-Roger method of computing the denominator degrees of freedom was used. The mean differences and their 95% CIs were exponentiated to provide point estimates of the ratio of geometric means and 95% CIs for these ratios, which can be interpreted as relative differences in percentages. $T_{1/2}$ was analysed by means of the Wilcoxon signed rank test and described with medians and interquartile ranges.

RESULTS

Patients

Twenty-nine patients were screened, of whom four were screen failures which were excluded from study participation (**Figure 1**). We randomized 25 patients to receive either cycles 1-3 with concomitant prednisone, and cycles 4-6 without prednisone (arm A, N=12), or *vice versa* (arm B, N=13). During treatment one patient withdrew consent in arm A, and six patients stopped treatment in arm B due to radiologic confirmed progression (*N*=3) or withdrawal of consent (*N*=3).

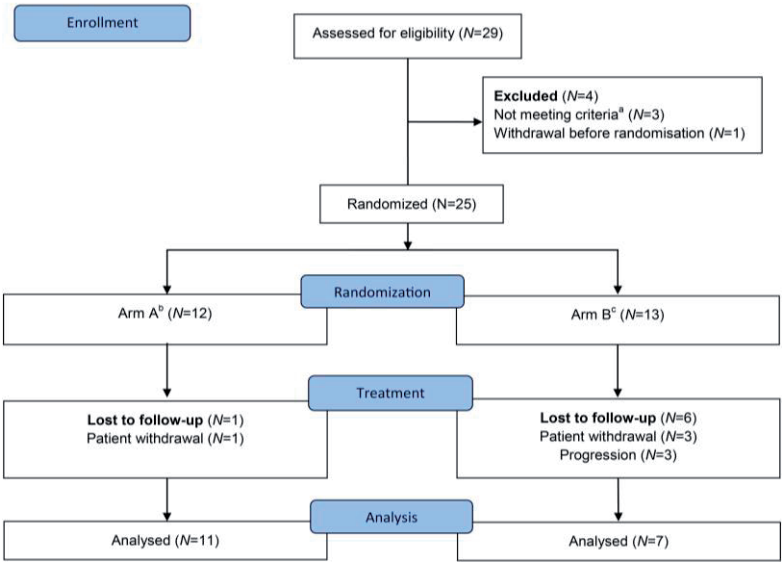


Figure 1. Flowchart
a. due to inadequate laboratory values
b. Arm A: Three cycles of docetaxel plusprednisone followed by three cycles of docetaxel alone
c. Arm B: Three cycles of docetaxel alone followed by three cycles of docetaxel plus prednisone

Baseline patient and disease characteristics are shown in **Table 2**. All patients, except three mHSPC patients, received the first cycle of docetaxel 4 weeks after initiation of ADT, to reach castration levels of testosterone. However, all patients received ADT for more than a month before PK samples during cycle 3 were withdrawn.

Pharmacokinetic parameters

The geometric mean exposure of docetaxel was not significantly different (1.9%, 95% CI -9.9% till 15.2%, *P*=0.75) during docetaxel with concomitant prednisone treatment ($AUC_{0\text{-}inf}$ of 2784 ng*h/mL, 95% CI 2436-3183 ng*h/mL) compared to docetaxel monotherapy ($AUC_{0\text{-}inf}$ of 2647 ng*h/mL, 95% CI 2377-2949 ng*h/mL). The pharmacokinetic variation, as expressed by coefficient of variation, was slightly higher in the docetaxel with prednisone arm as compared to docetaxel monotherapy (27% and 22% respectively). All pharmacokinetic parameters are shown in **Table 3** and were not significantly different for docetaxel with or without prednisone. Additionally, we graphically showed differences in exposure of docetaxel in mCRPC patients (blue line) and mHSPC patients (red line), separately in arm A and arm B; see **Figure 2**. We performed a t-test on the complete patient group (arm A and arm B combined) and found no significant (*P*=0.2) difference

Table 2. Patient and disease characteristics

Characteristic	***N* (%)**
Patients	18 (100)
Age (Median, IQR)	70 (62-73)
BMI (Median, IQR)	25.8(24.6-28.7)
WHO Performance Status	
- 0	8 (44)
- 1	10 (56)
Hormone Status	
- Hormone sensitive	11 (61)
- Castration resistant	7 (39)
Metastatic stage at screening	
- M0	5 (28)
- M1a	4 (22)
- M1b	8 (44)
- M1c	1 (6)
Gleason score at diagnosis	
- ≤ 7	4 (22)
- > 7	14 (77)
Type of castration	
- Bilateral orchidectomy	1 (6)
- LHRH analogues	17 (94)
Previous therapy	
- Radicale prostatectomy	1 (6)
- RTx prostate	3 (16)
- Hormone therapy	
Bicalutamide	6 (33)
Enzalutamide	2 (11)
- Radium-223	1 (6)
- Experimental therapy	1 (6)
Lab results at baseline	**Median (IQR)**
- PSA, µg/L	20 (3-87)
- Hb, mmol/L	8 (7-10)
- LDH, U/L	196 (178-216)
- AP, U/L	103 (70-160)
- Albumin, g/L	44 (43-46)

Abbreviations: IQR = Inter Quartile Range, BMI = Body Mass Index, WHO = World Health Organizations, LHRH = luteinizing hormone releasing hormone, RTx = radiotherapy, PSA = prostate specific antigen, Hb = hemoglobin, LDH = lactate dehydrogenase, AP = alkaline phosphatase

Table 3. Docetaxel pharmacokinetics

Docetaxel PK parameters	Docetaxel (*N*=18)	Docetaxel+Prednisone (*N*=18)	Relative difference (95% CI)	P-value
$AUC_{0\text{-}inf}$ [a] geomean ng*h/mL (CV%)	2647 (22)	2784 (27)	1.9% (-9.9 till 15.2)	0.75
C_{max} [a] geomean ng/mL (CV%)	2454 (26)	2505 (25)	-1.4% (-15.3 till 14.8)	0.85
CL [a] geomean, L/h (CV%)	55 (26)	53 (26)	-2.3% (-9.5 till 5-6)	0.53
$T_{1/2}$ [b] median, h (IQR)	12.6 (10.6-14.5)	13.7 (11.3-16.3)		0.31

Abbreviations: $AUC_{0\text{-}inf}$ = Area under curve timepoint zero until infinity, C_{max} = maximum concentration, CL = clearance, $T_{1/2}$ = half-life, geomean = geometric mean, CV% = coefficient of variation, CI = confidence interval, [a]= analyzed by means of a linear effect model, [b]= analyzed by means of Wilcoxon signed rank test.

between the exposure in mCRPC patients and mHSPC patients. Of note, we found a 13.4% (95% CI 2.1%-23.4%, *P*=0.025) lower exposure of docetaxel over time, independent from randomization or disease setting. This, so called, period-effect shows lower measured concentrations of docetaxel in cycle 6 compared to the concentrations in cycle 3, regardless of the addition of prednisone (**Figure 2**).

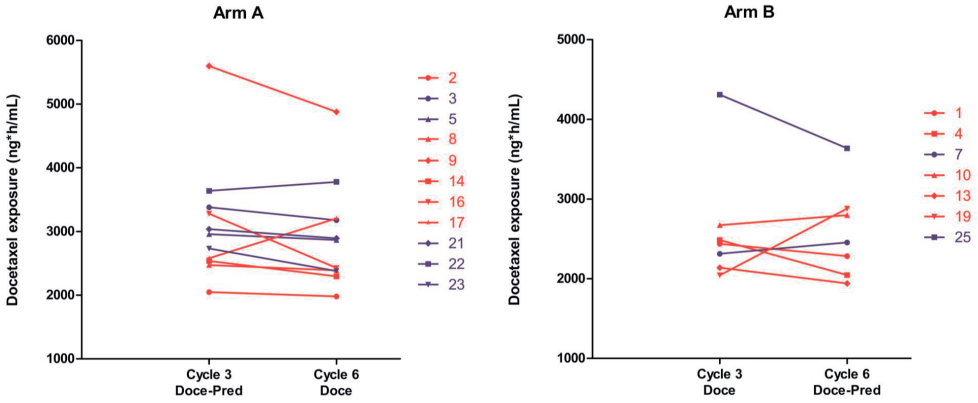


Figure 2. Docetaxel concentration by disease setting

Each line represents a patient for whom the measured docetaxel concentration (geomean $AUC_{0\text{-}inf}$) at cycle 3 and cycle 6 were connected with a line to visualize the concentration differences between the cycles. In the majority of the patients the measured concentration in cycle 6 is lower than in cycle 3, reflecting the period-effect observed in this study. The red lines represent patients in the hormone-sensitive disease setting and the blue lines represent the castration-resistant patients, subdivided by randomization arm.

Toxicity

Toxicity rates were similar between the cycles with and without prednisone, **Table 4**, except for neutropenia. A non-significant trend towards a higher rate of all grade (gr 1-4) neutropenia (*N*=12) was observed in patients treated without prednisone as compared to with prednisone (44 vs 22%, P=0.22). Seven patients (39%) experienced an episode of grade 3 – 4 neutropenia. Three febrile neutropenia hospitalizations were observed, two of which happened during co-administration of prednisone. There was no difference in the disease setting; toxicity was equally distributed in castration-resistant and hormone-sensitive setting (data not shown).

Table 4. Toxicity with or without prednisone

	All grades	
Toxicity	**With prednisone *N* (%)**	**Without prednisone *N* (%)**
Nausea	3 (17)	5 (28)
Mucositis	9 (50)	8 (44)
Diarrhea	5 (28)	2 (11)
Sens PNP	6 (33)	6 (33)
Fatigue	12 (67)	13 (72)
Neutropenia	4 (22)	8 (44)
Febrile neutropenia	1 (6)	2 (11)
Nail toxicity	5 (28)	6 (33)
Edema	0 (0)	1 (6)
Dysgeusia	1 (6)	1 (6)

Abbreviations: Sens PNP = sensory polyneuropathy

DISCUSSION

In this randomized study, the effects of prednisone on the pharmacokinetics of docetaxel were evaluated. No significant difference in docetaxel exposure with or without the administration of prednisone was observed. This is the first *randomized* pharmacokinetic study investigating the effects of prednisone on the pharmacokinetics of docetaxel. From a pharmacological perspective, we conclude that prednisone did not affect the exposure of the docetaxel regimen.

Glucocorticoids are classified as inducers of the CYP3A enzyme,[18] and docetaxel is metabolized primarily by this iso-enzyme. Previously, an interaction study of docetaxel and prednisone has been published in the Clinical Study Report (CSR) of docetaxel and no relevant drug-drug interaction was reported.[19] However, that PK-study was not randomized

and included only two docetaxel cycles; one with prednisone and one without, possibly not providing enough time for optimal CYP-induction by prednisone. Moreover, that study was limited by sparse PK-sampling (only 6 samples during each cycle) and by limited pharmacokinetic endpoints of docetaxel (clearance only). Therefore, in our study, we used a randomized cross-over design including 6 cycles of docetaxel (3 cycles in absence and 3 cycles in presence of prednisone), an enriched sampling scheme with more relevant pharmacokinetic endpoints.

Although we corrected for dose-reductions due to toxicity over time, we unexpectedly did find a significant period-effect in this study. This means that a decrease in docetaxel exposure occurred in the consecutive cycles independent of randomization or treatment. This might be an explanation for the trend towards an overall higher incidence of (febrile) neutropenia seen at the start of chemotherapy cycles. There are a few potential explanations for this phenomenon. First, a time-dependent induction of CYP3A4 by upregulation of Pregnane X receptor (PXR) due to repetitive docetaxel exposure could occur.[20-23] This phenomenon is called 'auto-induction' and is previously described with several other agents, e.g. dabrafenib.[24] A second possible explanation is an upregulation of ABCB1 (P-glycoprotein) by docetaxel. P-glycoprotein is an active drug-efflux transporter at the cell membrane of hepatocytes, kidney cells and intestine cells. Its upregulation leads to an increased efflux of docetaxel out of the circulation, resulting in decreased plasma concentrations.[25] This phenomenon could even lead to pharmacokinetic resistance to the drug.[26,27] This period effect is unlikely to be caused by castration-levels of the patients, since the maximum induction effect of ADT is reached after approximately 4 weeks , whereas in our study patients had received at least nine weeks of ADT at the time of PK sampling.

Interestingly, we observed no difference in docetaxel-induced toxicities in the absence or presence of prednisone, except for a non-significant difference in neutropenia. Because our study was not powered or designed for toxicity related questions, we can only conclude from a pharmacokinetic point of view that prednisone could be safely omitted from the docetaxel regimen.

The major benefit of administering docetaxel without prednisone could be a reduced treatment-period of prednisone for patients with metastatic prostate cancer. Long-term corticosteroid use, albeit in low dosage, may contribute to the development of severe toxicities, as mentioned before.[9] By excluding prednisone from the initial docetaxel chemotherapy regimen patients will no longer be unnecessarily exposed to these side-effects. Especially for those patients in the hormone-sensitive phase, who usually have a long life expectancy, excluding prednisone will be of relevance to avoid long-term toxicity

with unclear antitumor activity. In this light, Ghatalia *et al.* found no positive effect on survival nor on cabazitaxel-induced toxicity in patients with mCRPC.[28]

Limitations of our study include the administration of the standard pre-medication dexamethasone, which is another CYP3A inducer. We aimed to minimize the pharmacokinetic effect of dexamethasone on docetaxel by excluding the latest gift of dexamethasone before docetaxel infusion. Strengths of our study are the randomized design with extensive PK sampling at multiple time points.

In conclusion, we found no influence of prednisone on docetaxel pharmacokinetics. Docetaxel is registered with concomitant prednisone in the mCRPC setting. In metastatic hormone-sensitive disease, the use of prednisone should be supported by other arguments balancing the benefit of prednisone versus the potential long-term side effects of corticosteroid use.

REFERENCES

1. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Theodore C, James ND, Turesson I, Rosenthal MA, Eisenberger MA, Investigators TAX. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med 2004; 351: 1502-12.
2. Berthold DR, Pond GR, de Wit R, Eisenberger M, Tannock IF, Investigators TAX. Survival and PSA response of patients in the TAX 327 study who crossed over to receive docetaxel after mitoxantrone or vice versa. Ann Oncol 2008; 19: 1749-53.
3. Berry W, Dakhil S, Gregurich MA, Asmar L. Phase II trial of single-agent weekly docetaxel in hormone-refractory, symptomatic, metastatic carcinoma of the prostate. Semin Oncol 2001; 28: 8-15.
4. Beer TM, Pierce WC, Lowe BA, Henner WD. Phase II study of weekly docetaxel in symptomatic androgen-independent prostate cancer. Ann Oncol 2001; 12: 1273-9.
5. Kongsted P, Svane IM, Lindberg H, Daugaard G, Sengelov L. Low-dose prednisolone in first-line docetaxel for patients with metastatic castration-resistant prostate cancer: is there a clinical benefit? Urol Oncol 2015; 33: 494 e15-20.
6. De Santis M, Saad F. Practical Guidance on the Role of Corticosteroids in the Treatment of Metastatic Castration-resistant Prostate Cancer. Urology 2016; 96: 156-64.
7. Ndibe C, Wang CG, Sonpavde G. Corticosteroids in the management of prostate cancer: a critical review. Curr Treat Options Oncol 2015; 16: 6.
8. Venkitaraman R, Lorente D, Murthy V, Thomas K, Parker L, Ahiabor R, Dearnaley D, Huddart R, De Bono J, Parker C. A randomised phase 2 trial of dexamethasone versus prednisolone in castration-resistant prostate cancer. Eur Urol 2015; 67: 673-9.
9. Morgan CJ, Oh WK, Naik G, Galsky MD, Sonpavde G. Impact of prednisone on toxicities and survival in metastatic castration-resistant prostate cancer: A systematic review and meta-analysis of randomized clinical trials. Crit Rev Oncol Hematol 2014; 90: 253-61.
10. James ND, Sydes MR, Clarke NW, Mason MD, Dearnaley DP, Spears MR, Ritchie AW, Parker CC, Russell JM, Attard G, de Bono J, Cross W, Jones RJ, Thalmann G, Amos C, Matheson D, Millman R, Alzouebi M, Beesley S, Birtle AJ, Brock S, Cathomas R, Chakraborti P, Chowdhury S, Cook A, Elliott T, Gale J, Gibbs S, Graham JD, Hetherington J, Hughes R, Laing R, McKinna F, McLaren DB, O'Sullivan JM, Parikh O, Peedell C, Protheroe A, Robinson AJ, Srihari N, Srinivasan R, Staffurth J, Sundar S, Tolan S, Tsang D, Wagstaff J, Parmar MK, investigators S. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. Lancet 2016; 387: 1163-77.
11. Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, Eisenberger M, Wong YN, Hahn N, Kohli M, Cooney MM, Dreicer R, Vogelzang NJ, Picus J, Shevrin D, Hussain M, Garcia JA, DiPaola RS. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. N Engl J Med 2015; 373: 737-46.
12. Gravis G, Fizazi K, Joly F, Oudard S, Priou F, Esterni B, Latorzeff I, Delva R, Krakowski I, Laguerre B, Rolland F, Theodore C, Deplanque G, Ferrero JM, Pouessel D, Mourey L, Beuzeboc P, Zanetta S, Habibian M, Berdah JF, Dauba J, Baciuchka M, Platini C, Linassier C, Labourey JL, Machiels JP, El Kouri C, Ravaud A, Suc E, Eymard JC, Hasbini A, Bousquet G, Soulie M. Androgen-deprivation therapy alone or with docetaxel in non-castrate metastatic prostate cancer (GETUG-AFU 15): a randomised, open-label, phase 3 trial. Lancet Oncol 2013; 14: 149-58.
13. Pascussi JM, Drocourt L, Gerbal-Chaloin S, Fabre JM, Maurel P, Vilarem MJ. Dual effect of dexamethasone on CYP3A4 gene expression in human hepatocytes. Sequential role of glucocorticoid receptor and pregnane X receptor. Eur J Biochem 2001; 268: 6346-58.

14. de Graan AJ, Lancaster CS, Obaidat A, Hagenbuch B, Elens L, Friberg LE, de Bruijn P, Hu S, Gibson AA, Bruun GH, Corydon TJ, Mikkelsen TS, Walker AL, Du G, Loos WJ, van Schaik RH, Baker SD, Mathijssen RH, Sparreboom A. Influence of polymorphic OATP1B-type carriers on the disposition of docetaxel. Clin Cancer Res 2012; 18: 4433-40.
15. D. Schoenfeld MMGCRC. Statistical considerations for a cross-over study where the outcome is a measurement. In.
16. European Medicines Agency. Guideline on the investigation of bioequivalence. In.
17. Jones B, Kenward, M.G. Design and Analysis of Cross-Over Trials: Chapman&Hall/CRC monographs, 2014.
18. Quattrochi LC, Guzelian PS. Cyp3A regulation: from pharmacology to nuclear receptors. Drug Metab Dispos 2001; 29: 615-22.
19. Summary of Product Characteristics - Taxotere. In: EMA.
20. Harmsen S, Meijerman I, Beijnen JH, Schellens JH. Nuclear receptor mediated induction of cytochrome P450 3A4 by anticancer drugs: a key role for the pregnane X receptor. Cancer Chemother Pharmacol 2009; 64: 35-43.
21. Istrate MA, Nussler AK, Eichelbaum M, Burk O. Regulation of CYP3A4 by pregnane X receptor: The role of nuclear receptors competing for response element binding. Biochem Biophys Res Commun 2010; 393: 688-93.
22. Hilli J, Sailas L, Jyrkkio S, Pyrhonen S, Laine K. NCT01110291: induction of CYP3A activity and lowered exposure to docetaxel in patients with primary breast cancer. Cancer Chemother Pharmacol 2011; 67: 1353-62.
23. Luo G, Cunningham M, Kim S, Burn T, Lin J, Sinz M, Hamilton G, Rizzo C, Jolley S, Gilbert D, Downey A, Mudra D, Graham R, Carroll K, Xie J, Madan A, Parkinson A, Christ D, Selling B, LeCluyse E, Gan LS. CYP3A4 induction by drugs: correlation between a pregnane X receptor reporter gene assay and CYP3A4 expression in human hepatocytes. Drug Metab Dispos 2002; 30: 795-804.
24. Luke JJ, Ott PA. New developments in the treatment of metastatic melanoma - role of dabrafenib-trametinib combination therapy. Drug Healthc Patient Saf 2014; 6: 77-88.
25. Harmsen S, Meijerman I, Febus CL, Maas-Bakker RF, Beijnen JH, Schellens JH. PXR-mediated induction of P-glycoprotein by anticancer drugs in a human colon adenocarcinoma-derived cell line. Cancer Chemother Pharmacol 2010; 66: 765-71.
26. Kato T, Mizutani K, Kameyama K, Kawakami K, Fujita Y, Nakane K, Kanimoto Y, Ehara H, Ito H, Seishima M, Deguchi T, Ito M. Serum exosomal P-glycoprotein is a potential marker to diagnose docetaxel resistance and select a taxoid for patients with prostate cancer. Urol Oncol 2015; 33: 385 e15-20.
27. Shirakawa K, Takara K, Tanigawara Y, Aoyama N, Kasuga M, Komada F, Sakaeda T, Okumura K. Interaction of docetaxel ("Taxotere") with human P-glycoprotein. Jpn J Cancer Res 1999; 90: 1380-6.
28. Ghatalia P, Pond GR, Templeton AJ, Sonpavde G. Effect of Single-agent Daily Prednisone on Outcomes and Toxicities in Metastatic Castration-resistant Prostate Cancer: Pooled Analysis of Prospective Studies. Clin Genitourin Cancer 2018; 16: e277-e87.

Chapter 4

An open-label, multicenter, phase Ib study investigating the effect of apalutamide on ventricular repolarization in men with castration-resistant prostate cancer

Bodine P.S. Belderbos, Ronald de Wit, Caly Chien, Anna Mitselos, Peter Hellemans, James Jiao, Margaret K. Yu, Gerhardt Attard, Iurie Bulat, W. Jeffrey Edenfield, Fred Saad

Cancer Chemother Pharmacol. 2018 Sep; 457-468.

ABSTRACT

Purpose: Phase Ib study evaluating the effect of apalutamide, at therapeutic exposure, on ventricular repolarization by applying time-matched pharmacokinetics and electrocardiography (ECG) in patients with castration-resistant prostate cancer. Safety of daily apalutamide was also assessed.

Methods: Patients received 240 mg oral apalutamide daily. Time-matched ECGs were collected via continuous 12-lead Holter recording before apalutamide (Day –1) and on Days 1 and 57 (Cycle 3 Day 1). Pharmacokinetics of apalutamide were assessed on Days 1 and 57 at matched time points of ECG collection. QT interval was corrected for heart rate using Fridericia correction (QTcF). The primary endpoint was the maximum mean change in QTcF (ΔQTcF) from baseline to Cycle 3 Day 1 (steady state). Secondary endpoints were the effect of apalutamide on other ECG parameters, pharmacokinetics of apalutamide and its active metabolite, relationship between plasma concentrations of apalutamide and QTcF, and safety.

Results: Forty-five men were enrolled; 82% received treatment for ≥3 months. At steady state, the maximum ΔQTcF was 12.4 ms and the upper bound of its associated 90% CI was 16.0 ms. No clinically meaningful effects of apalutamide were reported for heart rate or other ECG parameters. A concentration-dependent increase in QTcF was observed for apalutamide. Most adverse events (AEs) (73%) were grade 1–2 in severity. No patients discontinued due to QTc prolongation or AEs.

Conclusion: The effect of apalutamide on QTc prolongation was modest and does not produce a clinically meaningful effect on ventricular repolarization. The AE profile was consistent with other studies of apalutamide.

INTRODUCTION

Prostate cancer is the second most common cancer in men worldwide, accounting for 15% of cancers diagnosed in men.[1] Metastatic castration-resistant prostate cancer (mCRPC) is associated with progressive morbidities, including skeletal-related events.[2] Because prostate cancer cells depend on the androgen receptor (AR) for survival and growth, treatment for recurrent or primary metastatic prostate cancer targets this receptor axis.[3] Despite initial therapies that target the AR, many patients progress to CRPC.[3] Apalutamide is an orally administered next-generation AR inhibitor currently approved in the United States for patients with nonmetastatic CRPC (nmCRPC).[4] It directly binds the ligand-binding domain of the AR, inhibits AR nuclear translocation and DNA binding, and impedes AR-mediated transcription.[5]

The efficacy and safety of apalutamide were demonstrated in patients with prostate cancer in the SPARTAN study, a randomized, double-blind, placebo-controlled, multicenter trial that evaluated apalutamide treatment in 1207 patients with high-risk nmCRPC.[6] This study was the first to demonstrate a significant longer median metastasis-free survival (MFS; 2 years over placebo) in apalutamide-treated patients compared with placebo-treated patients, with consistent benefit for apalutamide across all secondary endpoints, including time to symptomatic progression.[6] Minimal cardiac adverse events (AEs) were observed; atrial fibrillation was cited as the primary cardiac-associated AE reason for dose interruption and occurred in 0.7% and 0.5% of patients in the apalutamide and placebo arms, respectively. Based on these data, apalutamide was approved in February 2018 by the US Food and Drug Administration (FDA) for the treatment of men with nmCRPC.[4]

Apalutamide pharmacokinetics (PK) have been well characterized in clinical studies. Apalutamide is rapidly absorbed, with a median time to maximum observed plasma concentration (C_{max}) of 2–3 h after oral administration.[7] Additionally, PK were approximately proportional across dose levels, with a mean effective half-life of approximately 3 days after multiple doses (Data on file, Janssen). Steady-state exposure was achieved following 4 weeks of continuous, daily apalutamide administration.[4,7] For N-desmethyl apalutamide, a minimal peak to trough fluctuation ratio in plasma at steady state (≈1.3) was observed. Time to reach C_{max} (t_{max}) for N-desmethyl apalutamide at steady state was variable and typically occurred at around 1 or 24 h post dose (Data on file, Janssen).

The preclinical cardiovascular safety of apalutamide and its active metabolite N-desmethyl apalutamide has been assessed in in vitro and in vivo studies (Data on file, Janssen). Both apalutamide and N-desmethyl apalutamide inhibited human ether-à-go-go-related (hERG) gene current, with half maximal inhibitory concentration (IC_{50}) values of 6.17 μM and 4.56

μM, respectively, representing a safety margin of at least 7 relative to the anticipated C_{max} for unbound apalutamide and N-desmethyl apalutamide in patients at the clinical dose of 240 mg/day (Data on file, Janssen). No relevant effect was produced (no prolongation in action potential duration; no effect on resting membrane potential) in isolated canine Purkinje fibers with up to 30 μM of apalutamide or N-desmethyl apalutamide (Data on file, Janssen). Preclinical in vitro receptor binding assay testing did not reveal an effect of apalutamide on Na+ or Ca^2+ channels (Data on file, Janssen). No in vivo treatment-related cardiovascular effects (blood pressure, heart rate, body temperature, ECG lead II intervals, PR, QRS, QT, QTc, or ECG waveform morphology) were noted in a single-dose telemetry study in conscious dogs up to 40 mg/kg or after repeated dosing in good laboratory practice toxicology studies in dogs up to 10 mg/kg/day with exposures in the range of the clinical exposure for apalutamide and its metabolite N-desmethyl apalutamide (Data on file, Janssen). Overall preclinical cardiovascular safety assessment of apalutamide was not indicative of an increased risk for QTc prolongation in clinical use (Data on file, Janssen).

The effect of apalutamide on ventricular repolarization was previously evaluated as part of a phase I/II study[7] that included time-matched triplicate 12-lead electrocardiograms (ECGs) collected at baseline and at steady state in 12 patients with CRPC. The data showed no significant effect from apalutamide on ECG parameters, and there was no conclusive evidence for an increase in Fridericia corrected QT interval (QTcF) (Data on file, Janssen).

Androgen deprivation therapy (ADT) can increase cardiovascular risk because of its adverse effect on risk factors for cardiovascular disease.[8,9] Combination treatment with bicalutamide plus LHRH agonist therapy and 150-mg bicalutamide monotherapy may lead to QTc prolongation.[10-12] In AFFIRM, a randomized phase III study, the effect of enzalutamide (160 mg/day) on QTcF was assessed at steady state in 800 patients with CRPC.[13] No clinically meaningful changes were observed between the mean QTcF interval change from baseline in patients treated with enzalutamide versus those treated with placebo.[13] A recent post hoc analysis of the TERRAIN study suggests a higher risk for atrial fibrillation in patients with mCRPC taking enzalutamide (160 mg/day) versus bicalutamide (50 mg/day).[14]

Because drug-induced QT interval prolongation has been one of the most common causes of drug withdrawals or restrictions of already marketed drugs,[15,16] a thorough premarketing assessment of a drug's potential to cause ECG change or generate life-threatening arrhythmias is a regulatory requirement detailed in the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Harmonised Tripartite Guideline E14 (ICH E14).[17] Therefore, the current study evaluated the effect of therapeutic doses of apalutamide (240 mg) and its active metabolite, N-desmethyl apalutamide, on ventricular repolarization in patients with CRPC.

In accordance with the ICH E14 guideline, a thorough QT (TQT) study ideally has a four-way crossover design, including a therapeutic dose, a supratherapeutic dose, a placebo, and a positive control. In light of the absence of a preclinical QT signal and no conclusive evidence for QTc prolongation in a previous phase I/II study, combined with the need for ≥ 8 weeks of dosing with apalutamide to reach steady-state conditions, providing 8 weeks of placebo treatment in a cancer population would be unethical. The implementation of a positive control would have required the standalone administration of a positive control (e.g., moxifloxacin) and adequate washout prior to starting apalutamide treatment, to avoid any carry-over effect on the predose (Day –1) and Cycle 1 Day 1 (C1D1]) ECG assessments. Moreover, there is limited safety experience with apalutamide at a dose of >240 mg from previous clinical studies. Thus, an alternative multiple dose–dedicated QTc study design was chosen and customized for the oncological setting and for the PK characteristics of apalutamide.

MATERIAL AND METHODS

Patients

Enrolled patients were diagnosed with adenocarcinoma of the prostate and with either high-risk nmCRPC (prostate-specific antigen [PSA] doubling time ≤10 months) or mCRPC. Other inclusion factors were surgical or medical castration with testosterone levels <50 ng/dL, Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1, adequate bone marrow and organ function, QTcF ≤470 ms, and left ventricular ejection fraction of >45%. Key exclusion criteria included known brain metastases, prior treatment with enzalutamide or apalutamide, grade ≥2 electrolyte abnormalities (hypokalemia, hypocalcemia, hypomagnesemia), uncontrolled hypertension, significant cardiac function abnormalities on screening ECG, and history or evidence of certain cardiac conditions. Patients requiring concurrent therapy with medications known to be associated with QTc interval prolongation and an increased risk of torsades de pointes were excluded from the study. Per protocol, strong CYP3A4 inducers, strong CYP2C8 inducers (e.g., rifampin), and strong CYP2C8 inhibitors (e.g., gemfibrozil) were prohibited in order to not influence apalutamide exposure levels.

