Clinical and Preclinical Treatment Aspects of Castration Resistant Prostate Cancer (CRPC)

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Clinical and Preclinical Treatment Aspects of Castration Resistant Prostate Cancer (CRPC)

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Het leven is kort, de kunst duurt lang;
de gelegenheid gaat voorbij;
proefnemingen zijn gevaarlijk
en een oordeel vormen is moeilijk.

Hippocrates, 460-377 v. Chr.

Voor mijn ouders

Contents

Chapter 1

Introduction to the thesis

INTRODUCTION TO THE THESIS

In the Western countries prostate cancer is the most frequently diagnosed cancer, except for skin cancer, and the second leading cause of male cancer deaths[1]. Prostate cancer tends to develop in men over the age of fifty and although it is one of the most prevalent types of cancer in men, many patients may never have symptoms, undergo no therapy, and eventually die from other causes. This is because cancer of the prostate is, in many cases, slow-growing, symptom-free, and since men with the condition are older they often die from causes unrelated to the prostate cancer. Men diagnosed with clinically localized prostate cancer have several treatment options available, including watchful waiting, definitive radiation therapy and surgery. However, a significant proportion of patients who present with cancer that appears to be localized will eventually develop incurable metastatic disease and ultimately succumb to death as a result of advanced disease. In patients with advanced disease androgen-deprivation therapies typically result in rapid responses, but eventually nearly all patients develop progressive castration-resistant disease. Historically, clinical management for metastatic castration resistant prostate cancer (CRPC) has been primarily focused on relieving symptoms. In the last decade the therapeutic spectrum has changed dramatically, and our understanding of the biology of patients with prostate cancer has become far more sophisticated[2]. The treatment paradigm first changed with the publication of 2 pivotal randomized clinical trials in 2004, which demonstrated for the first time a survival benefit with docetaxel-based therapy in patients with metastatic CRPC[3,4]. Since then, docetaxel chemotherapy has become the standard first-line treatment in patients with CRPC **(Chapter 2)**. Recently, the landscape for CRPC treatment has changed again with the FDA approval of three additional therapies, sipuleucel-T[5], cabazitaxel[6] and abiraterone acetate[7]. While the addition of these new treatment options is a great advance for patients with metastatic CRPC, there are many new questions arising regarding the optimal sequencing of these treatments as well as potential combinations of and old drugs.

Also there is an armentarium of other promising novel agents (e.g. TAK 700, MDV 3100), which are currently being tested in the clinical setting[8]. The development of many of the novel agents being tested in patients with advanced disease reflects a change in the understanding of the biology of CRPC[2]. However, despite all remarkable advances in therapy and insight in the biological heterogeneity in prostate cancer over the past decade, the prognosis of patients with CRPC remains poor. The aim of this thesis is to investigate possible new treatment options, including targeted therapy and drug combinations, for the treatment of CRPC.

The skeleton is typically the first site of metastasis in patients with CRPC, and bone metastases can result in severe bone pain and potentially debilitating fractures. Zoledronic acid, a third generation bisphosphonate, has shown to delay the onset and the

incidence of skeletal related events (SREs)[9], and was approved and quickly became established as the standard of care in the United States in 2002. In several European countries the introduction of bisphosphonates took a slower pace since the delay of SREs was considered a weak primary endpoint to justify expensive medication. In 2004, when docetaxel based chemotherapy became the standard of care for patients with CRPC, the question arose in several European countries, including the Netherlands, what the role of bisphosphonates was relative to the initiation of effective chemotherapy. For this purpose, we investigated the efficacy and safety profile of the addition of risedronate, a third generation bisphosphonate, to docetaxel-based chemotherapy in patients with CRPC with bone metastases **(Chapter 3)**.

Aurora kinases are important regulators of mitosis that are frequently overexpressed in prostate cancer[10]. Since Aurora kinases contribute to the progression of prostate cancer, it is assumed that targeted inhibition of these kinases might work as a valuable target for prostate cancer treatment **(Chapter 4)**. We therefore investigated the efficacy and toxicity of danusertib, a pan- Aurora kinase inhibitor, in two different dosing schedules in patients with docetaxel refractory metastatic CRPC **(Chapter 5)**.

Since hormonal therapy is closely monitored with the use of prostate-specific antigen (PSA) as a proved biomarker to follow disease progression, there is a relatively large population of patients who are experiencing hormonal therapy failure with a rising PSA as the only evidence. The dilemma how to treat these mainly asymptomatic patients represents a clinical problem for the medical oncologist and urologist that need to be answered.

Further hormonal manipulations with anti-androgens alone, such as bicalutamide, may result in a PSA response, but mostly for a short period of time without clinically meaningful responses and without prolongation of survival. Bicalutamide alone therefore has little or no clinically significant effects. The lack of effective secondary hormonal treatments contributes to a large unmet medical need for patients with asymptomatic chemonaive metastatic CRPC. Genetic inactivation of PTEN through either gene deletion or point mutation is reasonably common in prostate cancer and the resulting activation of phosphoinositide 3-kinase, AKT and mTOR provides a major therapeutic opportunity[11]. Recent data indicate that there is a significant cross-talk between the PI3K/Akt/mTOR and androgen receptor signalling pathways. Preclinical evidence showed synergistic activity of the combination of an anti-androgen and mTOR inhibitor[12]. Therefore, we analysed the safety profile and possible pharmacokinetic interaction of combining ridaforolimus, an mTOR inhibitor, with bicalutamide, a non-steroidal anti-androgen, in patients with asymptomatic, metastatic chemonaive CRPC **(Chapter 6)**.

The orthotopic human prostate cancer xenograft model PC346C, provides a unique and representative reflection of the clinical setting of CRPC in preclinical experiments, and may allow an initial guidance to predict the efficacy of new agents and especially

combination of compounds in clinical trials. In order to translate preclinical findings into clinical practice we studied if the addition of zibotentan (ZD 4054) and/or lenalidomide to docetaxel was able to enhance the effect of the anti-prostate cancer activity of docetaxel in orthotopic human prostate cancer xenograft model PC346C **(Chapter 7)**.
Finally, the results of this thesis are discussed and suggestions for further research are mentioned **(Chapter 8)**.
In conclusion, this thesis entitled "Clinical and Preclinical Treatment Aspects of Castration Resistant Prostate Cancer" explores different treatment options for patients with CRPC at different stage of their disease. The ultimate goal of these studies is to expand treatment options for patients with castration resistant prostate cancer in order to improve overall survival.

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Chapter 2

Chemotherapy in patients with Castration Resistant Prostate Cancer

H.J. Meulenbeld, A.P. Hamberg and R. de Wit

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INTRODUCTION

Prostate cancer is, excluding non-melanoma skin cancer, the most common cancer among men, with approximately 301,500 new cases and 67,800 deaths annually in the European Union[1]. Prostate cancer is a major clinical problem, not only because of its high incidence and mortality, but also because of the severe morbidity associated with the advanced stages of this disease.

Treatment for clinically localised disease consists of early intervention with surgery, radiation therapy (external beam or brachytherapy), androgen suppression, or observation. Treatment for metastatic prostate cancer is palliative. In patients with metastatic prostate cancer, androgen ablation therapy is almost universally accepted as the initial treatment of choice, with a response rate ranging from 80% to 90%. However, these tumours, at a median of 18 months, become androgen-independent and grow despite androgen ablation[2]. Because patients may respond to second- and third-line hormonal therapies, the Prostate-Specific Antigen Working Group (PCWG 2) advised the classification of tumours that are progressing with castrate levels of testosterone (serum testosterone levels < 50ng/dL) as castration resistant[3]. Hormone-refractory prostate cancer (HRPC) arises when disease progression continues despite secondary hormonal manoeuvres that may include anti-androgen withdrawal[4]. The mechanisms of the development of hormone resistance are largely unknown, although the molecular changes of the androgen receptor, e.g. mutation or amplification, can explain some observations[5].

Historically, clinical management for advanced prostate cancer has been primarily focused on controlling symptoms. Over the last two decades, treatment options for patients with CRPC have changed notably.

Prostate cancer was considered resistant to chemotherapy until the mid-1990s, when randomised trials showed that mitoxantrone with prednisone resulted in prostate specific antigen (PSA) responses greater than 50%, pain relief and improved quality of life (QoL) more frequently than prednisone alone[6,7]. In 2004, two landmark trials, TAX 327[8] and Southwest Oncology Group (SWOG) 99-16[2], showed for the first time a survival benefit in patients with advanced CRPC by docetaxel-based chemotherapy as compared to mitoxantrone plus prednisone.

CHEMOTHERAPY FOR PROSTATE CANCER

Mitoxantrone

Cytotoxic chemotherapy has been studied in the treatment of CRPC for many years. Until 1996, when Tannock and colleagues[7] reported a randomised phase III trial defining

a clear role for mitoxantrone based chemotherapy, there was no convincing evidence to suggest that chemotherapy was of benefit to a meaningful proportion of patients with CRPC[9]. In the trial by Tannock and colleagues, 161 men were randomised to receive mitoxantrone plus prednisone or prednisone alone. Palliative response, defined as pain relief and/or reduction in analgesic requirement, was observed in 23 of 80 patients (29%) who received mitoxantrone plus prednisone, and in 10 of 81 patients (12%) who received prednisone alone. Most responding patients had an improvement in QoL and a non-significant decrease in PSA levels. There was no difference in overall survival. Toxicity of combined therapy included grade 3 or 4 neutropenia in 45% of all treatment courses, although only 1% was complicated by fever. These data were confirmed by the Cancer and Leukaemia Group B (CALGB) 9182 trial, in which 242 patients with CRPC were randomised between mitoxantrone plus hydrocortisone or hydrocortisone alone[10]. The PSA response was greater with the mitoxantrone plus hydrocortisone regimen, and there was a possible benefit with respect to pain control in those given mitoxantrone. However, no difference in overall survival was observed. Despite any survival benefit, the results of these trials led to the adoption of mitoxantrone plus prednisone as the standard regimen prior to the development of docetaxel-based chemotherapy.

Proof-of-concept trials using docetaxel

Shortly after the reports of the mitoxantrone studies became available, proof-of-concept trials were being conducted to assess the feasibility and therapeutic potential of the taxanes. Several docetaxel-based regimens were investigated: a 3-weekly regimen, a weekly regimen (owing to the assumption that this regimen would be better tolerated in an elderly population), and a combination of docetaxel and estramustine. The PSA response rates in these phase I-II trials evaluating docetaxel-based regimens were higher (41–68%) than those reported previously in the mitoxantrone trials[11,12,13,14,15,16]. In addition, these trials were the first to report objective response rates of approximately 20–50% in patients with measurable disease[11,12,13,14,15,16]. Furthermore, a median survival of up to 27 months was reported in patients who received 3-weekly docetaxel[11]. These results prompted the initiation of two large randomised phase III studies, TAX 327 and SWOG 99-16, to further evaluate the anti-tumour activity of docetaxel in this setting[2,8].

Phase III trials of docetaxel-based therapy in advanced castration-resistant prostate cancer

TAX 327[8]

The TAX 327 study was a large international randomised trial which compared the effectiveness of three schedules: 3-weekly mitoxantrone (12 mg/m^2), 3-weekly docetaxel

(75 mg/m^2) and weekly docetaxel (30 mg/m^2), all combined with prednisone (5 mg twice daily) in patients with CRPC[8]. The treatment duration was 30 weeks in all treatment schedules. A total of 1006 patients were randomised from March 2000 to June 2002. The median overall survival was 16.5 months in the mitoxantrone group, 17.4 months in the group given weekly docetaxel and 18.9 months in the group given docetaxel every 3 weeks. This improvement in overall survival was statistically significant (*P*=0.009) in the group given docetaxel every 3 weeks, with a significant reduction in the risk of death of 24%. A recent published updated survival analysis of the TAX 327 confirmed the significantly better survival with extended follow-up[17]. Median survival time was 19.2 months in the 3-weekly docetaxel arm, 17.8 months in the weekly docetaxel arm, and 16.3 months in the mitoxantrone arm. More patients survived ≥3 years in the groups treated with 3-weekly docetaxel and weekly docetaxel (18.6% and 16.6%, respectively) compared with patients treated with mitoxantrone (13.5%). Due to crossover between the treatment arms after disease progression in more than 30% of the patients the survival benefit of the docetaxel-based treatment groups is likely underestimated as compared to the mitoxantrone group. A subset analysis showed that the survival benefit with 3-weekly docetaxel remains in all subgroups; with similar trends in survival for patients above and below the age of 65 years, for those with or without pain at baseline, and for those with baseline PSA greater than or less than the median value of 115 ng/mL. Hence, there is no indication that specific subgroups (e.g. elderly patients, significant pain at entry) had less benefit from treatment with docetaxel-based chemotherapy. A reduction in pain was significantly more frequent among patients receiving docetaxel every 3 weeks than among patients treated with mitoxantrone (35% versus 22% respectively; *P*=0.01). The rates of PSA response were significantly higher in both docetaxel groups compared with mitoxantrone (docetaxel every 3 weeks, 45%, weekly docetaxel 48% and mitoxantrone 32%; *P*<0.001 for both comparisons). Also, the QoL assessment showed a significant improvement with the 3-weekly schedule of docetaxel compared to those treated with mitoxantrone (22% versus 13%; *P*=0.009).

Grade 3/4 neutropenia was significantly more common in patients treated with the 3-weekly docetaxel (32%) than for those patients receiving weekly docetaxel or mitoxantrone (2% and 22%, respectively), although the incidence of febrile neutropenia was less than 3% in all treatment arms. Nausea and vomiting were common with all regimens (38% to 42%) and diarrhoea was significantly more frequent with both docetaxel schedules. Discontinuation of treatment with docetaxel was incidentally due to fatigue, musculoskeletal events, nail changes, sensory neuropathy, and infection whereas for mitoxantrone cardiac dysfunction was the major reason to discontinue therapy.

SWOG 99-16[2]

The multicentre phase III study SWOG 99-16 was built on the prejudice that the combination of docetaxel plus estramustine had the greatest therapeutic potential. SWOG 99-16 compared docetaxel (60 mg/m^2, day 2) plus estramustine (280 mg, three times daily, days 1–5) with mitoxantrone (12mg/m^2, day1) plus prednisone (5 mg twice daily). Both were given on a 21-day cycle, and dose escalation to docetaxel 70 mg/m^2 or mitoxantrone to 14 mg/m^2 was allowed on cycle 2 if no grade 3/4 toxicities were detected in the first cycle. A total of 770 men were randomised; of whom 69 patients were found ineligible. Median overall survival was significantly longer in the group treated with docetaxel plus estramustine than the group treated with mitoxantrone plus prednisone, 17.5 months versus 15.6 months, respectively (P=0.020), with a 20% reduction in the risk of death in the group treated with docetaxel plus estramustine. The median time to progression was 6.3 months in the group given docetaxel and estramustine and 3.2 months in the group given mitoxantrone and prednisone (P<0.001). The rates of PSA response were significantly higher in the docetaxel plus estramustine group compared with the mitoxantrone plus prednisone group (50% versus 27%; P<0.001). Patients treated with docetaxel plus estramustine did not demonstrate statistically or clinically significant differences for pain palliation or improvement of global QoL when compared with patients treated with mitoxantrone plus prednisone. As the patients treated in SWOG 99-16 and TAX 327 had similar baseline characteristics, the reason for this difference in palliation may be attributed to incomplete QoL data collection in the SWOG 99-16, the continuous administration of prednisone in the TAX 327, and the additional toxicity caused by estramustine. As compared with the group given mitoxantrone and prednisone, the group given docetaxel plus estramustine had significantly higher rates of toxicity, with an increase in grade 3/4 nausea and vomiting (20% versus 5%, respectively; P<0.001), neutropenic fever (5% versus 2% respectively; P=0.01) and cardiovascular events (15% versus 7% respectively; P=0.001). The most frequent vascular toxicities were pulmonary embolism and thrombosis, which were attributable to the oestrogenic effects of estramustine[2].

