Chapter 1

General introduction

Prostate cancer is the second deadliest cancer in men in Western countries, being only surpassed by lung cancer.[1] Prostate tumors initially respond to hormonal therapy, radiotherapy and/or surgery, therefore, patients in early stages of disease have a five year overall survival rate of nearly 100%.[2] However, once the tumor becomes castrate-resistant and metastasizes, overall survival decreases, and treatment options are limited though expanding. Currently, first-line therapy in the Netherlands for patients with metastatic castrate-resistant prostate cancer (mCRPC) consists of docetaxel with prednisone.[3] Second-line therapies consist of cabazitaxel (with prednisone), abiraterone acetate (with prednisone) and enzalutamide.[4-7] Other therapies, such as tasquinimod, are in advanced stages of clinical development.[8] These improved therapies have contributed to the steady increase in the five year overall survival rate of prostate cancer patients from 63% to 86% in the Netherlands over the past two decades.[9] Nevertheless, the incidence and absolute mortality of prostate cancer are rising, attributed to ageing of the population and the introduction of PSA screenings.[10, 11] Therefore, research aimed at further improving mCRPC treatment in order to increase survival and/or quality of life for mCRPC patients, remains of crucial importance.

The mCRPC population is viewed as a homogenous population; all patients receive similar treatment. However, patient and/or disease characteristics, such as race, may influence the patient's response to mCRPC treatment.[12] Until such determinants are identified, it is important that clinical trials assessing novel prostate cancer treatment recruit participants who adequately reflect the population that will receive this therapy in clinical practice. **Chapter 2** addresses whether enrollment of racial minorities in prostate cancer studies reflects the racial distribution in the average prostate cancer population. Under-representation of racial minorities may question the scientific justification of use of studied therapies in minorities.

Despite the introduction of various targeted therapies, taxane chemotherapy remains crucial in the palliative treatment of mCRPC. Taxanes consist of docetaxel or cabazitaxel, and act on cancer cells by targeting and stabilizing microtubules.[13] The incorrect formation of microtubules leads to mitotic arrest in cancer cells, as mitotic spindles cannot be formed correctly.[14] Taxanes have other antitumor functions as well, such as a nuclear accumulation of p53, resulting in enhanced p53-induced apoptosis.[15]
Cabazitaxel has been used in Dutch clinics for mCRPC patients who progressed during or after docetaxel treatment since June 2011. Between June 2010, when the drug received approval from the United States (US) Food and Drug Administration (FDA), and May 2011, 51 patients were treated with cabazitaxel in the Netherlands via a compassionate use program. Such programs enable patients, for whom no good treatment alternative is currently available, to receive therapy when it is not yet reimbursed by insurance companies, but expected to be in the near future. Patients included in the compassionate use program are being monitored

to assess efficacy and safety of the therapy. These results were collected, analyzed and reported, and it was tested whether certain subgroups of mCRPC patients have a better outcome (**Chapter 3**).

In addition to cabazitaxel, abiraterone was recently approved as a second-line therapy in mCRPC patients after docetaxel treatment. The optimal treatment sequence has not been studied. Therefore, treatment decisions are currently based on the preference of physicians and/or patients.[16] In the CAST-study, the clinical outcome of mCRPC patients treated sequentially with cabazitaxel and abiraterone after docetaxel was retrospectively evaluated (**Chapter 4**).

The success of taxanes spurred the search for other compounds that target cancer cells in mitosis. As reviewed in **Chapter 5 section A**, this resulted amongst others in the development of Eg5-inhibitors, polo-like kinase 1 (Plk1-)inhibitors and aurora kinase (AK-)inhibitors. Early clinical studies with such inhibitors did not show significant antitumor activity, resulting in skeptical views from some researchers. However, improved inhibitors do show clinical potential, and are currently being tested in clinical trials involving mCRPC patients (**Chapter 5 section B**).