Study design

This was an open-label, multicenter, phase Ib study to investigate the effect of daily apalutamide (240 mg, orally) on ventricular repolarization (QTc). Approximately 42 patients

with high-risk nmCRPC (defined as having a PSA doubling time of ≤10 months) or mCRPC were planned to be enrolled, to ensure that at least 38 patients completed the study. The study consisted of screening, treatment, and follow-up phases. After providing informed consent, the patients entered a 28-day screening phase for determination of eligibility. If eligible, patients began the open-label treatment phase and were monitored for PK, pharmacodynamics ([PD] ECGs), and safety (including cardiac safety). Apalutamide was administered in continuous 28-day treatment cycles. The duration of the treatment phase for PD (ECG) and PK data collection was from baseline on C1D–1 until C3D1 (Day 57). Patients were allowed to continue apalutamide treatment after C3D1 until disease progression, withdrawal of consent, loss to follow-up, the occurrence of unacceptable toxicity, or loss of clinical benefit (investigator opinion). The follow-up phase for AEs lasted from discontinuation of apalutamide until 30 days after the last dose. Upon discontinuation of study drug, patients returned once for an end-of-treatment (EoT) visit ≤30 days after their last dose.

Pharmacodynamic (ECG) evaluations

Patients were admitted to the study center on C1D–1 (baseline), C1D1, and C1D3 for PK/PD evaluation. Study drug intake was planned at 9:00 AM on C1D1 and C1D3 after overnight fasting. Continuous 12-lead ECGs were collected by a Holter monitor on C1D–1, C1D1, and C3D1 from 8:00 AM to 3:00 PM. Triplicate 12-lead ECGs were obtained during a 10-min time interval at the following time points: at pre-dose (–0.5 h) and at 1, 2, 3, 4, and 5 h after apalutamide administration. This 10-min timeframe began 5 min before and ended 5 min after each scheduled time point. Holter recordings were sent to a blinded, third-party, central ECG contract laboratory for 12-lead ECG selection/extraction, ECG interval measurements, and ECG interpretation.

ECG parameters measured included QRS (the onset of ventricular depolarization), PR (the period extending from the beginning of the onset of atrial depolarization until the beginning of the QRS complex), RR (with R being the point corresponding to the peak of the QRS complex of the ECG wave, and RR being the interval between successive Rs), and QT (electrical depolarization and repolarization of the ventricles).

Pharmacokinetic evaluations

After apalutamide administration on C1D1 and C3D1, time-matched PK blood samples were collected ≤5 min after completion of the 10-min timeframe for planned ECG collection at pre-dose (–0.5 h) and at 1, 2, 3, 4, and 5 h post dose. To calculate an area under the curve (AUC) value over 24 h after the first dose (C1D1), a 24-h PK sample was also collected

during C1D2. Plasma concentrations of apalutamide and N-desmethyl apalutamide were determined using validated liquid chromatography/tandem mass spectrometry methods. The assay consisted of protein precipitation followed by liquid chromatography with tandem mass spectrometric detection. Stable isotope labeled internal standards were used for quantification. Chromatography was performed with a Waters Xbridge C18 column (50 × 2.1 mm, 3.5 µm) using a gradient with 0.1% formic acid and acetonitrile. An API5000 mass spectrometer in the negative ion mode with a Turbo-Ionspray Interface (AB SCIEX, MA, USA) was used. Multiple reaction monitoring (MRM) transitions were from m/z 476.1 to 419.1 and from 479.1 to 419.1 for apalutamide and the internal standard, respectively. For N-desmethyl apalutamide and the internal standard, respectively, MRM transitions were from m/z 464.1 to 235.0 and from 469.2 to 240.1. The quantification range was 0.0250-25.0 µg/mL for both analytes, and the assay performance was monitored using quality control samples. The recorded values all met the acceptance criteria.

PK parameters calculated for apalutamide and N-desmethyl apalutamide using noncompartmental analysis included C_{max}, t_{max}, AUC from time 0 to 24 h after dosing (AUC_{24h}), and the minimum observed plasma concentration (C_{min}). Additionally, the accumulation index (AI), metabolite:parent drug ratio, corrected for molecular weight (MPR; for N-desmethyl apalutamide only), and peak/trough ratio at steady state (PTR) were also calculated.

The PK/PD data collection sought to determine the potential relationship between change from baseline in QTc (ΔQTc) and the plasma concentrations of apalutamide and N-desmethyl apalutamide. The measured QT data were corrected for HR using Fridericia (QTcF),[18] Bazett (QTcB), and a study-specific correction Power (QTcP) correction method. The correlation between QTcF and RR was not significant, with a slope of 0.031 and a 90% CI (–0.01 to 0.07) that included zero. A similar analysis with the QTcP method also showed no statistically significant relationship between QTc and RR, whereas with the QTcB method a statistically significant relationship between QTc and RR was observed. Overall, these analyses support the use of the QTcF method as the primary correction method; thus, only QTcF data are reported herein. Baseline was defined as the mean QTc values of the triplicate ECG measurements taken at baseline. These baseline QTc values were time-matched with those on C1D1 and C3D1. The ΔQTc was calculated at each time point. The primary endpoint was the maximum mean change in QTc (ΔQTc) on C3D1, which was estimated by the mean ΔQTc at around t_{max} (i.e., steady-state). The duration of PD and PK assessments during the treatment phase was from baseline until C3D1.

Safety evaluations

Patients were monitored for safety during the screening, treatment, and follow-up phases until 30 days after the last dose of study drug. The safety evaluations included AE reporting, clinical laboratory safety evaluations, ECGs, multigated acquisition scan, or echocardiogram (screening only for determination of LVEF), ECOG performance status scores, vital signs, and physical examination. For patients who remained in the study after 3 cycles of apalutamide treatment, collection of AEs was limited to serious AEs (SAEs) and grade ≥3 AEs. Patients were followed for disease progression as clinically indicated per institutional guidelines, which could include PSA monitoring or imaging collected at the discretion of the investigator. The safety population included all patients who received at least one dose of apalutamide.

Statistical analysis

The clinical cutoff for the statistical analysis of the study was defined when the last patient enrolled had completed the C3D1 (Day 57) assessments. For the statistical analysis based on the primary correction method (QTcF), the mean changes from baseline (ΔQTcF) at each time point were summarized (mean, standard deviation [SD], median and range, two-sided 90% confidence interval [CI]). The primary endpoint analysis focused on the maximum mean ΔQTcF at C3D1, which was estimated by the mean QTcF change at around t_{max}, the time when C_{max} was reached. The mean ΔQTcF (± SD) over time was plotted. For each treatment and time point of measurement, HR, QRS, PR, and RR intervals, as well as the change from baseline in HR, QRS, PR, and RR (ΔHR, ΔQRS, ΔPR, ΔRR), were summarized using descriptive statistics (mean, SD, median, range, and 90% CI).

Individual plasma concentrations for apalutamide and its active metabolite N-desmethyl apalutamide were tabulated with descriptive statistics (including arithmetic mean, SD, coefficient of variation, median, minimum, and maximum) at each sampling time point for each visit. Individual and mean plasma concentration-time profiles were plotted.

A linear mixed-effects model was fitted to the ΔQTc data from C1D1 and C3D1 with either parent or metabolite concentration as a predictor and patient as a random effect. On the basis of these relationships, the predicted population average ΔQTc and its corresponding upper 90% two-sided CI bound were computed at the mean C_{max} of apalutamide and N-desmethyl apalutamide, or other concentrations of interest.

RESULTS

Patient and disease characteristics

At the time of clinical cutoff, 45 men enrolled at five study centers received at least one dose of apalutamide and were included in the safety analysis set. At study entry the majority (97.8%) of patients had mCRPC. One patient had high-risk nmCRPC. Forty-three of the 45 patients were considered study completers (defined as having completed C3D1 ECG collection and PK sampling procedures in the presence of adequate compliance with intake of the study drug during Cycles 1 and 2) and were included in the primary analysis set. Median age at study entry was 67 years (range, 52–86 years) (**Table 1**). All patients had received therapy for prostate cancer prior to study entry in addition to ADT or surgical castration; the most commonly prescribed prior therapies were bicalutamide (89%), abiraterone acetate (42%), and docetaxel (38%) (**Table 1**). Overall, study participants were largely compliant with avoidance of prohibited medications that could influence apalutamide or N-desmethyl apalutamide PK.

Table 1. Patient and disease characteristics

Baseline characteristic	**Total (*N* = 45)**
Median age, y (range)	67 (52–86)
Race, n (%)	
- White	42 (93)
- Black or African American	3 (7)
Median weight, kg (range)	80 (50–135)
Median time from initial diagnosis, mo (range)	68.2 (3.9–280.3)
Extent of disease, n (%)	
- Bone	40 (91)
- Soft tissue or node	14 (32)
- Liver	2 (4)
- Lung	1 (2)
- Other	3 (7)
- None	1 (2)
ECOG PS, n (%)	
- 0	27 (60)
- 1	18 (40)
Median testosterone, nM (range)	0.85 (0.07–1.63)
Prior therapy, n (%)	45 (100)
- Bicalutamide	40 (89)
- Abiraterone acetate	19 (42)
- Docetaxel	17 (38)
- Cabazitaxel	11 (24)

Abbreviations: ECOG PS=Eastern Cooperative Oncology Group performance status.

Patient disposition

At the time of clinical cutoff, the median treatment duration was 5 months (range, 2–8 months); 82% of patients had received treatment for ≥3 months. Furthermore, 13 of 45 patients had discontinued treatment. Treatment was discontinued due to progressive disease in 12 patients, and one patient withdrew consent.

Primary endpoint

A total of 831 evaluable ECGs were reviewed in this study, out of 855 expected ECG extractions. For each QTc correction method, the relation between QTc and RR at baseline was evaluated graphically by plotting the logarithm of baseline QTc values against the logarithm of corresponding RR intervals. This analysis supported the QTcF method being used as the primary correction method. The primary endpoint was maximum mean ΔQTcF on C3D1. There was no notable difference in QTcF intervals between baseline (Day –1) and after the first dosing (C1D1). For all corresponding time points on C3D1, there were mean increases from baseline in QTcF, but no obvious time-related trends over the course of the

Table 2. Mean ΔQTcF over time after single dose and steady state

	Absolute QTcF interval,[a] ms			ΔQTcF (LS mean) interval,[b] ms		
	N	**Mean (SD)**	**95% CI**	**N**	**Mean (SE)**	**90% CI**
C1D1						
Predose	43	428.7 (13.5)	(424.5–432.9)	43	–0.7 (1.59)	–3.4 to 1.9
1 h	42	430.1 (14.5)	(425.6–434.6)	41	–0.4 (1.62)	–3.1 to 2.3
2 h	43	432.4 (15.2)	(427.7–437.0)	42	1.9 (1.61)	–0.8 to 4.5
3 h	43	424.7 (13.9)	(420.4–429.0)	42	–3.1 (1.61)	–5.8 to –0.4
4 h	43	425.7 (14.5)	(421.3– 430.2)	42	–2.1 (1.61)	–4.8 to 0.6
5 h	43	422.4 (15.9)	(417.5–427.3)	41	–5.5 (1.62)	–8.2 to –2.8
C3D1						
Predose	42	441.6 (16.8)	(436.3–446.8)	42	12.0 (2.14)	8.4–15.5
1 h	42	442.7 (18.6)	(436.9–448.5)	41	12.3 (2.16)	8.7–15.9
2 h	43	442.9 (16.4)	(437.8–447.9)	42	12.4 (2.15)	8.8–16.0
3 h	42	439.3 (15.7)	(434.4–444.2)	41	10.9 (2.15)	7.3–14.5
4 h	42	436.5 (14.2)	(432.0–440.9)	41	8.2 (2.15)	4.6–11.8
5 h	42	436.0 (16.3)	(430.9–441.1)	40	8.0 (2.16)	4.4–11.6

[a]The time-matched baseline is defined as the mean values of the triplicate electrocardiographic measurements taken on C1D–1, at the time points matching with those on C1D1 and C3D1. [b]A repeated-measures mixed model was used with time point, and baseline value of QTc as fixed effect, and patient as a random effect. Abbreviations: CI confidence interval, LS least squares, SD standard deviation, SE standard error, C1D–1=Cycle 1 Day –1, C1D1=Cycle 1 Day 1, C3D1=Cycle 3 Day 1.

day. The least squares mean increases from baseline on C3D1 ranged from 8.0 to 12.4 ms (**Table 2**). The least squares mean (standard error) QTcF change at t_{max} on C1D1 and on C3D1 was +1.9 (1.6) ms and +12.4 (2.1) ms, respectively. The upper limit of the 90% CI of the least squares mean baseline corrected QTcF change at each postdose time point was below 10 ms for C1D1 (maximum of upper limits = 4.5 ms) and above 10 ms, for C3D1 (maximum of upper limits= 16.0 ms).

Patients with QTcF intervals exceeding the threshold values of 450, 480, and 500 ms are summarized in **Table 3**. Twelve patients had QTcF value >450 ms and ≤480 ms at baseline while the number increased to 20 patients on C3D1. One patient had a QTcF interval >480 and ≤500 ms at 1 h post dose on C3D1; this same patient also had a predose (C1D1, at –1 h) QTcF value of 469.3 ms. No QTcF intervals >500 ms were recorded. Numbers of patients with a QTcF interval change from baseline exceeding the threshold values of 30 or 60 ms are also summarized in **Table 3**. Two patients on C1D1 and nine patients on C3D1 had a QTcF interval change from baseline of >30 but ≤60 ms. Among the patients with QTcF interval changes from baseline of >30 ms but ≤60 ms, no association was observed with presence of underlying electrolyte abnormalities or significant cardiac medical history. One patient had a QTcF interval change from baseline of 60.4 ms at 1 h post dose on C3D1 (455.7 ms). The two patients with QTcF >480 ms or with QTcF increase of >60 ms from baseline did not have a significant cardiac medical history and did not use any concomitant medications with a liability for QTc prolongation. The patient with QTcF >480 ms on C3D1 showed grade 1 hypocalcemia at baseline and C3D1, but potassium and magnesium levels were normal.

Table 3. Categorical analysis of QTcF at baseline and post apalutamide treatment

	Baseline (*N* = 43)	**C1D1 (*N* = 43)**	**C3D1 (*N* = 43)**	**Total (C1D1 + C3D1) (*N* = 43)**
Pre- or post-apalutamide QTcF > 450 ms, n (%)[a]				
> 450 to ≤ 480 ms	12 (28)	6 (14)	20 (47)	20 (47)
> 480 to ≤ 500 ms	0	0	1 (2)	1 (2)
> 500 ms	0	0	0	0
QTcF increase from baseline > 30 ms, n (%)[b]				
> 30 to ≤ 60 ms	-	2 (5)	9 (21)	9 (21)
> 60 ms	-	0	1 (2)	1 (2)

[a]Percentages are calculated with the number of patients in primary analysis as denominators and only the worst value for a patient is presented; the C1D1 predose measurement and C1D–1 measurement are considered as baseline. [b]Percentages are calculated with the number of patients in primary analysis as denominators; time-matched baseline is defined as the mean values of the triplicate electrocardiographic measurements taken on C1D–1 (including pre-dose), at the time points matching with those on C1D1 (including pre-dose) and C3D1 (including pre-dose). Abbreviations: C1D–1=Cycle 1 Day –1, C1D1=Cycle 1 Day 1, C3D1=Cycle 3 Day 1.

Secondary endpoints

For all time points on C1D1, mean HR was increased from baseline (**Table 4**), without obvious time-related trends over the course of the day. The mean increases from baseline on C1D1 ranged from 0.1 to 2.5 bpm. For all time points on C3D1, there were mean decreases from baseline in HR, but with no obvious time-related trends over the course of the day. The mean decreases from baseline on C3D1 ranged from –0.4 to –3.5 bpm. The number of patients with any HR (>100 bpm [*n* = 3] or <50 bpm [*n* = 2]) was similar at baseline and on C1D1 and C3D1 (data not shown). Apalutamide did not have a clinically significant effect on HR.

For all time points on C1D1, there were mean decreases from baseline in RR interval but with no obvious time-related trends over the course of the day (**Table 4**). The decreases from baseline on C1D1 ranged from –6.4 to –21.5 ms. For all time points on C3D1, there were mean increases from baseline in RR interval, also with no obvious time-related trends over the course of the day. The increases from baseline on C3D1 ranged from 6.3 to 41.0 ms. The observations on the RR interval are inversely correlated with the observations on HR interval.

For mean PR interval over time compared with baseline, no obvious time-related trends were noted over the course of C1D1 or C3D1 (**Table 4**). The incidence count and percentage of patients with any PR interval >200 ms by study day and by time point was similar at baseline and on C1D1 or C3D1 (data not shown). No effect of apalutamide on the length of the PR interval was apparent. For all time points on C1D1 and C3D1, mean

Table 4. Change from baseline in heart rate, RR interval, PR interval, and QRS interval at C1D1, C3D1

	N	Mean ± SD	90% CI
Heart rate, bpm			
C1D1 Predose	43	0.6 ± 5.83	–0.9 to 2.1
1 h	41	0.7 ± 5.96	–0.9 to 2.3
2 h	42	2.4 ± 13.11	–1.0 to 5.8
3 h	42	2.5 ± 11.97	–0.6 to 5.7
4 h	42	0.1 ± 13.26	–3.4 to 3.5
5 h	41	2.2 ± 6.01	0.6–3.8
C3D1 Predose	42	–2.5 ± 6.09	–4.0 to –0.9
1 h	41	–1.7 ± 5.93	–3.2 to –0.1
2 h	42	–3.4 ± 6.60	–5.1 to –1.7
3 h	41	–1.3 ± 13.69	–4.9 to 2.3
4 h	41	–3.5 ± 14.82	–7.4 to 0.4
5 h	40	–0.4 ± 7.17	–2.3 to 1.5

Table 4. Change from baseline in heart rate, RR interval, PR interval, and QRS interval at C1D1, C3D1 (continued)

	N	Mean ± SD	90% CI
RR interval, ms			
C1D1 Predose	43	–7.5 ± 89.97	–30.6 to 15.5
1 h	41	–13.3 ± 92.29	–37.6 to 11.0
2 h	42	–16.1 ± 92.55	–40.1 to 8.0
3 h	42	–16.0 ± 83.55	–37.7 to 5.7
4 h	42	–6.4 ± 86.57	–28.9 to 16.1
5 h	41	–21.5 ± 63.13	–38.1 to 4.9
C3D1 Predose	42	39.0 ± 89.10	15.8–62.1
1 h	41	27.5 ± 84.93	5.2–49.9
2 h	42	41.0 ± 77.88	20.8–61.2
3 h	41	33.2 ± 143.13	–4.5 to 70.8
4 h	41	36.4 ± 141.19	–0.7 to 73.5
5 h	40	6.3 ± 79.87	–15.0 to 27.6
PR interval, ms			
C1D1 Predose	43	2.6 ± 12.34	–0.5 to 5.8
1 h	41	1.9 ± 13.01	–1.6 to 5.3
2 h	41	–0.7 ± 10.12	–3.4 to 2.0
3 h	41	–1.1 ± 9.78	–3.7 to 1.5
4 h	41	–1.8 ± 9.51	–4.3 to 0.7
5 h	41	–0.3 ± 6.31	–2.0 to 1.4
C3D1 Predose	42	2.2 ± 13.70	–1.3 to 5.8
1 h	41	0.1 ± 17.29	–4.4 to 4.7
2 h	42	1.6 ± 11.38	–1.4 to 4.5
3 h	40	–0.9 ± 15.72	–5.1 to 3.3
4 h	40	–2.7 ± 14.82	–6.6 to 1.3
5 h	40	0.6 ± 8.54	–1.7 to 2.8
QRS interval, ms			
C1D1 Predose	43	0.5 ± 3.36	–0.4 to 1.4
1 h	41	0.9 ± 3.35	0.0–1.8
2 h	42	0.5 ± 3.97	–0.5 to 1.6
3 h	42	0.8 ± 3.58	–0.2 to 1.7
4 h	42	0.9 ± 3.00	0.1–1.6
5 h	41	0.5 ± 3.53	–0.4 to 1.5
C3D1 Predose	42	2.4 ± 4.53	1.2–3.6
1 h	41	1.6 ± 5.05	0.3–2.9
2 h	42	1.9 ± 4.91	0.7–3.2
3 h	41	2.2 ± 4.88	0.9–3.4
4 h	41	1.6 ± 5.07	0.3–3.0
5 h	40	2.3 ± 4.71	1.1–3.6

Abbreviations: SD=standard deviation, CI=confidence interval, C1D–1=Cycle 1 Day –1, C1D1=Cycle 1 Day 1, C3D1=Cycle 3 Day 1

increases were observed from baseline in the QRS interval (**Table 4**). The mean increases from baseline on C1D1 ranged from 0.5 to 0.9 ms and on C3D1 from 1.6 to 2.4 ms. No patients had a QRS interval >110 ms at baseline or on C1D1. QRS intervals >110 ms but ≤115 ms on C3D1 were recorded in three patients. The largest mean change (increase) in QRS duration from baseline was 2.4 ms on C3D1 at pre-dose. Overall, no clinically relevant effects of apalutamide on the QRS interval were observed.

T-wave morphology was monitored, and the number of patients with flat, inverted, or biphasic T-waves was similar on pretreatment and post-treatment days. For most patients with T-wave abnormalities observed during the treatment phase, these observations were also noted on predose ECG before apalutamide administration. De novo T-wave abnormalities were observed in three patients (7%), which were absent at baseline, and no QTcF prolongation ≥480 ms was observed in these three patients. Apalutamide treatment did not have an apparent association with the appearance or worsening of T-wave abnormalities, and no U-waves were observed in any patient.

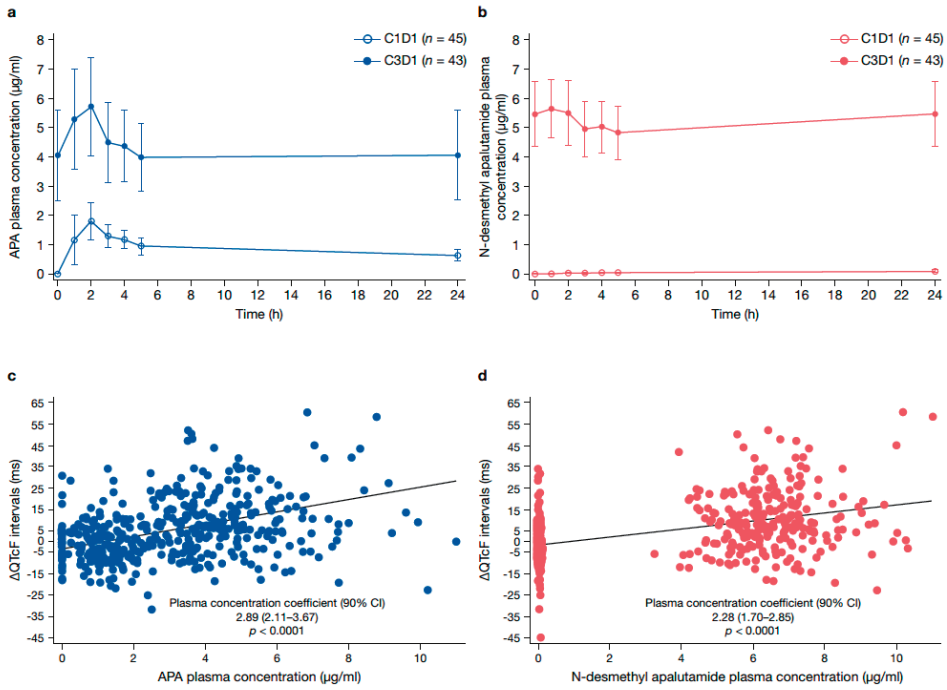


Figure 1. *Plasma concentration of apalutamide and N-desmethyl apalutamide and their association with QTcF:* ***a****. mean plasma concentration–time profiles of apalutamide after administration of 240 mg apalutamide on C1D1 and C3D1;* ***b.*** *mean plasma concentration–time profiles of N-desmethyl apalutamide after administration of 240 mg apalutamide on C1D1 and C3D1;* ***c.*** *scatter plot of the relationship between ΔQTcF and plasma concentration of apalutamide;* ***d****. scatter plot of the relationship between ΔQTcF and plasma concentration of N-desmethyl apalutamide*

Mean plasma concentrations over time for apalutamide and N-desmethyl apalutamide are shown in **Figure 1a** and **1b**. Repeated once-daily administration of 240-mg apalutamide under fasted conditions resulted in a three- and five-fold increase of C_{max} and AUC_{24h}, respectively, when comparing apalutamid e systemic exposure between C1D1 and C3D1 (**Table 5**). Median t_{max} was reached at approximately 2 h post dose on C1D1 and C3D1. At steady-state (C3D1), the active metabolite N-desmethyl apalutamide exhibited a flat PK profile with a mean PTR of 127%. The MPRs for C_{max} and AUC_{24h} were 105 ± 21% and 133 ± 28%, respectively (**Table 5**). A significant correlation was observed between the change in QTcF from baseline and the concentration of apalutamide (slope estimate, 2.89; 90% CI, 2.11–3.67; $p < 0.0001$) (**Figure 1c**). The predicted ΔQTcF (90% CI) at a mean C_{max} of 5.95 µg/mL was 13.8 ms (9.77-17.85). Likewise, a significant correlation was observed between the change in QTcF from baseline and the concentration of N-desmethyl apalutamide (slope estimate, 2.28; 90% CI, 1.70-2.85; $p < 0.0001$) (**Figure 1d**). For instance, on C3D1 the predicted ΔQTcF (90% CI) at a mean steady-state C_{max} of 5.84 µg/mL was 12.0 ms (8.58–15.38).

Table 5. Pharmacokinetics of apalutamide and N-desmethyl apalutamide

	Apalutamide		N-desmethyl apalutamide	
Parameter[a]	**C1D1 (*N* = 45)**	**C3D1 (*N* = 43)**	**C1D1 (*N* = 45)**	**C3D1 (*N* = 43)**
C_{max}, µg/mL	2.06 (0.58)	5.95 (1.66)	0.092 (0.057)	5.85 (1.04)
t_{max}, h	2.12 (1.08–5.10)	2.10 (1.00–4.17)	24.00 (4.10–24.58)	1.10 (0.00–4.17)
AUC_{24h}, µg·h/mL	21.1 (4.93)	100 (31.6)	1.41 (0.79)	124 (23.0)
C_{min}, µg/mL	–	3.72 (1.19)	–	4.66 (0.90)
PTR, %	–	163 (24.7)	–	127 (13.3)
$AI_{(Cmax)}$	–	3.09 (1.26)	–	82.1 (50.5)
$AI_{(AUC24h)}$	-	4.95 (1.69)	-	122 (108)
MPR C_{max}, % (SD)	-	-	-	105 (20.8)
MPR AUC_{24h}, % (SD)	-	-	-	133 (28.0)

[a] *All values are presented as the mean (SD) except for t_{max}, which is presented as the median (range), or as otherwise noted. Abbreviations: C1D1=Cycle 1 Day 1, C3D1=Cycle 3 Day 1, C_{max}=maximum observed plasma concentration, t_{max}=time to C_{max}, AUC_{24h}=AUC from time 0 to 24h after dosing, C_{min}=minimum observed plasma concentration, PTR=peak/trough ratio at steady state, AI=accumulation index, MPR=metabolite:parent drug ratio corrected for molecular weight.*

Safety

Dose modifications were allowed for toxicity attributed to apalutamide, and re-escalation was permitted if first discussed with and approved by the sponsor. The majority of patients had neither dose reduction (42 patients, 93%) nor dose interruption (38 patients, 84%). There were no dose re-escalations after initial dose reduction. Drug-related toxicities

leading to temporary dose interruption included grade 3 diarrhea and aspartate transaminase/alanine transaminase increase and grade 2 fatigue. Two patients required a dose reduction due to fatigue. Thirteen (29%) patients discontinued treatment, with 12 of those discontinuing due to progressive disease and one due to withdrawal of consent (no discontinuations were due to AEs).

Thirty-seven (82%) patients experienced at least one treatment-emergent AE (TEAE), most of which were grades 1–2. The most commonly reported TEAEs (≥10% of patients) were fatigue (40%), decreased appetite (24%), back pain (16%), diarrhea and dyspnea (13% each), rash/erythema (13%), and constipation and nausea (11% each). No treatment-emergent seizures were reported. The AEs recorded in this study were consistent with those observed in other published apalutamide studies.[6,7,19,20] Grade 3 TEAEs were reported in eight (18%) patients and grade 4 AEs in two (4%) patients. Grade 3 TEAEs reported in >1 patient were anemia (3 patients, 7%) and back pain (2 patients, 4%); these grade 3 TEAEs were not considered related to apalutamide treatment. Three patients reported grade 3 toxicities considered possibly or probably related to apalutamide, including fatigue, diarrhea, and aspartate transaminase/alanine transaminase increase. In one patient, grade 3 cardiac failure was reported on study Day 45 and was not considered related to apalutamide treatment. Grade 3 nervous system disorder and spinal cord compression were reported in two patients, were not considered related to apalutamide treatment, and occurred in an overall context of worsening vertebral metastatic disease. One patient experienced grade 4 thrombocytopenia, which was not considered related to apalutamide treatment and occurred in the context of progressive disease. Another patient had grade 4 neutropenia not considered related to apalutamide treatment that occurred while the patient took sulfamethoxazole plus trimethoprim for a bladder infection.

Five patients experienced ≥1 SAE, but none were considered related to apalutamide treatment; SAEs were grades 1–3, except for a grade 4 SAE of general health deterioration in one patient, who subsequently died from progressive disease. One patient experienced a grade 3 SAE of medullary compression, which was considered not related to apalutamide treatment and likely related to underlying metastatic disease in the vertebra. Another patient with bone metastases experienced a grade 3 SAE of progressive lower back pain, which was attributed to the magnetic resonance imaging–verified metastatic disease in the pelvis. One patient experienced multiple SAEs: grade 2 hypercalcemia, grade 3 jaw necrosis, grade 3 pain left hip, and grade 3 neurologic deficit due to spinal cord compression resulting from bone metastases; none were considered related to apalutamide treatment. The final patient, a 70-year-old man with a history of hypertension, mitral valve prolapse, and type 2 diabetes, experienced multiple SAEs, including grade 3 lower back pain, grade 2 infection, and grade 2 delirium, none of which were considered related to apalutamide

treatment. This patient also experienced a grade 3 SAE of heart failure caused by de novo atrial fibrillation that was not considered related to apalutamide treatment.

Laboratory values were collected over time (data not shown). Most patients had occasional changes in serum chemistry and some hematologic abnormalities, the majority of which were grade 0–2 in severity. Elevated thyroid-stimulating hormone (TSH) levels above the upper limit of normal during the study were observed in 13 patients (29%), were usually limited in magnitude and, in the majority of cases, thyroid hormone levels stayed within normal limits. In two patients (4%), significant TSH elevations were observed in combination with a decrease in thyroid hormone levels. One of these two patients had a medical history of hypothyroidism and required an increase in thyroid supplementation therapy in the course of the study.