These two independent studies represent an important therapeutic milestone by demonstrating that docetaxel-based chemotherapy compared with mitoxantrone improves overall survival in patients with advanced CRPC. Given that both docetaxel-based regimens resulted in a similar survival benefit, the combination of docetaxel plus prednisone is preferred in routine clinical practice, due to the avoidance of estramustine-related toxicity. Although several phase II studies and small randomised trials have suggested that the docetaxel plus estramustine combination may improve the PSA response rate compared with docetaxel alone, this was not supported by the results of the two pivotal phase III trials[15,16,18,19]. No clinically relevant advantage of the addition of estramustine to docetaxel has been observed in a randomised trial[20]. In this study, 150 patients were

randomised between docetaxel alone (35 mg/m^2 on days 2 and 9, every 3 weeks) or docetaxel in combination with estramustine (280 mg orally three times a day on days 1 to 5 and 8 to 12, every 3 weeks). All patients received prednisone (10 mg/day). The PSA response rate was not statistically different between the two groups. No significant differences were found for median time to PSA progression (docetaxel plus estramustine 6.9 months versus docetaxel 7.3 months) or median overall survival time (docetaxel plus estramustine 19.3 months versus docetaxel 21 months). More patients had at least one grade 3 or 4 toxicity with docetaxel plus estramustine (45%) compared with the docetaxel group (21%; *P*=0.005); mainly as a result of grade 3 or 4 gastrointestinal toxicity (*P*=0.05). Taking the data all together, in view of the apparent lack of superior activity and greater toxicity by the addition of estramustine, docetaxel every 3 weeks plus low-dose prednisone can be considered as the current standard treatment for patients with CRPC[21,22]. The optimal duration of docetaxel based chemotherapy for CRPC has not yet been established. In the TAX 327 patients were scheduled to receive 10 cycles of chemotherapy, in the SWOG 99-16 this was 12 cycles. As these studies are likely to define the standard of care in patients with CRPC, currently, standard practice is to treat patients with a fixed number of 10 cycles of chemotherapy.

The role of intermittent chemotherapy in the management of CRPC remains to be fully defined. A phase III trial of docetaxel plus either high-dose calcitriol or placebo permitted the use of intermittent chemotherapy and suggested that intermittent chemotherapy may be a feasible treatment strategy in selected patients who respond well on the initial cycles of chemotherapy, with treatment resuming when PSA begins to rise again[23]. However, there are no randomised phase III trials comparing intermittent versus continuous docetaxel-based chemotherapy. Further studies are needed to determine the value of this strategy on efficacy and cumulative toxicity of treatment.

PREDICTING OUTCOMES IN CRPC

CRPC is a heterogeneous disease with rather well characterised factors associated with outcome. Several prognostic models have been developed to estimate survival in patients with CRPC. The CALGB cooperative study group performed a pooled analysis combining data from six trials involving 1101 patients with metastatic CRPC treated between 1991 and 2001 and created a prognostic model for risk stratification of metastatic CRPC, by comparing the predicted probability with the actual survival probability on the basis of pre-treatment factors[24]. The factors used in this nomogram to estimate 12- and 24 month survival probability included the following: lactate dehydrogenase (LDH), PSA, alkaline phosphatase, Gleason score, performance status, haemoglobin, and the presence of visceral disease. Patients were classified into one of four risk groups.

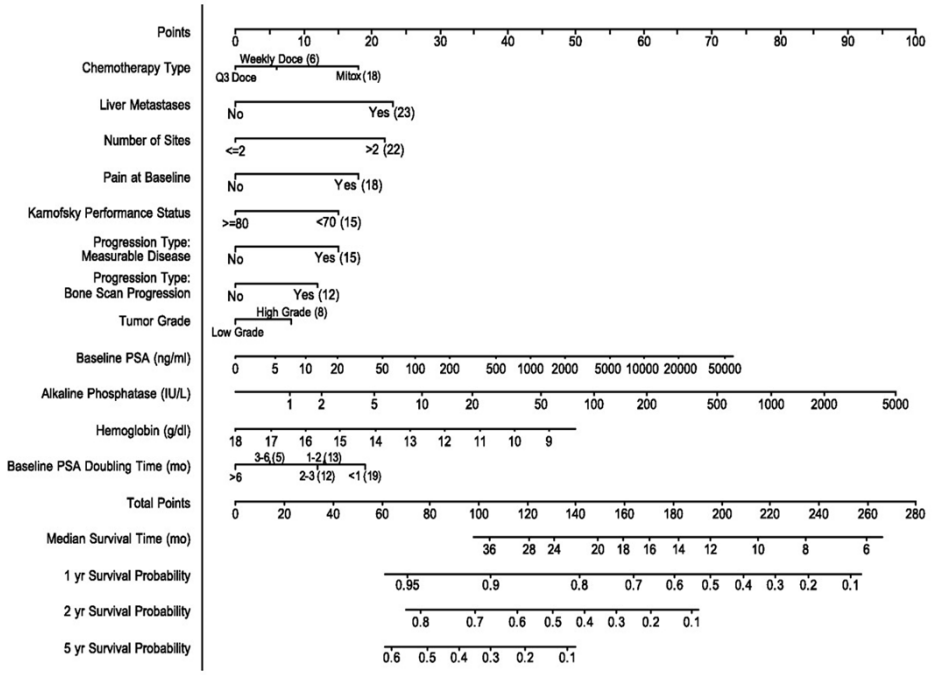

Figure 1. Nomogram for survival of patients treated with cytotoxic agents for progressive CRPC (689 patients, 518 mortality events).

Note: a pain intensity of ≥2 and/or an analgesic score of ≥10 were defined in the original protocol as indicative of the presence of significant pain. Reprinted with permission from Armstrong AJ and colleagues; A contemporary prognostic nomogram for men with hormone refractory metastatic prostate cancer: a TAX 327 study analysis. Clin Cancer Res 2007; 13: 6396–6403, fig. 3[26].

The observed median overall survival durations were 7.5, 13.4, 18.9, and 27.2 months for the first, second, third, and fourth risk groups, respectively. The corresponding median predicted overall survival times were 8.8, 13.4, 17.4, and 22.8 months for the four risk groups.

The significant better overall survival in the TAX 327 for patients without pain at entry[17], treated with docetaxel-based chemotherapy, was confirmed in a recent study, showing a median overall survival of 17.6 months and 10.2 months in men with low and high pain scores, respectively[25].

A subset analysis of the TAX 327 cohort, to investigate the significance of novel predictive variables, resulted in the TAX 327-based predictive nomogram (Fig. 1)[26]. Ten factors were associated with survival (Table 1)[26]. Univariately, PSA-doubling time (PSA-DT) is a prognostic factor for overall survival, but in the multivariate analysis PSA-DT retained only borderline significance (Fig. 2)[26]. As shown in the nomogram baseline PSA, alkaline phosphatase, visceral metastases, progression type, and haemoglobin at baseline have stronger predictive value (Fig. 1)[26].

Variable	Multivariate HR (95% CI)	*P*
Liver metastases	1.66 (1.09–2.54)	0.019
Number of metastatic sites (>2 vs. ≤2)	1.63 (1.23–2.15)	0.001
Pain at baseline	1.48 (1.23–1.79)	<0.001
Performance status (≤70 vs. ≥80)	1.39 (1.06–1.82)	0.016
Progression type		
Measurable disease	1.37 (1.10–1.70)	0.005
Bone scan progression	1.29 (1.06–1.57)	0.010
Baseline PSA-DT (<55 vs. ≥55 d)	1.19 (0.99–1.42)	0.066
Baseline log PSA (for every unit rise in log (PSA) in ng/dL)	1.17 (1.10–1.25)	<0.001
Tumour grade (Gleason ≥8 or WHO 3–4 vs. Gleason ≤7 or WHO 2–3)	1.18 (0.99–1.42)	0.069
Alkaline phosphatase, log scale (per log unit rise, IU/L)	1.27 (1.15–1.39)	<0.001
Haemoglobin (per unit decline, g/dL)	1.11 (1.03–1.19)	0.004

Table 1. A multivariate Cox proportional hazards analysis of ten independent prognostic markers showing a significant association with overall survival.

Adapted with permission from Armstrong and colleagues; A contemporary prognostic nomogram for men with hormone refractory metastatic prostate cancer: a TAX327 study analysis. Clin Cancer Res 2007; 13: 6396–6403, Table 3[26].

PSA-DT, prostate specific antigen-doubling time; vs., versus; d, days; PSA, prostate specific antigen; WHO: World Health Organisation; HR, hazard ratio; CI; 95% confidence interval.

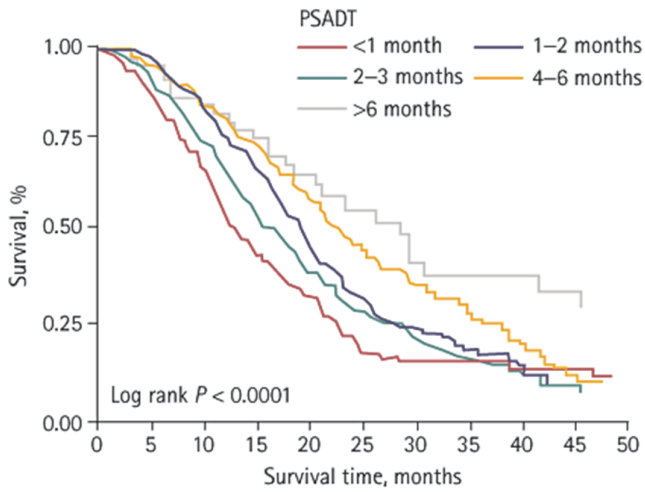

Figure 2. Kaplan–Meier estimates of overall survival according to PSA-doubling time in the TAX 327 cohort (*n* = 686, 518 mortality events).

The median PSA-DT in this study was 55 days. PSA-DT was separated into five cohorts: >6 months (*n* = 44, median OS not reached, mean OS 25 months), 3–6 months (*n* = 118, median OS not reached, mean OS 22.5 months), 2–3 months (*n* = 151, median OS 20.7 months), 1–2 months (*n* = 264, median OS 18.6 months), and <1 month (*n* = 109, median OS 13.3 months). Reprinted with permission from Armstrong and colleagues; A contemporary prognostic nomogram for men with hormone refractory metastatic prostate cancer: a TAX 327 study analysis. Clin Cancer Res 2007; 13: 6396–6403, fig. 1[26].

In contrast to the Halabi nomogram, which is regarded as a prognostic nomogram of the untreated underlying disease, the TAX 327 nomogram can be seen as a prognostic nomogram if it is assumed that the patient with CRPC is going to be treated with docetaxel-based chemotherapy. The TAX 327 nomogram should not be used for patients that do not receive treatment with docetaxel.

Recent data from TAX 327 risk group analysis, to develop and validate clinically applicable predictive factors for response-based endpoints and assess the performance of a risk-group based classification in predicting PSA declines and overall survival in CRPC, identified three risk groups on the basis of four independent risk factors[27]. In this multivariate analysis, based on the 3-month PSA decline data in patients enrolled in the TAX 327, four independent risk factors were identified that predicted for not reaching a ≥30% PSA decline in 3-month PSA: significant baseline pain, visceral metastases, anaemia (haemoglobin <13g/dl), and bone scan progression at baseline. These risk factors were combined to develop three risk groups; a low-risk group consisted of patients with 0–1 risk factors, an intermediate-risk group of patients with two risk factors and a high-risk group of patients with 3–4 risk factors, with a median overall survival of 25.7, 18.7 and 12.8 months, respectively, with a concordance index of 0.64 (P<0.0001 for trend).

WHEN TO START CYTOTOXIC THERAPY

The widespread use of PSA monitoring has resulted in earlier detection of CRPC, often in asymptomatic patients. An important question for the management of asymptomatic disease is whether to initiate chemotherapy or to wait until symptoms occur. Although chemotherapy may halt or reverse progression, associated toxicity of the chemotherapy might lead to deterioration of QoL. Comparative data evaluating the merits of delaying the initiation of chemotherapy are lacking. As previously mentioned, survival benefit from treatment with docetaxel-based chemotherapy is equal for all subgroups of patients[17]. Although the benefit is similar, there is a substantial difference in overall survival in patients with and without pain (14.4 months versus 21.3 months, respectively), but this does not necessarily imply benefit from early use of chemotherapy. Some patients had a decreased QoL after starting chemotherapy, and this was more often observed in patients with minimal symptoms[28]. Delaying cytotoxic therapy may be a suitable approach in CRPC patients with rather indolent disease, for which the following criteria were proposed: PSA only progression as a single sign of metastatic disease with low baseline PSA and a slow PSA-DT, a normal (or slightly raised) alkaline phosphatase and normal or (slightly lowered) haemoglobin. In patients who are more likely to develop symptoms and progression at an early stage (based on high PSA-DT and/or high base-

line PSA and/or bone scan progression and/or visceral progression), the start of chemotherapy should not be postponed[21].

Therefore, to optimise management of advanced CRPC, it becomes increasingly important to understand the predictive factors influencing the outcome. It is difficult to estimate the time expenditure in which the group of asymptomatic/low risk patients is likely to become symptomatic, or to develop other adverse prognostic features with associated poorer survival outcome. Postponing chemotherapy and gaining significant time without therapy, without impeding on survival expectancy when chemotherapy eventually starts, may be considered beneficial, whereas the risk of postponing treatment for only some months at which time the patient has obtained significant worse features and detrimental effects on survival probability must be avoided.

Furthermore, the data derived from patients classified as intermediate risk cannot easily be applied to patients whose disease characteristics worsen from low to intermediate risk (Fig. 3). As long as these two questions remain unanswered, the decision with regard to the introduction of cytotoxic therapy in asymptomatic men can only be based on the circumstantial evidence available.

ASSESSING RESPONSE FOR THERAPY

Assessing the response to treatment in prostate cancer is difficult as measurable disease, by standard oncologic criteria, occurs infrequently. The majority of men have bone metastases which are difficult to quantify objectively and reproducibly. The identification of surrogate endpoints, replacing solid endpoints, is crucial to the rapid evaluation of new cancer drugs. Recently, the PCWG 2 updated eligibility and outcome measures in trials that evaluate systemic treatment for patients with progressive prostate cancer and castrate levels of testosterone[3]. Treatment should be continued for at least 12 weeks to ensure adequate drug exposure. PCWG 2 defined criteria of progression on the basis of changes in PSA, bone metastases, and measurable disease. PSA progression has been defined as a 25% or greater increase and an absolute increase of 2 ng/mL or more from the lowest documented PSA level, which is confirmed by a second value obtained 3 or more weeks later. When the bone scan is the sole indicator of progression, PCWG 2 defines progression in bone when at least two or more new lesions are seen on a bone scan compared with prior scans. Trials collecting data on measurable lesions should follow Response Evaluation Criteria in Solid Tumours (RECIST)[29]. An analysis based on the PSA data in patients entered in the SWOG 99-16 trial has demonstrated that a decline in serum PSA of 30% at 12 weeks and post-treatment PSA velocity were the optimal surrogate markers for survival[30]. This finding has been validated by analysis of the TAX 327 database, in which a ≥30% PSA decline within 3 months of treatment initiation provides

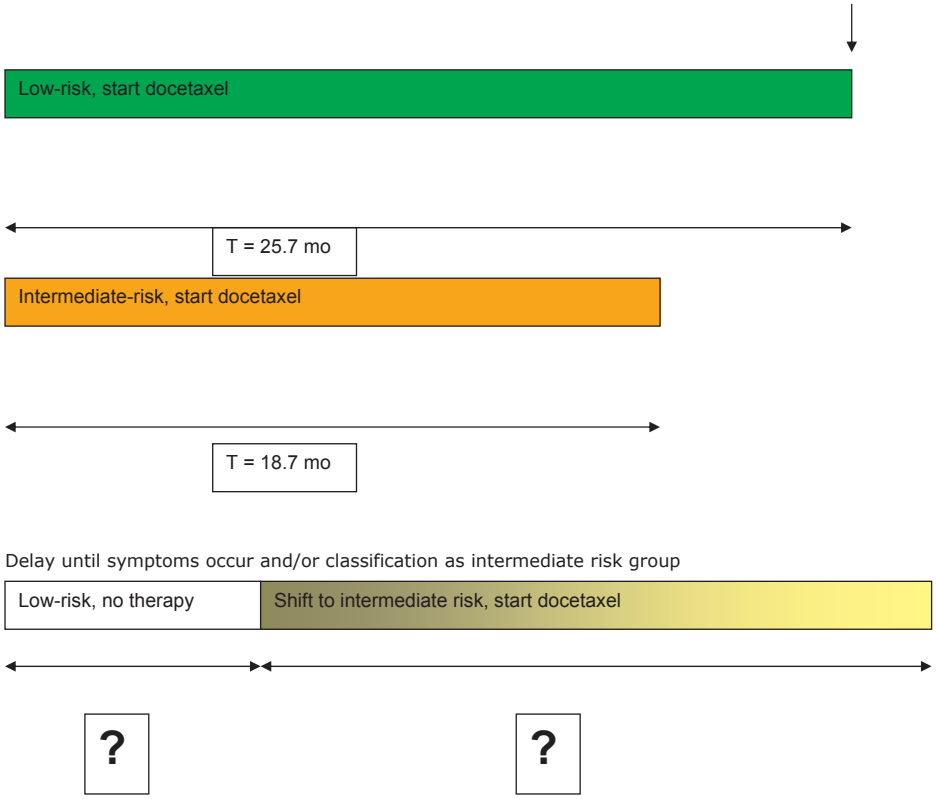

Figure 3. To estimate the time expenditure in which the group of asymptomatic/low-risk patients is likely to become symptomatic, or to develop other adverse prognostic features with associated poorer survival outcome.