Similar to other cancers, the concept that prostate cancer can be controlled or even cured by a single agent is being abandoned. This idea is being replaced by the hypothesis that it requires targeting of multiple cancer-related pathways with multiple therapies to control prostate cancer disease. Although testing of combination therapies in prostate cancer patients has steadily been growing, results of these studies have been disappointing.[17, 18] Most combination therapies were tested based on trial-and-error, and lacked a solid rationale. In **Chapter 6**, Analysis of Functional Annotation (AFA) was used to provide a rational combination strategy with histone deacetylase inhibitors (HDACIs) in prostate cancer. Histone deacetylases deacetylate lysine residues in N-terminal tails of histones, and overexpression of these enzymes results amongst others in decreased expression of tumor-suppressor genes and increased expression of oncogenes in prostate cancer.[19] Two HDACIs have been approved for their use in cutaneous T cell lymphomas, vorinostat (SAHA) and romidepsin; in solid tumors, HDACIs are not used outside the research setting yet. As HDACIs target many important cancer-related pathways, such as the DNA damage pathway,[20] this group of drugs has high potential for combination therapy. Therefore, HDACIs were used to identify potential novel combination therapies in the AFA study.
Based on the AFA results, it was hypothesized that combining HDACIs with agents that target mitotic enzymes such as Plk1- and AK-inhibitors may result in enhanced antitumor efficacy. Considering the potential of mitotic inhibitors in prostate cancer, combinations of HDACIs with Plk1- and AK-inhibitors were explored in preclinical studies (**Chapter 7** and **Chapter 8**,

respectively).

The development of inhibitors of the mitotic enzyme Eg5 was halted for prostate cancer treatment after a phase II trial with the Eg5-inhibitor ispinesib did not have significant effects on mCRPC patients.[21] Immunohistochemistry of tumor samples from these patients, who all had disease progression during or after docetaxel treatment, revealed that 15 out of 16 included patients did not have overexpression of Eg5 in the tumor. Therefore, it was concluded that Eg5-inhibitors would not be effective in mCRPC patients due to the lack of Eg5 overexpression.
However, a more recent study found that Eg5 is overexpressed in about 50% of all prostate cancers.[22] These results and the fact that Eg5 is mainly responsible for the separation of microtubules,[23] led to the rationale that Eg5 expression may be decreased upon docetaxel resistance, and may therefore be a marker for docetaxel response. This rationale is explored in **Chapter 9**.

Most prostate cancer-related deaths are contributed to cancer metastases.[2] Hence, treating metastases may be beneficial for mCRPC patients, reducing both morbidity and mortality of the disease. Radium-223 chloride is the first agent that has been shown to extend overall survival in cancer patients by exclusively targeting bone metastases.[24] It has been approved for the treatment of mCRPC patients with bone metastases by the US FDA, and has recently been introduced in Dutch clinical practice. In **Chapter 10**, the results leading to FDA approval of radium-223 are discussed, as well as the radionuclide's limitations.

Due to the clinical relevance of metastases, targeting genes involved in the formation and/or growth of prostate tumor metastases may result in significant clinical benefit for mCRPC patients. Two genes that play a crucial role in the formation, maintenance and/or growth of metastases, are N-myc downregulated gene 1 (NDRG1) and cyclin-dependent kinase 5 (CDK5). While NDRG1 is a metastasis suppressor gene in amongst others prostate cancer **(Chapter 11 section A)**,[25] CDK5 is necessary for metastasis formation in prostate and pancreatic cancer.[26, 27] In the final two chapters of this thesis, the Johns Hopkins Drug Library (JHDL) was used to perform a synthetically lethal high-throughput screen, thereby identifying small molecules that may target cancer cells based on the expression of NDRG1 **(Chapter 11 section B)** or CDK5 **(Chapter 12)**.

In conclusion, this thesis handles various aspects of research involved in the development of novel therapies to treat mCRPC patients. The scope of drug development ranges from basic research, by performing AFA on HDACI-treated cells, to clinical research, such as the CAST-study.

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