DISCUSSION

These data from an open-label, multicenter, phase Ib dedicated QT/QTc study that investigated the effect of apalutamide on ventricular repolarization and other ECG parameters confirm the absence of major effects from apalutamide on the QTc interval in men with CRPC. This modified QTc study, tailored to the oncologic setting and taking into account the PK characteristics of apalutamide, was rigorously executed with time-matched ECG and PK sample collection and central blinded ECG interval measurement and interpretation. Across all time points at steady state, the baseline-adjusted QTc intervals and the upper bounds of their associated 90% CIs were ≤20 ms following 240-mg once-daily doses of apalutamide. Consistent with the primary endpoint results, categorical analysis of absolute QTcF values revealed a slightly higher incidence of QTcF readings >450 but ≤480 ms on C3D1 compared with baseline and C1D1. These results indicate that the QTc increases observed with apalutamide become apparent at steady state (after minimally 4 weeks) but not after the first dose, likely because of an accumulation of apalutamide with repeat dosing.

There were two outliers with a larger QTc prolongation. One patient with a QTcF interval >480 and ≤500 ms also had a higher predose QTcF value (469.3 ms) and had a concurrent C3D1 observation of grade 1 hypocalcemia. A second patient had a large absolute ΔQTcF (60.4 ms) at 1 h post dose on C3D1 (QTcF 455.7 ms), which may have been related to exposure to apalutamide (6.84 μg/mL) or N-desmethyl apalutamide (8.21 μg/mL). However, the QTcF value was lower at 2 h post dose despite higher drug concentrations (QTcF of 446 ms [change of 58.3 ms]), with exposure of apalutamide and N-desmethyl apalutamide of 8.77 and 8.87 μ/mL, respectively, suggesting that the increase of more than 60.4 ms was

not consistent at similar exposure within the same individual. No patients discontinued treatment due to QTc prolongation, and no evidence of development of ventricular arrhythmias was observed that could be attributed to underlying QTc prolongation.

According to Sarapa et al., the magnitude of changes in QTcF interval (as observed in the present study) may be considered a mild to moderate QTc-prolonging effect for an anticancer agent, and these same authors have suggested that a dedicated QTc study for anticancer agents that excludes ΔQTc of <20 ms can be concluded as a negative study,[21] consistent with the present data showing no new clinical concerns.[4] Our data are supported by a small and voluntary QTc substudy of the SPARTAN trial,[6] which also revealed no patients in the apalutamide arm with a QTcF interval >480 ms. In the placebo arm of the SPARTAN study, two of six patients had at least one postdose QTcF interval >450 and ≤480 ms; one of these two patients had a baseline QTcF interval >450 ms.

De novo T-wave abnormalities were observed in three patients (7%) and were absent at baseline, and no QTcF prolongation ≥480 ms was observed in these three patients. No evidence for an apalutamide treatment effect was noted on the length of the PR interval in our study. The observed mean increases in QRS duration as observed on C3D1 were minimal (<2.5 ms) and are considered not clinically meaningful.

Overall, exposures of apalutamide and its extent of accumulation observed in this study are consistent with those previously reported.[7] To explore the relationship between apalutamide concentration at steady state and QTcF, PK (plasma concentration) and PD (change from baseline in QTcF) data were analyzed using a linear mixed-effects model. The analysis revealed an association between plasma concentration of apalutamide and QTcF and predicted a prolongation of 13.81 ms at C_{max} at steady state (C3D1), with an upper bound of two-sided 90% CI of 17.85 ms. Because of the correlation between apalutamide and N-desmethyl apalutamide exposures, a similar association between plasma concentration of N-desmethyl apalutamide and QTcF was detected. Based on the flat PK profile of N-desmethyl apalutamide at steady state, the apparent association between N-desmethyl apalutamide concentration and QTcF at steady state is considered less clinically meaningful compared with the parent drug. Overall, results of the PK/PD analysis indicated that a large effect on ΔQTcF is not expected at steady state following 240 mg daily dose of apalutamide.

These data from a Phase Ib QT/QTc study that investigated the effect of apalutamide on QTc intervals revealed no new safety signals associated with apalutamide treatment in men with CRPC. For the primary endpoint, no significant safety findings related to QT prolongation were documented and there were no observed arrhythmias related to

apalutamide. Overall, the safety profile observed in this study was as expected based on the known safety profile of apalutamide and results from other studies.[6,7,19,20]

Overall, these data demonstrate that apalutamide does not produce clinically meaningful changes in QTc interval or produce a concerning effect on ventricular repolarization in patients with CRPC.

REFERENCES

1. Ferlay J SI, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. (2012) GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Available from: http://globocan.iarc.fr, accessed on 28/2/2018. 2018
2. Gartrell BA, Saad F (2014) Managing bone metastases and reducing skeletal related events in prostate cancer. Nat Rev Clin Oncol 11 (6):335-345. doi:10.1038/nrclinonc.2014.70
3. Tan MH, Li J, Xu HE, Melcher K, Yong EL (2015) Androgen receptor: structure, role in prostate cancer and drug discovery. Acta Pharmacol Sin 36 (1):3-23. doi:10.1038/aps.2014.18
4. ERLEADA [Package Insert]. Janssen Products, LP (2018). Horsham PA
5. Clegg NJ, Wongvipat J, Joseph JD, Tran C, Ouk S, Dilhas A, Chen Y, Grillot K, Bischoff ED, Cai L, Aparicio A, Dorow S, Arora V, Shao G, Qian J, Zhao H, Yang G, Cao C, Sensintaffar J, Wasielewska T, Herbert MR, Bonnefous C, Darimont B, Scher HI, Smith-Jones P, Klang M, Smith ND, De Stanchina E, Wu N, Ouerfelli O, Rix PJ, Heyman RA, Jung ME, Sawyers CL, Hager JH (2012) ARN-509: a novel antiandrogen for prostate cancer treatment. Cancer Res 72 (6):1494-1503. doi:10.1158/0008-5472.CAN-11-3948
6. Smith MR, Saad F, Chowdhury S, Oudard S, Hadaschik BA, Graff JN, Olmos D, Mainwaring PN, Lee JY, Uemura H, Lopez-Gitlitz A, Trudel GC, Espina BM, Shu Y, Park YC, Rackoff WR, Yu MK, Small EJ, Investigators S (2018) Apalutamide Treatment and Metastasis-free Survival in Prostate Cancer. N Engl J Med. doi:10.1056/NEJMoa1715546
7. Rathkopf DE, Morris MJ, Fox JJ, Danila DC, Slovin SF, Hager JH, Rix PJ, Chow Maneval E, Chen I, Gonen M, Fleisher M, Larson SM, Sawyers CL, Scher HI (2013) Phase I study of ARN-509, a novel antiandrogen, in the treatment of castration-resistant prostate cancer. J Clin Oncol 31 (28):3525-3530. doi:10.1200/JCO.2013.50.1684
8. Levine GN, D'Amico AV, Berger P, Clark PE, Eckel RH, Keating NL, Milani RV, Sagalowsky AI, Smith MR, Zakai N (2010) Androgen-deprivation therapy in prostate cancer and cardiovascular risk: a science advisory from the American Heart Association, American Cancer Society, and American Urological Association: endorsed by the American Society for Radiation Oncology. CA Cancer J Clin 60 (3): 194-201. doi:caac.20061 [pii];10.3322/caac.20061 [doi]
9. Van Poppel H, Tombal B (2011) Cardiovascular risk during hormonal treatment in patients with prostate cancer. Cancer Manag Res 3:49-55. doi:10.2147/CMR.S16893
10. CASODEX [Package Insert]. AstraZeneca Pharmaceuticals LP (2017). Wilmington, DE
11. Garnick MB, Pratt CM, Campion M, Shipley J (2004) The effect of hormonal therapy for prostate cancer on the electrocardiographic QT interval: phase 3 results following treatment with leuprolide and goserelin, alone or with bicalutamide, and the GnRH antagonist abarelix. Journal of Clinical Oncology 22 (14_suppl):4578. doi:10.1200/jco.2004.22.14_suppl.4578
12. Casodex [Package Insert]. AstraZeneca UK Limited (2018). Bedforshire, UK
13. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, de Wit R, Mulders P, Chi KN, Shore ND, Armstrong AJ, Flaig TW, Flechon A, Mainwaring P, Fleming M, Hainsworth JD, Hirmand M, Selby B, Seely L, de Bono JS, Investigators A (2012) Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 367 (13):1187-1197. doi:10.1056/NEJMoa1207506
14. Siemens DR, Klotz L, Heidenreich A, Chowdhury S, Villers A, Baron B, van Os S, Hasabou N, Wang F, Lin P, Shore ND (2018) Efficacy and safety of enzalutamide vs bicalutamide in younger and older patients with metastatic castration resistant prostate cancer in the TERRAIN trial. J Urol 199 (1):147-154. doi: 10.1016/j.juro.2017.08.080
15. Camm AJ (2005) Clinical trial design to evaluate the effects of drugs on cardiac repolarization: current state of the art. Heart Rhythm 2 (2 Suppl):S23-29. doi:10.1016/j.hrthm.2004.09.019

16. Morganroth J (2004) A definitive or thorough phase 1 QT ECG trial as a requirement for drug safety assessment. J Electrocardiol 37 (1):25-29
17. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonized Tripartite Guideline E14: Clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. (2012). ICH 12
18. Fridericia LS (2003) The duration of systole in an electrocardiogram in normal humans and in patients with heart disease. 1920. Ann Noninvasive Electrocardiol 8 (4):343-351
19. Rathkopf DE, Antonarakis ES, Shore ND, Tutrone RF, Alumkal JJ, Ryan CJ, Saleh M, Hauke RJ, Bandekar R, Maneval EC, de Boer CJ, Yu MK, Scher HI (2017) Safety and Antitumor Activity of Apalutamide (ARN-509) in Metastatic Castration-Resistant Prostate Cancer with and without Prior Abiraterone Acetate and Prednisone. Clin Cancer Res 23 (14):3544-3551. doi:10.1158/1078-0432.CCR-16-2509
20. Smith MR, Antonarakis ES, Ryan CJ, Berry WR, Shore ND, Liu G, Alumkal JJ, Higano CS, Chow Maneval E, Bandekar R, de Boer CJ, Yu MK, Rathkopf DE (2016) Phase 2 Study of the Safety and Antitumor Activity of Apalutamide (ARN-509), a Potent Androgen Receptor Antagonist, in the High-risk Nonmetastatic Castration-resistant Prostate Cancer Cohort. Eur Urol 70 (6):963-970. doi:10.1016/j.eururo.2016.04.023
21. Sarapa N, Britto MR (2008) Challenges of characterizing proarrhythmic risk due to QTc prolongation induced by nonadjuvant anticancer agents. Expert Opin Drug Saf 7 (3):305-318. doi:10.1517/14740338.7.3.305

Chapter 5

Prognostic factors in men with metastatic castration-resistant prostate cancer treated with cabazitaxel

Bodine P.S. Belderbos, Ronald de Wit, Esther Oomen-de Hoop, Annemieke Nieuweboer, Paul Hamberg, Robbert J. van Alphen, André Bergman, Nelly van der Meer, Sander Bins, Ron H.J. Mathijssen, Robert J. van Soest

Oncotarget. 2017 Nov 16; 106468-106474

ABSTRACT

Background: Treatment selection for men with metastatic castration-resistant prostate cancer (mCRPC) has become increasingly challenging with the introduction of novel therapies at earlier disease stages. The purpose of this study was to identify prognostic factors for overall survival (OS) and PSA response in patients with mCRPC treated with cabazitaxel.

Methods: We performed a post-hoc analysis of a randomized phase II trial of mCRPC patients treated with cabazitaxel. Cox and logistic regression models were used to investigate the influence of clinical and biochemical variables on OS and PSA response. Nomograms were developed to estimate the chance of PSA response and OS.

Results: 224 mCRPC patients were included in the current analysis. In multivariable analysis, WHO performance status, baseline hemoglobin, alkaline phosphatase and albumin were all significantly associated with OS. Hemoglobin and alkaline phosphatase were significantly associated with PSA response.

Conclusion: This study identified prognostic factors for OS and PSA response of men with mCRPC treated with cabazitaxel. In an increasingly complicated treatment landscape with several treatment options available our findings might serve to estimate the chance of survival of men qualifying for treatment with second-line chemotherapy in daily practice. Furthermore, these data can be used to risk-stratify patients in clinical trials.

INTRODUCTION

With the introduction of novel agents, treatment options for metastatic castration-resistant prostate cancer (mCRPC) have notably evolved in the past few years. Since level one evidence supporting an optimal treatment sequence in mCRPC is lacking, the choice of treatment by the clinician has become increasingly challenging. Treatment decisions are generally made on the basis of clinical symptoms, comorbidities, expected side-effects and preferences by the patient and the treating physician. Risk classification and prognosis assessment remain critical before the start of a new therapy. Therefore, it is important to identify biomarkers, predictive and prognostic factors for clinical outcome, in order to simplify treatment choice and timing. Such factors may serve to predict individual prognosis at the start of therapy and to classify patients in different risk-groups, which can also be used for stratification in clinical trials.

Prognostic models and nomograms for mCRPC patients receiving first-line chemotherapy have been developed based on large phase III trials,[1-3] including parameters such as performance status, time since last docetaxel use, the presence of measurable disease, the presence of visceral disease, the presence of pain, duration of hormonal treatment, hemoglobin (Hb), prostate specific antigen (PSA) and alkaline phosphatase (AP). For second-line chemotherapy with cabazitaxel, prognostic factors were identified based on the TROPIC trial.[4,5] However, these findings have not been investigated in other datasets of men treated with cabazitaxel. In the current study, we aimed to identify prognostic factors for men with mCRPC receiving cabazitaxel. For this purpose, we used data from a multicenter randomized phase II trial.[6]

PATIENTS AND METHODS

Patients

We performed a post-hoc analysis of a randomized phase II trial (CABARESC, NTR2991). The CABARESC trial was designed to investigate the influence of budesonide (9mg daily) on cabazitaxel pharmacokinetics and cabazitaxel-induced diarrhea and was reported elsewhere.[6] Between December 2011 and October 2015, a total of 246 mCRPC patients were included in the study. The study was conducted in 22 Dutch hospitals and was approved by the ethics committee of the Erasmus MC (MEC 11-324) and all local institutional review boards. Written informed consent was obtained from all participants.

Full study details are described in the original paper.[6] Briefly, patients were eligible if they had metastatic castration-resistant adenocarcinoma of the prostate with disease progression during or after docetaxel therapy, defined as two consecutive rises in PSA (taken ≥ 1 week apart) or according to RECIST criteria. Full inclusion criteria are shown in **Supplementary 1: material and methods**. In this study, no significant impact of budesonide on the pharmacokinetics of cabazitaxel and cabazitaxel-induced diarrhea was found. We have previously shown that there was no influence of prior treatment with abiraterone acetate or enzalutamide on OS and PSA response of men treated with cabazitaxel.[13] Patients who were deemed inevaluable in the original CABARESC trial (N=19) (**Supplementary Table 2**) and patients with missing or inadequate PSA values were considered ineligible for the current analysis (N=3), leaving 224 patients evaluable for the current analysis.

Biomarker panel

From all evaluable patients, laboratory and clinical factors collected at baseline and during treatment were available. The biochemical parameters were collected at baseline included: PSA, lactate dehydrogenase (LDH), AP, albumin (Alb), Hb, derived neutrophil-lymphocyte ratio (dNLR), WHO performance status (0 vs 1), age, type of castration (GnRH analogues vs. surgical castration) and time since last chemotherapy cycle (>6 months vs ≤6 months). The dNLR was computed by the absolute neutrophil count (ANC) divided by the absolute white blood cell count (WBC) minus the ANC, (ANC/ (WBC-ANC)). The occurrence of ≥ grade 3 neutropenia during treatment was collected. Log transformation was applied to variables with a non-normal distribution.

Primary objective and definitions

The objective of the current analysis was to identify prognostic factors associated with OS and PSA response in mCRPC patients treated with cabazitaxel. OS was defined by time from randomization to death from any cause. Patients still alive at the end of the study were censored. Prostate Cancer Working Group 2 (PCWG2) criteria were used to define PSA response as a ≥ 50% decline from baseline measured twice 3 to 4 weeks apart.[14]

Statistical analysis

Descriptive statistics were used to summarize patients' characteristics. Univariable and multivariable Cox regression analyses were used to investigate the influence of laboratory and clinical parameters on OS. Univariable and multivariable logistic regression analysis were used with PSA response (≥50%) as the dependent variable and baseline parameters were included as covariates. Factors with a $p<0.10$ in univariable analysis were entered into the

multivariable analysis. The multivariable model was constructed using backward selection at the 5% level. Data were analyzed using STATA® version 14 (Stata-Corp LP TX, USA). Based on the multivariable model for OS and PSA response we have generated a nomogram for OS and PSA response. Software program 'R' was used to generate both nomograms.

RESULTS

Patient Characteristics

Baseline characteristics were available for 224 patients and are shown in **Table 1**. The characteristics were similar to other studies of men treated with second-line chemotherapy.[5]

Table 1. Baseline characteristics

Characteristic	Value No. (%)
All	224 (100)
Age, mean ± SD	68.8 ± 7.2
WHO	
- 0	92 (41)
- 1	130 (58)
- Missing	2 (1)
Type castration	
- Surgical	30 (13)
- LHRH-analogue	194 (87)
No. prior therapies	
- 1	204 (91)
- ≥ 2	20 (9)
Months since last chemotherapy	
- ≤6 months	102 (46)
- > 6 months	122 (54)
Baseline lab results	**Median (IQR)**
- Hb (mmol/L)	7.7 (6.8-8.2)
- Albumin (g/L)	39.0 (36.0-43.0)
- AP (U/L)	130.0 (86.0-262.0)
- LDH (U/L)	271.0 (210.0-392.0)
- ANC (10^9/L)	5.9 (4.4-7.6)
- PSA (µg/L)	154.1 (59.0-388.3)
- dNLR	2.7 (1.8-3.9)

Abbreviations: WHO= world health organization, Hb = hemoglobin, AP = Alkaline Phosphatase, LDH = lactate dehydrogenase, ANC= absolute neutrophil count, PSA= prostate specific antigen, dNLR= derived Neutrophil-Lymphocyte ratio

Overall survival

Median OS of patients in this dataset was 13.3 months (IQR 7.0 – 22.3). Results of the univariable and multivariable analyses for OS are shown in **Table 2**. Time since last chemotherapy, neutropenia grade 3/4, PSA and LDH were significantly associated with OS in univariable analysis, but not in multivariable analysis. In multivariable analysis, four parameters remained significantly associated with OS; WHO performance status (HR 1.49, 95% CI 1.04-2.13, p=0.028), Hb (HR 0.73, 95% CI 0.61-0.87, p=0.001), AP (HR 1.50, 95%CI 1.18-1.91, p=0.001) and Alb (HR 0.92, 95% CI 0.89-0.95, p<0.001). Of note, dNLR (p=0.45) was not associated with OS in univariable analysis. **Figure 1** presents a nomogram that is based on the multivariable model for OS. This nomogram can be used to predict the individual survival probability at 12 and 24 months. For example, a patient with a performance score of 1, an Hb level of 7.0 mmol/L, albumin level of 40 g/L and AP of 90 U/L (log_AP=4,5) has a 12-month survival probability of approximately 60% and a 24-month survival probability of approximately 20%.

Table 2. Univariable and multivariable model for OS

Variable	Univariable HR (95%CI)	P-value	Multivariable HR (95%CI)	P-value
WHO PS (1 vs 0)	1.57 (1.16-2.13)	0.004	1.49 (1.04-2.13)	0.028
Time since last therapy (>6 months)	0.59 (0.44-0.79)	<0.001		
Neutropenia (Gr. 3/4)	0.67 (0.50-0.90)	0.007		
Hb (mmol/L)	0.057 (0.49-0.67)	<0.001	0.73 (0.61-0.87)	0.001
PSA (log, µg/L)	1.12 (1.01-1.26)	0.040		
AP (log, U/L)	1.83 (1.49-2.25)	<0.001	1.50 (1.18-1.91)	0.001
LDH (log, U/L)	2.37 (1.73-3.25)	<0.001		
Albumin (g/L)	0.91 (0.89-0.94)	<0.001	0.92 (0.89-0.95)	<0.001

Univariable N=206-224 (because of missing values the number of included patients can differ per variable) Multivariable N=200. Abbreviations: WHO PS=world health organization performance status, Hb=hemoglobin, PSA=prostate specific antigen, AP=alkaline phosphatase, LDH=lactate dehydrogenase, log=log transformed variables when data were not normally distributed.

PSA response

Univariable analysis showed significant associations between PSA response and WHO performance status, Hb, AP and LDH. In the multivariable model Hb and AP remained significantly associated with PSA response and were taken into the final model (**Table 3**). Higher hemoglobin level before treatment (OR 1.48, 95%CI 1.05-2.07, p=0.024), and a lower AP level at the start of treatment (OR 0.61, 95%CI 0.39-0.96, p=0.034) resulted in a higher chance of PSA response. **Figure 2** displays a nomogram to calculate the chance of PSA response for an individual patient treated with cabazitaxel based on our multivariable model.

Table 3. Univariable and multivariable model for PSA response

Variable	Univariable OR (95%CI)	P-value	Multivariable OR (95%CI)	P-value
WHO PS (1 vs 0)	0.48 (0.27-0.84)	0.011		
Hb (mmol/L)	1.67 (1.22-2.29)	0.002	1.48 (1.05-2.07)	0.024
AP (log, U/L)	0.50 (0.33-0.77)	0.002	0.61 (0.39-0.96)	0.034
LDH (log, U/L)	0.49 (0.27-0.88)	0.016		

Univariable N= 217-224 (because of missing values the number of included patients can differ per variable) Multivariable N=215 Abbreviations: WHO PS=world health organization performance status, Hb=hemoglobin, AP=alkaline phosphatase, LDH=lactate dehydrogenase, log=log transformed variables when data were not normally distributed.

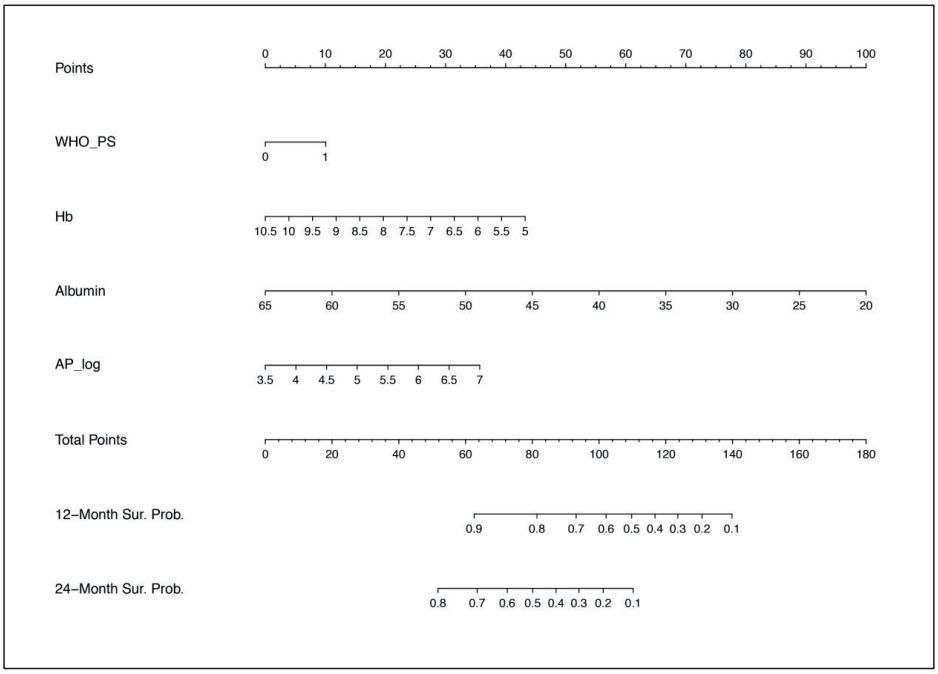


Figure 1. Nomogram for overall survival

Prognostic nomogram predicting overall survival probability. For each variable, starting with WHO performance score on the second axis, draw a vertical line up to the 'Points' axis (top line) to identify the number of prognostic points the patient receives for the value of this variable. Calculate the 'Total Points' by adding up the prognostic points for each variable. Determine the 12-month or 24-month overall survival by drawing a vertical line from the 'Total Points' axis down to the axis indicating the survival probabilty. WHO PS= world health organization performance status, Hb = hemoglobin, AP = alkaline phosphatase, log = log transformed variables when data were not normally distributed.

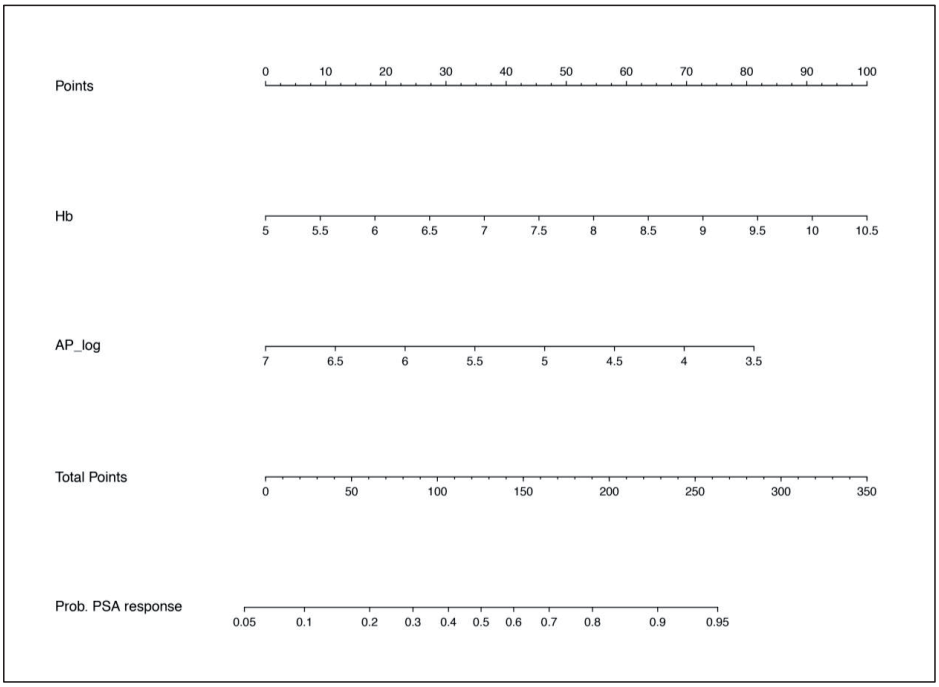


Figure 2. Nomogram for PSA response

Prognostic nomogram predicting PSA response. For both variables, Hb and AP_log, draw a vertical line from the absolute value of this variable up to the 'Points' axis (top line) to identify the number of prognostic points the patient receives for these variables. Calculate the 'Total Points' by adding up the prognostic points for both variables. Determine the probability of PSA response by drawing a vertival line form the 'Total Points' axis down to the axis of the probability of PSA response. Hb = hemoglobin, AP = alkaline phosphatase, log = log transformed variables when data were not normally distributed.

DISCUSSION

In this post-hoc analysis of a large phase II trial of patients with mCRPC treated with cabazitaxel, we found that WHO performance status, baseline Hb, AP and albumin were all significantly associated with OS. In addition, Hb and AP were identified as parameters to predict the probability of PSA response in mCRPC patients receiving second-line chemotherapy. To our knowledge, we are the first to report prognostic factors and a nomogram for OS and PSA response in men with mCRPC treated with cabazitaxel outside of the TROPIC registration trial.[5] Treatment selection of men with metastatic prostate cancer has become increasingly challenging with the introduction of novel therapies at earlier disease stages. In this changing treatment paradigm, predictors and prognostic factors of treatment response and outcome are still lacking. The current study shows that readily available, cheap and easy to use clinical parameters are of prognostic value for

estimating the chance of PSA response and OS using the presented nomograms (**Figure 1** and **2**). These individual probabilities of survival and response might help the physician to decide when to initiate treatment in daily clinical practice. As an illustration, if the identified prognostic factors follow a trend towards an unfavourable condition, it may help the physician to avoid missing the window of opportunity for cabazitaxel treatment and initiate therapy before the patients' condition is declining.

Several distinct predictors of OS and PSA response (e.g. albumin) were identified as compared to the models constructed from the TROPIC dataset.[5,7] The difference between the identified variables in the final model from Halabi et al. and our cohort may be due to a different population, different primary trial design and different model assumptions. The strength of our model is that it is based only on men treated with cabazitaxel. In contrast, the Halabi nomogram has been constructed based on the TROPIC trial and validated on the SPARC trial, including also men treated with mitoxantrone and satraplatin which are not used in the current treatment armamentarium.[5] Therefore, our study might represent a more contemporary real-world patient population. This study is limited by its post-hoc design. As a result, radiographic variables such as the presence of visceral disease were not collected in the primary trial.

dNLR, a parameter to determine the inflammatory response rate of the host, has been reported and validated as a prognostic factor for OS and response in mCRPC patients treated with docetaxel, cabazitaxel and abiraterone.[7-10] In addition, a high dNLR was associated with poor OS in other tumor types.[11] However, in our analyses no significant association between dNLR and OS, nor between dNLR and PSA response was found. Furthermore, in a post-hoc analysis of the TROPIC trial neutropenia during treatment with cabazitaxel has shown a significant relation with OS of men with mCRPC.[12] Although we had comparable treatment groups, no association between the occurrence of grade 3-4 neutropenia and OS or PSA response was found.

In conclusion, this study identified clinical and biochemical variables associated with OS and PSA response of patients with mCRPC treated with cabazitaxel. In an increasingly complicated treatment landscape with several treatment options available including chemotherapy and novel AR-targeted agents, our findings have prognostic value for treatment response and survival of men qualifying for treatment with second-line chemotherapy in daily practice. Furthermore, these data can be used to risk-stratify patients in future clinical trials.