T= Overall survival with docetaxel in patients with CRPC according to TAX 327 in different risk groups[27].

Postponing chemotherapy for the purpose of gaining some extra time without therapy, without impeding on survival expectancy when chemotherapy eventually starts, may be considered beneficial. The risk of postponing treatment for only some months at which time the patient has obtained significant worse features and associated decreased effects on survival probability must be avoided. Mo, months.

the highest degree of surrogacy for overall survival with a HR of 0.50 after adjusting for treatment effect[31]. Another analysis of the TAX 327 database revealed that both PSA response (HR 0.59; $P<0.001$) and pain response (HR 0.59; $P<0.001$) were associated with longer survival[28]. However, after adjusting for PSA and pain response, QoL response was not a significant predictor of survival (HR 0.97; $P=0.84$).

FUTURE DIRECTIONS

Different combinations of chemotherapy and numerous agents with novel mechanisms of anti-tumour activity have been studied in patients with CRPC; due to the rapid progress of this field it is beyond the scope of this review to cover all compounds under investigation.

Docetaxel-based combinations

Given the improved overall survival with docetaxel, several subsequent trials have administered docetaxel in combination with various agents to assess the additional benefit of these combinations relative to docetaxel alone.

Endothelin is a protein produced by vascular endothelium and is thought to play an important role in vascular homeostasis. The endothelin pathway is involved in several phases of prostate cancer development and progression[32].

Atrasentan is an oral selective inhibitor of endothelin-A receptor, and preclinical *in vivo* data have shown synergistic effects of atrasentan in combination with docetaxel chemotherapy[33]. A phase III trial comparing docetaxel with and without atrasentan, in patients with metastatic CRPC (SWOG S0421), is currently accruing patients.

Another endothelin receptor-A antagonist, ZD 4054, is also currently under investigation in a phase III study in combination with docetaxel for treatment of patients with metastatic CRPC.

Bevacizumab is a humanised monoclonal antibody to vascular endothelial growth factor (VEGF), a key activator of tumour angiogenesis. While single agent studies have failed to demonstrate significant results, a phase II study (CALGB 90006) added bevacizumab to docetaxel and estramustine in patients with metastatic CRPC with the result that 81% of the patients achieved a ≥50% PSA decline and overall median survival of 21 months[34]. The use of bevacizumab in the treatment of metastatic CRPC is currently being tested in a phase III trial comparing docetaxel with or without bevacizumab (CALGB 90401).

Several *in vitro* and *in vivo* models indicated that calcitriol (1,25-dihydroxyvitamin D3) inhibits the growth and stimulates the differentiation of prostate cancer cells[35,36]. The androgen-independent prostate cancer study of calcitriol enhancing docetaxel (ASCENT), a phase II trial of 250 patients with metastatic CRPC to compare docetaxel with or without high-dose calcitriol, suggested that the calcitriol-containing regimen was associated with improved survival, but the study was too underpowered to detect a survival difference and the primary endpoint PSA response did not reach statistical significance[37]. The subsequent phase III study (ASCENT-2) was closed by the Data and Safety Monitoring Board due to the higher number of deaths in the calcitriol plus docetaxel treatment group and the final analysis is awaited.

A Phase III study of Vaccine Immuno Therapy with Allogenic prostate cancer cell Lines 2 (VITAL-2), comparing docetaxel plus either prednisone or granulocyte-macrophage colony-stimulating factor gene transduced irradiated prostate cancer cells (GVAX), was recently closed because of a higher number of deaths in the docetaxel plus GVAX arm[38]. Bisphosphonates are inhibitors of osteoclast-mediated bone resorption that have been shown to decrease pain and the risk of skeletal complications by bone metastases in patients with breast cancer, prostate cancer and multiple myeloma[39]. Unlike bone metastases from breast cancer, most bone lesions in prostate cancer are osteoblastic. However, despite the osteoblastic nature of the metastatic bone lesions, morphologic studies suggest that most bone metastases from prostate cancer are characterised by excessive activity of both osteoblasts and osteoclasts[40,41]. Initial trials with first- and second-generation bisphosphonates (clodronate and pamidronate) have shown no significant effect on the prevention of skeletal-related events (SREs)[42,43]. In a phase III study zoledronic acid, a third generation bisphosphonate, significantly reduced SREs in patients with CRPC with bone metastases (P=0.021)[44]. A follow-up study of 24 months confirmed its long-term efficacy with a 36% (P=0.002) reduction in the ongoing risk of SREs in patients treated with zoledronic acid compared to the placebo group[45]. However, the study did not show a difference in survival or disease progression. Risedronate is an orally administered, third generation pyridinyl bisphosphonate, which reduces bone turnover and decreases resorption through osteoclastic effects, with no undesirable effects on cortical porosity or thickness or on cancellous bone volume[46]. Clinical studies with risedronate in patients with bone metastases have not been reported yet. Data from animal models have shown that risedronate can inhibit the formation and progression of bone metastases. In a mouse model, risedronate decreased breast cancer burden selectively in bone, which translated into a significantly longer survival for mice continuously treated with risedronate[47]. In another preclinical study, continuous administration of risedronate showed significant effects on the incidence and size of observed skeletal metastases in rats inoculated with ENU1564 mammary adenocarcinoma cells[48]. Pre-treatment of prostate and breast cancer cells with risedronate and other bisphosphonates resulted in inhibition of tumour cell adhesion to unmineralised and mineralised osteoblastic extracellular matrices in a dose-dependent manner[49]. There is strong pre-clinical evidence that bisphosphonates and paclitaxel induce apoptosis in breast cancer cells in a synergistic manner when they are combined[50]. Therefore, an ongoing multicentre phase III trial, the Netherlands Prostate Study Group (NePro) study, is currently enrolling patients with CRPC with bone metastases to evaluate the addition of risedronate to docetaxel-based chemotherapy[51]. The primary endpoint in this study will be time to progression. Secondary endpoints will be PSA response rate, pain response, toxicity profile, objective response by RECIST when measurable disease, duration of PSA response and overall survival.

SECOND-LINE TREATMENT

There are no approved agents for second-line therapy in patients with CRPC who progress after first–line docetaxel-based chemotherapy. Several options for these patients have been suggested e.g. clinical trials of novel agents, other cytotoxic agents, docetaxel retreatment, additional hormonal manipulations, and best supportive care[52]. However none of them have proven to improve QoL or overall survival. Being the previous standard of care, second-line mitoxantrone chemotherapy has been utilised after docetaxel-based chemotherapy. Retrospective studies have reported limited efficacy and tolerability, with PSA response rates in 10–20% of patients[53,54]. Retrospective crossover results of the TAX 327 trial identified 237 (23%) patients, among the 1006 patients, who received the other drug as second-line therapy off-study[55]. Eighty-nine men received 3-weekly docetaxel followed by mitoxantrone, 76 men received mitoxantrone after weekly docetaxel and 67 men received docetaxel after mitoxantrone. Median survival after crossover was 10 months and did not depend on direction of crossover. Data on PSA response were available for 96 patients: PSA response (≥50% reduction) occurred in 15% of 71 men receiving mitoxantrone after docetaxel and in 28% of 25 men receiving docetaxel after mitoxantrone. Median PSA progression-free survival was 3.4 months for mitoxantrone after docetaxel and 5.9 months for docetaxel after mitoxantrone. One prospective phase II trial tested second-line mitoxantrone chemotherapy for docetaxel-refractory CRPC in 41 patients[56]. In this study mitoxantrone also only had modest activity. In total, 20% of the patients treated with mitoxantrone and prednisone had a PSA decline ≥50%, the median time to PSA progression was 2.3 months, and the median response duration was 5.9 months. Median overall survival for patients treated with second-line mitoxantrone was 9.8 months. From these data, although mitoxantrone has a limited PSA response ranging from 0% to 20%, time to progression is short and toxicity is significant with no clear data on any palliative effect. Mitoxantrone should, therefore, be considered to have minimal activity after first-line docetaxel-based chemotherapy. The potential role of retreatment with docetaxel in docetaxel pre-treated patients relapsing after an initial successful series of cycles remains undefined. Data from the ASCENT-1 study support the utility of retreatment after a response in some selected patients[37].

Only one phase III randomised clinical trial has been completed in the second-line setting in CRPC, the Satraplatin and Prednisolone Against Refractory Cancer trial (SPARC). In the SPARC trial, patients with metastatic CRPC in whom one previous cytotoxic chemotherapy regimen had failed were randomised to prednisone with or without satraplatin, an oral platinum complex[57]. Treatment with satraplatin was associated with a statistically significant improvement in progression-free survival, PSA response, pain response, time to pain progression, and duration of pain response. However, since this

survival analysis, which was a co-primary endpoint, did not point towards a survival benefit, the Food and Drug Agency (FDA) and European Medicine Agency (EMEA) did not approve satraplatin in CRPC[58]. The survival data may have been adversely influenced by a greater number of patients in the placebo group who received a subsequent line of chemotherapy including docetaxel. Final data analysis and subset analysis is pending. Abiraterone acetate, a 17-hydroxylase and C17,20-lyase inhibitor, to decrease serum androgen to undetectable levels, has shown activity in patients previously treated with docetaxel chemotherapy[59,60]. This agent is currently being explored in a phase III trial for patients with metastatic CRPC who have failed docetaxel-based chemotherapy, using overall survival as primary endpoint.

CONCLUSIONS

Following the results of the two landmark phase III studies, TAX 327 and SWOG 99-16, the combination of 3-weekly docetaxel plus low-dose prednisone has become standard treatment for patients with CRPC. These two independent randomised studies have demonstrated that 3-weekly docetaxel significantly improves overall survival compared with mitoxantrone-based chemotherapy, with acceptable toxicity. An updated survival analysis of data from TAX 327 showed that survival benefit was sustained at 3 years. The 3-weekly regimen with docetaxel also showed statistical significant improvement of pain and QoL in symptomatic patients.

An important question is when to start chemotherapy in asymptomatic patients. Delaying cytotoxic therapy may be a suitable approach in patients who are asymptomatic and at low risk for rapid progression and developing symptoms. In contrast, in those patients with fast disease progression the initiation of cytotoxic therapy should not be delayed. Several variables including baseline PSA and PSA-DT have been identified as prognostic parameters for overall survival and could facilitate the decision as to when to start cytotoxic therapy. Until our understanding of CRPC expands, nomograms may help to guide management in CRPC.

There is a need for robust clinical outcome measures in clinical trials for patients with CRPC.

Although an important and clinically meaningful first step, the impact on survival of docetaxel is modest and median overall survival for patients with advanced CRPC is still around 19 months. Novel agents in combination with docetaxel may provide further avenues through which CRPC can be treated more effectively. There are no second-line treatment options with demonstrated effectiveness and drug development is needed in this setting. There are numerous unanswered questions in the management of CRPC and patients should be enrolled in clinical trials whenever available.

CONFLICT OF INTEREST STATEMENT

Dr de Wit has received research grant support and consultancy fees from Sanofi-Aventis. Dr Meulenbeld and Dr Hamberg have no conflicts of interest to report.

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Chapter 3

Randomized phase II/III study of Docetaxel with or without Risedronate in patients with metastatic Castration Resistant Prostate Cancer (CRPC), the Netherlands Prostate Study (NePro)

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ABSTRACT

Background:

This multicentre, randomized, open label, phase II/III study aimed to investigate the potential benefit of adding risedronate (R) to docetaxel (D) in patients with metastatic Castration Resistant Prostate Cancer.

Patients and Methods:

CRPC patients with bone metastasis were randomly assigned to receive D 75 mg/m² every 3 weeks and prednisone as first line chemotherapy, with or without R 30 mg oral once daily. The primary endpoint was Time to Progression (TTP). A composite endpoint of objective progression by RECIST criteria, PSA progression, or pain progression, whichever occurred first, was applied. The study had 80% power to detect an improvement of 30% in median TTP in the DR group (two-sided $\alpha = 0.05$).

Results:

Five hundred and ninety-two men (301 D versus 291 DR) were randomized. TTP was 7.4 [D] versus 6.5 [DR] months (p=0.75). PSA and pain response rates were similar, 66.3% [D] versus 65.9% [DR] and 27.9% [D] versus 31.2% [DR], respectively. Median overall survival (OS) was 18.4 [D] versus 19.2 [DR] months (p=0.33). There were no differences in toxicity.

Conclusion:

The addition of the third generation bisphosphonate, risedronate, in the setting of effective first line docetaxel based chemotherapy did not increase efficacy, as indicated by the lack of improvement in TTP, OS, PSA- and pain response.

Keywords: bisphosphonates, bone metastases, castration resistant prostate cancer, docetaxel, risedronate.

INTRODUCTION

Approximately 80% of patients with advanced Castration Resistant Prostate Cancer (CRPC) develop bone metastases that often lead to severe bone pain, hypercalcaemia and skeletal-related events (SREs)[1,2]. Despite the osteoblastic appearance of prostate cancer bone metastases, there is increasing evidence that most metastases are characterized by excessive activity of both osteoblasts and osteoclasts[3,4,5,6].

Bisphosphonates are potent inhibitors of osteoclast-mediated bone resorption. In CRPC zoledronic acid has shown to delay the onset and the incidence of SREs[2,7]. Zoledronic acid was approved and quickly became established as the standard of care in the United States in 2002. In several European countries the introduction of zoledronic acid took a slower pace since the delay of SREs was considered a weak primary endpoint to justify expensive medication. In 2004, the treatment paradigm of metastatic CRPC changed after two landmark trials[8,9], demonstrated for the first time a survival benefit in patients with metastatic CRPC utilizing docetaxel based chemotherapy, setting a new standard of care for patients with CRPC. With the results of these studies the question arose in Europe what the role of bisphosphonates was relative to the initiation of effective chemotherapy.

An emerging body of preclinical evidence indicate that bisphosphonates also exhibit direct anti-tumour activity[10,11,12]. *In vitro* data have shown that bisphosphonates directly inhibit breast and prostate carcinoma cell invasion[13,14].

However, clinical data of bisphosphonates, on anti-tumour efficacy are limited, and provide conflicting evidence. Risedronate is an oral third generation pyridinyl bisphosphonate, which reduces bone turnover and reduces osteoclast-mediated resorption[15].

Data from animal models have shown that risedronate and docetaxel act synergistically to decrease tumour burden of established bone metastases from breast cancer cells[16].

Since both *in vivo* and *in vitro* studies have shown synergistic action of zoledronate and taxanes[17,18,19], there is a rationale to conduct this study to evaluate the efficacy and safety profile of adding risedronate to docetaxel in patients with CRPC with bone metastases.

MATERIALS AND METHODS

Patients

This randomized, open label, phase II/III trial was undertaken at 45 centres in the Netherlands and one in Norway. Patients had histologically proven prostate cancer with progression during prior castration and castration levels of testosterone. Progressive disease was defined as PSA progression documented by at least two consecutive increases relative to a reference value measured at least a week apart. The initial protocol

included all patients with metastatic CRPC, but during the early phase of the trial, the eligibility criteria were modified so that only patients with bone metastases were to be included. Patients without bone metastases at randomization were excluded from the efficacy analysis. Eligible patients were ≥18 years, with an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2, and adequate haematological, hepatic, and renal function. Patients with disease related pain at entry must have been on a stable analgesic regimen ≥ 1 week prior to registration. Prior antiandrogen withdrawal followed by progression had to have taken place at least 4 weeks prior to randomization (6 weeks for bicalutamide). LHRH analogues were continued, unless patients were surgically castrated. Any prior or concomitant use of bisphosphonates was excluded. Patients who had received radiotherapy within 4 weeks before enrolment, CNS involvement, or other serious illness (including secondary cancer) were excluded.
The study was conducted according to the Declaration of Helsinki and in compliance with Good Clinical Practice and local ethical and legal requirements. Written informed consent was obtained from all participants.

Study design

Patients were randomly assigned to receive docetaxel 75 mg/m² intravenously, every 3 weeks and prednisone 5 mg bid, with or without risedronate 30 mg oral od. Chemotherapy was continued until progression, unacceptable toxicity, or withdrawal of consent for a maximum of 10 cycles. In the DR group, R and prednisone was continued until progression. At the time of progression, further treatment was at the discretion of the investigator. Upon progression patients in the D alone arm were offered R. Treatment delays of up to two weeks and up to two dose reductions were allowed.