REFERENCES

1. Halabi S, Small EJ, Kantoff PW, Kattan MW, Kaplan EB, Dawson NA, Levine EG, Blumenstein BA and Vogelzang NJ. Prognostic model for predicting survival in men with hormone-refractory metastatic prostate cancer. J Clin Oncol. 2003; 21(7):1232-1237.
2. Smaletz O, Scher HI, Small EJ, Verbel DA, McMillan A, Regan K, Kelly WK and Kattan MW. Nomogram for overall survival of patients with progressive metastatic prostate cancer after castration. J Clin Oncol. 2002; 20(19):3972-3982.
3. Armstrong AJ, Garrett-Mayer ES, Yang YC, de Wit R, Tannock IF and Eisenberger M. A contemporary prognostic nomogram for men with hormone-refractory metastatic prostate cancer: a TAX327 study analysis. Clin Cancer Res. 2007; 13(21):6396-6403.
4. de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, Gravis G, Bodrogi I, Mackenzie MJ, Shen L, Roessner M, Gupta S, Sartor AO and Investigators T. 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.
5. Halabi S, Lin CY, Small EJ, Armstrong AJ, Kaplan EB, Petrylak D, Sternberg CN, Shen L, Oudard S, de Bono J and Sartor O. Prognostic model predicting metastatic castration-resistant prostate cancer survival in men treated with second-line chemotherapy. J Natl Cancer Inst. 2013; 105(22):1729-1737.
6. Nieuweboer AJ, de Graan AM, Hamberg P, Bins S, van Soest RJ, van Alphen RJ, Bergman AM, Beeker A, van Halteren H, Ten Tije AJ, Zuetenhorst H, van der Meer N, Chitu D, de Wit R, Mathijssen RH and Dutch Uro-Oncology S. Effects of Budesonide on Cabazitaxel Pharmacokinetics and Cabazitaxel-Induced Diarrhea: A Randomized, Open-Label Multicenter Phase II Study. Clin Cancer Res. 2017; 23(7):1679-1683.
7. Lorente D, Mateo J, Templeton AJ, Zafeiriou Z, Bianchini D, Ferraldeschi R, Bahl A, Shen L, Su Z, Sartor O and de Bono JS. Baseline neutrophil-lymphocyte ratio (NLR) is associated with survival and response to treatment with second-line chemotherapy for advanced prostate cancer independent of baseline steroid use. Ann Oncol. 2015; 26(4):750-755.
8. Sonpavde G, Pond GR, Armstrong AJ, Clarke SJ, Vardy JL, Templeton AJ, Wang SL, Paolini J, Chen I, Chow-Maneval E, Lechuga M, Smith MR and Michaelson MD. Prognostic impact of the neutrophil-to-lymphocyte ratio in men with metastatic castration-resistant prostate cancer. Clin Genitourin Cancer. 2014; 12(5):317-324.
9. van Soest RJ, Templeton AJ, Vera-Badillo FE, Mercier F, Sonpavde G, Amir E, Tombal B, Rosenthal M, Eisenberger MA, Tannock IF and de Wit R. 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. Ann Oncol. 2015; 26(4):743-749.
10. Cao J, Zhu X, Zhao X, Li XF and Xu R. Neutrophil-to-Lymphocyte Ratio Predicts PSA Response and Prognosis in Prostate Cancer: A Systematic Review and Meta-Analysis. PLoS One. 2016; 11(7): e0158770.
11. Templeton AJ, McNamara MG, Seruga B, Vera-Badillo FE, Aneja P, Ocana A, Leibowitz-Amit R, Sonpavde G, Knox JJ, Tran B, Tannock IF and Amir E. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst. 2014; 106(6):dju124.
12. Meisel A, von Felten S, Vogt DR, Liewen H, de Wit R, de Bono J, Sartor O and Stenner-Liewen F. Severe neutropenia during cabazitaxel treatment is associated with survival benefit in men with metastatic castration-resistant prostate cancer (mCRPC): A post-hoc analysis of the TROPIC phase III trial. Eur J Cancer. 2016; 56:93-100.

13. van Soest RJ, Nieuweboer AJ, de Morree ES, Chitu D, Bergman AM, Goey SH, Bos MM, van der Meer N, Hamberg P, de Wit R, Mathijssen RH and Dutch Uro-Oncology S. The influence of prior novel androgen receptor targeted therapy on the efficacy of cabazitaxel in men with metastatic castration-resistant prostate cancer. Eur J Cancer. 2015; 51(17):2562-2569.
14. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, Eisenberger MA, Higano C, Bubley GJ, Dreicer R, Petrylak D, Kantoff P, Basch E, Kelly WK, Figg WD, Small EJ, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. J Clin Oncol. 2008; 26(7): 1148-1159.

SUPPLEMENTARY

Supplementary 1: materials and methods

Inclusion and exclusion criteria CABARESC study:

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 (ALT) and aspartate aminotransferase (AST) ≤2.5x ULN, in case of liver metastasis < 5 ULN; alkaline phosphatase (AP) < 5x ULN) In case of bone metastasis, AP < 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

Supplementary Table 2. Ineligible patient of the CABARESC trial

Reason for exclusion	Group CABA	Group BUD	Post-hoc	Total
No cabazitaxel (in study context)[a]	3	7	0	10
Initial cabazitaxel dose <25mg/m^2	5	1	0	6
Treatment started before randomization	1	0	0	1
Death before start therapy	1	0	0	1
Long treatment delay after randomization[b]	1	0	0	1
Missing laboratory values	0	0	3	3
Total	11	8	3	22

[a] *due to disease progression and worsening of patient conditions: patient did not receive treatment or not in context of this study.*

[b] *due to ASAT and ALAT > 2 upper limit of normal without liver metastases there was a time of two months between randomization and start of cabazitaxel therapy.*

Chapter 7

Associations between *AR-V7* Status in Circulating Tumor Cells, Circulating Tumor Cell Count and Survival in Men with Metastatic Castration-Resistant Prostate Cancer

Bodine P.S. Belderbos, Anieta M. Sieuwerts†, Esther Oomen-de Hoop, Bianca Mostert, Jaco Kraan, Paul Hamberg, Mai N. Van, Corine M. Beaufort, Wendy Onstenk, Robert J. van Soest, John Martens, Stefan Sleijfer, Ronald de Wit, Ron H.J. Mathijssen, Martijn P. Lolkema.

Eur J Cancer. 2019 sep 19; 48-54

ABSTRACT

Background: The interpretation of the presence of *AR-V7* in circulating tumor cells (CTCs) in men with metastatic castration-resistant prostate cancer (mCRPC) remains to be elucidated. *AR-V7* may hold promise as a predictive biomarker, but there may be prognostic impact of *AR-V7* positivity as well. To investigate the clinical value of *AR-V7*, we determined whether *AR-V7* detection in CTCs in mCRPC patients is associated with CTC counts and survival.

Methods: Between December 2011 and January 2019 three prospective clinical trials collected clinical data of mCRPC patients, who progressed after docetaxel and/or enzalutamide or abiraterone. Baseline (and follow-up) blood samples were withdrawn determining CTC count and *AR-V7* expression. The majority of patients started cabazitaxel as next line of treatment following *AR-V7* characterization.

Results: A total of 127 samples were evaluable for the analysis of CTC count versus *AR-V7* status. Although an association was observed between *AR-V7* and CTC count in all mCRPC patients (p=0.017), no such association was found in the prognostic unfavorable subgroup of patients with ≥5 CTCs. After adjusting for clinical prognostic factors, *AR-V7* expression in CTCs was not associated with overall survival (HR=1.33, 95%CI 0.81-2.15, p=0.25).

Conclusion: We found that *AR-V7* expression in CTCs had no additional prognostic value in mCRPC patients, mostly treated with cabazitaxel. In mCRPC patients with a predefined worse prognosis of higher CTC count (≥5), a predictive biomarker is an important unmet medical need. Prospective trials should investigate whether *AR-V7* detection in CTCs may guide treatment selection for these adverse prognosis patients.

INTRODUCTION

AR-V7 is a mRNA splice variant of the androgen receptor (AR) translating to a constitutively active receptor that lacks the ligand binding domain.[1] Several studies investigating the correlation between the presence of *AR-V7* in circulating tumor cells (CTCs) of metastatic castration-resistant prostate cancer (mCRPC) patients and treatment response found that *AR-V7* positive patients had decreased sensitivity to AR targeted therapy (ART), like enzalutamide and abiraterone, but not to taxanes.[2,3] Therefore, *AR-V7* expression is being explored as a potential predictive biomarker for ART. However, *AR-V7* positivity has also been related to unfavorable baseline characteristics, therefore it may merely reflect a higher tumor burden.[4-6] This could be of influence on how to interpreted the presence of AR-V7 in circulating tumor cells, as it suggests a prognostic value of *AR-V7*. To improve our understanding of the clinical value of *AR-V7*, we performed a post-hoc analysis evaluating the relationship between *AR-V7* status in CTCs, CTC count and overall survival (OS).

METHODS

Patients and trials

Patients with mCRPC post-docetaxel included in one of three different trials performed in The Netherlands between December 2011 and January 2019: The PRELUDE trial (MEC-2015-353), the CABARESC trial[7] or the CABA-V7 trial (EudraCT number 2016-002993-11). The PRELUDE and CABA-V7 trial communicated the *AR-V7* test results to the treating physician before new treatment was started. All patients in the CABARESC trial received cabazitaxel. The design of each study is described in the **Supplementary Methods/Supplementary Table 1**. These trials were performed according to the Declaration of Helsinki and all participants gave written informed consent.

Outcomes

The primary objective was to investigate associations between *AR-V7* status in CTCs and CTC count in all mCRPC patients and in patients with an unfavorable prognosis based on CTC count ≥5. Additionally, OS was compared between *AR-V7* positive and negative patients in all mCRPC patients and the patients with ≥5 CTCs to investigate its potential prognostic value. OS was calculated from the date when blood samples were withdrawn till date of death from any cause or end of the study, whichever came first.

Sample processing and quality control

Two blood samples (one CellSave tube for CTC count and one EDTA tube for mRNA profiling) were withdrawn from mCRPC patients and processed within 96 hours (CTC count) or 24 hours (for RNA profile) at the laboratory of Translational Cancer Genomics and Proteomics of the Department of Medical Oncology, Erasmus MC Cancer Institute. CTC enrichment occurred from 7,5 mL of peripheral blood using the CellSearch System. After CTC enrichment from the EDTA tube, cells were lysed and RNA isolation followed by RT-qPCR. Processing of the samples occurred via standard operation procedures in a pre-PCR environment and was approved by an independent external audit. The steps of our quality control are described by Sieuwerts et al.[8] In short, the first checkpoint was blood collection of at least 7,5 mL. To ensure we could compare the experiments of different trials and different processing sessions with each other, a calibrator of cultured VCaP RNA with known expression of our gene expression markers had to be included in all RT-qPCR sessions for evaluation of these samples. If samples were analyzed without a VCaP calibrator, these samples were excluded from analysis. By the usage of the average Cq value of three references genes (GUSB, HMBS, HPRT1), the quantity and quality of RNA was checked. Only samples with an average reference gene Cq value <26.5 were considered to be of sufficient quality and quantity for a meaningful *AR-V7* analysis. The Cq values measured for *AR-V7* were normalized by the mean Cq value of two epithelial genes (EPCAM, KRT19) to correct for the number of epithelial CTCs present in the sample. Only samples with Cq value <26.0 for the average of the epithelial genes were considered to have enough epithelial load for a meaningful *AR-V7* analysis. If a sample had sufficient Cq values for the reference and epithelial targets, but did not produce a quantitative PCR signal for *AR-V7* within 8.5 cycles after the average Cq of the epithelial markers, the sample was considered *AR-V7* negative. The rationale for this cut-off has been explained in detail before.[8]

Statistical analysis

AR-V7 status was tested for associations with CTC count, using a Mann-Whitney U test. Log-ranktesting was applied to perform OS analysis and Kaplan-Meier curves were drafted to visualize OS differences. Prognostic factors for survival (OS) were identified by univariate and multivariate Cox regression analysis. All factors with a P-value <0.05 detected in univariate analyses were included in multivariate analyses together with *AR-V7* as variable of main interest. A backward selection method was used for the multivariate model where a threshold of P <0.05 was applied. All tests were two-sided and a p-value of <0.05 was considered statistically significant. Statistical analyses were performed using STATA, SPSS version 24 or Graphpad Prism 5.

RESULTS

In this post-hoc analysis 212 patient samples of three trials were included; the CABARESC trial (n=109), the PRELUDE trial (n=31) and the CABA-v7 trial (n=72). Eighty-five samples had insufficient epithelial signal to reliably give a result on *AR-V7* status. A total of 127 samples were evaluable and included in the current analysis. For the survival analysis, data of 94 patients were available (**Figure 1**). Baseline characteristics, prior and subsequent life prolonging therapies are shown in **Table 1**.

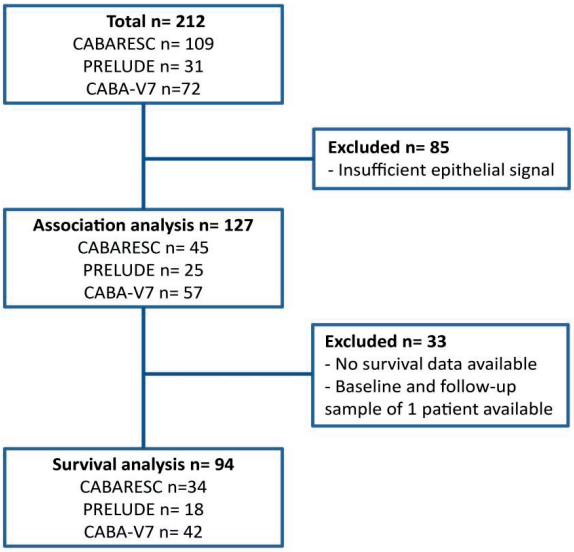


Figure 1. Flowchart

AR-V7 status and CTC count

Of the 127 evaluable samples, presence of *AR-V7* was detected in 45 patients (35%). Despite the correction for epithelial signal a significantly higher median CTC count of 25 CTCs/7.5 mL blood was detected in *AR-V7* positive patients compared to a median CTC count of 10 CTCs/7.5 mL blood in *AR-V7* negative patients (p=0.017, **Figure 2a**). In patients with ≥5 CTCs no significant difference in median CTC count could be detected between *AR-V7* positive (n=36) and negative (n=50) patients (34 and 33, p=0.24, **Figure 2b**). In the 0-4 CTC group, no association between *AR-V7* positivity and CTC count was detected (**Supplementary Figure 1**).

Table 1. Baseline characteristics

	Total (n=94) (median, IQR)	*AR-V7* pos (n=34) (median, IQR)	*AR-V7* neg (n=60) (median, IQR)
Age (years)	69 (65 – 75)	68 (64 – 73)	73 (66 – 76)
WHO PS, n (%)			
- 0	34 (36%)	13 (38%)	21 (35%)
- 1	43 (46%)	15 (44%)	28 (47%)
- 2	1 (1%)	0 (0%)	1 (2%)
- Missing	16 (17%)	6 (18%)	10 (17%)
Prior therapies, n(%)			
- Docetaxel	94 (100%)	34 (100%)	60 (100%)
- Enzalutamide	27 (29%)	14 (41%)	13 (22%)
- Abiraterone	13 (14%)	5 (15%)	8 (13%)
Hb, mmol/L	7.7 (6.9 – 8.2)	7.3 (6.8 – 8.2)	7.8 (7.1 – 8.2)
- Missing	n=15 (16%)	n=4 (12%)	n=12 (20%)
PSA, µg/L	186 (67 – 356)	171 (68 – 358)	198 (61- 373)
- Missing	n=39 (41%)	n=9 (26%)	n=30 (50%)
Subsequent Treatment after *AR-V7* determination			
- Cabazitaxel	73 (78%)	32 (94%)	41 (68%)
- Enzalutamide	11 (12%)	2 (6%)	9 (15%)
- Abiraterone	4 (4%)		4 (7%)
- Apalutamide	1 (1%)		1 (1.5%)
- Radium-223	1 (1%)		1 (1.5%)
- None	4 (4%)		4 (7%)

Abbreviations: pos = positive, neg = negative, IQR = inter quartile range, WHO PS = World Health Organization Performance Score, Hb = Hemoglobin, PSA = prostate specific antigen

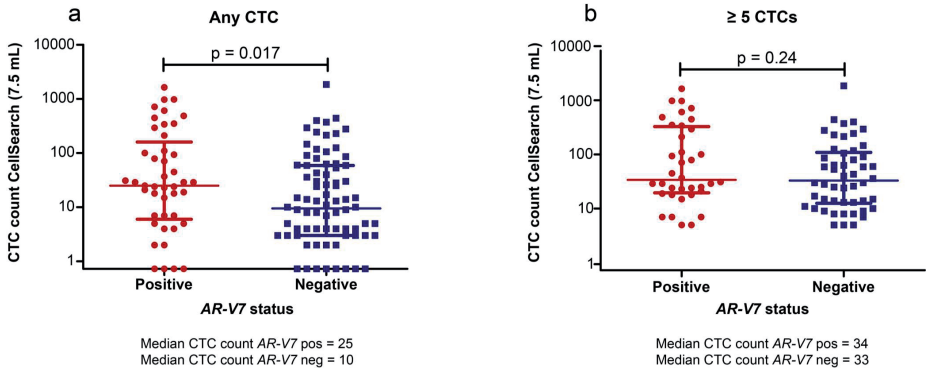


Figure 2. Relation between *AR-V7* status and CTC count.

All samples are quality controlled checked. Blue dots are AR-V7 negative and red dots are AR-V7 positive. ***a*** *samples with all CTC counts are included, p=0.017.* ***b*** *Samples with at least 5 CTCs are included, p=0.24*

AR-V7 status and OS

After adjusting for CTC count and clinical prognostic factors no difference in survival between *AR-V7* positive and negative patients was observed (**Figure 3a**/**Supplementary Table 2**). As expected, patients with ≥5 CTCs had a significantly worse prognosis with a median survival of 6.9 months (IQR 4.3-13.8) compared to patients with <5 CTCs (median 22.3 months, IQR 19.2-34.6, **Figure 3b**). In this subgroup also no difference in survival was observed between *AR-V7* positive and *AR-V7* negative patients (HR of 1.1, 95%CI 0.6-1.9, p=0.78, **Figure 3c**).

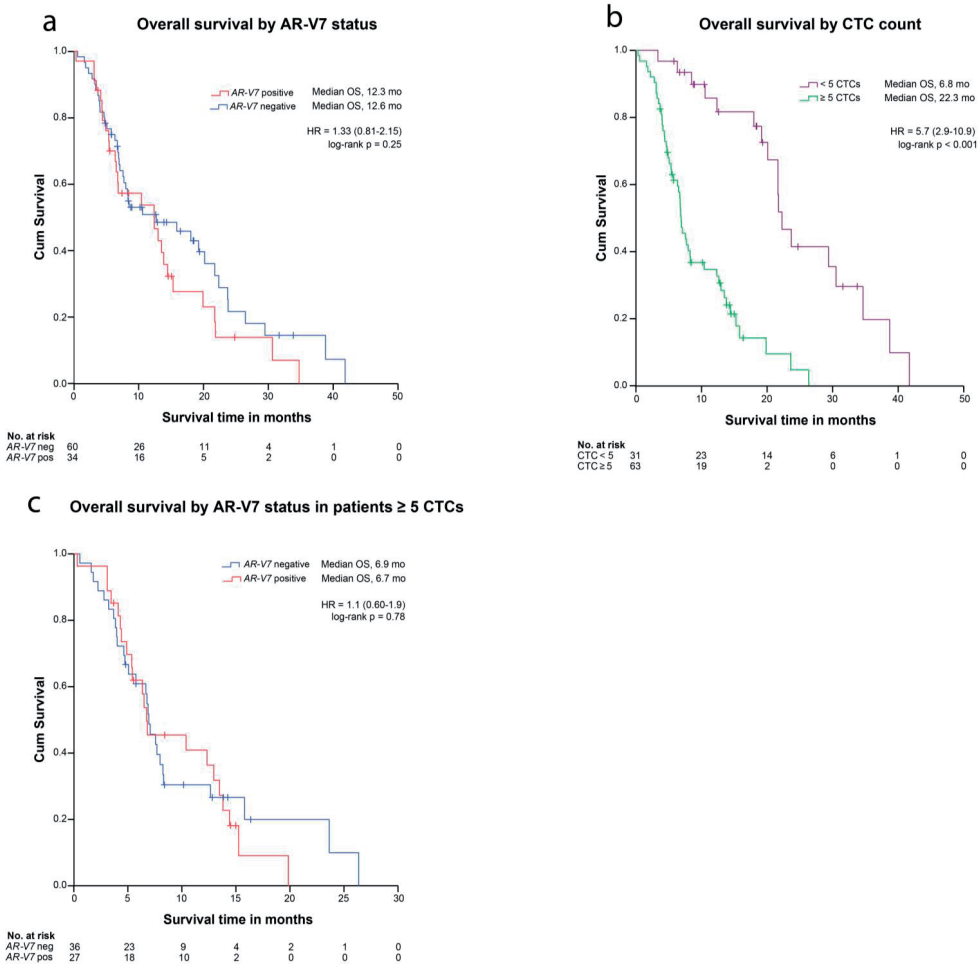


Figure 3. Survival curves
a. *All patients stratified by AR-V7 status.* ***b.*** *All patients stratified by CTC count.* ***c.*** *Patients with ≥ 5 CTCs stratified by AR-V7 status.*

DISCUSSION

In this post-hoc analysis of mCRPC patients enrolled in 3 clinical trials post-docetaxel, we evaluated associations between the *AR-V7* expression in CTCs, CTC count and OS. A significantly higher median CTC count was observed in *AR-V7* positive patients compared to *AR-V7* negative patients. However, this association was not found in the unfavorable prognosis patient group with ≥5 CTCs. Moreover, *AR-V7* positivity in CTCs was not associated with a worse prognosis in mCRPC men mostly treated with cabazitaxel.

We observed an association between *AR-V7* status and median CTC count in all mCRPC patients, which was probably driven by a lack of *AR-V7* positivity in patients with 4 or less CTCs. Lower incidence of *AR-V7* positivity in patients with a low CTC count could potentially be explained by intra-patient heterogeneity of *AR-V7* expression in CTCs and the lack of specificity of the test in samples with a low CTC count (or no CTCs at all).[9,10] Other platforms for *AR-V7* determination such as nuclear *AR-V7* protein expression in tissue/CTCs, mRNA expression in extracellular vesicles like exosomes/peripheral blood mononuclear cells/ whole blood face the same issue of low specificity to a lesser or greater extent.[10-13] The availability of all these different testing methods indicates that *AR-V7* testing is far from standardized. Consensus on the analytical method of testing is needed before clinical implementation is possible.

AR-V7 may also be a biomarker that identifies a subgroup of patients with advanced disease and thus may be underrepresented in the good prognosis group. Therefore, *AR-V7* determination in the low (<5) CTC population may not be reliable nor relevant for clinical decision making. The ADNA test, often used to determine *AR-V7* expression in CTCs, has no ability to count the CTCs. The CTC count could potentially be relevant to discriminate in which patients *AR-V7* determination is clinically relevant, therefore it is suggested to incorporated the CTC count in the *AR-V7* testing. The recent study of Sharp et al. did incorporate the CTC count by Cellsearch and combined this with the *AR-V7* expression.[14] We showed similar results (**Figure 2** of both studies), as a significant difference in CTC count between *AR-V7* positive and negative patients was found in both studies. In addition, similar to our results most patients with a low to zero CTCs are tested *AR-V7* negative. It remains to be determined if these patients are truly *AR-V7* negative, which seems debatable as some of the CTC based *AR-V7* negative patients in the study of Sharp et al. have *AR-V7* protein expression in the tumor biopsies and these patients had limited response to ART. In additional to their work, we investigated the association in the poor prognosis group of mCRPC patients with ≥5 CTCs.

We confirmed the prognostic value of CTC count at the cut-off of 5 CTCs in this patient cohort.[15] The sub-group with ≥5 CTCs had a median OS of 6.9 months, in which the presence of *AR-V7* did not further impact survival. The short survival time of patients with ≥5 CTCs emphasizes the need to carefully select treatment as the 'window of opportunity' to administer an effective treatment is relatively small. Our CTC based AR-V7 characterization test has a turnaround time of less than 2 weeks enabling early treatment advice in this specific sub-group.[8] Therefore, we propose that the value of *AR-V7* should be further assessed in patients with ≥5 CTCs, as they have the highest need for rapidly available accurate predictive biomarkers.

The predictive value of *AR-V7* could not be further addressed in this study since no control group was available. However, *AR-V7* positivity in CTCs of mCRPC patients, mostly treated with cabazitaxel was not associated with worse outcomes. Recently, the negative prognostic value of *AR-V7* has been validated prospectively in abiraterone and enzalutamide treated patients, indicating that AR-V7 positivity was associated with worse outcomes with these novel ART.[16] A limitation of the study by Armstrong et al. was the heterogeneity of the patient cohort and the treatment selection. In our study most patients started cabazitaxel, sometimes based on the *AR-V7* result. This may confound the potential prognostic value of *AR-V7*, as it may correct for an otherwise poor prognosis and potentially influence our results. However, the majority - of *AR-V7* negative patients - also received cabazitaxel.

CONCLUSION

In conclusion, detection of *AR-V7* in CTCs has no additional prognostic value in mCRPC patients, who were mostly treated with cabazitaxel and prospective validation is needed to investigate if *AR-V7* could fulfill the criteria for a useful predictive biomarker.

REFERENCES

1 Antonarakis ES, Armstrong AJ, Dehm SM, Luo J. Androgen receptor variant-driven prostate cancer: clinical implications and therapeutic targeting. Prostate Cancer Prostatic Dis. 2016;19:231-41.

2 Antonarakis ES, Lu C, Wang H, Luber B, Nakazawa M, Roeser JC, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med. 2014;371:1028-38.

3 Onstenk W, Sieuwerts AM, Kraan J, Van M, Nieuweboer AJ, Mathijssen RH, et al. Efficacy of Cabazitaxel in Castration-resistant Prostate Cancer Is Independent of the Presence of AR-V7 in Circulating Tumor Cells. Eur Urol. 2015;68:939-45.

4 Tagawa ST, Antonarakis ES, Gjyrezi A, Galletti G, Kim S, Worroll D, et al. Expression of AR-V7 and ARv567es in circulating tumor cells correlates with outcomes to taxane therapy in men with metastatic prostate cancer treated in TAXYNERGY. Clin Cancer Res. 2018.

5 Plymate SR, Sharp A, de Bono JS. Nuclear Circulating Tumor Cell Androgen Receptor Variant 7 in Castration-Resistant Prostate Cancer: The Devil Is in the Detail. JAMA Oncol. 2018;4:1187-8.

6 Scher HI, Graf RP, Schreiber NA, Jayaram A, Winquist E, McLaughlin B, et al. Assessment of the Validity of Nuclear-Localized Androgen Receptor Splice Variant 7 in Circulating Tumor Cells as a Predictive Biomarker for Castration-Resistant Prostate Cancer. JAMA Oncol. 2018;4:1179-86.

7 Nieuweboer AJ, de Graan AM, Hamberg P, Bins S, van Soest RJ, van Alphen RJ, et al. Effects of Budesonide on Cabazitaxel Pharmacokinetics and Cabazitaxel-Induced Diarrhea: A Randomized, Open-Label Multicenter Phase II Study. Clin Cancer Res. 2017;23:1679-83.

8 Sieuwerts AM, Mostert B, van der Vlugt-Daane M, Kraan J, Beaufort CM, Van M, et al. An In-Depth Evaluation of the Validity and Logistics Surrounding the Testing of AR-V7 mRNA Expression in Circulating Tumor Cells. J Mol Diagn. 2018;20:316-25.

9 Crespo M, van Dalum G, Ferraldeschi R, Zafeiriou Z, Sideris S, Lorente D, et al. Androgen receptor expression in circulating tumour cells from castration-resistant prostate cancer patients treated with novel endocrine agents. Br J Cancer. 2015;112:1166-74.

10 Zhu Y, Sharp A, Anderson CM, Silberstein JL, Taylor M, Lu C, et al. Novel Junction-specific and Quantifiable In Situ Detection of AR-V7 and its Clinical Correlates in Metastatic Castration-resistant Prostate Cancer. Eur Urol. 2018;73:727-35.

11 Sharp A, Coleman I, Yuan W, Sprenger C, Dolling D, Rodrigues DN, et al. Androgen receptor splice variant-7 expression emerges with castration resistance in prostate cancer. J Clin Invest. 2019;129: 192-208.

12 Qu F, Xie W, Nakabayashi M, Zhang H, Jeong SH, Wang X, et al. Association of AR-V7 and Prostate-Specific Antigen RNA Levels in Blood with Efficacy of Abiraterone Acetate and Enzalutamide Treatment in Men with Prostate Cancer. Clin Cancer Res. 2017;23:726-34.

13 Todenhofer T, Azad A, Stewart C, Gao J, Eigl BJ, Gleave ME, et al. AR-V7 Transcripts in Whole Blood RNA of Patients with Metastatic Castration Resistant Prostate Cancer Correlate with Response to Abiraterone Acetate. J Urol. 2017;197:135-42.

14 Sharp A, Welti JC, Lambros MBK, Dolling D, Rodrigues DN, Pope L, et al. Clinical Utility of Circulating Tumour Cell Androgen Receptor Splice Variant-7 Status in Metastatic Castration-resistant Prostate Cancer. Eur Urol. 2019.

15 Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med. 2004;351:781-91.

16 Armstrong AJ, Halabi S, Luo J, Nanus DM, Giannakakou P, Szmulewitz RZ, et al. Prospective Multicenter Validation of Androgen Receptor Splice Variant 7 and Hormone Therapy Resistance in High-Risk Castration-Resistant Prostate Cancer: The PROPHECY Study. J Clin Oncol. 2019:JCO1801731.

SUPPLEMENTARY

Supplementary methods – *Design of the studies*

The CABARESC study was a multicenter, pharmacodynamic study investigating the effects of budesonide on cabazitaxel-induced diarrhea in which, as secondary objective, baseline CTC samples were collected.[7] In the primary study clinical details and survival data of the patients had already been collected. All patients received cabazitaxel treatment.

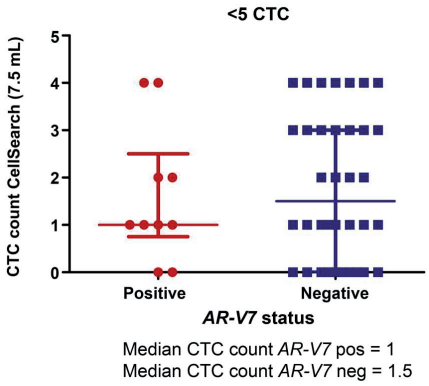
The PRELUDE study was a feasibility study across six different hospitals in the Netherlands to determine the logistics of reporting the results of *AR-V7* determination in CTCs to different hospitals around the nation. Since this feasibility study did not include clinical or survival data of the patients, these had to be retrospectively collected. The protocol of this post-hoc analysis was reviewed by the Ethical Committee of the Erasmus MC Cancer Institute and was assessed as no subject to Medical Research. After the positive report by the Ethical Committee, clinical and survival data were collected with consent of the patient if possible, e.g. if the patient was alive. The test results had been communicated to the treating physician before treatment start, therefore patients in the PRELUDE trial had been treated with hormonal therapy or cabazitaxel therapy.

The CABA-V7 study is still actively recruiting and prospectively investigates PSA response rates in *AR-V7* positive patients treated with cabazitaxel. In this trial all clinical data needed for this post-hoc analysis had already been collected, including the data of *AR-V7* negative patients. All *AR-V7* negative patients were allowed treatment with hormonal therapies as well as cabazitaxel. The CABA-V7 trial communicated the *AR-V7* test results to the treating physician before new treatment was started.

All patients had given written informed consent before participation to one of the studies, which included a paraphrase referring to the potential use of clinical and collected data for future research. Although all trials had slightly different primary objectives, they all included baseline CTC count and AR-V7 quantification by RT-qPCR in CellSearch enriched CTC fractions. The CABARESC and CABA-v7 trial also included CTC response with *AR-V7* characterization at cabazitaxel cycle 3 or 4, which we took into account for the association between *AR-V7* and CTC count.

Supplementary Table 1. Study details

Study	Recruitment period	Survival data collection	Patients eligible for survival analysis (n)	Median survival (months, 95%CI)
CABARESC	Dec 2011 – Oct 2016	Oct 2016	34	12.3 (7.5-15.8)
PRELUDE	Sep 2015 – Mar 2016	Jan 2019	18	15.8 (7.6-21.7)
CABA-V7	Feb 2017 – Jan 2019	Jan 2019	42	8.0 (4.0-19.9)



Supplementary Figure 1. Association between *AR-V7* positivity and CTC count in the 0-4 CTC group.