Follow-up and outcomes

Pre-treatment evaluations included a medical history, ECOG performance status, physical examination, laboratory screening, serum PSA and testosterone concentration, chest X-ray or CT scan, abdominal CT scan, and a bone scan. Pain and analgesic consumption was assessed at baseline, every three weeks, at end of study and then every month until pain progression or further anti-tumour therapy. Pain was assessed with Present Pain Intensity (PPI) scale from the McGill-Melzack questionnaire[20]. Physical examinations and blood tests, including PSA, were repeated before each infusion of docetaxel and at the end of treatment, and then every month during 6 months and every 2 months thereafter until PSA progression or further anti-tumour therapy.
Bone scans were performed before study entry, after week 30, to confirm a response, and at study discontinuation. In patients with measurable disease, CT scans were re-

peated at intervals of nine weeks, when clinical progression was suspected, and at end of chemotherapy. All assessments were repeated to confirm a response at least 4 weeks later.

The primary end point of the phase II part was the rate of objective (PSA) responses to treatment. Per an attained phase III design, at least 26 responses out of 69 evaluable patients must have been observed in the experimental arm, to continue the study as a randomized phase-III study. In the phase-III part the primary endpoint was TTP. A composite endpoint of objective progression by Response Evaluation Criteria in Solid Tumours (RECIST)[21] criteria, PSA progression, or pain progression, whichever occurred first, was applied. PSA progression was defined as an increase of ≥25% over nadir PSA concentration provided that the increase in the absolute PSA value was ≥5 ng/ml for men without PSA response, or ≥50% over nadir for PSA responders. Pain progression was defined as an increase in median PPI score of ≥1 point from the nadir, increase in analgesics class compared to nadir, or requirement for palliative radiotherapy.

Secondary endpoints included PSA response, duration of PSA response, and pain response. PSA response was defined by ≥ 50% decline in serum PSA concentration compared to baseline in patients with a baseline value of ≥20 µg/L, confirmed at least 4 weeks later. Pain response was defined as ≥ 2 point reduction from baseline median PPI score, without increase in analgesic class, or a decrease in analgesic class without an increase in PPI score, maintained for 2 consecutive evaluations at least three weeks apart. Other secondary endpoints were toxicity, response by RECIST, and overall survival (OS). Toxicity was assessed before each treatment cycle and graded according to the National Cancer Institute Common Terminology Criteria for adverse events (version 2).

On the basis of emerging guidelines recommending the delivery of 12 weeks of treatment before adjustment of therapy for metastatic CRPC[22], an amendment was made to the trial protocol after 115 patients had been enrolled to ensure that PSA increase only did not qualify for progression within the first 4 cycles of treatment.

Statistical Analysis

This study consisted of a phase II and a phase III part. In the randomized phase II part, Simon's two-stage minimax design was used[23], where the experimental treatment would be declared of insufficient activity if 25 or fewer PSA responses would be observed out of 69 evaluable patients in the experimental arm with 90% power at $\alpha = 0.1$. If this criterium was met, the study was extended into phase III, with TTP as the primary endpoint. A sample size of 589 patients was required (456 events), to detect an improvement of 30% in median TTP in the DR group relative to the D group (HR = 0.77) with 80% power, with a two-sided significance level of 0.05, assuming a median TTP of 6 months in the D group. Patients were randomized between two groups (1:1 ratio), stratifying according

to measurable versus non measurable disease and median PPI at baseline (≥2 versus < 2). TTP, OS, and duration of response were analyzed using the Kaplan-Meier method, with log rank comparison. Response rates were calculated as percentage of evaluable patients along with binomial confidence intervals. TTP was calculated from the date of randomisation to the date of RECIST, PSA, or pain progression, whichever occurred first. Patients without progression at death or last follow-up were censored. TTP was also calculated for the composite endpoints separately, censoring patients for a particular type if they experienced another type first.
Safety analyses included all randomized men. Hazard ratios (HR) and 95% CIs were calculated with a Cox proportional hazards model. A separate analysis for TTP was performed excluding the group of patients with disease progression only based on PSA increase during the first 4 cycles of treatment. SAS software (version 9.2) and R (version 2.14.0) were used for all analyses.

RESULTS

Patients and treatment

From January 2004 to April 2010, 592 patients were enrolled and randomly assigned to one of the study arms (301 D and 291 DR). Patient demographics and baseline characteristics were well balanced between the two groups (table 1).There were 23 patients without bone metastases enrolled before the amendment to require evidence of bone metastases. Exposure to docetaxel was similar between the two groups, with a median of 9 cycles. Most discontinuations were because of end of protocol treatment (277 of 592; 47%) and were balanced between the two groups (table 2). The median follow-up was 42 months.

Efficacy

At data cut off 1st July 2011 about 86% of the patients in both groups had investigator determined progressive disease (table 3). Upon progression 97 patients in the D alone arm were treated with R. Median TTP (a composite endpoint) was 7.4 vs 6.5 months (HR 1.04; 95%CI 0.87-1.24) for D and DR, respectively (Fig 1). Furthermore; there were no differences in time to progression by the separate endpoints of pain progression, PSA progression and time to objective progression according to RECIST criteria (Fig 2). The adjusted analysis excluding the 2 patients (both in the D group) with disease progression only based on PSA increase during the first 4 cycles of treatment, showed a median TTP of 7.5 vs 6.5 months for the D and DR group respectively. The Kaplan-

Characteristic	Docetaxel (N=301)	Docetaxel and Risedronate (N=291)
Age (years)		
Median [range]	69 [46-84]	68 [46-89]
WHO PS		
0 or 1	88%	89%
2	6%	5%
NA	6%	7%
PSA (ng/ml)		
Median [range]	168 [2-8046]	206 [8-11443]
Disease location		
Bone	95%	97%
Node	42%	45%
Visceral	16%	18%
Measurable disease		
Measurable	58%	58%
Non-measurable	42%	42%
Alkaline Phosphatase (U/L)		
Median [range]	195 [40-8130]	201 [45-6045]
Albumin (g/L)		
Median [range]	40 [25-50]	40 [20-50]
Baseline PPI		
0-1	76%	76%
≥2	24%	24%
Class of analgetics		
Non-narcotic analgetics(I+II)	62%	68%
Mild opiates (III)	8%	9%
Opiates (IV+V)	23%	18%
NA	7%	5%

Table 1. Baseline characteristics.

PS: performance score; NA: not applicable; PSA: prostate specific antigen; PPI: present pain intensity score.

Meier analysis showed no difference in overall survival. Median OS was 18.4 months for D and 19.2 months for DR (HR= 1.09; p=0.33; fig 3). The objective response according to RECIST, pain response and/or PSA response were similar in both groups (table 4). Eighteen patients (9 D vs 9 DR) required palliative radiotherapy within 3 months after last chemotherapy.

Toxicity

No significant differences were observed between the D and DR arm in the incidence grade 3/4 toxicity. The most frequent non-haematological grade 3 or higher adverse events were neurotoxicity, diarrhea and nausea (table 5). The information on the frequency of grade 3-4 haematological toxicity is limited since weekly blood counts were

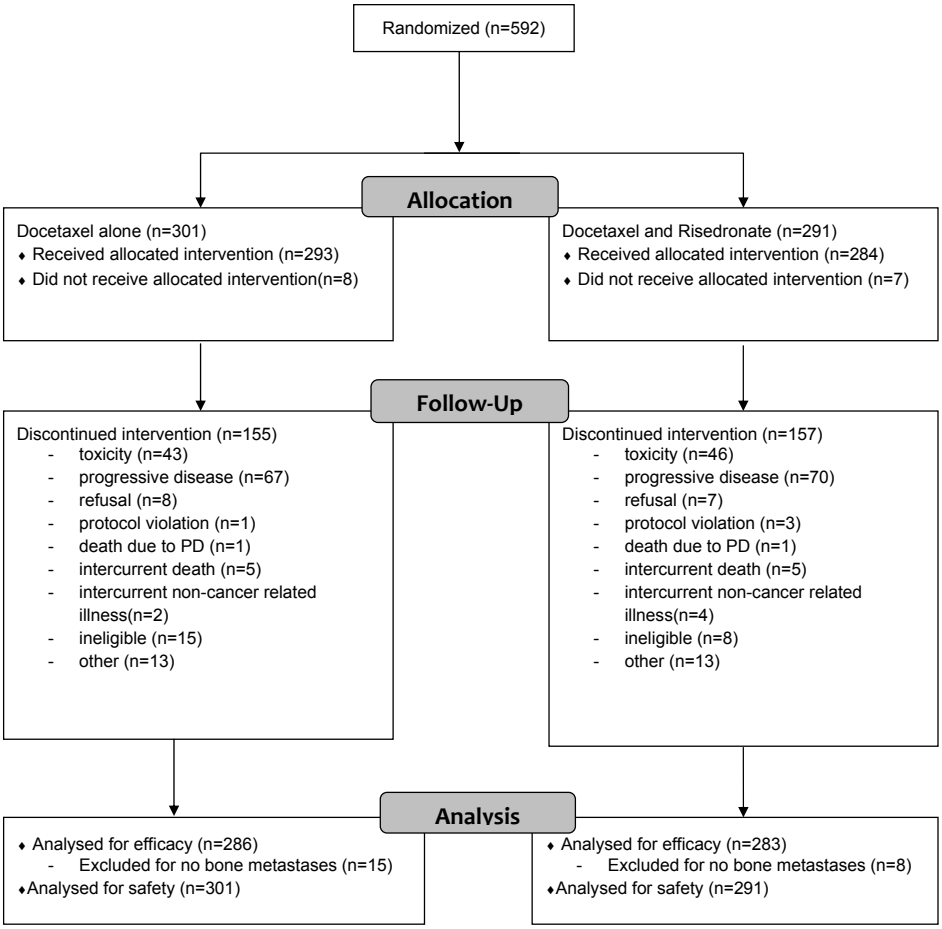

Table 2. Consort diagram.

	Docetaxel (N=286)	Docetaxel and Risedronate (N=283)
Total no. of patients with progression	248 (87%)	242 (86%)
During docetaxel	91 (37%)	106 (44%)
Within 3 months after docetaxel	67 (27%)	73 (30%)
Between 3 and 6 months after docetaxel	54 (22%)	29 (12%)
Later than 6 months after docetaxel	33 (13%)	31 (13%)
Progression, but date end of chemotherapy missing	3 (1%)	3 (1%)

Table 3. Time to progression of eligible patients (excluding patients without bone metastases).

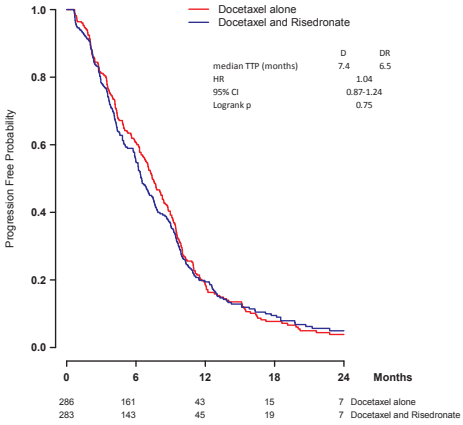

Figure 1. Time to Progression.

D: docetaxel; DR: docetaxel and risedronate; TTP: time to progression; HR: Hazard ratio; CI: confidence interval.

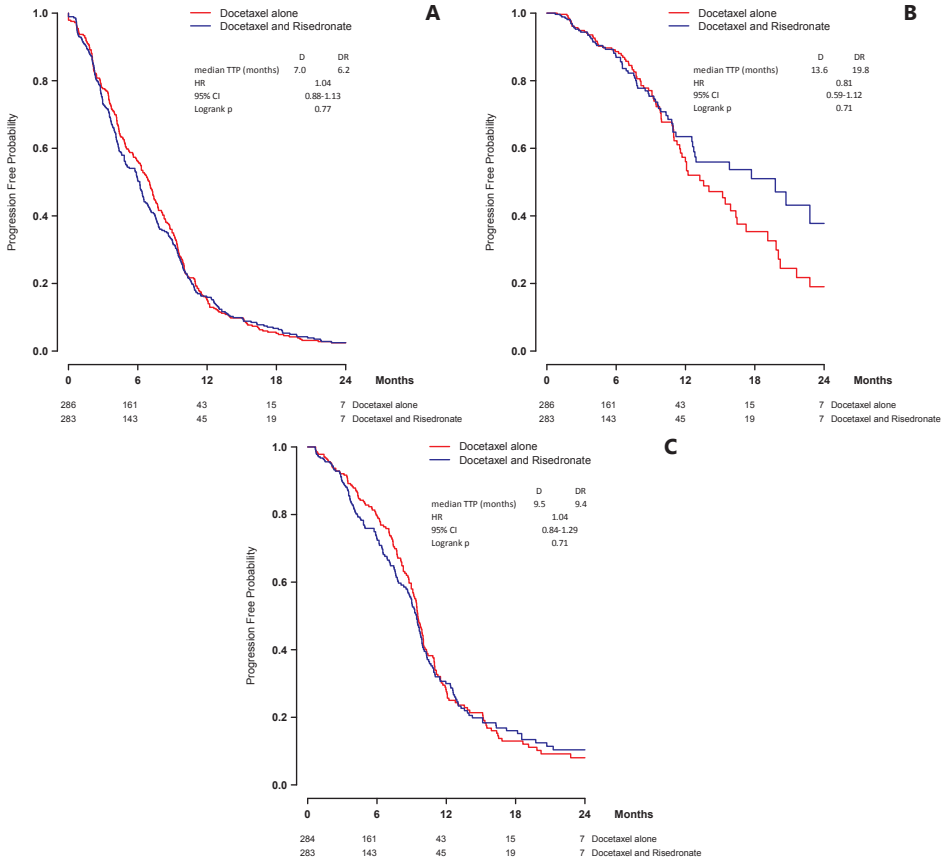

Figure 2. Kaplan Meier curves for separate progression endpoints. (A) Pain progression; (B) progression according to RECIST; (C) PSA progression.

D: docetaxel; DR: docetaxel and risedronate; TTP: time to progression; HR: Hazard ratio; CI: confidence interval.

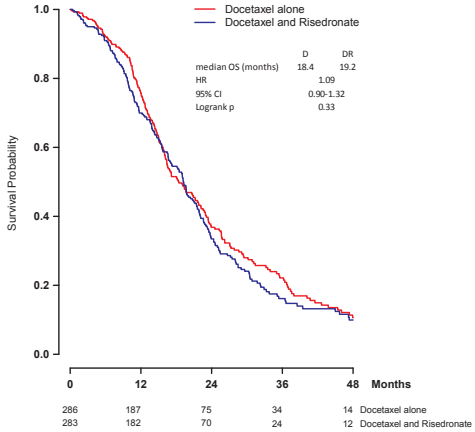

Figure 3. Overall Survival.

D: docetaxel; DR: docetaxel and risedronate; OS: overall survival; HR: Hazard ratio; CI: confidence interval.

	D	DR	Hazard Ratio (95%CI)
Tumor assessment			
Response rate*	20.8%	25.1%	
Duration of response (months)	9.4	9.5	1.05 (0.70-1,57)
PSA assessment			
Response rate*	66.3%	65.9%	
Duration of response (months)	8.0	8.1	0.94 (0.73-1.20)
Pain assessment			
Response rate*	27.9%	31.2%	
Duration of response (months)	5.5	3.4	1.27 (0.84-1.92)

Table 4. Response rate and duration of response.

D: docetaxel; DR: docetaxel and risedronate; CI: confidence interval.

*Determined only for subjects with at baseline measurable disease,
PSA ≥20 ng/ml, or median PPI >2 on McGill-Melzack scale, respectively.

	D (N=301)		DR (N=291)	
	All grades	**Grade ≥3**	**All grades**	**Grade ≥3**
Any adverse event	289 (96%)	163 (54%)	284 (98%)	161 (55%)
Febrile neutropenia		15 (5%)		23 (8%)
Diarrhea	86 (29%)	9 (3%)	96 (33%)	6 (2%)
Neurotoxicity	139 (46%)	11 (4%)	149 (51%)	10 (3%)
Nausea	101 (34%)	3 (1%)	112 (38%)	3 (1%)
Hypocalcaemia	0 (0%)	0 (0%)	3 (1%)	1 (0.3%)

Table 5. Adverse events.

D: docetaxel; DR: docetaxel and risedronate.

not mandatory. Neutropenic fever was observed in 5% versus 8% of the patients in the D and DR group, respectively. No cases of osteonecrosis of the jaw were observed. Of the 436 deaths, 215 and 221 deaths occurred in the D group and DR group, respectively. Twelve patients (6 DR and 6 D) died within 30 days after the last cycle of docetaxel treatment. The most frequent cause of death was related to disease progression. There were 2 treatment related deaths (all in the D group) 1 due to neutropenic sepsis and 1 patient died from sepsis during docetaxel treatment but was not neutropenic. During the follow-up phase similar number of patients in the D and DR group received second line antineoplastic therapy (33% D versus 36% DR).

DISCUSSION

When bisphosphonates, such as zoledronic acid, were introduced in Europe in the early 2000s, to delay skeletal related events in the setting of CRPC, the results became available of the phase III docetaxel studies that showed survival benefit[8,9]. In Europe many patients did not yet routinely receive zoledronic acid until they had reached a mCRPC status. The obvious question therefore was whether bisphosphonates would provide an additional benefit at the time of initiation of effective docetaxel chemotherapy.