Supplementary Table 2. Univariate and Multivariate analyses of baseline prognostic factors

	Univariate analysis			Multivariate analysis		
Variable	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
AR-V7	1.33	0.81-2.18	0.253			
CTC ≥ 5	5.66	2.92-10.95	< 0.001	7.36	2.71-20.05	< 0.001
WHO PS						
0 vs 1/2	2.27	1.26-4.07	0.006	2.66	1.39-5.09	0.003
Hb (mmol/L)	0.67	0.49-0.93	0.016			
AP (U/L)	1.002	1.001-1.003	< 0.001	1.001	1.000-1.002	0.013
LDH (U/L)	1.001	1.001-1.002	0.001	1.002	1.001-1.002	0.001

Abbreviations: HR = hazard ratio, CI = confidence interval, AR-V7 = androgen-receptor splice variant 7, CTC = circulating tumor cells, WHO = world health organization performance status, Hb = hemoglobin, AP = alkaline phosphatase, LDH = lactate dehydrogenase

Chapter 9

Novel Treatment Options in the Management of Metastatic Castration-naïve Prostate Cancer; Which Treatment Modality to choose?

Bodine P.S. Belderbos, Ronald de Wit, Martijn P.J. Lolkema, Ron H.J. Mathijssen and Robert J. van Soest

Ann Oncol. 2019 Jul 24; Epub ahead of print

ABSTRACT

Androgen-deprivation therapy (ADT) has been the mainstay of treatment for metastatic prostate cancer since the first report of its hormonal dependence in the 1940s. Since 2015, the addition of docetaxel and the addition of abiraterone to ADT have demonstrated to confer significant overall survival benefit in men with metastatic castration-naïve prostate cancer (mCNPC). The shift of these treatment options for metastatic prostate cancer from the castration-resistant setting to the castration-naïve setting has led to new challenges in the management of this disease. It remains to be determined which patient may benefit most from either early concomitant docetaxel, or from abiraterone, since biomarkers for early therapy response and risk stratification are currently lacking. Therefore, the ability to generate personalized medicine is hampered. Furthermore, the earlier detection of metastatic prostate cancer by using new imaging modalities makes the application of clinical trial results in daily practice increasingly challenging. Recently, both local radiotherapy to the primary tumor combined with ADT, and abiraterone combined with ADT showed a survival benefit in low-volume disease patients. The latest data also demonstrated a survival benefit with the addition of apalutamide or enzalutamide to ADT. The extent of metastatic disease may become one of the most important factors to determine treatment choice. In this review article we summarize trial data to provide guidance for treatment selection in metastatic castration-naïve prostate cancer.

INTRODUCTION

In the past 15 years, several new treatment options for metastatic castration-resistant prostate cancer (mCRPC) have been approved including docetaxel, cabazitaxel, abiraterone, enzalutamide and radium-223.[1-8] Recently, major shifts in the treatment of metastatic prostate cancer have occurred, since significant survival benefit has been obtained with docetaxel and abiraterone in addition to the standard of ADT in metastatic castration-naïve prostate cancer (mCNPC).[9-12] The challenge is now to identify the mCNPC patients who will benefit most from combination strategies (ADT+docetaxel or ADT+abiraterone/prednisone). The trials were conducted with slightly different patient populations. Unfortunately, no biomarkers are available, leaving the treatment choice based on clinical symptoms, patients' preference, experience, toxicity, and costs. However, stratification of patients by disease burden seems to be a possible factor for treatment selection, since the recently published post-hoc analysis of the STAMPEDE abiraterone trial showed a survival benefit in low-volume (LV) mCNPC patients, which has not been significantly established by the addition of docetaxel.[13] The latest insights include the benefit of local radiotherapy to the prostate in LV disease patients, and the OS benefit obtained by the addition of apalutamide or enzalutamide to ADT in mCNPC patients.[14-16] In this review article we aim to provide guidance for treatment selection of the approved drugs in mCNPC patients.

METHODS

The methods are described in online-only **Supplementary Data.**

ADT + DOCETAXEL

The addition of docetaxel to ADT has been investigated in three large phase III trials; the GETUG-AFU15, the CHAARTED and the STAMPEDE trial (docetaxel comparison).[9,10,17] All trials compared overall survival (OS) in mCNPC patients, who were treated with ADT alone versus the combination of ADT plus docetaxel. The study design, included patients and primary outcomes are shown in **Tables 1, 2** and **3**. The first trial, GETUG-AFU15 (N=385), showed significant longer biochemical and clinical progression-free survival in the combination group, but failed to show an OS benefit (OS 58.9 months (95%CI 50.8-69.1) versus 54.2 months (95%CI 42.2-not reached,P=0.95).[17] Based on these results, the authors did not recommend the addition of docetaxel to standard ADT in the castration-naïve setting. However, the sample size of the GETUG-AFU15 was relatively small and the larger CHAARTED and STAMPEDE trials shed a new light on this conclusion. The mature analysis of

Table 1. Design of included randomized controlled trials and primary outcomes of agents included in the guidelines

	GETUG-AFU15	CHAARTED	STAMPEDE Docetaxel*	LATITUDE	STAMPEDE Abiraterone*	HORRAD	STAMPEDE Radiotherapy
Treatment arm (*N*)	ADT+Doce (192)	ADT+Doce (397)	ADT+Doce (362)	ADT+Abi (597)	ADT+Abi (500)	ADT+RTx (216)	ADT+RTx (1032)
Treatment	Docetaxel 9 cycles 75 mg/m^2 No prednisone	Docetaxel 6 cycles 75 mg/m^2 Daily prednisone not required	Docetaxel 6 cycles 75mg/m^2 Prednisone 10 mg/day	Abiraterone 1000 mg/day Prednisone 5 mg/day	Abiraterone 1000 mg/day Prednisolone 5 mg/day	Radiotherapy 70 Gy in 35 fractions of 2 Gy, OR 57.76 Gy in 19 fractions of 3.04 Gy	Radiotherapy 36 Gy in 6 fractions of 6 Gy/week OR 55 Gy in 20 fractions of 2,75 Gy/day +/- Docetaxel 75 mg/m^2
Control arm (*N*)	ADT (193)	ADT (393)	ADT (724)	ADT (602)	ADT (502)	ADT (216)	ADT (1029)
Randomization	1:1	1:1	2:1	1:1	1:1	1:1	1:1
Primary Endpoint	OS	OS	OS	OS, rPFS	OS	OS	OS, FFS
Secondary Endpoints	cPFS, bPFS	Time to CRPC Time to clinical progression PSA <0.2ng/mL at 6 months PSA <0.2ng/mL at 12 months	FFS Time to any treatment for progression Time to any life-prolonging therapy	Time to next SRE Time to PSA progression Time to next therapy Time to next chemotherapy Time to pain progression	FFS PFS Prostate cancer-specific survival Symptomatic SRE Toxicity Quality of Life	Time to PSA progression	PFS Metastatic PFS
Accrual	October 2004 - December 2008	July 2006 - November 2012	October 2005 - March 2013	February 2013 - December 2014	November 2011 - January 2014	November 2004 - September 2014	January 2013 - September 2016
Treatment start	Docetaxel start no longer than 2 mo after ADT	Docetaxel start no longer than 4 mo after ADT	Enrollment within 12 weeks of ADT start	Enrollment within 12 weeks of ADT start	Enrollment within 12 weeks of ADT start	RTx start no longer than 3 mo after ADT	RTx start as soon as practicable after randomization, and within 3-4 weeks after last docetaxel cycle.
Median FU (months)	84	28.9	43	30	40	47	37
Primary Outcome (HR, 95%CI)	0.90 (0.70-1.2)	0. 72 (0. 59-0.89)	0.76 (0.62-0.92)	0.66 (0.56-0.78) 0.47 (0.39-0.55)	0. 61 (0. 49-0.75)	0.90 (0.70-1.14)	0.92 (0.80-1.06) 0.76 (0.68-0.84)

** Data shown correspond to subgroup of metastatic patients in the STAMPEDE trials.*

Table 2. Overview of selected patients per randomized controlled trial of the agents included in the guidelines

	GETUG-AFU15	CHAARTED	STAMPEDE Docetaxel	LATITUDE	STAMPEDE Abiraterone	HORRAD	STAMPEDE Radiotherapy
Included patients	385	790	1776	1199	1917	432	2061
Metastatic disease	385 (100%)	790 (100%)	1086 (61%)	1199 (100%)	1002 (52%)	432 (100%)	2061 (100%)
Node positive	-	-	257 (15%)	-	369 (19%)	-	-
High risk locally advanced	-	-	433 (24%)	-	546 (28%)	-	-
Prior local therapy	108 (28%)	214 (27%)	95 (5%)	-	98 (5%)	0 (0%)	0 (0%)
High-volume/ high risk	183 (48%)[a,]b	512 (65%)	NR	1199 (100%)	499 (55%)b,d/ 473 (52%)c,d	272 (63%)e	1120 (54%)b
Gleason 8-10	216 (56%)	484 (61%)	1246 (70%)	1170 (98%)	1436 (75%)	286 (66%)	1630 (79%)
WHO PS 0	357 (93%)[a]	549 (69%)	NR	NR	1489 (78%)	363 (84%)	1466 (71%)

Abbreviations: NR = not reported, WHO PS = world health organization performance score. Missing data of each study are not presented nor incorporated in the table.

[a] *Based on post-hoc analysis by Gravis et al (23).*

[b] *Based on CHAARTED high volume criteria, see Table 3.*

[c] *Based on LATITUDE high risk criteria, see Table 3*

[d] *Based on post-hoc analysis of only metastatic patients in STAMPEDE Abiraterone trial, diagnosed with conventional imaging and available Gleason score, n=901. Analysis was performed by Hoyle et al (16).*

[e] *High volume was defined as ≥ 5 metastases, see Table 3*

the CHAARTED trial (N=790) showed an OS benefit of 10.4 months for patients treated with ADT plus docetaxel compared to ADT alone (57.6 months versus 47.2 months, HR=0.72, 95%CI 0.59-0.89,P=0.0018).[10,18] In addition, this trial conducted a preplanned subgroup analysis based on disease burden. The majority of patients (65%) had high-volume (HV) disease, which involved presence of visceral metastases or ≥4 bone metastases of which at least 1 was beyond pelvis and vertebrae (**Tables 2** and **3**). In the HV disease group (N=513) an even larger survival benefit of almost 17 months was observed in the patients treated with ADT plus docetaxel (51.2 months versus 34.4 months, HR=0.63, 95%CI 0.50-0.79,P<0.001). A survival benefit was absent in the LV disease group (N=277) (HR=1.04, 95%CI 0.70-1.55,P=0.86).[18] Thereby, the mature results of CHAARTED have demonstrated the survival benefit of docetaxel was only obtained in patients with HV metastatic disease. A post-hoc analysis of the CHAARTED and GETUG-AFU15 trials, confirmed the results of the mature analysis of CHAARTED.[19] The pooled risk of death was 32% lower with the combination of ADT plus docetaxel in the HV disease group (HR=0.68, 95%CI 0.56-0.82,P<0.001).

The STAMPEDE trial has a unique multi-arm, multi-stage design to evaluate survival benefit in patients commencing ADT for prostate cancer including patients with both metastatic and high-risk locally advanced disease with the addition of several treatments [e.g. docetaxel, bisphosphonates, zoledronic acid, abiraterone, prostate radiotherapy] to ADT.[9] For this review we only included the study details of metastatic patients (**Tables 1** and **2**). The STAMPEDE docetaxel comparison included 1086 patients with mCNPC, and showed a survival benefit of 15 months in favor of the combination group (60 months versus 45 months, HR=0.76, 95%CI 0.62-0.92,P=0.005). The investigators did not stratify for LV or HV disease in the docetaxel comparison of the STAMPEDE trial.

Both STAMPEDE and CHAARTED studies showed a longer survival with the addition of docetaxel to ADT compared to ADT alone in the total patient cohort including both HV and LV disease. Only in CHAARTED, stratification based on disease volume was conducted suggesting the survival benefit is mainly driven by metastatic HV disease patients. Following the results of these trials, the addition of chemotherapy became the new standard of care in HV mCNPC patients, while the benefit in LV disease patients had not firmly been established.

ADT + CYP17 INHIBITION (ABIRATERONE)

The addition of abiraterone and prednisone to ADT in CNPC patients has been investigated in two large trials: LATITUDE and STAMPEDE abiraterone comparison.[11,12] The design and

Table 3. Definition of metastatic burden

	CHAARTED	LATITUDE	HORRAD
High-volume/ high-risk	presence of visceral metastases or ≥ 4 bone metastases of which at least 1 was beyond pelvis and vertebrae	Metastatic disease with at least two of the following risk factors; Gleason score ≥ 8, and/or ≥3 bone lesions, and/or presence of visceral metastases	≥ 5 metastases
Low-volume/ low-risk	No visceral metastases and no bone metastases outside the pelvis and vertebrae or less than 4 bone metastases	All patients with less than two of the above mentioned risk factors	< 5 metastases

patients included in these trials are shown in **Tables 1** to **3**. Briefly, LATITUDE only included patients with metastatic HV disease (involving two or more of the following risk factors; Gleason≥8, bone lesions≥3, visceral metastases), whereas STAMPEDE also included LV disease patients and patients with locally advanced disease. The final analysis of the LATITUDE trial (N=1199) showed a 34% reduction in the risk of death in the abiraterone group (HR=0.66, 95%CI 0.56-0.78,P<0.001).[20] Although the benefit of the addition of abiraterone to ADT in the trial was evident, the lack of cross-over to abiraterone in the placebo group at the time of disease progression to mCRPC was a serious confounder of this study. Due to the double-blinding nature of the study, the treating physician was unaware of the received treatment. As a result, only 11% of the placebo treated patients received abiraterone at the time of mCRPC. Other subsequent life-prolonging therapies at disease progression were docetaxel (40%), enzalutamide (16%), radium-223 (6%) and cabazitaxel (6%). Interestingly, 21% of the placebo treated patients did not receive any subsequent life-prolonging therapy. Consequently, one could argue that the LATITUDE trial tested early abiraterone versus no abiraterone, rather than early abiraterone versus abiraterone at the time of mCRPC.[4,21] Therefore, the design of the trial evokes concern, since the use of abiraterone in the mCRPC setting has demonstrated survival benefit and should have been available as a standard treatment option.[4,21] However, based on the results of the LATITUDE trial, EMA and FDA have licensed abiraterone for HV mCNPC.

In STAMPEDE, abiraterone was added in the multi-arm trial design of the study to investigate its addition to ADT in CNPC (N=1002).[12] The median OS was not reached in the combination group, but there was survival benefit in the combination group with 150 deaths compared to 218 deaths in the ADT alone group (HR=0.61, 95%CI 0.49-0.75). Toxicity occurred more frequent in the abiraterone group (grade 3-5, 47% versus 33%, **Table 6**), but was similar to toxicity rates observed in the COU-AA-301 and COU-AA-302 studies.[4,5,21] In this study, 6% of patients did not receive any subsequent life-prolonging treatment at the time of progression, while others received docetaxel (37%), enzalutamide (26%), abiraterone (22%), cabazitaxel (5%) or radium-223 (4%).

The STAMPEDE investigators recently conducted a post-hoc analysis of all metastatic patients enrolled into the abiraterone comparison, classifying patients by metastatic extent according LATITUDE and CHAARTED criteria (**Table 3**).[13] The post-hoc analysis demonstrated that, according to the LATITUDE criteria, 428 patients (48%) were identified as having LV disease, of whom 220 received ADT and 208 received ADT plus abiraterone. Patients treated with ADT plus abiraterone in this LV disease group showed a 34% reduced risk of death compared to the ADT alone group (HR=0.66, 95%CI 0.44-0.98, P=0.041), resulting in a difference of 4% in 3-year OS rates. However, the median OS in this LV patient group had not yet been reached during this follow-up period. More events are needed to show the real magnitude of the survival benefit. At the time of this post-hoc analysis the median OS was reached in the HV disease group, confirming the benefit in HV patients (HR=0.54, 95%CI 0.41-0.70,P<0.001).

ADT + RADIOTHERAPY TO THE PRIMARY TUMOR

In 2018 two large trials, HORRAD and STAMPEDE radiotherapy comparison, reported a potential survival benefit by prostate radiotherapy (RT) in addition to ADT in patients with newly diagnosed LV metastatic disease.[14,22] The STAMPEDE investigators investigated the effect of prostate radiotherapy by adding an extra comparison in their multi-arm, multi-stage trial design, enrolling newly diagnosed metastatic prostate cancer patients (N=2061,**Tables 1** and **2**). Patients were randomized in a 1:1 ratio to receive either ADT+/-docetaxel (during the trial the addition of docetaxel became the new standard of care) or ADT+/-docetaxel plus prostate radiotherapy (RT). Radiotherapy was commenced as soon as possible after randomization, or within 3-4 weeks after the last docetaxel cycle. The results showed no significant OS benefit from the addition of local RT in the complete patient group (HR=0.92, 95%CI 0.80-1.06,P=0.27). However, there was a robust improvement in FFS (HR=0.76, 95%CI 0.68-0.84,P<0.001). A directionally hypothesized and pre-specified subgroup analysis based on disease burden was conducted prior to study analysis, but not prior to accrual. The results revealed that LV patients defined by CHAARTED, obtained OS benefit when local RT was added to ADT+/-docetaxel (HR=0.68, 95%CI 0.52-0.90,P=0.009). This study was the first to show a survival benefit by treatment of the primary tumor in metastatic prostate cancer and is truly practice changing.

The HORRAD trial included 432 patients with newly diagnosed metastatic disease confirmed by bone-scintigraphy and randomized patients (1:1) to receive either ADT plus prostate RT (N=216) or ADT alone (N=216).[22] The study showed no OS benefit of the addition of prostate radiotherapy. Although not statistically significant, a trend towards survival benefit was seen in LV disease patients with a HR of 0.68 (95%CI 0.42-1.10),

concordant with the HR of the STAMPEDE study. In the HORRAD trial the majority of included patients had HV disease (63%) and the trial was not powered to detect a difference in the subgroup of LV patients. Therefore, the trend in LV patients observed in this trial supports the benefit of radiotherapy in LV patients obtained in the STAMPEDE study.

In an ongoing 4-arm trial, PEACE-1, the question of the addition of prostate radiotherapy to ADT plus docetaxel in mCNPC will again be explored (NCT01957436). In this study, mCNPC patients are randomized to either standard of care (SOC = ADT+Docetaxel), SOC plus abiraterone, SOC plus prostate radiotherapy, or SOC plus abiraterone and prostate radiotherapy. This study will conclude on the extent of the benefit of prostate radiotherapy in LV disease patients, as well as whether combining treatments may lead to additional survival benefit.

ADT + NOVEL AR TARGETED THERAPIES

Recently, the addition of novel AR targeted therapies enzalutamide and apalutamide to ADT have also shown survival benefit in the castration-naïve setting. The ENZAMET trial showed additional benefit of enzalutamide plus ADT compared to ADT plus standard antiandrogen therapy (bicalutamide/flutamide/nilutamide) in mCNPC.[15] Patients (N=1125) were stratified according to disease volume by CHAARTED definition. The study was amended to allow patients to receive early docetaxel (up to two cycles before randomization), as this was established as the new standard of care. A 33% reduced risk of death in the combination group of ADT plus enzalutamide compared to the standard antiandrogen group was shown (HR=0.67, 95%CI 0.52-0.86, p=0.002).[15] Early docetaxel was planned in 45% of patients. All six cycles were delivered in 65% of patients in the enzalutamide group and 76% in the standard antiandrogen group. A small majority (52%) of patients had HV-disease. Although the trial was not powered for subgroup analysis, it showed smaller effects on OS in patients who received early docetaxel (HR=0.90, 95%CI 0.62-1.31), and in HV disease patients (HR=0.80, 95%CI 0.59-1.07). The overall benefit of the addition of enzalutamide to ADT was supported by the interim results of the ARCHES trial presented at the ASCO GU 2019.[23] In ARCHES, 1150 patients were randomized to receive enzalutamide versus placebo combined with standard ADT in men with mCNPC. The primary endpoint radiographic PFS (rPFS) was significantly longer in the enzalutamide group compared to placebo (HR=0.39, 95%CI 0.30-0.50, p<0.0001). The publication of final data of the ARCHES needs to be awaited.

In addition, the TITAN study showed OS benefit of ADT combined with apalutamide, a new AR targeted agent with a similar working mechanism as enzalutamide, compared to ADT plus placebo.[16] The TITAN trial (N=1052) investigated ADT plus apalutamide versus

ADT plus placebo in men with mCNPC. Prior docetaxel (6 cycles) was allowed and patients were stratified according to disease volume by CHAARTED, resulting in a total of 113 patients (11%) who had received prior docetaxel, and 660 patients (63%) with HV disease. The results showed that patients treated with apalutamide plus ADT had a 52% lower risk of radiographic progression compared to ADT plus placebo (HR=0.48, 95%CI0.39-0.60, p<0.001). Preliminary analysis of OS showed a 32% decreased risk of death among patients treated with apalutamide (HR=0.68, 95%CI 0.51-0.90, p=0.005). The favorable effect of apalutamide on rPFS and OS was consistent across all subgroups.

CONSIDERATIONS BASED ON THE TRIAL DATA OF CURRENT AGENTS INCLUDED IN THE GUIDELINES

Comparisons of treatment options

Thus far, no direct prospective trials have been conducted between the available therapy options for mCNPC, in part because some therapy options were introduced only recently. Hereunder, we will discuss treatment comparisons and considerations based on the agents included in the current guidelines (docetaxel and abiraterone).

Although STAMPEDE was not designed to directly compare the docetaxel arm and the abiraterone arm, the investigators performed a retrospective analysis of those patients who had been accrued and treated in the overlapping period from November 2011 to March 2013.[24] A total of 566 patients had been allocated to receive either docetaxel (N=189) or abiraterone (N=377). With a median follow-up of 4 years, no difference was found in OS (HR=1.16, 95%CI 0.82-1.65,P=0.4) or prostate cancer-specific survival (HR=1.02, 95%CI 0.7-1.49). Also, metastasis-free survival and symptomatic skeletal-related events were similar. A advantage of abiraterone was seen on failure-free survival (HR=0.51, 95%CI 0.39-0.67,P<0.001) and progression-free survival (HR=0.65, 95%CI 0.48-0.88,P=005). In contrast, OS was in favor of patients treated with docetaxel, likely due to the larger number of non-prostate cancer related deaths in the abiraterone arm (19% in abiraterone arm versus 9% in docetaxel arm).[24] Considering similar efficacy, but larger non-prostate cancer related deaths in the abiraterone comparison, docetaxel might be the preferred treatment in these patients.

Additionally, several meta-analyses have made some cautious, indirect comparisons on the best treatment for mCNPC patients in terms of OS and other endpoints.[25-32] A 23-27% reduction in the risk of death was observed when ADT was combined with docetaxel compared to ADT alone with pooled HRs of 0.73-0.77 and the addition of abiraterone to ADT

Table 4. Overview of included meta-analyses and pooled results of overall survival

Meta-analysis/ Review	Year	Trials included	Pooled HR (95%CI) ADT+Doce	Pooled HR (95%CI) ADT+Abi	Pooled HR (95%CI) ADT+prostate Rtx
Vale *et al.* **[25]**	2016	GETUG-AFU15 - CHAARTED - STAMPEDE (doce)	0.77 (0.68-087)		
Tucci *et al.* **[26]**	2016	GETUG-AFU15 – CHAARTED - STAMPEDE (doce)	0.73 (0.60-0.90)		
Gravis *et al.* **[27]**	2017	GETUG-AFU15 – CHAARTED - STAMPEDE (doce)	0.77 (0.68-0.87)		
McNamara *et al.* **[28]**	2017	GETUG-AFU15 – CHAARTED - STAMPEDE (doce) LATITUDE - STAMPEDE (abi)	0.73 (0.60-0.90)		
Wallis *et al.* **[29]**	2017	GETUG-AFU15 – CHAARTED - STAMPEDE (doce) LATITUDE - STAMPEDE (abi)	0.75 (0.63-0.91)	0.63 (0.55-0.72)	
Vale *et al.* **[30]**	2018	GETUG-AFU15 – CHAARTED - STAMPEDE (all arms[a]) LATITUDE – CALGB - ZAPCA	0.77 (0.68-0.87)	0.61 (0.53-0.71)	
Tan *et al.* **[31]**	2018	GETUG-AFU15 – CHAARTED - STAMPEDE (all arms[a]) LATITUDE – CALGB – ZAPCA - MRC PR05	0.74 (0.63-0.86)	0.60 (0.50-0.70)	
Burdett *et al.* **[32]**	2019	HORRAD – STAMPEDE (Rtx)			0.92 (0.81-1.04)

Abbreviations: HR = Hazard ratios, CI = Confidence Interval, ADT = androgen-deprivation therapy, Doce = docetaxel, Abi = abiraterone, Rtx = radiotherapy

[a] without Rtx comparison, as this comparison had not yet been added to the multi-arm, multi-stage design of STAMPEDE trial.

resulted in a 37-40% reduction in risk of death with pooled HRs of 0.60-0.63 (**Table 4**). Pooled results of ADT + prostate radiotherapy showed no OS improvements in the total patient population, although patients with low tumor burden (<5) had improved OS (HR=1.47, 95%CI 1.11-1.94,P=0.007). Nonetheless, across trial comparisons remain difficult due to different inclusion criteria and patient populations. Even when only the HV patients of CHAARTED and LATITUDE were compared, still 18% of patients had been risk-stratified differently based on disease volume, using both studies inclusion criteria (**Table 3**).[13] While this field is pending the results of the direct comparison of the ongoing trials, considerations extracted from the subgroup/post-hoc analyses of the CHAARTED/STAMPEDE/LATITUDE trials is the best available evidence but should be interpreted with caution. In **Table 5** recommendations from current international guidelines and consensus conferences are described.

Table 5. Recommendations from international guidelines concerning therapy of mCNPC patients, assessed June 2019

Guidelines	Docetaxel in mCNPC	Abiraterone in mCNPC	Local radiotherapy in mCNPC
NICE - Prostate cancer guideline	Offer 6 cycles of 75mg/m^2 docetaxel in newly diagnosed metastatic prostate cancer patients who do not have significant co-morbidities		
ESMO	In newly diagnosed metastatic CNPC patients, who are fit enough for chemotherapy, ADT plus docetaxel is recommended as first-line treatment	In patients with metastatic CNPC ADT plus abiraterone/prednisone may be considered as first-line treatment	In patients with newly diagnosed low-volume metastatic prostate cancer, local prostate radiotherapy is recommended in addition to ADT
EAU	Offer ADT plus docetaxel in all patients with newly diagnosed metastatic CNPC, who are fit enough for chemotherapy	Offer ADT plus abiraterone/ prednisone to all patients with newly diagnosed metastatic CNPC and who are fit enough for the regimen	Offer ADT plus prostate radiotherapy to patients with newly diagnosed metastatic CNPC and who have low-volume disease according to CHAARTED
NCCN	Offer ADT plus docetaxel in high-volume patients with metastatic CNPC, who are fit enough for chemotherapy	Offer ADT plus abiraterone/ prednisone in all patients with metastatic CNPC	Offer ADT plus prostate radiotherapy for low-volume metastatic disease
APCCC 2017	Offer ADT plus docetaxel in high-volume (according to CHAARTED) patients with newly diagnosed metastatic CNPC or relapsing disease after prior local therapy, who are fit enough for chemotherapy		Data not yet published. But a rather large percentage of panel would consider treatment of the primary tumor in some patients with metastatic disease.

Disease burden

As mentioned GETUG-AFU15, CHAARTED, LATITUDE, STAMPEDE and HORRAD applied different definitions of disease burden and different criteria for inclusion, resulting in a different patient selection (**Table 2**). It is important to be aware of these differences when translating the study results into clinical practice. Based on the CHAARTED and GETUG-AFU15 studies, only patients with HV disease benefited from the addition of docetaxel. In the analysis of the mature datasets of both studies, the initial trend towards benefit in the LV group was lost with a HR of 1.04.[10,19] Unfortunately, there had been no post-hoc collection of information on the extent of disease in the STAMPEDE (docetaxel comparison) trial. The STAMPEDE investigators did however conduct a post-hoc analysis of disease extent in patients enrolled into the abiraterone comparison, classifying patients according to LATITUDE and CHAARTED criteria.[13] As expected these data showed a benefit by the

addition of abiraterone in the HV group, but also LV patients benefit the addition.[13] The STAMPEDE (radiotherapy comparison) had demonstrated that metastatic burden is also a discriminating factor for additional radiotherapy to the prostate since survival benefit was only obtained in LV disease patients. These STAMPEDE comparisons were the first to show survival benefit in LV patients with newly diagnosed metastatic disease.

Taken together, based on disease burden, patients with HV metastatic disease should receive either ADT plus 6 cycles docetaxel or long-term abiraterone/prednisone. The extent of the benefit by the addition of docetaxel or abiraterone in HV patients is similar based on the indirect comparison in STAMPEDE.[24] Patients with LV metastatic disease can be treated with either ADT plus abiraterone or ADT plus prostate radiotherapy.

Time of metastatic presentation

Some mCNPC patients have newly diagnosed metastatic disease, while others may present with recurrent disease after previous local therapy. Both patient groups were included in most above-mentioned trials, albeit patients with metastases after local therapy in smaller numbers (**Table 2**). In general, patients with previous local treatment had longer OS compared to those with newly diagnosed metastatic disease.[33] In CHAARTED a preplanned subgroup analysis was conducted, showing no statistically significant benefit by the addition of docetaxel to ADT in the patient group with recurrent disease after prior local therapy (HR=0.55, 95%CI 0.23-1.31,P>0.05).[10,19] However, the trial was not sufficiently powered for a subset analysis of this much smaller subgroup (27%).[10,27] Since patients recurring after local therapy are not well represented, the benefit of early docetaxel in this patient group is not known. Therefore, treatment decisions might be preferably based on disease volume instead of prior local therapy. As a result of close follow-up, patients who underwent prior local therapy have a tendency to develop LV metastatic disease at recurrence, and thus are not likely to benefit from docetaxel. However, if a patient might develop HV metastatic disease after prior local therapy, chemotherapy might still be considered. Similarly, numbers of patients with previous local therapy treated with abiraterone were very small (5%, **Table 2**). The additional benefit of abiraterone to ADT in this patient group is therefore unknown.

Patients with high-risk locally advanced or lymph node positive disease without previous local treatment may sometimes rapidly progress (3 to 6 months) to metastatic disease, or disease may become apparent as a result of clinical or radiological flare effects. Although these patients are not strictly newly diagnosed metastatic patients, it is conceivable that they might also benefit from early combined therapy.

Imaging modalities

In the herewith presented phase III trials, conventional imaging (bone-, CT-scan) was used for the diagnosis, the extent of metastatic disease, and the inclusion in studies. Despite being standard modalities for years, CT and bone scans lack the ability to accurately detect small volume metastases, recurrent disease (especially with low PSA values) and differentiate high- from low-grade disease.[34] New imaging techniques have become available representing more sensitive methods for staging at first diagnosis and at biochemical recurrence.[35] Upcoming modalities are whole body diffusion weighted MRI (WB-MRI) and PET CT-scan with various potential tracers, e.g. ^{18}F-Choline, ^{68}Ga-PSMA.[36] WB-MRI has a pooled sensitivity of 95% and specificity of 92% to detect bone and lymph node metastases at primary diagnosis.[37] Although WB-MRI can detect metastases at very low PSA values, there was no consensus by international experts for its use at biochemical recurrence.[38,39] The most promising imaging modality to replace conventional imaging at biochemical recurrence is ^{68}Ga-PSMA PET/CT, with a sensitivity of 64% and specificity 95%.[38,40] Prostate-specific membrane antigen (PSMA) is a cell transmembrane protein which is overexpressed in prostate cancer cells and ^{68}Ga-PSMA has a remarkable affinity to the extracellular domain of this peptide. The ^{68}Ga-PSMA tracer excels by having a higher detection rate even with low PSA values; a pooled analysis showed 42% sensitivity in PSA values <0.2 ng/mL compared to a detection rate of 30-40% in PSA values <3 ng/mL for other PET-CT tracers.[40,41] The sensitivity for CT/MRI to detect lymph node (LN) metastases, based on the morphological changes of the LN, ranges from 30-80% as compared to 63-86% for PSMA-PET.[41,42]

To date, the use of ^{68}Ga-PSMA PET/CT is recommended in the EAU-guidelines only for the purpose of detecting recurrent disease after local treatment.[43] But with the expanded sensitivity of ^{68}Ga-PSMA PET/CT and WB-MRI compared to conventional imaging, it will be increasingly utilized as an imaging tool in the initial diagnosis and staging of prostate cancer, although it is not approved by regulatory authorities yet.[39,40] The early detection of (oligo-)metastatic disease may allow for early and metastasis-directed therapy. However, the randomized clinical trials (RCTs) concerning early therapy in mCNPC patients were based on diagnosis by conventional imaging and the majority of the included patients have overtly newly diagnosed metastatic disease. Therefore, to date it remains unclear how the detection of more extensive metastatic disease in newly diagnosed metastatic patients by PSMA PET/CT scan imaging should be interpreted, and hence treated.

In conclusion, for the purpose of risk stratifying patients for treatment decisions based on disease volume, bone and CT scan remain the gold standard. In the STAMPEDE (radiotherapy comparison) study bone scans demonstrated to be predictive for the benefit

of local RT combined with ADT.[14] Therefore, the majority of patients diagnosed with LV disease on bone scan, should not be denied prostate radiotherapy, even when PSMA scan shows HV disease. Yet, personalized exceptions are possible, together with a well-informed patient shared decision making could provide decisive guidance.

Toxicity and Quality of Life

When comparing the early addition of AR-targeted therapy or docetaxel to ADT for mCNPC patients, it is important to be aware of the incidence and severity of toxicities of these regimens. In addition, the quality of life (QoL) of these patients during and after their treatment should be taken into account. The highest-grade toxicities observed per treatment and per phase III trial are shown in **Table 6**. It has been suggested that the risk of febrile neutropenia is higher in the mCNPC setting as compared to the mCRPC setting.[2]

An additional analysis of the STAMPEDE trial compared toxicity of treatment with docetaxel and abiraterone during the overlapping inclusion period in the STAMPEDE trial.[24] In this analysis the incidence of adverse events was similar with 50% grade 3-5 adverse events in the docetaxel comparison and 48% in the abiraterone comparison. However, the types of adverse events differed with more (febrile) neutropenia occurring in docetaxel treated patients (13% versus 1%), and more cardiovascular disorders occurring with abiraterone treatment (9% versus 3%). After 2 years, the prevalence of grade 3-4 toxicity in patients without a prior FFS event was 11% for docetaxel as well as for abiraterone. However, it is likely that after 14 months docetaxel treatment will have benefits over abiraterone treatment in terms of toxicity, since at this time point, docetaxel therapy is completed (5 months) and the patients have regained their QoL (6-9 months after treatment completion). Docetaxel is generally followed by a certain period of time without any treatment (except for ADT), which regains and restores QoL. In contrast, abiraterone has to be administered for about 2-3 years or until disease progression, in which patients are constantly exposed to therapy and thus more prone to toxicity, which may affect QoL.

Because of the increased cardiovascular risk observed in patients treated with abiraterone, patients should be carefully selected. In view of the modest survival gain of abiraterone in LV disease patients, those patients who are at risk for cardiovascular morbidity or mortality, especially those who have a history of cardiovascular disease, should not be treated with abiraterone. Such patients would appear excellent candidates for the addition of radiotherapy to the prostate. In HV disease patients with a cardiovascular risk profile, docetaxel is the preferred treatment.

Table 6. Frequent adverse events per treatment and phase III trial of the agents included in the guidelines

		Grade 3/4 adverse events		
Docetaxel	**TAX327**	**GETUG-AFU15**	**CHAARTED**	**STAMPEDE Docetaxel[a]**
Any grade 3/4 event	NR	NR	114 (29%)	288 (49%)
Neutropenia	32%	61 (32%)	47 (12%)	66 (11%)
Febrile neutropenia	3%	14 (7%)	24 (6%)	84 (14%)
Anemia	5%	4 (2%)	5 (1%)	NR
Peripheral Neuropathy	30%	3 (2%)	2 (<1%)	19 (3%)
Endocrine disorders (hot flushes, impotence)	NR	24 (13%)	NR	57 (10%)
Grade 5 events	0.3%	2 (1%)	1 (<1%)	4 (<1%)
Abiraterone	**COU-AA-302**	**LATITUDE**		**STAMPEDE Abiraterone[a]**
Any grade 3/4 event	NR	374 (63%)		443 (47%)
Hypertension	10 (1%)	131 (22%)		44 (5%)
Hypokaliemia	30 (4%)	70 (12%)		12 (1%)
Increased ALAT/ASAT	NR	61 (10%)		63 (6%)
Any cardiac disorder	33 (4%)	23 (4%)		92 (10%)
Edema	63 (19%)	5 (1%)		5 (1%)
Grade 5 events		38 (6%)		9 (1%)
Radiotherapy		**STAMPEDE Radiotherapy[b]**		
Any grade 3/4 event (bladder/bowel)		51 (6%)		
Diarrhea		12 (1%)		
Proctitis		11 (1%)		
Cystitis		7 (1%)		
Haematuria		6 (1%)		
Bowel obstruction		1 (<1%)		
Grade 5 events		0 (0%)		

Abbreviations: NR= Not Reported. Horrad study did not present any adverse events. Quality of Life evaluation concerning these patients will follow.

[a] STAMPEDE docetaxel and abiraterone comparisons present the worst grade events, while other studies present all grade 3/4 events.

[b] STAMPEDE Radiotherapy only presents adverse events related to radiotherapy and no adverse events related to docetaxel treatment in patients who also had additive docetaxel. Adverse events of both radiotherapy schedules are merged.

FUTURE PERSPECTIVES

The field of mCNPC treatment is rapidly evolving and there is an ongoing effort to establish the efficacy of other treatment options in the same disease phase. After the positive results of the addition of enzalutamide to ADT in the ENZAMET and the ARCHES trial, the

STAMPEDE enza comparison (NCT00268476), will soon report their results which also involve the efficacy of enzalutamide with or without abiraterone in mCNPC. Besides the forthcoming results of the PEACE-1 trial, other trials will report results in the near future.[44] Other systemic therapies currently under investigation are ADT+docetaxel+darolutamide (ARASENS, NCT02799602) and ADT+TAK-700 (SWOG1216, NCT01809691). For oligometastatic disease the benefit of metastatic directed therapy is investigated in STOMP (NCT01558427), ORIOLE (NCT02680587) and PLATON (NCT03784755). Finally, effects of radiotherapy or surgery of the prostate is investigated in PEACE-1 (NCT01957436), g-RAMPP (NCT02454543), TRoMbone (ISRCTN15704862) and SWOG1802 (NCT03678025).

CONCLUSION

In patients with mCNPC, the addition of docetaxel or abiraterone to ADT significantly improved survival as compared to ADT alone. Survival benefits obtained are much larger compared with these drugs given at later disease stages, i.e. CRPC. Most recent data showed longer survival with prostate radiotherapy, enzalutamide, or apalutamide in combination with ADT, of which the last two await official approval. Prostate cancer remains a very heterogeneous disease, and while pending molecular biomarkers to individualize treatment choice, clinical factors may provide guidance to risk stratify patients; such as metastatic extent and comorbidities. LV mCNPC patients appear to obtain survival benefit from novel AR targeted therapies or prostate radiotherapy. The role of docetaxel is currently uncertain in LV disease. However, this might be soon addressed by a metastatic burden analysis of the STAMPEDE docetaxel comparison. In HV disease patients no apparent difference in OS benefit was observed between the addition of docetaxel or abiraterone to ADT. However, patients with a history of cardiovascular disease or those with increased risk of cardiovascular morbidity may not be served best by the addition of abiraterone, but should receive radiotherapy or docetaxel depending on the metastatic extent of the disease. Moreover, the benefit of shorter treatment periods with docetaxel/radiotherapy could outweigh the longer treatment period of abiraterone and its adverse events affecting QoL of these patients. Further research is needed to determine the impact of recurrent disease, the role of new imaging techniques, the effect of the combination of agents, and development of predictive molecular biomarkers.

REFERENCES

1. Lorente D, Fizazi K, Sweeney C, de Bono JS. Optimal Treatment Sequence for Metastatic Castration-resistant Prostate Cancer. Eur Urol Focus 2016; 2: 488-498.
2. 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: 1502-1512.
3. 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: 1147-1154.
4. de Bono JS, Logothetis CJ, Molina A et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011; 364: 1995-2005.
5. Ryan CJ, Smith MR, de Bono JS et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med 2013; 368: 138-148.
6. Scher HI, Fizazi K, Saad F et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 2012; 367: 1187-1197.
7. Beer TM, Armstrong AJ, Rathkopf DE et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med 2014; 371: 424-433.
8. Parker C, Nilsson S, Heinrich D et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. N Engl J Med 2013; 369: 213-223.
9. James ND, Sydes MR, Clarke NW et al. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. Lancet 2016; 387: 1163-1177.
10. Sweeney CJ, Chen YH, Carducci M et al. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. N Engl J Med 2015; 373: 737-746.
11. Fizazi K, Tran N, Fein L et al. Abiraterone plus Prednisone in Metastatic, Castration-Sensitive Prostate Cancer. N Engl J Med 2017; 377: 352-360.
12. James ND, de Bono JS, Spears MR et al. Abiraterone for Prostate Cancer Not Previously Treated with Hormone Therapy. N Engl J Med 2017; 377: 338-351.
13. Hoyle AP, Ali SA, James ND et al. LBA4Effects of abiraterone acetate plus prednisone/prednisolone in high and low risk metastatic hormone sensitive prostate cancer. Annals of Oncology 2018; 29.
14. Parker CC, James ND, Brawley CD et al. Radiotherapy to the primary tumour for newly diagnosed, metastatic prostate cancer (STAMPEDE): a randomised controlled phase 3 trial. Lancet 2018; 392: 2353-2366.
15. Davis ID, Martin AJ, Stockler MR et al. Enzalutamide with Standard First-Line Therapy in Metastatic Prostate Cancer. N Engl J Med 2019.
16. Chi KN, Agarwal N, Bjartell A et al. Apalutamide for Metastatic, Castration-Sensitive Prostate Cancer. N Engl J Med 2019.
17. Gravis G, Fizazi K, Joly F et al. Androgen-deprivation therapy alone or with docetaxel in non-castrate metastatic prostate cancer (GETUG-AFU 15): a randomised, open-label, phase 3 trial. Lancet Oncol 2013; 14: 149-158.
18. Kyriakopoulos CE, Chen YH, Carducci MA et al. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer: Long-Term Survival Analysis of the Randomized Phase III E3805 CHAARTED Trial. J Clin Oncol 2018; 36: 1080-1087.
19. Gravis G, Boher JM, Chen YH et al. Burden of Metastatic Castrate Naive Prostate Cancer Patients, to Identify Men More Likely to Benefit from Early Docetaxel: Further Analyses of CHAARTED and GETUG-AFU15 Studies. Eur Urol 2018; 73: 847-855.

20. Fizazi K, Tran N, Fein L et al. Abiraterone acetate plus prednisone in patients with newly diagnosed high-risk metastatic castration-sensitive prostate cancer (LATITUDE): final overall survival analysis of a randomised, double-blind, phase 3 trial. Lancet Oncol 2019.
21. Ryan CJ, Smith MR, Fizazi K et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol 2015; 16: 152-160.
22. Boeve LMS, Hulshof M, Vis AN et al. Effect on Survival of Androgen Deprivation Therapy Alone Compared to Androgen Deprivation Therapy Combined with Concurrent Radiation Therapy to the Prostate in Patients with Primary Bone Metastatic Prostate Cancer in a Prospective Randomised Clinical Trial: Data from the HORRAD Trial. Eur Urol 2019; 75: 410-418.
23. Armstrong A SR, Petrylak DP, et al. Phase 3 study of androgen deprivation therapy with enzalutamide or placebo in metastatic hormone-sensitive prostate cancer: The ARCHES trial. In ASCO GU 2019. 2019.
24. Sydes MR, Spears MR, Mason MD et al. Adding abiraterone or docetaxel to long-term hormone therapy for prostate cancer: directly randomised data from the STAMPEDE multi-arm, multi-stage platform protocol. Ann Oncol 2018; 29: 1235-1248.
25. Vale CL, Burdett S, Rydzewska LHM et al. Addition of docetaxel or bisphosphonates to standard of care in men with localised or metastatic, hormone-sensitive prostate cancer: a systematic review and meta-analyses of aggregate data. Lancet Oncol 2016; 17: 243-256.
26. Tucci M, Bertaglia V, Vignani F et al. Addition of Docetaxel to Androgen Deprivation Therapy for Patients with Hormone-sensitive Metastatic Prostate Cancer: A Systematic Review and Meta-analysis. Eur Urol 2016; 69: 563-573.
27. Gravis G, Audenet F, Irani J et al. Chemotherapy in hormone-sensitive metastatic prostate cancer: Evidences and uncertainties from the literature. Cancer Treat Rev 2017; 55: 211-217.
28. McNamara M, Sweeney C, Antonarakis ES, Armstrong AJ. The evolving landscape of metastatic hormone-sensitive prostate cancer: a critical review of the evidence for adding docetaxel or abiraterone to androgen deprivation. Prostate Cancer Prostatic Dis 2018; 21: 306-318.
29. Wallis CJD, Klaassen Z, Bhindi B et al. Comparison of Abiraterone Acetate and Docetaxel with Androgen Deprivation Therapy in High-risk and Metastatic Hormone-naive Prostate Cancer: A Systematic Review and Network Meta-analysis. Eur Urol 2018; 73: 834-844.
30. Vale CL, Fisher DJ, White IR et al. What is the optimal systemic treatment of men with metastatic, hormone-naive prostate cancer? A STOPCAP systematic review and network meta-analysis. Ann Oncol 2018; 29: 1249-1257.
31. Tan PS, Aguiar P, Jr., Haaland B, Lopes G. Addition of abiraterone, docetaxel, bisphosphonate, celecoxib or combinations to androgen-deprivation therapy (ADT) for metastatic hormone-sensitive prostate cancer (mHSPC): a network meta-analysis. Prostate Cancer Prostatic Dis 2018; 21: 516-523.
32. Burdett S, Boeve LM, Ingleby FC et al. Prostate Radiotherapy for Metastatic Hormone-sensitive Prostate Cancer: A STOPCAP Systematic Review and Meta-analysis. Eur Urol 2019.
33. Finianos A, Gupta K, Clark B et al. Characterization of Differences Between Prostate Cancer Patients Presenting With De Novo Versus Primary Progressive Metastatic Disease. Clin Genitourin Cancer 2017.
34. Moghanaki D, Turkbey B, Vapiwala N et al. Advances in Prostate Cancer Magnetic Resonance Imaging and Positron Emission Tomography-Computed Tomography for Staging and Radiotherapy Treatment Planning. Semin Radiat Oncol 2017; 27: 21-33.
35. Mertan FV, Lindenberg L, Choyke PL, Turkbey B. PET imaging of recurrent and metastatic prostate cancer with novel tracers. Future Oncol 2016; 12: 2463-2477.

36. Lindenberg ML, Turkbey B, Mena E, Choyke PL. Imaging Locally Advanced, Recurrent, and Metastatic Prostate Cancer: A Review. JAMA Oncol 2017; 3: 1415-1422.
37. Liu LP, Cui LB, Zhang XX et al. Diagnostic Performance of Diffusion-weighted Magnetic Resonance Imaging in Bone Malignancy: Evidence From a Meta-Analysis. Medicine (Baltimore) 2015; 94: e1998.
38. Lecouvet FE, Oprea-Lager DE, Liu Y et al. Use of modern imaging methods to facilitate trials of metastasis-directed therapy for oligometastatic disease in prostate cancer: a consensus recommendation from the EORTC Imaging Group. Lancet Oncol 2018; 19: e534-e545.
39. Fanti S, Minozzi S, Antoch G et al. Consensus on molecular imaging and theranostics in prostate cancer. Lancet Oncol 2018; 19: e696-e708.
40. Perera M, Papa N, Christidis D et al. Sensitivity, Specificity, and Predictors of Positive (68)Ga-Prostate-specific Membrane Antigen Positron Emission Tomography in Advanced Prostate Cancer: A Systematic Review and Meta-analysis. Eur Urol 2016; 70: 926-937.
41. Eiber M, Maurer T, Souvatzoglou M et al. Evaluation of Hybrid (6)(8)Ga-PSMA Ligand PET/CT in 248 Patients with Biochemical Recurrence After Radical Prostatectomy. J Nucl Med 2015; 56: 668-674.
42. Giesel FL, Fiedler H, Stefanova M et al. PSMA PET/CT with Glu-urea-Lys-(Ahx)-[(6)(8)Ga(HBED-CC)] versus 3D CT volumetric lymph node assessment in recurrent prostate cancer. Eur J Nucl Med Mol Imaging 2015; 42: 1794-1800.
43. Mottet N. http://uroweb.org/guideline/prostate-cancer/. In. EAU 2018.
44. Hamid AA, Morris MJ, Davis ID. Clinical Trials of Metastatic Castration-sensitive Prostate Cancer: Recent Progress and New Horizons. Eur Urol Focus 2019; 5: 165-167.

SUPPLEMENTARY METHODS

We performed a non-systematic review of literature from January 2013 to June 2019 by searching Medline and Embase with the keywords: metastatic castration-naïve prostate cancer, metastatic hormone-naïve prostate cancer, metastatic castration-sensitive prostate cancer, metastatic hormone-sensitive prostate cancer, docetaxel, abiraterone, local radiotherapy. All clinical trials and review articles written in English were reviewed. Conference abstracts were also included and cross-matching references were used to find additional articles. Only articles that clearly defined the mCNPC study population, clinical endpoints, and methods were included in this review. In total, we included 12 articles that investigated the treatment of mCNPC.

Chapter 10

General discussion and summary

In this thesis two main issues regarding the treatment of metastatic prostate cancer are addressed. At first, we raise the importance of pharmacological changes in drug treatment in patients with metastatic castration-resistant prostate cancer (mCRPC). Next, the thesis continues on identifying factors determining treatment selection or treatment response in the wide landscape of therapies in mCRPC as well as in metastatic hormone-sensitive prostate cancer (mHSPC).

PERSPECTIVE ON PHARMACOLOGY

Clinical pharmacology aims to improve efficacy and safety of drug treatment by increasing knowledge on pharmacokinetics and pharmacodynamics. *Pharmacokinetics* describe how drugs are metabolized in the body, based on administration, distribution, metabolism and elimination of the medication. Interference of these processes can potentially result in clinical relevant changes in concentrations of the drugs. Simple patient-related factors, like ageing, organ failure, pregnancy or malnutrition can alter the pharmacokinetic processes. Moreover, food, herbal supplements (e.g. grapefruit, St. John's Wort, curcumin) and co-medication can also distort this process.[1] For instance, NSAIDs may lower kidney function and affect the excretion of lithium, resulting in higher levels of lithium. Especially in agents with a small therapeutic window, like most oncological agents, slight changes may already result in sub-optimal or toxic levels of medication, which should be avoided.

Since nearly two decades the treatment possibilities for metastatic prostate cancer have evolved rapidly. With the introduction of docetaxel, cabazitaxel, abiraterone, enzalutamide and radium-223 in the mCRPC setting, the challenge on treatment choice and sequencing treatment has augmented.[2-8] Moreover, metastatic prostate cancer is a heterogeneous disease. Thus, combining therapies with different complementary mechanisms of action has the potential for additional survival benefit and is therefore increasingly investigated.[9,10] In the pre-clinical setting the enhanced efficacy of taxanes in combination with androgen-suppressing agents has been shown.[11] This confirms the active role of the androgen receptor (AR) pathway in mCRPC and enhances a rationale for combining taxanes with novel AR-targeted therapies (ART). Combining anti-cancer agents also means an increased risk for clinical relevant pharmacokinetic drug-drug interactions. Taxanes of interest, docetaxel and cabazitaxel, are extensively metabolized by the cytochrome P450 iso-enzyme CYP3A4 in the liver, which is prone for inhibition/induction from other drugs. Therefore, combinations involving taxanes warrant thorough pharmacokinetic evaluation to ensure safety of the combination. For instance, enzalutamide, an orally available AR-targeted agent, is a strong inducer of the CYP3A4 enzyme. When combining enzalutamide with cabazitaxel, we observed a decline in geometric mean exposure of 22%

(95%CI 9-34%, p=0.005) in cabazitaxel concentration (**Chapter 2**). The clinical relevance of this interaction lies in the magnitude of the interaction in combination with a recent study showing non-inferiority in terms of overall survival (OS) between the registered 3-weekly dose of cabazitaxel (25 mg/m^2) and a 20% lower dose (20 mg/m^2).[12] As the lower dose has led to less toxicity, while maintaining its efficacy, it is currently widely used. However, when the promising combination with enzalutamide is further investigated, it is not desirable to lower the cabazitaxel dose to 20 mg/m^2 on forehand as this might lead to sub-optimal concentrations of cabazitaxel, due to the found drug-drug interaction. In previous research, the effect of enzalutamide has also been investigated in combination with the other taxane used in prostate cancer; docetaxel. In that study, only a modest interaction with 12% decrease in docetaxel concentration was observed.[13] The variety of the interaction of both combinations might lie in the different taxanes that were used. Moreover, the observation period in the study investigating the combination of enzalutamide with docetaxel was shorter. Although the drug-drug interaction is larger in combination with cabazitaxel, this combination has a greater potential to be used in the mCRPC setting, as nowadays docetaxel is often used in the hormone-sensitive setting of prostate cancer. In this setting, docetaxel has been investigated in two large trials; one used docetaxel in combination with prednisone, while the other used docetaxel without prednisone.[14,15] Prednisone is a moderate CYP3A4 inducer, so could potentially also influence docetaxel concentrations. Since the registration of docetaxel in 2004 it has been combined with prednisone. Prednisone's impact on prostate cancer itself remains to be elucidated. It has been suggested that prednisone lowers the testosterone production via the adrenal glands, affects PSA, and reduces taxane-induced toxicity.[16-18] However, no effect of prednisone on OS has been observed and in a recent phase III trial (CHAARTED) docetaxel-induced toxicity did not increase without prednisone.[14] On the contrary, long-term use of corticosteroids is accompanied by negative adverse events like osteoporosis, which especially in prostate cancer patients with bone-metastases is an adverse event to avoid. Therefore, the omission of prednisone from standard docetaxel regimens would be beneficial, particularly in mHSPC patients who have not yet been introduced to prednisone therapy. In a randomized pharmacokinetic cross-over trial we investigated the effect of prednisone on docetaxel concentrations. We observed no substantial differences in docetaxel concentration when combined with prednisone compared to docetaxel monotherapy (**Chapter 3**). From a pharmacokinetic perspective prednisone could be eliminated from the regimen, although the benefits of the omission should outweigh the positive effects of prednisone therapy before definite exclusion of prednisone can be suggested. Therefore, the individual impact of prednisone on metastatic prostate cancer needs to be clarified and it should be confirmed that non-inferiority in terms of incidence of docetaxel-induced toxicities holds with or without prednisone.

Pharmacodynamics describe how drugs have an effect on organ function (and the tumor of the patient). Most extensively investigated (prior to clinical implementation of new drugs) is the influence of a drug on cardiac repolarization. Measurement of the QT-interval in electrocardiography (ECG) indicates ventricular repolarization, and it is corrected for the heart rate (QTc). Prolongation of ventricular repolarization can potentially result in arrhythmias, or even cardiac arrest. Therefore, caution is needed and development of new drugs always includes QTc-prolongation studies. One of the new developed AR-targeted agents for metastatic prostate cancer is apalutamide (240 mg). Apalutamide is a new anti-androgen, directly binding to the ligand binding domain of AR, inhibiting AR nuclear translocation and DNA binding and impedes AR-mediated transcription.[19] In an international, multicenter trial we found a moderate, clinically irrelevant effect of apalutamide on ventricular repolarization in mCRPC and non-metastatic CRPC patients (**Chapter 4**). Extensive ECG measurements at cycle 1 and cycle 3, when steady state of apalutamide was reached, showed QTc intervals and their associated 90% confidence interval (CI) changed ≤20 ms from baseline. These results may be considered mild for an anti-cancer agent and an extensive QTc study like this is therefore considered negative when showing these results.[20] Patients treated in this pharmacodynamic study were allowed to continue treatment when effective, resulting in remarkable long responses to apalutamide indicating its potential as additional therapy option, or in combination with other treatment options. Apalutamide has been approved in the USA for non-metastatic CRPC, and recent results have suggested survival benefit in mHSPC patients.[21,22]

PERSPECTIVE ON TREATMENT SELECTION - mCRPC

A major challenge for physicians treating metastatic prostate cancer patients remains the choice of treatment and sequence of treatments. Both abiraterone and enzalutamide have shown to be effective before or after docetaxel.[4,5,7,8] Efficacy of cabazitaxel treatment was not different in patients who had received prior ART.[23] In addition, cabazitaxel has shown similar efficacy to docetaxel as first-line chemotherapy for mCRPC patients; yet cabazitaxel is also an effective agent in the post-docetaxel mCRPC setting.[3,24] Therefore, it is preferably used as second-line chemotherapy. Since no predictive biomarkers are currently available in clinical practice, it is challenging to determine which patient would benefit from which treatment. Over the past couple of years, the predictive value of the presence of androgen receptor splice variant 7 (*AR-V7*) has increasingly been investigated and was recently validated, showing a decreased response to ART, enzalutamide and abiraterone. However, consensus needs to be achieved on outstanding issues regarding the right assay and criteria to allocate a patient *AR-V7* positive before clinical implementation is accessible. Meanwhile, research on *AR-V7* continues to improve the knowledge on this potential

predictive biomarker. Apart from that, cohort studies and post-hoc analyses of large phase III trials (to determine response biomarkers) provide the best available evidence for treatment choice. Here we discuss some considerations to guide treatment choice in mCRPC patients based on this thesis and other literature.

Prognostic factors

Currently, treatment choice is often based on patients' preferences, co-morbidities and toxicity profile. Prognostic factors might help to determine treatment choice as well as treatment start. In mCRPC patients scheduled to start chemotherapy, prognostic factors identified include the WHO performance status, presence of visceral metastases, pain, duration of androgen-deprivation therapy (ADT), Gleason score, circulating tumor cells (CTCs) and laboratory results: hemoglobin (Hb), alkaline phosphatase (AP), lactate dehydrogenase (LDH), prostate specific antigen (PSA) and derived neutrophil/lymphocyte ratio (dNLR).[25-30] In a large prospectively collected dataset of mCRPC patients treated with cabazitaxel (the CABARESC trial),[31] we confirmed some of these prognostic factors including WHO performance status, Hb, AP and complemented these by albumin as additional prognostic factor for overall survival (OS). Moreover, we found Hb and AP predictive of PSA response in this patient cohort. With these prognostic factors we designed a nomogram for OS and PSA response, which could be easily implemented in clinical practice. A clear overview on prognosis might help the physician to determine when treatment start could be extended or when intervention is needed, before a patients' condition deteriorates (**Chapter 5**). In our cohort, we were not able to analyze pain, Gleason score, visceral metastases and duration of ADT as potential prognostic factor since these data were not collected in the CABARESC trial. The parameter for an inflammatory response rate of the host, dNLR, was collected, however it was not correlated with OS in our cohort. Nevertheless, its relevance as prognostic factor in prostate cancer patients has been established based on other post-hoc analyses and it is advised to be incorporated in prospective randomized controlled trials as stratification factor.[29,32]

In addition to these baseline clinical and laboratory factors, genetic variation appears to be of prognostic value. Besides being able to estimate the probability for survival, genetic alterations (single-nucleotide polymorphisms; SNPs) can help identifying patients with an increased risk of developing adverse events. Taxanes' main dose limiting toxicity is the development of neutropenia, which could potentially result in life-threatening febrile neutropenia. In consequence to neutropenia the dose is reduced, treatment is interrupted, or may even be discontinued. Identification of factors predicting for occurrence of toxicity, especially neutropenia, in taxane treated patients would therefore be of great value. For cabazitaxel therapy SNPs have been identified which were related to toxicity and activity

in 45 patients with advanced urothelial cell carcinoma. Polymorphisms in *CYP3A5* and *ABCB1* were identified as potentially related to altered adverse events profile and efficacy of cabazitaxel.[33] In this thesis we aimed to identify genetic variations associated with toxicity, survival or pharmacokinetics in mCRPC patients in an explorative analysis (**Chapter 6**). A large, prospectively collected, dataset of mCRPC patients treated with cabazitaxel was used for this analysis, complemented by data of two smaller pharmacokinetic trials (n=24) investigating cabazitaxel concentrations in mCRPC patients. We genotyped 128 bloodsamples of men with mCRPC treated with cabazitaxel for 7 SNPs in 6 genes (*CYP3A4, CYP3A5, SLCO1B1, SLCO1B3, ABCB1* and *TUBB1*), which are involved in the metabolism and working mechanism of cabazitaxel. The haplotype of *SLCO1B1*15* was significantly associated with a lower incidence of leukopenia and neutropenia. The isolated hepatic expression of OATP1B1, the influx transporter encoded by *SLCO1B1,* strongly suggests a pharmacokinetic rationale for the influence of these SNPs on toxicity. However, none of the SNPs were associated with pharmacokinetic changes. Moreover, the observed associations were opposite to the expected working mechanism of the genetic variated influx transporter.[34,35] Therefore, the observed associations of *SLCO1B* SNPs to lower incidence of neutropenia and leukopenia in cabazitaxel treated mCRPC are likely to be false-positive and imply that studying *SLCO1B1* SNPs does not seem indicated in future cabazitaxel research.