There are clinical and preclinical data suggesting that bisphosphonates have osteoclast-independent effects that can be associated with an anti-tumour effect.

Data from animal models have shown that risedronate and docetaxel act synergistically to protect bone and decrease tumour burden of established bone metastases from breast cancer cells[16]. The current study is the first prospective study evaluating the effectiveness of the third generation bisphosphonate risedronate in combination with docetaxel in CRPC. Our results demonstrate that the addition of risedronate to docetaxel, although well tolerated, has no impact on disease progression and overall survival. We found neither reduction in pain scores with the addition of risedronate to docetaxel in our study. The median OS in this study is comparable to previous studies with docetaxel based chemotherapy.

We found a higher incidence of neutropenic fever in our study compared to previous studies[8,9]. This might be attributable to differences in patient populations. As a result of the established OS benefit and the previously reported low incidence of neutropenic fever, in recent years there may have been a shifting threshold in treating more frail patients with more advanced disease with an associated higher risk of neutropenic complications.

The addition of risedronate to docetaxel did not increase toxic effects associated with standard docetaxel and could be safely administered.

Four parameters should be considered when selecting a bisphosphonate: efficacy, compliance, adherence, and safety. The choice of risedronate in our study was for an oral rather than an intravenous route of administration. Risedronate is the most potent oral nitrogen containing bisphosphonate available and there is strong pre-clinical evidence of a possible efficacy of the drug in skeletal metastases[15,24]. Patient's adherence to drug intake was monitored at each study visit by directly interviewing patients if they had been taking their prescribed medication, both by the treating physicians and at the time of collecting the patient diaries by the oncology nurses.

A potential confounder of this trial was its open-label design. Pain scores, analgesic use, and quality of life are difficult to objectively measure and may be confounded by the absence of blinding and could have potentially introduced observer and patient biases. However, since the study is entirely negative we do not believe that the results have been subjected by bias, as we found neither differences in palliative outcome measures, nor an improvement in TTP and OS by adding risedronate to docetaxel.

In patients with hormone-sensitive prostate cancer and bone metastases, sodium clodronate, an oral first generation bisphosphonate, may improve overall survival when given in addition to standard hormone therapy[25]. Thus far this is the only trial that has shown such benefit in patients with prostate cancer. The effects of zoledronic acid are currently being evaluated in patients with metastatic hormone-sensitive prostate cancer who are receiving androgen deprivation therapy in the Cancer and Leukemia Group B (CALGB) 90202 trial. Results from this trial may provide further insights into the potential benefits of bisphosphonates in the setting of hormone-sensitive disease[26].

Our study though, demonstrates that the addition of the third generation risedronate to docetaxel in patients with CRPC with bone metastases, although well tolerated, have no effect on TTP, PSA- and pain response and overall survival. Therefore, the addition of this bisphosphonate to docetaxel based chemotherapy cannot be recommended.

Acknowledgements

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Conflict of interest statement

This work was supported by Sanofi-Aventis, Gouda, the Netherlands. Ronald de Wit has consultant/advisory relationships with Sanofi-Aventis and has received honoraria and research funding from Sanofi-Aventis. He has no employment or leadership position, stock ownership, research funding, expert testimony or other remuneration disclosures. The other authors have no conflict of interest.

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Chapter 4

Danusertib, an Aurora Kinase Inhibitor

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ABSTRACT

Introduction

Drugs that interfere with the normal progression of mitosis belong to the most successful cytotoxic agents currently used for anti-cancer treatment. Aurora kinases are serine/threonine kinases that function as key regulators of mitoses and are frequently overexpressed in human cancers. The use of several small molecule aurora kinase inhibitors as potential anticancer therapeutic is being investigated. Danusertib (formerly PHA-739358) is a small ATP competitive molecule that inhibits Aurora A, B and C kinases. Interestingly, danusertib also inhibits several receptor tyrosine kinases such as Abl, Ret, FGFR-1 and TrkA. These tyrosine kinases are involved in the pathogenesis of a variety of malignancies and the observed multitarget inhibition may increase the antitumor activity resulting in extending the indication. Danusertib was one of the first Aurora kinase inhibitors to enter the clinic and has been studied in phase I and II trials.

Area covered

This review provides an updated summary of preclinical and clinical experience with danusertib up to July 2011.

Expert opinion

Future studies with danusertib should focus on the possibility of combining this agent with other targeted anticancer agents, chemotherapy, or radiotherapy. As a single agent, danusertib may show more promise in the treatment of leukemias than in solid tumors.

Keywords: aurora kinase, aurora kinase inhibitor, danusertib, mitosis, cancer, review.

1. INTRODUCTION

Mitosis is a key step in the cell cycle and is tightly controlled by the interplay of many proteins; abnormalities in any of these could result in uncontrolled or aberrant mitosis, leading to defects in the genetic material transfer to the daughter cells[1]. Most human cancer cells are characterized by hyperproliferation and changes in the DNA content due to errors in mitosis resulting in chromosome instability and aneuploidy[2]. Drugs that interfere with the normal progression of mitosis such as the taxanes and vinca alkaloids belong to the most successful cytotoxic agents currently used in cancer treatment. Anti-mitotic drugs either inhibit microtubule dynamics, or target proteins in the mitotic spindle. Classically, these drugs inhibit the function of the mitotic spindle by way of binding to the microtubule, and halt the cell cycle in mitosis and to induce apoptosis in tumor cells. However, these compounds do not selectively act on proliferating tumor cells, but exhibit substantial side effects on non-proliferating cells including neurons that are highly dependent on intracellular transport processes mediated by microtubules[3]. Hence, there is a need for more specific targets interfering with mitosis to avoid the side effects. Considering the complexity of mitosis multiple checkpoint systems have been identified that ensure proper coordination. Progression through mitosis depends on three regulatory mechanisms: protein localization, proteolysis and phosphorylation performed by several serine/threonine kinases, known as mitotic kinases[4,5.] Several mitotic kinases are known to date including the aurora kinase family.

Aurora kinases are serine/threonine kinases that play a crucial role in chromosome segregation and cytokinesis required for genome stability. The first aurora kinase was originally discovered in 1995 during a phenotype screening for defects at the mitotic spindles in Drosophila mutants[6]. The loss of function of a serine-threonine protein kinase led to failure of the centrosomes to separate and to form a bipolar spindle. Three years later the human homologue was described and a first link to cancer was established[7]. Shortly thereafter, two members of the aurora kinase family were discerned in mammals, aurora A and B[8].

The aurora family has been conserved throughout eukaryotic evolution. The evolutionary relationship between the aurora kinase proteins across species such as budding yeast (IpL1), fission yeast (Ark1), *Caenorhabditis elegans*, *Drosophila melanogaster*, *Xenopus laevis*, rat, mouse and human is complex. Mammals uniquely have three Aurora kinases, while for other metazoans, only aurora-A and aurora-B kinases are known. The fungi have only one aurora-like homolog[9].

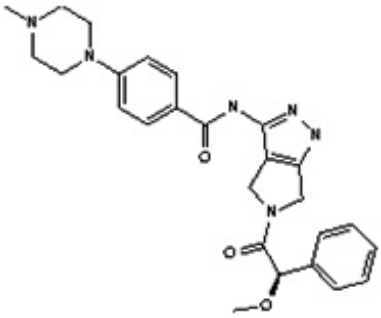
In the human genome three members of the aurora kinase family, aurora A, B and C have so far been identified. These three mammalian aurora paralogs are very similar in sequence, in particular within the carboxyterminal catalytic domain[10]. Surprisingly, given this level of similarity, the three mammalian aurora kinases have very distinct localiza-

tions and display distinct roles during mitosis, which are reflected in their subcellular locations. The three human aurora kinases range from 309 to 403 amino acids and share 67-76% amino acid sequence identity in their catalytic domains with little similarity in their N-terminus that provide the molecular basis for specific but diversified interactions with different effector proteins[11,12]. These aurora-effector protein interactions may account for their distinct subcellular localization on the mitotic spindle. Aurora A kinase is associated with centrosome maturation and separation and thereby regulates spindle assembly and stability, whereas aurora B kinase is a chromosome passenger protein and regulates chromosome segregation and cytokinesis[13].

Aurora kinases are frequently overexpressed in a wide range of human cancers and thus identified as potential new mitotic targets. Elevated expression has been associated with chromosome instability and poor prognosis exemplifying their significance for tumor formation and progression[7,14,15]. In a systemic analysis of expression levels of aurora A, B and C mRNA in multiple primary tumors, aurora A and B were significantly overexpressed compared with normal controls[13].

Therefore, Aurora kinases have become an attractive target for new anticancer treatments and development of small molecule inhibitors was initiated.

This review discusses the role of danusertib, a small-molecule pan-aurora kinase inhibitor, in the treatment of malignant disorders (Box 1). Several other aurora kinase inhibitors entered clinical development but are out of the scope of this review.

Drug name	Danusertib
Phase	Phase II
Indication	Malignancies
Pharmacology description	Aurora kinase inhibitor
Route of administration	intravenously
Chemical structure	
	N-{5-[2R)-2-methoxy-2-phenylacetyl]-1,4,5,6-tetrahydropyrrolo[3,4-c] pyrazol-3-yl}-4-(4-methylpiperazin-1-yl)benzamide
Pivotal trial(s)	[32,45,46,52-54]

Box 1. Drug summary.

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2. AURORA KINASE INHIBITORS

2.1 Aurora A

The gene for aurora A is located on chromosome 20q13.2, a locus that is frequently amplified in a variety of malignant tumors, including upper gastro-intestinal adenocarcinomas, breast, prostate and ovarian cancers[16]. In head and neck squamous cell carcinoma, a negative correlation between aurora A expression levels and tumor progression and overall survival has been demonstrated[17]. Although aurora A mRNA and protein are frequently overexpressed in several tumors, this is not necessarily correlated with gene amplification[18.]

Overexpression of an active mutant of aurora A in rat-1 cells induced neoplastic transformation indicating that it may function as an oncogene. Apart from gene amplification, transcriptional activation and inhibition of protein degradation could also contribute to the elevated levels of such overexpression, and promote tumorigenesis.

The oncogenic potential of aurora A probably results from the two different functions of the kinase; (1)chromosome segregation as well as control of genomic stability and (2) regulation of entry in mitosis[19]. To exert its functions aurora A is associated with different proteins, many of which are substrates and many of which are altered in cancer such as BRCA1, Lats2, NM-23, p53, or TACC[20,21,22].

The localisation of aurora A differs during progression of the cell cycle. During interphase it is localized on duplicated centrosomes and moves to the spindle poles in early mitoses (Figure 1). Aurora A plays a major role in centrosome maturation by recruitment of multiple proteins and participation in spindle assembly and stability. By binding to its substrate, aurora A is activated by autophosphorylation[5]. The aurora A substrate conglomerate prevents aurora A from being dephosphorylated by a type I phosphatase that associates with the kinase. The carefully orchestrated balance between aurora A kinase and its activator substrates and inhibitors is extremely important for normal mitosis. Therefore increase as well as decrease of aurora A kinase activity can cause errors of mitosis[4].

Moreover, overexpression of Aurora-A leads to genetic instability, characterized by centrosome amplification, chromosome tetraploidization and premature sister chromatid segregation at stages before tumor formation[23].

2.2 Aurora B

The aurora B gene maps to chromosome region 17p13.1 and like aurora A, aurora B kinase is overexpressed in tumor cells. Aurora B kinase is a chromosomal passenger protein localized to centromeres in metaphase and remaining associated with the spindle

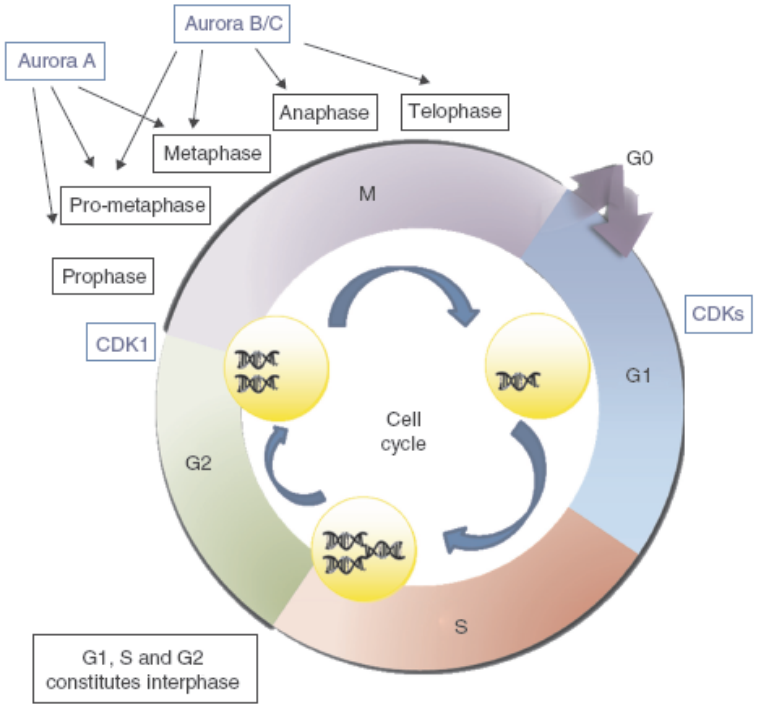

Figure 1. Involvement of aurora kinases A, B and C in the progression of the mitotic phase of the cell cycle. Reproduced from Cheung et al (2009)[1]. Adapted by permission from Informa Healthcare: Investig Drugs, copyright 2009.

midzone in anaphase (Figure 1)[1]. Aurora B together with three other proteins, inner centromere protein (INCENP), survivin and borealin, forms the chromosal passenger complex (CPC). This complex controls the accurate segregation of the chromatids at mitosis, histone modification and cytokinesis[24]. Aurora B is the key component of the CPC, with inhibition of aurora B resulting in impaired CPC function. Aurora B phosphorylates the INCENP, survivin and borealin; moreover, aurora B activity and localization during mitosis is tightly regulated by its complex interaction with these CPC partners. Phosphorylation of the inner centromere protein by aurora B induces a conformational change that, in turn, facilitates aurora B phosphorylation and full activation of the kinase. Survivin phosphorylation by aurora B at threonine 117 is involved in regulating localization. Aurora B also phosphorylates histone H3, a protein involved in chromosome condensation, at serine 10 and 28 during mitosis. Selective inhibition of aurora B results in polyploidy, inhibition of histone H3 phosphorylation at serine 10 and apoptosis[24,25]. In addition, aurora B also phophorylates mitotic centromere-associated kinesin (MCAK) involved in the spindle checkpoint correcting the improper attachments of microtubles to the kinetochores[26].

Finally aurora B is very important for proper cytokinesis. In the absence of aurora B mediated phosphorylation of Ser72 in vimentin, the two daughter cells remain attached to each other through bridges of cytoplasm and cytokinesis fails[27]. Other aurora B regulated proteins involved in cytokinesis include MgcRacGap, MKLP-1 and condensin 1[28].

2.3 Aurora C

The aurora C gene maps to chromosome region 19q13. Characterization of aurora C has been rather limited and unlike aurora A and B, which are ubiquitously expressed in many tissues, particularly in mitotically dividing cells, aurora C is predominantly expressed in the testis and is mainly restricted to meiotically dividing spermatocytes and oocytes[29,30]. Aurora C is also associated with INCENP in male spermatocytes. Increased expression of both aurora C and INCENP will lead to increased phosphorylation of histone H3. This observation leads to the hypothesis that aurora C is a chromosome passenger protein and can act in a similar fashion as aurora B, although little is known about its functional role[31].

3. DANUSERTIB

Danusertib is a potent small-molecule 3-aminopyrazole derivative developed by Nerviano Medical Sciences S.r.l.- Milan, Italy. Danusertib inhibits the adenosine triphosphate (ATP) site of all three members of the aurora kinase family, aurora A, B, and C, with IC_{50} of 13, 79, and 61 nM, respectively[32]. The molecular structure of danusertib is shown in Box 1. The major route of metabolism of danusertib involves the formation of the N-oxide derivative, mainly through the enzyme flavin containing monooxygenase 3. The N-oxide metabolite has less than 1% potency of the parent compound. Danusertib does not inhibit cytochrome P450 isoenzymes or ABCB1 (P-glycoprotein)[33]. Preclinical pharmocokinetics (PKs) of danusertib are dose proportional and time independent. Cells treated with danusertib showed the expected inhibition of phosphorylation of histone H3[32], which effect can be assessed as biomarker and clinical proof of principle of danusertib activity.