Additionally, an extensively investigated prognostic factor in patients with several solid tumor types is the enumeration of circulating tumor cells (CTCs). Men with a baseline 'favorable' CTC count of <5 CTCs/7.5mL blood have a better survival compared to patients with an 'unfavorable' CTC count of ≥5 CTCs/7.5mL blood at baseline.[28] CTCs after treatment/ at progression have also been shown to be prognostic for survival. Post-treatment CTC count was even more informative for prognosis than a ≥50% change in PSA.[27,36] In addition, CTC responses defined as the decline of an unfavorable CTC count (≥5) to a favorable CTC count (<5), during treatment have been associated with OS.[27] The use of CTC enumeration as prognostic factor at the start of a new treatment should be incorporated in daily practice and the presence of CTCs offers opportunities for additional analysis to unravel tumor information (**Chapter 7-8**).

Predictive markers

Besides CTC enumeration, molecular characterization of CTCs harvests great interest. The presence of *AR-V7* is associated with decreased response to ART, but not to taxanes, as shortly mentioned above.[37-40] *AR-V7* is a mRNA splice variant of the androgen receptor (AR) that translates into a constitutively active AR in absence of ligands.[41] Its expression is increased in patients with more advanced disease, who have been treated before with

abiraterone or enzalutamide. Therefore, it has been suggested that *AR-V7* represents a greater disease burden, rather than its predictive value to ART.[42] This was supported by data showing decreased responses to taxanes and showing PSA responses to ART in *AR-V7* positive patients.[43,44] To improve our understanding of the clinical value of *AR-V7* we have conducted a post-hoc analysis of three clinical trials to investigate the association of *AR-V7* in CTCs with CTC count and OS (**Chapter 7**). An association was observed between CTC count and *AR-V7* status. However, this association was not found in patients with an unfavorable prognosis due to a baseline CTC count ≥5 CTCs. In addition, in our cohort of mCRPC patients who were mostly treated with cabazitaxel, *AR-V7* positivity was not associated with a worse prognosis. In an ongoing prospective clinical trial (the CABA-V7 study) the PSA response and OS in *AR-V7* positive men with mCRPC treated with cabazitaxel will be investigated. Moreover, another recent prospective trial validated *AR-V7* as predictive factor in patients treated with abiraterone and enzalutamide.[45] Therefore, it seems that *AR-V7* expression will keep its predictive value in mCRPC patients treated with ART.

Treatment response markers

Optimal guidance of metastatic prostate cancer patients during treatment requires early response markers. Nowadays PSA and radiographic responses are primarily used to determine treatment response, albeit with some limitations. CTC enumeration and CTC responses are extensively incorporated in prospective phase III trials in combination with PSA and radiology to improve our knowledge on its role as potential response indicators. Yet it is unclear if a failure to show CTC response would imply to directly change treatment for an individual patient. As an example; a patients' CTC count can decline from 100 to 10 during treatment, without having formally achieved a CTC response, while a decrease in CTC count from 7 to 4 CTCs is referred to as a CTC response. This indicates the difficulty in CTC responses as a treatment response marker. Therefore, in my opinion, individual disease monitoring by CTC enumeration/response should be in combination with the absence of other signs of progression to generally indicate a treatment response and to determine if treatment can be continued.

In this thesis we describe the results of the interim analysis of the CABA-V7 study, focusing on CTC response rates in *AR-V7* positive men with mCRPC who are treated with cabazitaxel (**Chapter 8**). We found a moderate 15% CTC response rate after 3 cabazitaxel cycles (9 weeks). Additionally, we observed 45% of patients converted from *AR-V7* positive CTCs to *AR-V7* negative during these 3 cycles of cabazitaxel. While the preliminary results of CTC response in this cohort were disappointing and less informative, the conversion of *AR-V7* is an interesting observation, since it was also associated with survival. In this study,

AR-V7 expression was determined at baseline and already after 3 cycles (9 weeks after start of treatment), suggesting the potential of characterization of CTCs as early treatment biomarker in *AR-V7* positive patients treated with cabazitaxel. Without prejudice to the final results of the CABA-V7 trials, I think it would be interesting to investigate if *AR-V7* converted patients would again be sensitive to ART.

PERSPECTIVE ON TREATMENT SELECTION – mHSPC

For years, the only treatment of mHSPC (equally referred to as metastatic castration-naïve prostate cancer; mCNPC) has been androgen-deprivation therapy (ADT). Recently, major shifts in the treatment of mHSPC have occurred. Several trials, CHAARTED, STAMPEDE, LATITUDE and HORRAD, have shown survival benefit in mHSPC patients treated more aggressively, with additional therapy combined with ADT.[14,15,46-50] The additional therapy options in mHSPC have expanded with docetaxel, abiraterone, prostate radiotherapy, enzalutamide and apalutamide, of which the last two await official approval and incorporation into the international guidelines.[21,51] With the introduction of these additional therapies to the hormone sensitive setting, the challenge of individualizing therapies has also been introduced in this setting, as it is in the castration-resistant phase. Pending molecular biomarkers to individualize treatment choice, clinical factors may provide guidance to risk stratify patients; such as metastatic extent and comorbidities. Low-volume patients, according to the definition by CHAARTED, appear to obtain survival benefit with novel ART or local radiotherapy to the prostate.[50,52] At this moment, there is no role for docetaxel in low-volume patients. In high-volume patients, both docetaxel and abiraterone has shown large survival benefit.[14,52] In this patient group, the comorbidities can be decisive for treatment choice. As abiraterone showed increased cardiovascular toxicity, patients with cardiovascular risk or morbidity might not be served best with abiraterone. Moreover, the treatment period of docetaxel, or radiotherapy in low-volume patients, is substantially shorter compared to abiraterone treatment, which could potentially affect quality of life and can be taken into account to determine treatment choice. In **Chapter 9** we provide a comprehensive overview of the current available treatment options for mHSPC and aim to provide some tools to guide treatment selection in this patient group.

FUTURE PERSPECTIVES

In the near future, new therapy options will be explored and the timing of treatment will require additional awareness.[53-56] Moreover, interesting treatment strategies of novel ARTs/local prostate treatment in combination with taxanes/other ARTs will supervene

to show potential additive or synergistic effect of both treatment options in mCRPC/mHSPC patients. By doing so, pharmacokinetic studies will continue to be important to ensure a safe administration of combination of agents. In both disease phases, mCRPC and mHSPC, increasing effort will be made to individualize treatment by determination of molecular biomarkers. While pending results on predictive biomarkers, clinical factors of post-hoc analyses and cohort studies are the best we have to select the right treatment for an individual patient. The treatment landscape of metastatic prostate cancer continues to evolve and new perceptions on treatment selection and sequence of treatment will continue to be shared. Therefore, it is important to be up to date and to continue to treat metastatic prostate cancer patient in a multidisciplinary team, to merge the latest insights of each medical specialization.

REFERENCES

1. Mathijssen RH, Sparreboom A, Verweij J. Determining the optimal dose in the development of anticancer agents. Nat Rev Clin Oncol. 2014;11(5):272-281.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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.
7. Beer TM, Armstrong AJ, Rathkopf DE, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med. 2014;371(5):424-433.
8. Ryan CJ, Smith MR, Fizazi K, et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol. 2015;16(2):152-160.
9. Sternberg CN. Improving Survival for Metastatic Castrate-resistant Prostate Cancer: Will Combination Therapy Help Us To Move Forward? Eur Urol. 2016;70(5):722-723.
10. Corn PG, Agarwal N, Araujo JC, Sonpavde G. Taxane-based Combination Therapies for Metastatic Prostate Cancer. Eur Urol Focus. 2017.
11. Mout L, de Ridder C, Stuurman D, et al. Testosterone abolishes cabazitaxel efficacy in an in vivo model of prostate cancer and affects intratumoral concentrations. AACR AACR 2017 #1099.
12. Eisenberger M, Hardy-Bessard AC, Kim CS, et al. Phase III Study Comparing a Reduced Dose of Cabazitaxel (20 mg/m2) and the Currently Approved Dose (25 mg/m2) in Postdocetaxel Patients With Metastatic Castration-Resistant Prostate Cancer-PROSELICA. J Clin Oncol. 2017;35(28):3198-3206.
13. Morris MJ, Rathkopf DE, Novotny W, et al. Phase Ib Study of Enzalutamide in Combination with Docetaxel in Men with Metastatic Castration-Resistant Prostate Cancer. Clin Cancer Res. 2016;22(15): 3774-3781.
14. Sweeney CJ, Chen YH, Carducci M, et al. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. N Engl J Med. 2015;373(8):737-746.
15. James ND, Sydes MR, Clarke NW, et al. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. Lancet. 2016;387(10024):1163-1177.
16. Kongsted P, Svane IM, Lindberg H, Daugaard G, Sengelov L. Low-dose prednisolone in first-line docetaxel for patients with metastatic castration-resistant prostate cancer: is there a clinical benefit? Urol Oncol. 2015;33(11):494 e415-420.
17. De Santis M, Saad F. Practical Guidance on the Role of Corticosteroids in the Treatment of Metastatic Castration-resistant Prostate Cancer. Urology. 2016;96:156-164.
18. Ndibe C, Wang CG, Sonpavde G. Corticosteroids in the management of prostate cancer: a critical review. Curr Treat Options Oncol. 2015;16(2):6.

19. Clegg NJ, Wongvipat J, Joseph JD, et al. ARN-509: a novel antiandrogen for prostate cancer treatment. Cancer Res. 2012;72(6):1494-1503.
20. Sarapa N, Britto MR. Challenges of characterizing proarrhythmic risk due to QTc prolongation induced by nonadjuvant anticancer agents. Expert Opin Drug Saf. 2008;7(3):305-318.
21. Chi KN, Agarwal N, Bjartell A, et al. Apalutamide for Metastatic, Castration-Sensitive Prostate Cancer. N Engl J Med. 2019.
22. Smith MR, Saad F, Chowdhury S, et al. Apalutamide Treatment and Metastasis-free Survival in Prostate Cancer. N Engl J Med. 2018;378(15):1408-1418.
23. van Soest RJ, Nieuweboer AJ, de Morree ES, et al. The influence of prior novel androgen receptor targeted therapy on the efficacy of cabazitaxel in men with metastatic castration-resistant prostate cancer. Eur J Cancer. 2015;51(17):2562-2569.
24. Oudard S, Fizazi K, Sengelov L, et al. Cabazitaxel Versus Docetaxel As First-Line Therapy for Patients With Metastatic Castration-Resistant Prostate Cancer: A Randomized Phase III Trial-FIRSTANA. J Clin Oncol. 2017;35(28):3189-3197.
25. Halabi S, Lin CY, Kelly WK, et al. Updated prognostic model for predicting overall survival in first-line chemotherapy for patients with metastatic castration-resistant prostate cancer. J Clin Oncol. 2014; 32(7):671-677.
26. Halabi S, Lin CY, Small EJ, et al. Prognostic model predicting metastatic castration-resistant prostate cancer survival in men treated with second-line chemotherapy. J Natl Cancer Inst. 2013;105(22): 1729-1737.
27. de Bono JS, Scher HI, Montgomery RB, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res. 2008;14(19):6302-6309.
28. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med. 2004;351(8):781-791.
29. van Soest RJ, Templeton AJ, Vera-Badillo FE, et al. 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. Ann Oncol. 2015;26(4):743-749.
30. 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. 2014;66(2):330-336.
31. Nieuweboer AJ, de Graan AM, Hamberg P, et al. Effects of Budesonide on Cabazitaxel Pharmacokinetics and Cabazitaxel-Induced Diarrhea: A Randomized, Open-Label Multicenter Phase II Study. Clin Cancer Res. 2017;23(7):1679-1683.
32. Dolan RD, Laird BJA, Horgan PG, McMillan DC. The prognostic value of the systemic inflammatory response in randomised clinical trials in cancer: A systematic review. Crit Rev Oncol Hematol. 2018; 132:130-137.
33. Duran I, Hagen C, Arranz JA, et al. SNPs associated with activity and toxicity of cabazitaxel in patients with advanced urothelial cell carcinoma. Pharmacogenomics. 2016;17(5):463-471.
34. Oshiro C, Mangravite L, Klein T, Altman R. PharmGKB very important pharmacogene: SLCO1B1. Pharmacogenetics and genomics. 2010;20(3):211-216.
35. Ieiri I, Higuchi S, Sugiyama Y. Genetic polymorphisms of uptake (OATP1B1, 1B3) and efflux (MRP2, BCRP) transporters: implications for inter-individual differences in the pharmacokinetics and pharmacodynamics of statins and other clinically relevant drugs. Expert Opin Drug Metab Toxicol. 2009;5(7):703-729.
36. Scher HI, Jia X, de Bono JS, et al. Circulating tumour cells as prognostic markers in progressive, castration-resistant prostate cancer: a reanalysis of IMMC38 trial data. Lancet Oncol. 2009;10(3): 233-239.

37. Antonarakis ES, Lu C, Luber B, et al. Androgen Receptor Splice Variant 7 and Efficacy of Taxane Chemotherapy in Patients With Metastatic Castration-Resistant Prostate Cancer. JAMA Oncol. 2015; 1(5):582-591.
38. Antonarakis ES, Lu C, Wang H, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med. 2014;371(11):1028-1038.
39. Onstenk W, Sieuwerts AM, Kraan J, et al. Efficacy of Cabazitaxel in Castration-resistant Prostate Cancer Is Independent of the Presence of AR-V7 in Circulating Tumor Cells. Eur Urol. 2015;68(6):939-945.
40. Scher HI, Graf RP, Schreiber NA, et al. Assessment of the Validity of Nuclear-Localized Androgen Receptor Splice Variant 7 in Circulating Tumor Cells as a Predictive Biomarker for Castration-Resistant Prostate Cancer. JAMA Oncol. 2018;4(9):1179-1186.
41. Antonarakis ES, Armstrong AJ, Dehm SM, Luo J. Androgen receptor variant-driven prostate cancer: clinical implications and therapeutic targeting. Prostate Cancer Prostatic Dis. 2016;19(3):231-241.
42. Plymate SR, Sharp A, de Bono JS. Nuclear Circulating Tumor Cell Androgen Receptor Variant 7 in Castration-Resistant Prostate Cancer: The Devil Is in the Detail. JAMA Oncol. 2018;4(9):1187-1188.
43. Tagawa ST, Antonarakis ES, Gjyrezi A, et al. Expression of AR-V7 and ARv567es in circulating tumor cells correlates with outcomes to taxane therapy in men with metastatic prostate cancer treated in TAXYNERGY. Clin Cancer Res. 2018.
44. To SQ, Kwan EM, Fettke HC, et al. Expression of Androgen Receptor Splice Variant 7 or 9 in Whole Blood Does Not Predict Response to Androgen-Axis-targeting Agents in Metastatic Castration-resistant Prostate Cancer. Eur Urol. 2018;73(6):818-821.
45. Armstrong AJ, Halabi S, Luo J, et al. Prospective Multicenter Validation of Androgen Receptor Splice Variant 7 and Hormone Therapy Resistance in High-Risk Castration-Resistant Prostate Cancer: The PROPHECY Study. J Clin Oncol. 2019;37(13):1120-1129.
46. Gravis G, Fizazi K, Joly F, et al. Androgen-deprivation therapy alone or with docetaxel in non-castrate metastatic prostate cancer (GETUG-AFU 15): a randomised, open-label, phase 3 trial. Lancet Oncol. 2013;14(2):149-158.
47. Fizazi K, Tran N, Fein L, et al. Abiraterone acetate plus prednisone in patients with newly diagnosed high-risk metastatic castration-sensitive prostate cancer (LATITUDE): final overall survival analysis of a randomised, double-blind, phase 3 trial. Lancet Oncol. 2019.
48. James ND, de Bono JS, Spears MR, et al. Abiraterone for Prostate Cancer Not Previously Treated with Hormone Therapy. N Engl J Med. 2017;377(4):338-351.
49. Boeve LMS, Hulshof M, Vis AN, et al. Effect on Survival of Androgen Deprivation Therapy Alone Compared to Androgen Deprivation Therapy Combined with Concurrent Radiation Therapy to the Prostate in Patients with Primary Bone Metastatic Prostate Cancer in a Prospective Randomised Clinical Trial: Data from the HORRAD Trial. Eur Urol. 2019;75(3):410-418.
50. Parker CC, James ND, Brawley CD, et al. Radiotherapy to the primary tumour for newly diagnosed, metastatic prostate cancer (STAMPEDE): a randomised controlled phase 3 trial. Lancet. 2018; 392(10162):2353-2366.
51. Davis ID, Martin AJ, Stockler MR, et al. Enzalutamide with Standard First-Line Therapy in Metastatic Prostate Cancer. N Engl J Med. 2019.
52. Hoyle AP, Ali SA, James ND, et al. LBA4Effects of abiraterone acetate plus prednisone/prednisolone in high and low risk metastatic hormone sensitive prostate cancer. Annals of Oncology. 2018; 29(suppl_8).
53. Ramakrishnan Geethakumari P, Schiewer MJ, Knudsen KE, Kelly WK. PARP Inhibitors in Prostate Cancer. Curr Treat Options Oncol. 2017;18(6):37.
54. De Velasco MA, Uemura H. Prostate cancer immunotherapy: where are we and where are we going? Curr Opin Urol. 2018;28(1):15-24.

55. Fizazi K, Smith MR, Tombal B. Clinical Development of Darolutamide: A Novel Androgen Receptor Antagonist for the Treatment of Prostate Cancer. Clin Genitourin Cancer. 2018;16(5):332-340.
56. Dellis AE, Papatsoris AG. Perspectives on the current and emerging chemical androgen receptor antagonists for the treatment of prostate cancer. Expert Opin Pharmacother. 2019;20(2):163-172.

Chapter 11

Nederlandse samenvatting

Prostaatkanker heeft een hoge incidentie met meer dan 12.000 nieuwe gevallen per jaar in Nederland. De vijfjaarsoverleving van patiënten met prostaatkanker (prostaatcarcinoom) bedraagt circa 90%.[1] Prostaatkanker, welke nog niet uitgezaaid is, kan in het overgrote deel genezen worden door het chirurgisch verwijderen van de prostaat (en eventuele lymfklieren), of door lokale bestraling van de prostaatloge. Indien de ziekte terugkeert met uitzaaiingen (metastasen) of wanneer ten tijde van de diagnose de ziekte al gemetastaseerd is, wordt systemische behandeling ingezet om de prostaatkanker te onderdrukken en de overleving te verlengen. Systemische behandeling is een behandeling die alle cellen in het hele lichaam bereikt, zoals bijvoorbeeld chemotherapie. Sinds 1941 is bekend dat het onderdrukken van de testosteron productie in de testis door chirurgische castratie de prostaatkanker gemiddeld 20 maanden onder controle kan houden, daarna wordt de ziekte castratieresistent.[2] Later werd anti-hormonale medicatie ontdekt, die de hypofyse-hypothalamus-gonaden as verstoort, wat chemische castratie of androgeen-deprivatie therapie (ADT) wordt genoemd.[3] Jarenlang is castratie de enige behandeloptie voor gemetastaseerde prostaatkanker geweest.

Vanaf 2004 is de eerste additionele systemische therapie, het chemotherapeuticum docetaxel geïntroduceerd.[4] In de daaropvolgende jaren zijn er meer antikankerbehandelingen voor gemetastaseerd prostaatkanker ontwikkeld, zoals het nieuwe chemotherapeuticum cabazitaxel, de nieuwe anti-hormonale middelen enzalutamide en abiraterone en verder radioactief radium-223.[4-9] Taxanen (docetaxel en cabazitaxel) stabiliseren de celdeling door in te grijpen op de structuur binnen de cel, wat leidt tot natuurlijke celdood (apoptose). Daarnaast remmen ze het transport van de androgeenreceptor naar de celkern.[10] Anti-hormonale middelen zijn voornamelijk effectief door het verstoren van de werking van de AR. Zo bindt enzalutamide aan de AR en deactiveert hierdoor de werking van de AR. Abiraterone vermindert de productie van testosteron uit de bijnier en in de tumor zelf door het remmen van het CYP17-enzym, dat essentieel is voor de omzetting van progesteron naar testosteron. De anti-hormonale middelen richten zich voornamelijk op de androgeenreceptor (AR), omdat deze een belangrijke rol speelt bij progressie van ziekte ondanks het castratieniveau van testosteron (<50 ng/dL). Radium-223 wordt alleen ingezet bij patiënten met botmetastasen. Radioactief radium-223 werkt door afgifte van lokale straling nadat het door het lichaam is ingebouwd in de botten, voornamelijk op plekken waar uitzaaiingen zitten. Deze behandelopties kunnen in de castratieresistente fase van de ziekte worden ingezet en laten in toenemende mate additionele effectiviteit zien in de hormoonsensitieve fase (wanneer de ziekte nog gevoelig is voor castratie) van de ziekte.

Momenteel wordt onderzocht of verschillende combinaties van deze therapieën, met verschillende werkingsmechanismen, een mogelijk toegevoegd effect hebben op

overleving of progressievrije overleving in patiënten met gemetastaseerd castratieresistent prostaatcarcinoom (mCRPC).[11] Echter, bij combinatietherapieën is het van essentieel belang om te weten of er geneesmiddeleninteracties met klinische relevante gevolgen optreden. Zo kan er sprake zijn van toxische (te hoge) of subtherapeutische (te lage) levels van medicatie, indien het metabolisme (omzetting/verwerking) van het desbetreffende middel geremd of gestimuleerd wordt. Taxanen worden in de lever grotendeels gemetaboliseerd door het cytochroom-P450 iso-enzym CYP3A4.[12,13] Enzalutamide is bekend als een sterke CYP3A4-inductor (activator). Daarom hebben wij in **hoofdstuk 2** van dit proefschrift onderzocht of de preklinisch veelbelovende combinatie van enzalutamide en cabazitaxel invloed heeft op de concentratie van cabazitaxel in het bloed van de patiënt. Daaruit bleek dat bij gelijktijdige behandeling de concentratie van cabazitaxel met 22% daalt. Recent is gebleken dat een lagere dosering (20 mg/m^2) van cabazitaxel niet minder effectief is dan een hogere dosering (25 mg/m^2), maar wel minder bijwerkingen oplevert.[14] Daarom wordt momenteel de lagere dosering wereldwijd veelvuldig toegepast. Indien de combinatie verder onderzocht gaat worden in klinische trials, dient rekening gehouden te worden met de interactie en lijkt het niet gewenst om de concentratie van cabazitaxel bij voorbaat te verlagen door een lagere dosering.

Uitgaande van hetzelfde mechanisme hebben we in **hoofdstuk 3** onderzocht of de standaardcombinatie van docetaxel met prednison een interactie geeft. Prednison is een milde inductor van het CYP3A4 enzym, waardoor docetaxel gemetaboliseerd wordt. Zowel bij patiënten met gemetastaseerd hormoonsensitief prostaatcarcinoom (mHSPC) als mCRPC wordt vanaf de registratie van docetaxel, prednison aan de behandelstrategie toegevoegd. Prednison zelf heeft een effect op de PSA (prostate-specific antigen) respons, beinvloedt de productie van bijniertestosteron en vermindert mogelijk de bijwerkingen van docetaxel.[15,16] Echter brengt langdurig prednison gebruik ook nadelige effecten met zich mee, zoals osteoporose (botontkalking) en bijnierschorsinsufficiëntie. In een recent onderzoek met mHSPC patiënten is daarom docetaxel ook zonder prednison is gegeven, zonder duidelijke toename van docetaxel-geïduceerde bijwerkingen.[17] De resultaten van onze interactiestudie lieten geen significant effect van prednison op de concentratie van docetaxel zien. Vanuit farmacotherapeutisch oogpunt is er dus geen argument voor het weglaten dan wel toevoegen van prednison aan de chemotherapie. De mogelijk beschermende werking van prednison tegen docetaxel-geïnduceerde bijwerkingen en de individuele impact van prednison als therapie dient verder onderzocht te worden. Bij het ontbreken van een significant toegevoegde waarde van prednison dient dit afgewogen te worden tegen de nadelige bijwerkingen van langdurig prednison gebruik. Zeker voor mHSPC patiënten, die over het algemeen nog geen prednison gebruiken, kan het weglaten van prednison en daarmee het verkorten van de behandelperiode met prednison voordelig zijn.

Naast combinaties van bestaande middelen worden ook nieuwe middelen ontwikkeld. Apalutamide, een nieuw anti-hormonaal middel, heeft een vergelijkbare werking als enzalutamide.[18] Voor de veilige ontwikkeling van een nieuw middel dient men op de hoogte te zijn van de farmacodynamische effecten van het middel op het lichaam. In **hoofdstuk 4** hebben we in een internationale multi-centerstudie onderzocht wat het effect van apalutamide was op de geleidingstijden van het hart. Daaruit bleek een milde, niet klinisch relevant effect op de duur van het elektrisch herstel van het hart bij steady-stateconcentraties van apalutamide. Er werden geen andere essentiële veranderingen op het hartfilmpje (electrocardiogram; ECG) geconstateerd, waarna het middel verder ontwikkeld kon worden. Momenteel is apalutamide in de VS goedgekeurd als therapie in niet-gemetastaseerd CRPC. In Nederland heeft apalutamide op dit moment nog geen plek binnen de behandeling van gemetastaseerd prostaatkanker gekregen vanwege gebrek aan bewijs voor een daadwerkelijke overlevingswinst ten opzichte van andere behandelingen.[19] In de registratietrial van apalutamide is alleen aangetoond dat de metastase-vrije overleving verlengd wordt bij gebruik van apalutamide ten opzichte van placebo in patiënten met niet-gemetastaseerd CRPC.[20] Deze fase van de ziekte komt in Nederland bijna niet voor omdat patiënten die een PSA stijging hebben maar geen tekenen van gemetastaseerde ziekte, in Nederland niet gecastreerd worden. In Amerika wordt dat al wel gedaan waardoor patiënten dus castratieresistent kunnen raken alvorens zij radiologisch gemetastaseerd zijn. In mei 2019 is vanuit een klinische fase III trial gebleken dat apalutamide wel overlevingswinst geeft bij patiënten met mHSPC.[21] Hiermee bestaat de mogelijkheid dat apalutamide binnenkort toch op de Nederlandse markt beschikbaar wordt.

Om uit de vele middelen die beschikbaar zijn voor prostaatkanker de juiste keuze voor een individuele patiënt te kunnen maken wordt gezocht naar 'prognostische' en 'predictieve' factoren die respectievelijk de prognose en de respons op een bepaalde therapie kunnen voorspellen. In een grote dataset (van de eerdere CABARESC-studie[22]) met mCRPC patiënten die behandeld waren met cabazitaxel, hebben we gekeken of we prognostische factoren konden identificeren en eerder beschreven prognostische factoren konden bevestigen (**hoofdstuk 5**). Daaruit bleken baselinefactoren zoals WHO performance status (WHO PS; conditie van de patiënt), hemoglobine (Hb), alkalisch fosfatase (AF) en albumine voorspellend voor overleving. Daarnaast gaven Hb en AF ook een inzicht in de kans op PSA respons. Dit komt overeen met voorgaande publicaties, maar parameters als PSA, neutrofielen/lymfocyten ratio en tijd sinds vorige chemotherapie konden we in onze dataset niet valideren als voorspellers voor overleving.[23-25] Desalniettemin kan de situatie van een individuele patiënt aan de hand van de geïdentificeerde baselinefactoren op dat moment worden ingeschat, hierdoor kan de prognose maar vooral de timing van de behandeling bepaald worden. Als voorbeeld; wanneer de geïdentificeerde prognostische

factoren een ongunstige verandering laten zien, kan dit de behandelend arts helpen om tijdig te starten met de cabazitaxel behandeling waardoor de kans op deze behandeling niet gemist wordt door een te snel verslechterde conditie van de patiënt.

Naast voorspellers voor overleving kunnen patiëntfactoren ook voorspellend zijn voor de ontwikkeling van bijwerkingen. Binnen de oncologie is bijvoorbeeld bekend dat dragers van genetische variaties in het *DPYD*-gen een verhoogde kans op toxiciteit hebben wanneer ze met fluoropyrimidines worden behandeld.[26] Voor prostaatkanker patiënten zijn dergelijke genetische factoren helaas nog niet bekend, maar het belang ervan is des te groter gezien de verscheidenheid aan behandelmogelijkheden. In **hoofdstuk 6** hebben we daarom gekeken naar de associatie tussen veel voorkomende genetische variaties ('single-nucleotide polymorphisms'; SNPs) en de hoogte van de concentratie van cabazitaxel in het bloed, het ontstaan van bijwerkingen en de overleving bij mCRPC patiënten die behandeld worden met cabazitaxel. Geen van de onderzochte SNPs was geassocieerd met de farmacokinetiek van cabazitaxel of overleving. Voor het ontstaan van bijwerkingen was er in ons cohort een associatie met genetische variaties in genen coderend voor transporteiwitten (het *SLCO*-gen). Echter konden we deze associatie niet ondersteunen met farmacologische veranderingen of verklaren op basis van het veranderde werkingsmechanisme van het eiwit. Op basis hiervan lijken de gevonden resultaten vals-positief en impliceren dat er in de toekomst geen plaats is voor *SLCO1B1* bepaling ter voorspelling van therapierespons of bijwerkingen bij mCRPC patiënten die behandeld worden met cabazitaxel.[27,28]

Een veelvuldig onderzochte prognostische factor bij patiënten met kanker is de hoeveelheid circulerende tumorcellen. Circulerende tumor cellen (CTCs) zijn kankercellen die losgekomen zijn van de primaire tumor of uitzaaiingen en via het bloed in andere organen in het lichaam terecht komen, waar ze nieuwe uitzaaiingen kunnen vormen. Via een simpele bloedafname (liquid biopsy) kunnen in ongeveer 60% van de patiënten met uitgezaaide epitheliale kanker CTCs geïdentificeerd worden. Bij bepaalde tumoren, waaronder prostaatkanker, is aangetoond dat het aantal CTCs voorspellend is voor overleving zowel voor als na een nieuwe behandeling.[29] Een 'unfavorable' CTC aantal van 5 of meer (per 7.5mL bloed) geeft een slechtere prognose dan een CTC aantal van minder dan 5/7.5 mL bloed.

Naast de voorspellende waarde van het aantal CTCs, bezitten de CTCs ook genetische informatie van de tumor. Deze kan over de tijd en onder invloed van anti-kankermedicatie veranderen. Momenteel gaat veel aandacht uit naar het identificeren van genetische tumoreigenschappen die voorspellend zijn voor de respons op bepaalde therapie, waardoor een geïndividualiseerde therapiekeuze gemaakt kan

worden. Zoals eerder benoemd is de AR van belang voor progressie van ziekte en zijn de meeste behandelmethodes erop gericht de functie van de AR te remmen. Een resistentiemechanisme tegen anti-kankermiddelen is het vormen van genetische variaties van de AR, zoals de androgeen-receptor splice variant 7 (*AR-V7)*. Deze variant van de AR blijft ook in de afwezigheid van androgenen actief. Anti-hormonale behandeling met enzalutamide en abiraterone blijkt minder effectief bij patiënten met *AR-V7*, terwijl die niet geldt voor behandeling met taxanen.[30-32] Daarom wordt bij prostaatkanker *AR-V7* status als mogelijk predictieve factor gezien. Echter blijft de klinische waarde van *AR-V7* onzeker. Er zijn namelijk aanwijzingen dat *AR-V7* meer gerelateerd is aan de mate van ziekte-uitbreiding en dat suggereert dat *AR-V7* positieve patiënten überhaupt een slechtere prognose hebben, ongeacht de behandeling.[33-35] Om de waarde van *AR-V7* beter te kunnen begrijpen hebben we in **hoofdstuk 7** de associatie tussen *AR-V7* status, het aantal CTCs en overleving uiteengezet bij patiënten die voornamelijk behandeld zijn met cabazitaxel. *AR-V7* blijkt in deze groep geen additionele prognostische waarde te hebben ten opzichte van bekende prognostische factoren. En hoewel er in het gehele cohort een associatie tussen *AR-V7* en het aantal CTCs werd gevonden, was de associatie niet aanwezig bij patiënten met ≥5 CTCs/7.5 mL bloed. Deze groep heeft bij voorbaat een slechtere prognose, met een gemiddelde overleving van circa 7 maanden. Zij zullen daarom extra gebaat zijn bij een marker die therapierespons kan voorspellen, zodat direct de juiste therapie gestart kan worden. Aanvullend onderzoek in de toekomst zal moeten uitwijzen of *AR-V7* deze rol in deze patiëntengroep daadwerkelijk kan vervullen.