3.1 Preclinical studies with danusertib

3.1.1 In vitro studies

In vitro studies showed strong antiproliferative effects of danusertib in a wide range of tumor cell lines, with accumulation of tetraploid cells in G1-like growth arrest or cells > 4N DNA as a feature of endoreduplication. These different effects of danusertib might be

due to the genetic background of the tumor cells tested, reflecting different requirements for aurora kinase activity. Most probably they depend on the status of p53-dependent mitotic checkpoint, since treatment with danusertib of cells with wild-type p53 resulted in a growth arrest with 4N DNA content, whereas cells with defective p53 were more prone to progress through the cell cycle after failed cytokinesis and accumulated with >4N DNA content[32,34,35]. As expected, cells treated with danusertib showed decreased phosphorylation of histone H3 and a reduction in aurora A auto-phosphorylation, suggesting a potent inhibition of both kinases at nano-molar concentrations in cells.

Tested in a panel of 32 kinases, danusertib showed also cross-reactivity with other kinases, most importantly Abl (IC_{50} 25 nM), including the T315I mutant, as well as Ret (IC_{50} 31 nM), Trk-A (IC_{50} 30 nM), and fibroblast growth factor receptor-1 (FGFR-1; IC_{50} 47 nM). Cross-reactivity with Abl was seen at a 2-fold higher IC_{50} compared with aurora A, whereas 28 other kinases displayed at least a 10-fold selectivity[33]. The observed cross-reactivities may guide assessment of clinical activity in specific tumors.

Given the key role of the oncogenic Bcr-Abl tyrosine kinase in chronic myelogenous leukemia (CML) and a subset of acute lymphoblastic leukemias (ALL)[36], danusertib was tested and showed inhibited the growth of $CD34^+$ cells derived from imatinib resistant CML patients with wild type or mutated Bcr-Abl, including the T315I mutation[34].

Overexpression of the Abcg2 efflux transporter was identified and functionally validated as the predominant mechanism of acquired danusertib resistance in Bcr-Abl-positive cells[37]. The combined treatment with imatinib and danusertib significantly reduced resistance emergence.

Expression of Ret has been linked to thyroid carcinoma and recently has been identified as one of the genes most altered in breast cancer[38,39]. Expression of Trk-A has been reported in prostate and thyroid carcinoma[40].

The Ret kinase inhibitory activity of danusertib was tested in cells which contained a Ret allele with a consecutively activating mutation in the extracellular domain and which can be used to determine receptor autophosphorylation[32,41]. Inhibition of ligand-induced Trk-A phosphorylation was evaluated in PC-12 cells, a NGF-responsive cell line established from a rat pheochromocytoma. Both Ret kinase and Trk-A kinase were inhibited at low micromolar concentrations of danusertib, although sensitivity was lower as compared to aurora inhibition. Furthermore, danusertib had an effect on mitogen-activated protein kinase (MAPK) activation induced by FGF, but not by EGF, demonstrating selectivity for inhibition of the FGFR-1 pathway[32].

3.1.2 In vivo studies

The antitumor activity of danusertib was examined in several solid human tumor xenografts models[32]. Significant tumor growth inhibition in models of ovarian carcinoma, colon carcinoma, and acute myelogenous leukemia[32], showed dose-dependency in bal-

Model	Dose and schedule	Maximal TGI, % (day)	Maximal weight loss (%)
A2780 human ovarian carcinoma xenograft in nude mice	30 mg/kg i.v. qD, D1-10	80 (19)	18
HCT-116 human colon carcinoma xenograft in nude mice	30 mg/kg i.v. bd, D1-5	66 (17)	22
HL-60 human acute myelogenous leukemia xenograft in SCID mice	30 mg/kg i.v. bd, D1-5	98 (22)*	16
DMBA-induced mammary carcinoma in rat	25 mg/kg i.v. bd, D1-3 q wk x 2	75 (10)†	10
MMTV-RAS transgenic mammary carcinoma in mice	30 mg/kg i.v. bd, D1-3 q wk x 2	68 (23)	15
TRAMP transgenic prostate carcinoma in mice	30 mg/kg i.v. bd, D1-5	3/16 PR 10/16 SD 3 progressions	ND

NOTE: n = 8-10 animals/study. The TGI (%) was calculated according to the equation % TGI = 100 – (mean tumor weight of treated group/mean tumor weight of control group) x 100.

Abbreviations: ND, not determined; qD, once a day.

*Two of eight animals showed complete regression.

†One of 10 animals showed complete regression; 1/10 death at day 5.

Table 1. *In vivo* activity of danusertib. Reproduced from [32].

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ance with a good safety profile (Table 1). There was also evidence of tumor regression and occasional cures.

In two breast tumor models, namely a DMBA (7,12-dimethylbenz[α]anthracene)-induced mammary carcinoma in rats and activated Ras-driven mammary carcinoma in transgenic mice, danusertib resulted also in significant tumor growth inhibition[32].

In the transgenic mouse prostate (TRAMP) carcinoma model magnetic resonance imaging revealed tumor regressions and disease stabilizations induced by danusertib[32,42].

Neuroendocrine prostate cancer (NEPC) is a distinctly different disease from prostate adenocarcinoma and also showed significant molecular differences[43]. Aurora kinase A and N-myc were overexpressed in NEPC vs prostate adenocarcinoma ($P<0.001$), and both genes were amplified in 35% of NEPC, 5% of prostate adenocarcinoma, and none of benign prostate. Transfection of MYCN induced aurora kinase A expression and kinase activity in vitro, and aurora kinase A induced MYCN. Enhanced in vitro and in vivo sensitivity to danusertib was observed in NCI-H660 (NEPC) compared to LNCaP and VCaP (prostate adenocarcinoma), with > 50% tumor shrinkage in NEPC and minimal to no effect in prostate adenocarcinoma. Histone H3 phophorylation was inhibited in the treated NCI-H660 and not in prostate adenocarcinoma.

In a study by Benten et al.[44], the efficacy and toxicity of danusertib was evaluated in subcutaneous hepatocellular carcinoma xenograft models showing high antiproliferative activity at well-tolerated doses. Inhibition of tumor growth in rapidly proliferat-

ing Huh-7 tumors was highly significant although tumors continued to grow at a very slow rate. Antiproliferative efficacy was even more pronounced in moderately growing HepG2 tumors, although no significant tumor regression was observed. The combination of danusertib with sorafenib showed a synergistic effect and when tumors restarted to grow under sorafenib monotherapy, subsequent treatment with danusertib induced tumor shrinkage by up to 81%.
Based on the results from preclinical testing, danusertib was progressed to phase I clinical investigation.

3.2 Clinical trials with danusertib

3.2.1 Phase I clinical trials
Two parallel phase I dose escalation studies were performed. The first study evaluated danusertib administered IV on days 1, 8, and 15 every 4 weeks in 6-hour and 3-hour infusion schedules at dose ranges 45-400 mg/m^2, in 50 patients with solid tumors[45]. The main dose limiting toxicity (DLT) observed was grade 3-4 neutropenia. The most frequent non-haematologic adverse events were mainly grade 1 and 2 fatigue, nausea, diarrhea and anorexia. Stable disease was observed in 24% of the evaluable patients, in five of whom the disease stabilization lasted more than 6 months. The systemic exposure to the parent compound increased linearly with dose and was not influenced by the infusion duration. Biomarker analysis showed inhibition of histone H3 phosphorylation in skin biopsies starting at a dose of 190 mg/m^2. The recommended phase II dose was 330 mg/m^2 danusertib administered over 6 hours on days 1, 8 and 15 every 28 days.
The second study tested 24h infusion in a 2-week cycle in patients with advanced solid tumors[46]. In the first part of the study 40 patients were treated without granulocyte colony-stimulating factor (G-CSF) and 7 dose levels were explored (45-650 mg/m^2). Again, principal DLTs were grade 3-4 neutropenia, diarrhea, nausea, vomiting and fatigue. Non-haematological toxicities were mostly mild and included fatigue, anorexia, vomiting, diarrhea, constipation and pyrexia. Eleven of the 40 patients showed disease stabilization. One patient with refractory small cell lung cancer had an objective response lasting 23 weeks. Post-therapy skin biopsies showed decreased level of histone H3 phosphorylation starting at 500 mg/m^2. The recommended phase II dose was established at 500 mg/m^2 without G-CSF. In the second part of the study further dose escalation (580-1000 mg/m^2) was performed with co-administration of G-CSF in 16 patients. Renal toxicity became dose limiting and the maximum tolerated dose (MTD) was set at 750mg/m^2 i.v. over 24 hours every 14 days. Pharmacokinetics (PK) of Danusertib are summarized in Table 2.
In an exploratory study on patients of a phase I trial and subsets of two phase II trials no significant associations between polymorphisms in genes coding for drug metabolizing

Reference	Subject and Regimen	Cmax (µmol/L)	Cl (L/h)	V_d (L)	$T_{1/2}$ (h)	AUC (µmol/L*h)
[45] Steeghs, et al, 2009	50 patients with solid tumors					
	Group 1: 6 hour IVS, d1,8,15 every 28 days D1 dose, mg/m^2 (n)					
	45 (3)					
	90 (7)					
	135 (4)	0.83±0.3	33.4± 11.0	857± 312	17.6 ± 0.8	5.9 ± 2.2
	190 (4)	2.25± 0.6	27.4± 6.8	1010± 725	27.2 ± 22	14.0 ± 3.0
	250 (10)	2.56± 1.4	38.3± 10.5	1041± 198	19.5 ± 3.9	13.9 ± 3.6
	330 (7)	3.86± 1.1	30.0± 7.6	1085± 565	24.4 ± 7.8	27.5 ± 6.2
	400 (4)	4.75± 1.6	35.1± 11.8	1272± 645	25.1 ± 13	30.8 ± 9.2
	45-400 (39)	5.62± 2.5	38.3± 12.5	1832± 933	33.3 ± 17	38.5 ± 11
		6.31± 2.3	35.5± 9.8	1872±1030	37.7 ± 22	49.3 ± 11
	Group 2: 3 hour IVS, d1,8,15 every 28 days. D1 dose, mg/m^2 (n)	4.00± 2.3	34.0±10.4	1312±752	27.0 ±15.4	27.1 ±15.4
	250 (2)					
	330 (6)					
	250-330 (8)					
		7.10± 0.9	38.7± 10.4	1787± 400	32.3± 1.5	28.7 ± 2.4
		10.1± 1.7	32.8± 14.8	1386± 762	28.5± 9.8	52.7 ± 30
		9.34± 2.0	34.3± 13.4	1487± 687	29.4± 8.5	46.7 ± 28
		Cmax (µmol/L)	**CL (L/h/kg)**	**V_d (L/kg)**	**$T_{1/2}$ (h)**	**AUC (µmol/L*h)**
[46] Cohen, et al, 2009	56 patients with solid tumors 24 hour IVS, d 1 every 14 days D1 dose, mg/m^2 (n)					
	45 (3)	0.27 ± 0.03	0.39 ± 0.05	9.9 ± 2.6	18 ± 4	6.2 ± 0.9
	90 (3)	0.60 ± 0.17	0.36 ± 0.10	9.9 ± 4.1	19 ± 8	12 ± 2.9
	180 (3)	1.09 ± 0.42	0.43 ± 0.11	13 ± 5.3	22 ± 8	23 ± 6.2
	360 (6)	2.06 ± 0.63	0.46 ± 0.15	16 ± 9.2	23 ± 7	45 ± 14
	500 (12)	3.20 ± 1.29	0.40 ± 0.14	14 ± 8.9	23 ± 8	74 ± 25
	580 (9)	4.13 ± 0.77	0.33 ± 0.05	12 ± 2.9	25 ± 5	91 ± 11
	650 (7)	4.25 ± 0.71	0.36 ± 0.9	12 ± 4.8	24 ± 8	104 ± 20
	750 (6)	4.50 ± 1.39	0.38 ± 0.13	14 ± 5.7	26 ± 2	106 ± 34
	11000 (7)	6.56 ± 2.59	0.40 ± 0.10	15 ± 3.7	25 ± 3	147 ± 38

Table 2. Summary of danusertib pharmacokinetics, during cycle 1.

Abbreviations: Cmax, maximum concentration level; Cl, systemic clearance; V_d, volume of distribution; $T_{1/2}$, terminal half –life; AUC, area under the curve; IVS, infusion schedule; d, day; n, number of patients.

enzyme, for transporter proteins and clearance of danusertib, between target receptor polymorphisms and toxicity of danusertib and between polymorphisms in the aurora kinase B receptor and the extent histone H3 phophorylation were observed[47]. These clinical findings are notably different to the previously mentioned *in vitro* data in which overexpression of Abcg2 efflux transporter was correlated with resistance to danusertib, whereas in this study no apparent association between danusertib clearance and genetic polymorphisms in AbcG2 was observed[37].

Danusertib was also explored in combination with bevacizumab in a phase I study[48]. Grade 3 diarrhea, nausea, vomiting and fatigue were dose-limiting. Treatment-related grade 3/4 haematological toxicitiy was represented by neutropenia in 65% of the patients. The most frequent treatment-related events were usually of grade 1/2 severity, reversible and easily manageable and included fatigue, nausea, diarrhea, anorexia, mucosal inflammation and vomiting. PK parameters were dose and time independent and characterized by limited interpatient variability. Bevacizumab did not alter the PK profile of danusertib. The recommended dose for phase II was 250 mg/m^2 of danusertib and 15 mg/kg of bevacizumab.

The above mentioned studies were all performed in patients with solid tumors. In addition, 23 patients with CML and Philadelphia chromosome positive ALL were enrolled in a phase I study of danusertib administered via 3 hours infusion daily for 7 consecutive days every 14 days[49]. Fourteen out of 24 patients carried a confirmed T315I BCR-Abl mutation, 2 patients demonstrated hematologic response and 9 patients demonstrated a hematological improvement. The MTD was not yet determined at the time of reporting. A phase II clinical trial treating patients with CML completed accrual and report of the results are expected soon[50].

3.2.2 Phase II clinical trials

The last mentioned phase II study is performed in patients with CML relapsing on imatinib or other c-ABL therapy, and explored danusertib at two dose levels, 250 or 330 mg/m^2/day, given as a weekly 6-hour infusion for 3 consecutive weeks, every 4 weeks. A very preliminary abstract reported on the first seven CML patients (1 in chronic phase, 1 in accelerated phase, 5 in blast phase) enrolled[51]. Six out of seven patients had the BCR-ABL T315I mutation. Two patients with T315I mutated BCR-ABL achieved a complete hematologic response (CHR) associated to a complete cytogenetic response and a minor cytogenetic response (one case each). The Cmax at the effective dose of 330 mg/m^2/day was 4-6 uM/h. Modulation of histone H3 phosphorylation was observed in 3 of 5 evaluable patients.

In another phase II trial, evaluating the 4 months progression-free rate, 42 patients with breast cancer and 34 patients with ovarian cancer, progressing after 2 prior chemotherapy lines for advanced/metastatic disease, were enrolled[52]. Danusertib was ad-

Subject and Regimen	7 patients with CML 250 or 330 mg/m^2 6 hour IVS, d1,8,15 every 28 days		76 patients with advanced BC or OC 500 mg/m^2 24 hour IVS, d 1 every 14 days		68 patients with advanced CRC or PC 500 mg/m^2 24 hour IVS, d 1 every 14 days		81 patients with advanced CRPC A:330 mg/m^2 6 hour IVS, d1,8,15 every 28 days or B: 500 mg/m^2 24 hour IVS, d 1,15 every 28 days	
Reference	[51]		[52]		[53]		[54]	
Adverse Events	**All grades**	**Grades 3/4**	**All grades**	**Grades 3/4**	**All grades**	**Grades 3/4**	**All grades**	**Grades 3/4**
	No.(%)	**No.(%)**	**No.(%)**	**No.(%)**	**No.(%)**	**No.(%)**	**No.(%)**	**No.(%)**
neutropenia	.	1 (14)	74 (97)	66 (87)	.	55 (81)	A:93 B:84	A:39 B:34
Febrile neutropenia	.	.	5 (7)	5 (7)	.	6 (9)	.	.
lymphocytopenia	.	.	.	.	.	10 (15)	.	.
thrombocytopenia	.	.	8 (10.5)	1 (1.3)	.	1 (1.5)	.	.
anemia	.		.55 (72.3)	3 (4)	.	3 (4.4)	.	.
fatigue	.	0	.25 (33)	3 (4)	.	7 (10.3)	≥10	.
diarrhea	.	0	.33 (43.4)	2 (3)	.	5 (7.3)	≥10	.
pyrexia	.	0	.13 (17)	2 (3)	.	.	≥10	.
anorexia	.	0	.15 (20)	.	.	.	≥10	.
vomiting	.	0	.21 (28)	.	.	.	≥10	.
constipation	.	0	.23 (30)	.	.	.	≥10	.
hypertension	.	0	.5 (7)	.	.	.	≥10	.
phlebitis	.	.	.5 (7)	.	.	.	≥10	.
infusion-related reaction	.	1 (14)	.	.	.	.	.	.
nausea	.	.	44 (58)	.	.	.	.	.

Table 3. Summary of danusertib related side effects in phase II clinical trials.

Abbreviations: CML, Chronic myelogenous leukemia; BC, breast cancer; OC, ovarian cancer; CRC, colorectal cancer; PC, pancreatic cancer; CRPC, castration resistant prostate cancer; IVS, infusion schedule; No., number of patients.

ministered i.v. over 24 hours every 14 days at 500 mg/m^2. In breast cancer 7 out of 38 evaluable patients were progression free at 4 months. Best response was stable disease in 11 patients with a median duration of 20 weeks. In ovarian cancer, 4 patients out of 34 were free from progression at 4 months. Best response was a confirmed partial response in 1 patient. There were 10 patients with stable disease (SD). Side effects from all phase II trials are tabulated in Table 3. The most frequent grade 3/4 hematological toxicity consisted of neutropenia (86%). The study was closed in ovarian cancer patients after stage one because the efficacy of danusertib did not meet the predefined study endpoint boundaries.

In a phase II study by Laffranchi et al.[53], patients with advanced/metastatic pancreatic and colorectal cancers (CRC) danusertib was also administered every 14 days, over 24-hour i.v. infusions of 500 mg/m^2. The primary endpoint was progression-free rate at 4 months.

Thirty-three patients with CRC and 2 prior lines of chemotherapy were included. No patient was progression free at 4 months. Thirty –five patients with pancreatic cancer, relapsing after 1 prior chemotherapy line for advanced/metastatic disease were enrolled. Three of the 31 evaluable patients were free from progression at 4 months, and had stable disease for 6 to 8.5 months. Danusertib plasma concentrations at the end of the infusions and pre-dose in different cycles were in line with those obtained in phase I studies.

In a randomized phase II study in patients with metastatic castration-resistant prostate (CRPC) progressing after first line docetaxel based chemotherapy, danusertib was administered using 2 different schedules with equivalent dose intensity [54]. The primary endpoint was 50% prostate specific antigen (PSA) response rate at 3 months. Forty-three patients (30 evaluable patients) were treated with danusertib 330 mg/m^2 over 6 hours i.v. on Days 1, 8, 15, every 4 weeks (A) and 37 patients (28 evaluable patients) were treated with danusertib 500 mg/m^2 over 24 hours i.v. on Days 1, 15, every 4 weeks (B). There were no statistical significant differences between the 2 study arms. One patient per arm achieved a PSA response. Best overall response according to Response Evaluation Criteria in Solid Tumors (RECIST)[55] was stable disease in 26% (A) and 43% (B) of the patients, respectively. Clinically relevant disease stabilizations lasting ≥6 months were reported in 12 patients (5 in the first arm and 7 in the second arm). Median progression free survival was 12 weeks in both arms. Both schedules showed acceptable toxicity. Uncomplicated neutropenia was the most frequent treatment emergent hematologic adverse event. Neutropenic fever was observed in 3 cases. Non-haematologic adverse events were mostly grade 1 and 2, consisting of fatigue, nausea, diarrhea, anorexia, pyrexia, vomiting, constipation, hypertension, and phlebitis, which was insufficient to activate the second stage of the study in both schedules tested.

3.3 Combination treatment

Data on combination treatments involving danusertib are scanty and mainly from preclinical studies. As previously mentioned synergistic effects have been shown in leukemia cells treated with danusertib and imatinib and in HCC xenograft models treated with danusertib in combination with sorafenib[37,44]. Moreover intraperitoneal administration of danusertib at a dose of 15 mg/kg (twice daily and continuously for 9 days) combined with imatinib at a dose of 100 mg/kg clearly demonstrated the synergistic in tumor growth inhibition as compared to the monotherapy in mice implanted with

K562 cancer cells. On the other hand, combined treatment using danusertib at a dose of 15 mg/kg (twice a day, continuously for 10 days) with bevacizumab at a dose of 20 mg/kg (once per day for 3 days) has been shown to induce synergistic tumor growth inhibition in mice implanted with human DU145 prostate cancer cells[56].
The only clinical information yet available is from the phase I trial treating patients with solid tumors with danusertib in combination with bevacizumab which was well tolerated[48].

4. CONCLUSIONS

Aurora A, B and C, are members of the serine/threonine kinase family, and play an important role in mitosis. They are essential for spindle assembly, centrosome maturation, chromosomal segregation and cytokinesis during mitosis. Overexpression/amplification of aurora kinases has been implicated in oncogenic transformation, including the development of chromosomal instability in cancer cells. Since their discovery aurora kinases have been identified as a potential target in anticancer therapy and currently, many aurora-selective small molecule kinase inhibitors are in development, undergoing preclinical and clinical studies. The interest in designing drugs against aurora kinase family members stems from the facts that these kinases are not only vitally important regulators of mitosis but have also been shown to functionally interact with multiple critical oncoproteins and tumor suppressor proteins.
Danusertib inhibits the ATP site of all three members of the aurora kinase family, and was in fact the first aurora kinase inhibitor to be tested in the clinic[45,46] and it remains the most advanced in clinical development. *In vitro* studies using a broad panel of different human cancer cell lines showed strong antiproliferative effects of danusertib treatment. *In vivo* administration of danusertib exhibited significant antitumor activity at the tolerated doses in several human tumor xenografts as well as spontaneous and transgenic mouse and rat tumor models of CML, ovarian, colon, mammary and hepatocellular carcinomas.
In phase I studies clinically relevant disease stabilizations were observed in several patients. Danusertib was well tolerated, with neutropenia as the principal toxicity.
However, the preliminary data of the available phase II studies showed limited activity of danusertib in patients with solid tumors and the studies were discontinued after the first part because of this.
Albeit that synergism in preclinical studies has only infrequently translated into additive activity in human beings, it can not be excluded that, given its side effect profile and based upon the preclinical studies, danusertib may yield more activity in the treatment of leukemias than in solid tumors. Combinations of aurora kinase inhibitors and exist-

ing cytotoxic compounds could be more beneficial than single agent treatment, and are therefore logical next step in future development.

5. EXPERT OPINION

Since their discovery, aurora kinases emerged as important enzymes involved in the cell cycle regulation and have shifter to become interesting targets of anticancer therapy. Currently various aurora kinase inhibitors are in clinical development. Danusertib, which inhibits all three aurora kinases, is a highly innovative drug and was in fact the first aurora kinase inhibitor to be tested in human. Phase I studies with danusertib showed tumor stabilization as the major clinical response, which stresses the need for biomarkers as a helpful tool to guide decisions for further development. At present, only modulation of histone H3 phosphorylation has been demonstrated to be useful as a pharmacodynamic biomarker for target modulation of aurora B inhibitors, whereas the degree of autophosphorylation on Threonine 288 constitutes a biomarker for aurora A activity.
Involvement of aurora kinases in deregulating multiple tumor suppressor and oncogenic pathways together with the preclinical findings on the efficacy of aurora kinase inhibitors in attenuating growth of tumor cells render these molecules as potentially active anticancer drugs. However, in contrast to the yet limited but interesting results in haematological malignancies, phase II trials with danusertib as monotherapy in patients with solid tumors did not demonstrate the required activity, although safety was established. This might be partly explained by the relative low percentage of tumor cells in mitosis in most solid tumors. In addition emerging data demonstrate an important role for microtubule trafficking in cell survival. Many crucial oncoproteins such as p53, BRCA1, Rb and androgen receptor are associated with microtubules. Inhibition or interference with the ability of these proteins to traffic on microtubules could result in cell death and might constitute an advantage of the classical tubule binding agents like the taxanes over aurora kinase inhibitors and other mitosis-specific inhibitors[57].
Aurora kinases also have been found physically associates with multiple significant cancer-related proteins. Inhibitions of aurora kinases have been shown to result in cell death or enhance cytotoxic effect induced by other anticancer agents through these pathways. Combinations of danusertib with additional drugs targeting other oncogenic deregulated pathways, existing cytotoxic compounds and/or radiotherapy therefore deserves attention in future studies.
As with other molecular targeted anticancer therapies, the key question to be addressed will concern the selection of the patient population with the highest probability of responding to danusertib. A predictive biomarker that can be used for this purpose has not yet been identified

Danusertib may yield more activity in the treatment of leukemias than in solid tumors. However, currently it is not clear if the preliminary therapeutic activity of danusertib in leukaemia is primarily due to aurora inhibition or due to the multi-kinase targeting of BCR-ABL kinase. The most interesting application for danusertib appears to be in Philadelphia positive ALL and imatinib-resistant CML particularly involving the T315I mutation.

In conclusion, the clinical activity of single agent danusertib in phase II trials in patients with solid tumors has been rather disappointing. Preclinical data support combination of the agents with other targeted anticancer agents, cytotoxic, or radiotherapy. Future single agent studies, danusertib should focus on leukemias rather than solid tumors. Development of specific biomarkers predictive for response may enable a more focused evaluation of this class of anticancer drugs.

DECLARATION OF INTEREST

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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Chapter 5

Randomized phase II study of Danusertib in patients with metastatic Castration Resistant Prostate Cancer after Docetaxel failure

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ABSTRACT

Objectives:

To determine the efficacy and toxicity of danusertib (formerly PHA-739358) administered i.v. over two different dosing schedules with equivalent dose intensity in patients with metastatic castration-resistant prostate cancer with progressive disease after docetaxel-based treatment.

Patients and Methods:

In this open-label, multicentre phase II trial 88 patients were randomly assigned (1:1 ratio) to receive either danusertib 330 mg/m^2 over 6 h i.v. on days 1, 8, and 15 (arm A; *n*=43) or 500 mg/m^2 over 24 h i.v. on days 1, 15 (arm B; *n*=38), every 4 weeks. The primary endpoint chosen for this exploratory study was PSA response rate at 3 months.

Results:

Sixty patients (31/43 in arm A and 29/38 in arm B) were evaluable for the primary endpoint. Median progression-free survival was 12 weeks in both arms. PSA response occurred in one patient in each arm; best overall response was stable disease in eight (18.6%) and 13 (34.2%) patients in arm A and B, respectively. Eleven out of 81 (13.6%) treated patients had stable disease for ≥ 6 months. Danusertib was generally well tolerated; the most common grade 3 and 4 drug-related adverse event was neutropenia which occurred in 37.2% (arm A) and 15.8% (arm B) of the patients.

Conclusions:

Danusertib monotherapy shows minimal efficacy in patients with castration-resistant prostate cancer. Further studies are required to establish specific biomarkers predictive for either response or prolonged disease stabilization.

Keywords: aurora kinase, aurora kinase inhibitor, castration-resistant prostate cancer danusertib, PHA-739358

INTRODUCTION

For more than 70 years androgen deprivation therapy (ADT) has been the standard of care in patients with advanced prostate cancer, but eventually patients uniformly progress to a state of castration-resistant prostate cancer (CRPC) [1]. At the initiation of this study the only approved therapy that had been shown to prolong survival in patients with CRPC was docetaxel-based chemotherapy [2]. Treatment options for patients whose disease progresses after docetaxel treatment was an unmet medical need. Recently, this clinical landscape has been altered by the results of phase III trials with cabazitaxel [3], abiraterone acetate [4], enzalutamide [5] and radium-223 chloride [6]. Although treatment options have increased, patients still have a poor prognosis and novel rational approaches are needed.

Aurora-kinases play an essential role as key mitotic regulators, controlling entry into mitosis, centrosome function, chromosome assembly, and segregation [7]. Since Aurora kinases are typically overexpressed in prostate cancers [8], and probably facilitate progression of prostate cancer, targeted inhibition of these kinases may offer therapeutic benefit. Danusertib (formerly PHA-739358) is a potent small-molecule 3-aminopyrazole derivative that inhibits all Aurora kinases [9]. *In vivo* data in the transgenic mouse prostate carcinoma model revealed tumour regressions of >80% in three out of 16 animals and disease stabilizations in 10 out of 16 animals treated with danusertib [10,11]. Two parallel phase I dose escalation studies with danusertib in patients with advanced solid tumours were performed [12,13]. Two phase II doses and schedules were established. A weekly regimen applies 330 mg/m^2 administered over 6 hours i.v. and a 2-weekly regimen applies 500 mg/m^2 administered over 24 hours i.v. The main dose limiting toxicity (DLT) observed was grade 3-4 neutropenia. Based on these considerations and preclinical data supporting substantial efficacy of danusertib in prostate cancer the current study was designed to assess the antitumour activity of danusertib in patients with docetaxel-refractory metastatic CRPC.

MATERIALS AND METHODS

Patients

Eligible patients had metastatic CRPC with disease progression after treatment with docetaxel chemotherapy. For patients with measurable disease, progressive disease was defined according to the Response Evaluation Criteria in Solid Tumours (RECIST) [14]. For patients without measurable disease, progressive disease was defined as appearance of new metastases on bone scan in combination with PSA progression, according the criteria of the PSA Working Group [15].

Additional eligibility criteria included Eastern Cooperative Oncology Group performance status (ECOG PS) ≤ 2; age ≥ 18 years; life expectancy ≥ 3 months, castrate levels of testosterone; and haematological and chemical laboratory values that met predefined criteria.
Patients receiving bisphosphonates must have been on stable doses for at least 4 weeks. Exclusion criteria were more than one previous chemotherapy regimen, treatment within the previous 28 days with radiotherapy or investigational drugs, CNS involvement, uncontrolled infection, clinically significant vascular disease, uncontrolled hypertension and other prior malignancy except for non-melanoma skin cancer or superficial bladder cancer.
Corticosteroids had not to be initiated during the study period unless mandated by patient clinical condition. Initiation or increase in dose of corticosteroids during the study period, as they might reduce PSA levels, rendered the patient not evaluable for the primary end-point.
Patients provided written informed consent before enrollment. The study was conducted according to the Declaration of Helsinki and in compliance with good clinical practice and local ethical and legal requirements.

Study design

This open-label, non-comparative, multicentre, randomized phase II study was designed in two sequential parts. The first part, presented here, was performed to select the best dose schedule of danusertib based on PSA responses. If there were three or more out of 29 evaluable patients per arm with a PSA response, the second part would be initiated. The second part would consist of a randomized, open-label comparison of the selected dose schedule of danusertib with mitoxantrone or an approved second-line treatment if any agent was, at the time of initiating the second part of the study, was approved for this indication. Patients were centrally randomly assigned in a 1:1 ratio to treatment to either danusertib 330 mg/m^2 over 6 h i.v. on days 1, 8 and 15 (arm A) or danusertib 500 mg/m^2 over 24 h through a central venous catheter, on days 1 and 15 (arm B), every 4 weeks. Patients were stratified by PSA response to prior docexatel chemotherapy (≥ 50% PSA decline vs < 50% PSA decline). Danusertib was continued until disease progression, unacceptable toxicity or patient refusal.

Outcome analysis

The primary endpoint was PSA response rate within the first three months of treatment. PSA response was defined as ≥ 50% decline in serum PSA concentration compared with baseline, confirmed at least 4 weeks later. Secondary endpoints included duration of

PSA response, 30% PSA reduction rate, and objective tumour response rate according to RECIST criteria. All responses had to be confirmed. Analyses of PSA endpoints were only performed in patients with documented PSA progression at study entry.
Other secondary endpoints were progression free survival (PFS), clinical benefit rate and safety. For patients without measurable disease, progressive disease was defined as appearance of more than one new lesion outside the bone or two or more new bone lesions or one new bone lesion associated with PSA progression. For patients with measurable disease, progression was defined according to RECIST criteria.
To take the new recommendation of the Prostate Cancer Clinical Trials Working Group 2 [16] into consideration the protocol was amended to change the definition of PSA progression. PSA progression was defined as increase of ≥25% over nadir PSA concentration provided that the increase in the absolute PSA value was ≥2 ng/ml. PSA increase only did not qualify for progression within the first 12 weeks of treatment.
The clinical benefit was assessed based on evaluation of tumour pain, analgesic consumption, and performance status. Pain was assessed with a standard 10-point pain numerical scale and analgesic consumption with a five-point analgesic scale [17]. Clinical benefit was defined as a ≥2-point decrease in pain score with stable or reduced analgesic score or a ≥1-point decrease of analgesic score with stable or reduced pain score compared to baseline lasting ≥2 weeks and without deterioration in performance score. Toxicity was evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) (Version 3.0).

Assessments

Tumour assessments consisting of a bone scan, chest X-ray or CT scan, and abdominal CT scan or MRI were performed at baseline, every three cycles and at the end of treatment. PSA was measured before each cycle. All assessments were repeated to confirm a response 4 weeks after the response was observed. Pain and analgesic consumption were assessed at baseline, on day 1 and 15 every cycle, at the end of treatment, and then every month until progression or further anti-tumour therapy. Safety evaluations were performed throughout the study.