Buiten de zoektocht naar factoren die overleving of therapierespons bij voorbaat kunnen voorspellen, zijn er ook indicatoren nodig die tijdens een behandeling eenvoudig kunnen weergeven of een patiënt op de behandeling reageert. Wanneer tijdens een behandeling het CTC aantal daalt van ≥5 CTCs naar <5 CTCs wordt dat CTC-respons genoemd. Echter is nog onduidelijk wat de waarde van CTC-respons tijdens de behandeling is, en of dit beleidsconsequenties heeft. Om meer inzicht te krijgen in de waarde van CTC-respons hebben we in **hoofdstuk 8** in een kleine groep van 20 mCRPC patiënten met zeer uitgebreide voorgeschiedenis die destijds met cabazitaxel behandeld werden, de CTC-respons geobserveerd. Er bleek na 9 weken behandeling in 15% van de patiënten sprake van een CTC-respons. Er waren te weinig patiënten met een CTC-respons om dit te correleren aan overleving. Daarnaast hebben we de *AR-V7* status bepaald voor en tijdens de behandeling en zagen we in 45% van de patiënten een conversie van *AR-V7* positief naar *AR-V7* negatief na 9 weken behandeling met cabazitaxel. Dit wekt de suggestie dat cabazitaxel de tumor mogelijk weer gevoelig kan maken voor anti-hormonale behandeling.

In **hoofdstuk 9** richten we ons tenslotte in een uitgebreid overzichtsartikel (review) op de recente ontwikkelingen in de behandeling van hormoonsensitief prostaatkanker.

In grote gerandomiseerde studies (CHAARTED, STAMPEDE, LATIDUDE) is een langere overlevingswinst gebleken wanneer docetaxel en abiraterone in combinatie met ADT gebruikt worden in de hormoonsensitieve fase ten opzichte van de castratieresistente fase.[17,36-38] Daarnaast blijkt lokale radiotherapie op de prostaat ook overlevingswinst te geven in patiënten met mHSPC en onlangs zijn daar apalutamide en enzalutamide aan toegevoegd.[21,39,40] Ondanks de duidelijke overlevingswinst bij het vervroegen van additionele therapie naast ADT, blijft onduidelijk welke patiënt het meeste gebaat is bij welke therapie. Vooralsnog is de mate van uitzaaiing de meest relevante factor voor het bepalen van de behandeling. In nagenoeg alle trials werd onderscheid gemaakt tussen hoog- en laag-volume ziekte. Hoewel de definitie hiervan per trial verschillend was, wordt over het algemeen de CHAARTED definitie aangehouden waarbij de aanwezigheid van uitzaaiingen in andere organen of de aanwezigheid van 4 of meer uitzaaiingen in het bot (waarvan tenminste 1 buiten het bekken) als hoog-volume ziekte wordt gezien. Patiënten met hoog-volume ziekte bleken voornamelijk baat te hebben bij behandeling met ADT + docetaxel/abiraterone, terwijl lokale radiotherapie/abiraterone in combinatie met ADT ingezet kan worden bij laag-volume patiënten. Daarnaast kan de co-morbiditeit van de patiënt een aanvullende bepalende factor zijn. Aangezien meer cardiale events voorkwamen bij behandeling met abiraterone, heeft het de voorkeur om patiënten met een cardiale voorgeschiedenis of verhoogd risico op cardiale events te behandelen met docetaxel.

In de nabije toekomst zullen nieuwe behandelingen en behandelcombinaties hun intrede doen, waardoor de behandeling van patiënten met gemetastaseerd prostaatkanker zich blijft ontwikkelen en een multidisciplinaire aanpak vergt voor het beste advies. Onderzoek naar farmacologische effecten van de (combinaties van) medicatie is daarbij belangrijk om de effectiviteit en veiligheid te kunnen waarborgen. Daarnaast zal wereldwijd veel aandacht uitgaan naar identificeren van biomarkers via translationeel onderzoek. Hoewel er nog een hoop openstaande issues zijn, blijft het vermoeden dat *AR-V7* van predicitieve waarde zal zijn voor mannen met mCRPC. Er zal daarbij een internationale consensus bereikt moeten worden over de manier waarop *AR-V7* wordt bepaald, en wat de strikte criteria worden om een patient *AR-V7* positief of negatief te noemen. Daarnaast heeft CTC-respons met het bepalen van de genetische informatie van de CTC, potentie om als early biomarker voor treatment respons in klinische trials gebruikt te gaan worden, maar daarvoor zal eerst meer bekend moeten worden over de kinetiek (natuurlijk beloop) van CTCs en blijft de discussie of surrogaateindpunten ooit overall survival kunnen vervangen. Voor de hand ligt dat aanvullend onderzoek naar de waarde van liquid biopsies in de komende jaren zoveel informatie gaat opleveren dat het standaard behandelbeleid van gemetastaseerde prostaatkanker-patiënten omgezet kan worden naar een geïndividualiseerd behandeladvies, dat in samenspraak met een goed geïnformeerde patiënt kan leiden tot een weloverwogen behandelplan.

REFERENCES

1. IKNL. Cijfers over Kanker. 2019; https://www.cijfersoverkanker.nl/. Accessed 30-05-2019, 2019.
2. Higgins C, Hodges CO. Studies on prostatic cancer. 1. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res. 1941;1:293-297.
3. Schally AV, Arimura A, Baba Y, et al. Isolation and properties of the FSH and LH-releasing hormone. Biochem Biophys Res Commun. 1971;43(2):393-399.
4. 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.
5. 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.
6. 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.
7. 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.
8. Beer TM, Armstrong AJ, Rathkopf DE, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med. 2014;371(5):424-433.
9. 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.
10. van Soest RJ, van Royen ME, de Morree ES, et al. Cross-resistance between taxanes and new hormonal agents abiraterone and enzalutamide may affect drug sequence choices in metastatic castration-resistant prostate cancer. Eur J Cancer. 2013;49(18):3821-3830.
11. Sternberg CN. Improving Survival for Metastatic Castrate-resistant Prostate Cancer: Will Combination Therapy Help Us To Move Forward? Eur Urol. 2016;70(5):722-723.
12. Pean E, Demolis P, Moreau A, et al. The European Medicines Agency review of cabazitaxel (Jevtana(R)) for the treatment of hormone-refractory metastatic prostate cancer: summary of the scientific assessment of the committee for medicinal products for human use. Oncologist. 2012;17(4):543-549.
13. Nieuweboer AJ, de Morree ES, de Graan AJ, Sparreboom A, de Wit R, Mathijssen RH. Inter-patient variability in docetaxel pharmacokinetics: A review. Cancer Treat Rev. 2015;41(7):605-613.
14. Eisenberger M, Hardy-Bessard AC, Kim CS, et al. Phase III Study Comparing a Reduced Dose of Cabazitaxel (20 mg/m2) and the Currently Approved Dose (25 mg/m2) in Postdocetaxel Patients With Metastatic Castration-Resistant Prostate Cancer-PROSELICA. J Clin Oncol. 2017;35(28):3198-3206.
15. Sartor O, Weinberger M, Moore A, Li A, Figg WD. Effect of prednisone on prostate-specific antigen in patients with hormone-refractory prostate cancer. Urology. 1998;52(2):252-256.
16. Hu J, Chen Q. The role of glucocorticoid receptor in prostate cancer progression: from bench to bedside. Int Urol Nephrol. 2017;49(3):369-380.
17. Sweeney CJ, Chen YH, Carducci M, et al. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. N Engl J Med. 2015;373(8):737-746.
18. Koshkin VS, Small EJ. Apalutamide in the treatment of castrate-resistant prostate cancer: evidence from clinical trials. Ther Adv Urol. 2018;10(12):445-454.
19. BOM. Apalutamide of enzalutamide bij het niet-gemetastaseerd castratieresistent prostaatcarcinoom. 2019; https://www.nvmo.org/bom/apalutamide-of-enzalutamide-bij-het-niet-gemetastaseerd-castratieresistent-prostaatcarcinoom/?meta. Accessed 31-05-2019, 2019.

20. Smith MR, Saad F, Chowdhury S, et al. Apalutamide Treatment and Metastasis-free Survival in Prostate Cancer. N Engl J Med. 2018;378(15):1408-1418.
21. Chi KN, Agarwal N, Bjartell A, et al. Apalutamide for Metastatic, Castration-Sensitive Prostate Cancer. N Engl J Med. 2019.
22. Nieuweboer AJ, de Graan AM, Hamberg P, et al. Effects of Budesonide on Cabazitaxel Pharmacokinetics and Cabazitaxel-Induced Diarrhea: A Randomized, Open-Label Multicenter Phase II Study. Clin Cancer Res. 2017;23(7):1679-1683.
23. Halabi S, Lin CY, Small EJ, et al. Prognostic model predicting metastatic castration-resistant prostate cancer survival in men treated with second-line chemotherapy. J Natl Cancer Inst. 2013;105(22): 1729-1737.
24. Halabi S, Lin CY, Kelly WK, et al. Updated prognostic model for predicting overall survival in first-line chemotherapy for patients with metastatic castration-resistant prostate cancer. J Clin Oncol. 2014; 32(7):671-677.
25. van Soest RJ, Templeton AJ, Vera-Badillo FE, et al. 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. Ann Oncol. 2015;26(4):743-749.
26. Henricks LM, Lunenburg C, de Man FM, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. Lancet Oncol. 2018; 19(11):1459-1467.
27. Duran I, Hagen C, Arranz JA, et al. SNPs associated with activity and toxicity of cabazitaxel in patients with advanced urothelial cell carcinoma. Pharmacogenomics. 2016;17(5):463-471.
28. Marsh S. Taxane pharmacogenetics. Per Med. 2006;3(1):33-43.
29. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med. 2004;351(8):781-791.
30. Antonarakis ES, Lu C, Wang H, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med. 2014;371(11):1028-1038.
31. Antonarakis ES, Lu C, Luber B, et al. Androgen Receptor Splice Variant 7 and Efficacy of Taxane Chemotherapy in Patients With Metastatic Castration-Resistant Prostate Cancer. JAMA Oncol. 2015; 1(5):582-591.
32. Onstenk W, Sieuwerts AM, Kraan J, et al. Efficacy of Cabazitaxel in Castration-resistant Prostate Cancer Is Independent of the Presence of AR-V7 in Circulating Tumor Cells. Eur Urol. 2015;68(6):939-945.
33. Plymate SR, Sharp A, de Bono JS. Nuclear Circulating Tumor Cell Androgen Receptor Variant 7 in Castration-Resistant Prostate Cancer: The Devil Is in the Detail. JAMA Oncol. 2018;4(9):1187-1188.
34. Abida W, Cyrta J, Heller G, et al. Genomic correlates of clinical outcome in advanced prostate cancer. Proceedings of the National Academy of Sciences. 2019:201902651.
35. Tagawa ST, Antonarakis ES, Gjyrezi A, et al. Expression of AR-V7 and ARv(567es) in Circulating Tumor Cells Correlates with Outcomes to Taxane Therapy in Men with Metastatic Prostate Cancer Treated in TAXYNERGY. Clin Cancer Res. 2019;25(6):1880-1888.
36. James ND, Sydes MR, Clarke NW, et al. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. Lancet. 2016;387(10024):1163-1177.
37. James ND, de Bono JS, Spears MR, et al. Abiraterone for Prostate Cancer Not Previously Treated with Hormone Therapy. N Engl J Med. 2017;377(4):338-351.
38. Fizazi K, Tran N, Fein L, et al. Abiraterone acetate plus prednisone in patients with newly diagnosed high-risk metastatic castration-sensitive prostate cancer (LATITUDE): final overall survival analysis of a randomised, double-blind, phase 3 trial. Lancet Oncol. 2019;20(5):686-700.

39. Parker CC, James ND, Brawley CD, et al. Radiotherapy to the primary tumour for newly diagnosed, metastatic prostate cancer (STAMPEDE): a randomised controlled phase 3 trial. Lancet. 2018; 392(10162):2353-2366.
40. Davis ID, Martin AJ, Stockler MR, et al. Enzalutamide with Standard First-Line Therapy in Metastatic Prostate Cancer. N Engl J Med. 2019.

Appendices

List of publications
Co-author affiliations
Curriculum Vitae
Dankwoord
PhD portfolio

LIST OF PUBLICATIONS

Belderbos BPS, de Wit R, Hoop EO, Nieuweboer A, Hamberg P, van Alphen RJ, Bergman A, van der Meer N, Bins S, Mathijssen RHJ, van Soest RJ.
Prognostic factors in men with metastatic castration-resistant prostate cancer treated with cabazitaxel.
Oncotarget. 2017 Nov 16;8(63):106468-106474.

Belderbos BPS, Bins S, van Leeuwen RWF, Oomen-de Hoop E, van der Meer N, de Bruijn P, Hamberg P, Overkleeft ENM, van der Deure WM, Lolkema MP, de Wit R, Mathijssen RHJ.
Influence of Enzalutamide on Cabazitaxel Pharmacokinetics: a Drug-Drug Interaction Study in Metastatic Castration-resistant Prostate Cancer (mCRPC) Patients.
Clin Cancer Res. 2018 Feb 1;24(3):541-546.

Belderbos BPSI, de Wit R, Chien C, Mitselos A, Hellemans P, Jiao J, Yu MK, Attard G, Bulat I, Edenfield WJ, Saad F.
An open-label, multicenter, phase Ib study investigating the effect of apalutamide on ventricular repolarization in men with castration-resistant prostate cancer.
Cancer Chemother Pharmacol. 2018 Sep;82(3):457-468. doi: 10.1007/s00280-018-3632-6.

Belderbos BPS, Hussaarts KGAM, van Harten LJ, Oomen-de Hoop E, de Bruijn P, Hamberg P, van Alphen RJ, Haberkorn BCM, Lolkema MP, de Wit R, van Soest RJ, Mathijssen RHJ.
Effects of prednisone on docetaxel pharmacokinetics in men with metastatic prostate cancer: A randomized drug-drug interaction study.
Br J Clin Pharmacol. 2019 May;85(5):986-992.

Belderbos BPS, de Wit R, Lolkema MPJ, Mathijssen RHJ, van Soest RJ.
Novel Treatment Options in the Management of Metastatic Castration-naïve Prostate Cancer; Which Treatment Modality to choose?
Ann Oncol. 2019 Jul 24. Epub ahead of print.

Belderbos BPS, Sieuwerts AM, Oomen-de Hoop E, Mostert B, Kraan J, Hamberg P, Van MN, Beaufort CM, Onstenk W, van Soest RJ, Martens J, Sleijfer S, de Wit R, Mathijssen RHJ, Lolkema MPJ.
Associations between *AR-V7* Status in Circulating Tumor Cells, Circulating Tumor Cell Count and Survival in Men with Metastatic Castration-Resistant Prostate Cancer.
Eur J Cancer. 2019 Sep 19;121:48-54.

Belderbos BPS, de With M, Singh RK, Agema BC, El Bouazzaoui S, Oomen – de Hoop E, de Wit R, van Schaik RHN, Mathijssen RHJ, Bins S.

The of Single-Nucleotide Polymorphisms and Toxicity Cabazitaxel Treated Patients with Metastatic Castration-Resistant Prostate Cancer.

Submitted for publication

CO-AUTHOR AFFILIATIONS

Co-author	Chapter	Affiliations
Anieta M. Sieuwerts[†]	7	Erasmus MC, Rotterdam
Anna Mitselos	4	Janssen Research & Development, Beerse, Belgium
André Bergman	5	Antoni van Leeuwenhoek, Amsterdam
Annemieke Nieuweboer	5	Erasmus MC, Rotterdam
Bianca Mostert	7	Erasmus MC, Rotterdam
Bram C. Agema	6	Erasmus MC, Rotterdam
Brigitte C.M. Haberkorn	3	Maasstad Ziekenhuis, Rotterdam
Caly Chien	4	Janssen Research & Development, Spring House, USA
Corine M. Beaufort	7	Erasmus MC, Rotterdam
Esther Oomen-de Hoop	2,3,5,6,7	Erasmus MC, Rotterdam
Esther N.M. van Overkleeft	2	Ikazia ziekenhuis, Rotterdam
Fred Saad	4	Centre Hospitalier de l'Université de Montréal/CRCHUM, Montreal, Canada
Gerard Attard	4	Research Department of Oncology, UCL Cancer Institute, London, UK
Iurie Bulat	4	ARENSIA Exploratory Medicine's Research Unit, The Institute of Oncology, Chişinău, Republic of Moldova
Jaco Kraan	7	Erasmus MC, Rotterdam
James Jiao	4	Janssen Research & Development, Raritan, USA
Jeffrey W. Edenfield	4	GHS Cancer Institute, Greenville, USA
Joan B. Bolt-de Vries	8	Erasmus MC, Rotterdam
John Martens	7	Erasmus MC, Rotterdam
Koen G.A.M. Hussaarts	3	Erasmus MC, Rotterdam
Leonie J. van Harten	3	Erasmus MC, Rotterdam
Mai N. Van	7	Erasmus MC, Rotterdam
Martijn P. Lolkema	2,3,7,8,9	Erasmus MC, Rotterdam
Margaret K. Yu	4	Janssen Research & Development, Los Angeles, USA
Mirjam de With	6	Erasmus MC, Rotterdam
Nelly van der Meer	3,5	Erasmus MC, Rotterdam
Paul Hamberg	3,5,7	Sint Franciscus Vlietland Groep, Rotterdam
Peter de Bruijn	2,3	Erasmus MC, Rotterdam
Peter Hellemans	4	Janssen Research & Development, Beerse, Belgium
Rajbir K. Singh	6	Erasmus MC, Rotterdam
Robbert J. van Alphen	2,5	Elisabeth Twee Steden Ziekenhuis, Rotterdam
Robert J. van Soest	2,3,5,7,9	Erasmus MC, Rotterdam
Roelof W.F. van Leeuwen	2	Erasmus MC, Rotterdam
Ron H.J. Mathijssen	2,3,5,6,7,9	Erasmus MC, Rotterdam
Ron H.N. van Schaik	6	Erasmus MC, Rotterdam
Ronald de Wit	2,3,4,5,6,7,9	Erasmus MC, Rotterdam

Co-author	Chapter	Affiliations
Samira El Bouazzaoui	6	Erasmus MC, Rotterdam
Sander Bins	2,5,6	Erasmus MC, Rotterdam
Stefan Sleijfer	7	Erasmus MC, Rotterdam
Wendy Onstenk	7	Erasmus MC, Rotterdam
Wendy M. van der Deure	2	Groene Hart Ziekenhuis, Gouda

CURRICULUM VITAE

Bodine Pauline Sophie Ida Belderbos was born on November 19th 1988 in Breda, the Netherlands. After graduating from secondary school (De Nassau Scholengemeenschap, Breda) in 2007, she studied medicine at Erasmus University Rotterdam. In 2014, she obtained her medical degree. From November 2014 until December 2015 she worked as a Resident at the Department of Urology at the St. Franciscus Gasthuis in Rotterdam. She then started her PhD in January 2016 at the Department of Medical Oncology and Translation Pharmacology at the Erasmus MC Cancer Institute, Rotterdam, under supervision of Prof. Dr. R. de Wit and prof. Dr. A.H.J. Mathijssen. With her special interest in genitourinary tumors, the PhD focused on the pharmacological and biological perspectives of metastatic prostate cancer. As part of her Urology traineeship, she is currently working as a Resident at the General Surgery Department of the IJsslland Ziekenhuis in Rotterdam, supervised by Dr. P.G. Doornebosch. From 2021, she will continue her Urology residency in the Erasmus MC, under the supervision of Dr. J.R. Scheepe.

DANKWOORD

'Winnen doe je met zijn allen' (Johan Cruijff), oftewel het dankwoord.

Vol goede moed en enthousiasme stapte ik zo'n 4 jaar geleden dit promotietraject in. Mijn wetenschappelijke kennis reikte op dat moment niet verder dan wat retrospectief statusonderzoek op de Intensive Care in het kinderziekenhuis van Melbourne, waarbij ik me met name de fantastische ervaringen buiten het ziekenhuis nog kan herinneren. Een echte uitdaging dus, maar juist het gebrek aan wetenschappelijke ervaringen was mijn grootste drijfveer om veel te leren en dit promotietraject succesvol af te ronden. Echter werden de strubbelingen van een promotie mij ook al snel duidelijk. Het lijkt of geen promovendus zich kan onttrekken aan langzaam verlopende inclusies (die bij voorbaat altijd positiever worden ingeschat dan uiteindelijk blijken), duizelingwekkende databases (waar je met 4 weken statistiek van de opleiding geneeskunde echt niks van kan maken) en eindeloze submissies (die je subtiel doch duidelijk de impact van jouw onderzoek in de wetenschappelijke wereld laten zien). Des te belangrijker waren in deze tijd de mensen om me heen.

Als eerst wil ik alle patiënten bedanken die hebben deelgenomen aan de verschillende studies beschreven in dit boekje. Uw inzet voor de wetenschap, in een moeilijk periode van uw eigen leven is bewonderenswaardig. Zonder uw bijdrage, valt geen onderzoek te voltooien.

Mijn beide promotoren wil ik bedanken. Prof. dr. de Wit, Beste Ronald, hoewel ik nooit aan jouw wetenschappelijke standaard heb kunnen tippen, heb ik de afgelopen jaren een hele hoop geleerd, zowel op professioneel als op persoonlijk vlak. Dankzij jouw expertise en internationale aanzien heb ik de kans gekregen aan mooie studies mee te werken. Bedankt dat je mij de mogelijkheid hebt gegeven kennis te nemen van de wetenschappelijke wereld.
Prof. dr. Mathijssen, Beste Ron, de manier waarop jij je promovendi begeleidt is bewonderenswaardig. Binnen een paar jaar is de onderzoeksgroep translationele farmacologie uitgegroeid van een paar promovendi tot één groot team. De lat ligt daarbij hoog, maar de drempel is laag. Bedankt dat ik afgelopen paar jaar deel heb mogen uitmaken van dat team.

Dr. Lolkema, Beste Martijn, het enthousiasme waarmee je je vak uitoefent en je kennis overbrengt is aanstekelijk. Jouw didactische vaardigheden en geduld, maakten dat zelfs ik begreep wat je bedoelde. Bedankt voor de wijze lessen, ik zal ze missen.

Dr. Van Soest, Beste Robert, je telefoontje om te peilen of ik interesse had voor een promotietraject kan ik me nog goed herinneren. Dank voor je vertrouwen om deze stap te maken en je geduldige begeleiding waar ik op elk moment in de afgelopen jaren op terug kon vallen. Ik heb veel gehad aan je kritische blik en de manier waarop je het overbrengt, en ik hoop dat ik op een later moment in mijn opleiding nog meer van je mag leren. Succes om je ambities waar te maken, daar heb ík alle vertrouwen in.

Dr. Bins, Beste Sander, jij hebt me wegwijs gemaakt in de eerste stappen van mijn promotieonderzoek. Het was bijna irritant om te zien met welk gemak je analyses uitvoerde, figuren maakte en argumenten formuleerde in een pittige discussie, maar dat natuurlijk vooral uit bewondering. Ik heb genoten van onze relativerende gesprekken op de momenten dat het even moeilijk was. Ik vind het heel leuk dat je vandaag in de grote commissie wilde plaatsnemen.

De leden van de leescommissie wil ik bedanken voor het lezen van het manuscript en de bereidheid om van gedachte te wisselen.

Voor de patiënte inclusie, dataverzameling en logistieke zaken rondom de verschillende studies heb ik veel hulp gehad vanuit alle hoeken.
Allereerst wil ik de oncologen en arts-assistenten van het Erasmus MC bedanken voor hun bijdrage aan de inclusies en follow-up van studie patiënten. De artsen en onderzoekers van het maandelijkse prostaatkanker PhD overleg, dank voor jullie kritische vragen en opmerkingen die mijn studies ten goede kwamen. De verpleegkundigen van de afdeling B0-zuid in het oude Daniel den Hoed en de huidige oncologie afdeling in het nieuwe Erasmus MC, dank voor jullie lieve zorg voor studie patiënten en tijdige bloedafnames. Collega's van back&frontoffice, dank voor de verwerking van deze bloedsamples.
Dr. Hamberg, Beste Paul, en Suzan Ras, de goedlopende logistiek en samenwerking op uw afdeling/poli maakten het SFVG een van de best includerende ziekenhuizen voor de CABARESC, CABENZA, Doce-Pred en CABA-v7 studie. Dank daarvoor.
Alle medewerkers van het Clinical Trial Center die hebben bijgedragen aan de uitdagende, logistieke zaken rondom de CABARESC, Doce-Pred, CABA-v7 studie, wat hebben jullie mij veel werk uit handen genomen, dank voor jullie hulp!
Dank aan de medewerkers van de verschillende secretariaten voor het organiseren van afspraken met patiënten en (co-)promotoren.

Lieve collega's van het farmacolab, bedankt voor jullie advies wanneer ik even vastliep, voor jullie hulp wanneer ik het niet wist, maar vooral voor het lachen! Ik heb genoten van de maandagochtend koffietjes en de lunches in het park, de vrijdagmiddagborrels vanaf half 4 en de gezellige uitjes de afgelopen jaren.

Lieve uro-collega's, ook al kwam ik maar sporadisch boven, het was er altijd erg gezellig. Misschien maar goed dat ik daar niet zat, want anders was er niks van dit proefschrift terecht gekomen. Dank voor de introductie bij Dennis en de gezellig RUAG borrels, die we natuurlijk gewoon zullen blijven voortzetten!

Lieve Bruno, wat ben ik blij dat ik jou heb leren kennen tijdens mijn promotietijd. In een wereld waar iedereen maar door raast en meer wilt, is het heerlijk om thuis te komen bij iemand die je een andere kant van het leven laat zien. We hebben genoten van de vrijheid, en jij maakte deze periode voor mij echt een heel stuk leuker. Bedankt voor al je begrip en lieve zorgen de laatste hectische tijd voor het afronden van dit boekje en het starten van de opleiding. Ik kan altijd op je terugvallen.

Lieve Tim en Bob, dank dat jullie vandaag mijn paranimfen willen zijn. Het onaantastbare gevoel wat jullie mij geven wanneer we samen zijn, kan ik vandaag extra goed gebruiken. Wie doet me wat als ik jullie heb? Jullie vertrouwen, liefde en humor heeft me al zo ver gebracht en brengt mij vandaag de dag wel door. Tim, mijn grote broer waar ik altijd tegenop kijk, die altijd alles net een beetje sneller en beter kan. Je bent daarmee voor mij een enorme drijfveer. Ik bewonder hoe je alle ballen zo ogenschijnlijk makkelijk hoog houdt, ik geniet van je lieve fantastische gezinnetje en ik luister graag naar je advies. Bobba, mijn kleine grote broer, streeft me inmiddels aan alle kanten voorbij. Je bijzondere verschijning, je wijsheid en je humor maken dat iedereen zich jouw na een eerste ontmoeting nog kan herinneren. Ik kan altijd bij je terecht, voor ontspanning en een goed gesprek. Ik heb fantastische herinneringen aan onze mooie reis samen, en geniet altijd van je aanwezigheid.

Lieve pap en mam, bedankt voor de bijzonder liefdevolle thuishaven waar ik altijd steun kan vinden. In de vorm van een goed gesprek of een warme knuffel wijzen jullie mij de juiste richting. Wat jullie mij hebben gegeven zal ik nooit kunnen terug geven, maar ik zal proberen hetzelfde door te geven.
Ik ben jullie voor altijd dankbaar.

Persoonlijk dankwoord

PHD PORTFOLIO

Name: Bodine P.S.I. Belderbos
Department: Medical Oncology
Research School: MolMed

PhD period: January 2016 – June 2019
Promotors: Prof. R. de Wit; Prof. A.H.J Mathijssen
Co-promotors: Dr. M.P.J. Lolkema; Dr. R.J. van Soest

1. PhD training	Year	Workload (ECTS)
General Courses		
- Basiscursus Regelgeving Klinisch Onderzoek (BROK)	2016	1.5
- Scientific Integrity	2017	0.3
- Biostatistical Methods I: Basic Principles	2016	5.7
Specific Courses		
- CPO minicourse in methodology	2016	0.3
- Genetics for Dummies	2016	0.6
- MS Excel: Basic workshop	2016	0.3
- MS Excel: Advanced workshop	2016	0.4
- Basic introduction on SPSS	2017	1.0
- Biomedical English writing	2017	2.0
- Basic and Translation Oncology	2017	1.8
- English Biomedical Writing and Communication	2017	3.0
- Introduction in GraphPad Prism	2018	0.3
Seminars and Workshops		
- Training Open Clinica	2017	0.5
- Vena Workshop 'Pitch jezelf'	2017	0.5
- Department of Urology Symposia (Refereeravonden)	2016-2019	1.0
- Department of Oncology Symposia (Jong Oncologen avonden)	2016-2019	1.0
Presentations		
- Translational pharmacology meetings, Erasmus MC	2016-2019	1.5
- Medical oncology research meetings, Erasmus MC	2017-2019	1.5
- Annual meeting, Astellas ISR	2016-2017	1.0
- Annual meeting ASCO GU, Orlando	2017	0.5
- Expert meeting ASCO GU, Orlando	2017	0.5
- Scientific meeting, Erasmus MC	2017	0.5
- Annual meeting NFKVB, Utrecht	2018	0.5
- Annual meeting ESMO, Munchen	2018	0.5
- Annual meeting ICPAD, Amsterdam	2018	0.5
- Meeting Tour d'Europe, Rotterdam	2018	0.5
(Inter)national conferences		
- Annual meeting ASCO GU	2016	2.0
- Annual meeting NFKVB	2016-2018	1.5

1. PhD training	Year	Workload (ECTS)
- Annual meeting EAU	2018	2.0
- Annual meeting ESMO	2018	2.0
- Annual meeting ICPAD	2018	0.5
- Tour d'Europe	2018	1.0
Other		
- Scientific meeting, Medical Oncology, Erasmus MC	2016-2018	1.0

2. Teaching	Year	Workload (ECTS)
Supervising Master's Thesis		
- Leonie van Harten	2017	1.0
- Raji Singh	2019	1.0
Lecturing		
- Clinical examination, Anatomy and General Urology for medical students/ interns and OR assistants	2017	0.5
Total		**40.2**