Statistical analysis

Simon's two-stage Mini Max design was used to determine the sample size and interim decision criteria [18]. Assuming that a PSA response rate of 20 % in evaluable patients would indicate potential usefulness, whereas a rate of 10% would be the lower limit of interest, with $\alpha = 0.10$ and $\beta = 0.20$, the estimated accrual number for the first part was 29 evaluable patients for each randomized schedule. In the case of three or more PSA

responses out of 29 evaluable patients in one of the arms in the first part, the second part would be initiated. In the second part of the study 54 additional patients would be randomized between treatment with the selected dose schedule of danusertib (27 patients) or mitoxantrone or an approved second-line treatment (27 patients). If nine or more out of the total of 56 patients treated with the selected dose schedule of danusertib had a PSA response after this second step, it was to be concluded that the efficacy of the regimen warranted further investigation.
PFS and duration of PSA response were estimated by Kaplan-Meier method. No comparative analyses were performed to detect a statistical difference between treatment arms.

RESULTS

Patients and treatment

From September 2007 through October 2009, 88 patients were randomized and 81 were treated. The main reasons for not receiving study treatment were deterioration of the laboratory values or health condition and withdrawal of consent (Fig 1).
Of the 81 treated patients, 60 patients (31/43 arm A and 29/38 arm B), were evaluable for the primary endpoint. The major reasons for non-evaluability were one or no PSA assessment on treatment, increase in dose of corticosteroids on treatment and radiological progression but no PSA progression before study entry (Fig. 1). The two dose groups were well balanced for baseline characteristics, except for the number of patients with liver metastases and corticosteroids and bisphosphonates use (Table 1).
PSA response to last prior docetaxel line was achieved by 56% and 58% of patients in arm A and B, respectively. The median duration of treatment was 8.4 (range 0.14-37.14) weeks in arm A and 9.93 (range 0.14-132.14) weeks in arm B, with a median dose intensity of 184.8 mg/m^2/week and 215.2 mg/m^2/week, respectively. Treatment modifications, mainly dose delays and omissions, were more frequent in Arm A (74.3%) than in arm B (36%) (Table 2). The main reason for dose delays and reductions was grade ≥ 3 neutropenia.

Efficacy

PSA response
Of the 60 evaluable patients, two patients, one in each arm, had a confirmed PSA response, lasting 8.3 weeks and 33.6 weeks for the patient in arm A and B, respectively (Table 3). Both patients with a PSA response had also a PSA response during prior

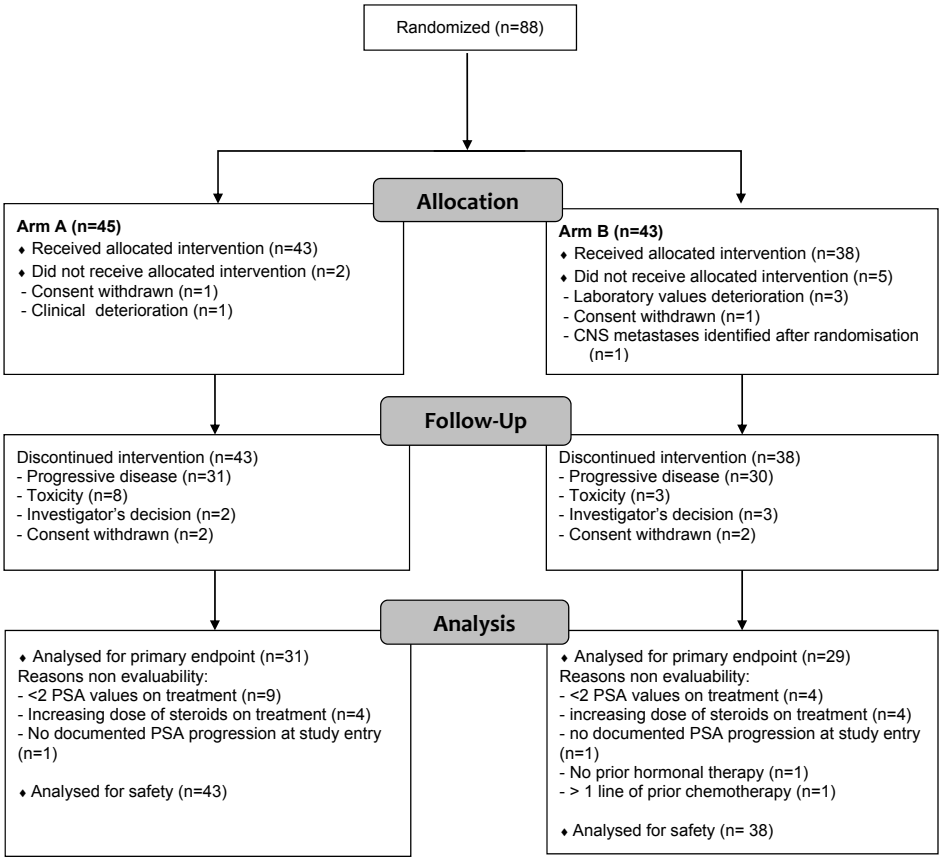


Figure 1. Consort diagram. Arm A: danusertib 330 mg/m^2, 6h i.v.; day 1,8,15, q4wks; Arm B: danusertib 500 mg/m^2, 24h i.v.; day 1,15, q 4wks. n: number of patients.

docetaxel chemotherapy. The two study groups did not meet the criteria for continuation into the second part of the study.

Tumour response

There were no objective tumour responses according to modified RECIST and PSA Working Group criteria. The best overall response was disease stabilisation in 8/43 (18.6%) patients in arm A and 13/38 (34.2%) in arm B. Clinically relevant disease stabilizations (lasting ≥6 months) were reported in 4/43 (9.3%) patients in Arm A and in 7/38 (18.4%) in Arm B (Table 3). Three patients, all in arm B, had disease stabilization lasting ≥ 20 months.

Patients Characteristics	Arm A (N= 43)	Arm B (N=38)
Age (years)		
Median (range)	68.3 (48 -80)	67.9 (50-80)
≥ 65 years	33 (76.7%)	29 (76.3%)
Gleason score (%)		
≤ 7	5 (11.6%)	2 (5.2%)
8-10	35 (81.4%)	32 (84.2%)
Not Applicable	3 (7.0%)	4 (10.5%)
ECOG PS		
0	16 (37.2%)	18 (47.4%)
1	24 (55.8%)	18 (47.4%)
2	3 (7.0%)	2 (5.3%)
Serum baseline PSA		
Median (range)	158.9 (4.1-5314)	197 (27-2182)
< 20 ng/mL	4 (9.4%)	0
≥ 20 ng/mL	39 (90.7%)	38 (100%)
Extent of disease		
Bone metastases only	15 (34.9%)	15 (39.5%)
Visceral metastases only	2 (4.7%)	3 (7.9%)
Bone and visceral metastases	26 (60.5%)	20 (52.6%)
Liver metastases	8 (18.6%)	0
Lung metastases	2 (4.7%)	2 (5.3%)
Lymph nodes metastases	23 (53.5%)	21 (55.3%)
Prior docetaxel lines		
1	39 (90.7%)	36 (94.7%)
2	4 (9.3%)	1 (2.6%)
3	0	1 (2.6%)
PSA response to docetaxel		
Responder	24 (55.8%)	22 (57.9%)
Non responder	19 (44.2%)	16 (42.1%)
Bisphosphonate at baseline		
Yes	20 (46.5%)	12 (31.6%)
No	23 (53.5%)	26 (68.4%)
Corticosteroid at baseline		
Yes	22 (51.2%)	16 (42.1%)
No	21 (48.8%)	22 (57.9%)
Pain score at baseline*		
<2	19 (44.2%)	15 (39.5%)
≥ 2	21 (48.8%)	22 (57.9%)
Oncologic assessment**		
Target and non target lesions	25 (58.1%)	19 (50.0%)
Target lesions (only)	1 (2.3%)	2 (5.3%)
Non target lesions (only)	17 (39.5%)	17 (44.7%)

Table 1. Baseline characteristics. Arm A: danusertib 330 mg/m^2 6h i.v.; day 1,8,15, q 4wks; Arm B: danusertib 500 mg/m^2 24h i.v.; day 1,15, q 4 wks. N: number of patients; ECOG PS: Eastern Cooperative Oncology Group performance status; PSA: prostate specific antigen; * According to National Comprehensive Cancer Network, Clinical Practice Guidelines in Oncology; ** According to RECIST criteria.

	Arm A (N=43)	Arm B (N=38)
Treatment cycles		
Number	113	164
Median (range)	2 (1-8)	3 (1-33)
Treatment duration (weeks)		
Median (range)	8.43 (0.14-37.14)	9.93 (0.14-132.14)
Dose intensity (mg/m^2/week)		
Intended dose intensity	247.50	250.00
Median (range)	184.81 (76.70-251.04)	215.15 (124.68-257.29)
Relative dose intensity	74.67%	86.06%
Cumulative dose (mg/m^2)		
Median (range)	1970.95 (306.8-8076.5)	2620.86 (498.7-32660.1)
Treatment Delay		
Number of patients	34	25
Number of cycles	71	52
Number of events	92	58
Reasons for delay		
Hematological toxicity	50	19
Non-Hematological toxicity	20	11
Other	28	28
Dose Omissions		
Number of patients	15	7
Number of cycles	21	7
Number of events	22	7
Reasons for omission		
Hematological toxicity	8	1
Non-Hematological toxicity	10	5
Other	6	1
Dose reductions		
Number of patients	4	4
Number of cycles	4	4
Number of events	4	4
Reasons for reduction		
Hematological toxicity	3	4
Non-Hematological toxicity	1	0

Table 2. Treatment details and dose modifications.

Arm A: danusertib 330 mg/m^2 6h i.v.; day 1,8,15, q 4wks; Arm B: danusertib 500 mg/m^2 24h i.v.; day 1,15, q 4 wks. N: number of patients.

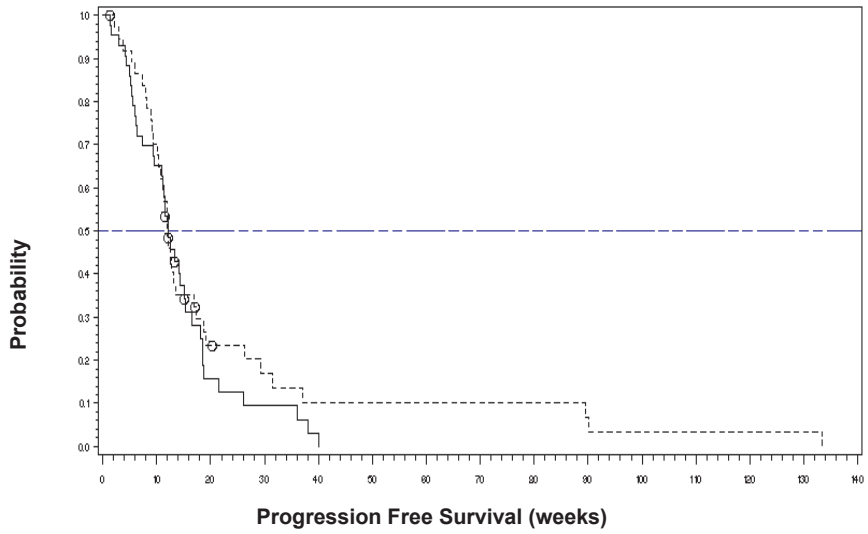
Clinical benefit

Fifty-two (64.2%) patients were evaluable for clinical benefit. Six out of 28 (21.4%) patients in Arm A and 4/24 (16.7%) patients in Arm B had a clinical benefit on treatment (Table 3).

	Arm A (N=43)	Arm B (N=38)
PSA assessment		
Evaluable patients	31	29
>50% PSA response	1 (3.2%)	1 (3.4%)
>30% and <50% PSA response		
confirmed	0	1 (3.4%)
unconfirmed	1 (3.2%)	2 (6.9%)
Tumor assessment*		
Evaluable patients	43	38
Stable disease (overall)	8 (18.6%)	13 (34.2%)
Stable disease ≥ 6 months	4 (9.3%)	7 (18.4%)
Progressive disease	23 (53.5%)	17 (44.7%)
Not evaluable	12 (27.9%)	8 (21.1%)
Clinical Benefit assessment		
Evaluable patients	28	24
Pain and analgesic score	4 (14.3%)	3 (12.5%)
Pain score improvement (only)	1 (3.6%)	1 (4.2%)

Table 3. Efficacy Results.

Arm A: danusertib 330 mg/m^2 6h i.v.; day 1,8,15, q 4wks; Arm B: danusertib 500 mg/m^2 24h i.v.; day 1,15, q 4 wks.
*According to modified RECIST criteria and recommendations of the PSA working group. N: number of patients.



--- Arm A: danusertib 330 mg/m^2 6h i.v.; days 1,8,15, q4wks – Median PFS (95% CI): 12.14 wks (10.86 – 15.14)
— Arm B: danusertib 500 mg/m^2 24h i.v.; days 1, 15, q4wks – Median PFS (95% CI): 12.14 wks (10.29 – 17.00)
oo Censored patients

Figure 2. Kaplan-Meier Curve for progression free survival.

Median progression free survival was 12.14 weeks (95%CI; 10.86 –15.14) for arm A and 12.00 weeks (95%CI; 10.29 –17.00) for arm B.

Arm A: danusertib 330 mg/m^2 6h i.v.; day 1,8,15, q 4wks; Arm B: danusertib 500 mg/m^2 24h i.v.;day 1,15, q 4 wks.

Progression free survival

At the time of data analysis 30/43 (86.1%) patients in arm A and 26/38 (92.1%) patients in arm B had investigator determined progressive disease. Median PFS was 12.1 weeks (95% confidence interval (CI); 10.9 –15.1) for arm A and 12.0 weeks (95%CI; 10.3 –17.0) for arm B (Fig. 2).

Safety and tolerability

Both schedules showed acceptable toxicity. In all, 93% of all patients experienced at least one drug-related adverse event. The most frequent grade ≥3 drug-related adverse events were neutropenia in 22 patients (16 arm A; six arm B), fatigue in five patients (four arm A; one arm B) and neutropenic fever (two patients in each arm) (Table 4). Irrespective of the treatment arm, the most common drug-related adverse events for all grades involved gastrointestinal disorders (61.7%), general disorders and administration site conditions (60.5%), and blood and lymphatic system disorders (50.6%) (Table 4). No drug-related deaths were reported. One patient died on study due to pneumonia, unlikely to be related to the study drug.

DISCUSSION

Aurora kinases are frequently overexpressed in prostate cancer and therefore these proteins are an attractive target for treatment in patients with CRPC [19]. Danusertib showed *in vivo* antitumour activity in prostate cancer models. This randomized phase II study demonstrated that danusertib has minimal single agent activity in patients with docetaxel-refractory CRPC. Due to the modest efficacy, the trial did not meet the criteria to enter the second stage of the study. Both schedules demonstrated a manageable toxicity profile consistent with earlier clinical trials.

While PSA response was observed in only two patients, 11 patients (13.6%) had sustained stable disease lasting ≥ 6 months. In the absence of a placebo control arm, it may be difficult to discriminate whether a stable disease is attributable to the treatment efficacy or an indolent behaviour of the disease. However, all evaluable patients had documented disease progression before entering the trial. As with other molecular targeted therapies, the key question to be addressed will concern to identify the subset of patients with the highest probability of responding to danusertib. A predictive biomarker that can be used for this purpose has not yet been identified.

As discussed, this study enrolled an unselected population with progressive metastatic CRPC after docetaxel. It has recently been demonstrated that a previously underrecognized sub-population of prostate cancer, termed neuroendocrine prostate cancer

Event	CTC Grade	Arm A (N= 43)		Arm B (N= 38)		Total (N=81)	
		n	%	n	%	n	%
Any Term	1-4	42	97.7	33	86.8	75	92.6
	3-4	27	62.8	10	26.3	37	45.7
Nausea	1-4	16	37.2	19	50.0	35	43.2
	3-4	1	2.3	-	-	1	1.2
Neutropenia	1-4	23	53.5	8	21.1	31	38.3
	3-4	16	37.2	6	15.8	22	27.2
Diarrhoea NOS	1-4	10	23.3	18	47.4	28	34.6
	3-4	-	-	1	2.6	1	1.2
Fatigue	1-4	14	32.6	12	31.6	26	32.1
	3-4	4	9.3	1	2.6	5	6.2
Asthenia	1-4	11	25.6	13	34.2	24	29.6
	3-4	2	4.7	1	2.6	3	3.7
Anorexia	1-4	13	30.2	7	18.4	20	24.7
	3-4	2	4.7	-	-	2	2.5
Pyrexia	1-4	10	23.3	6	15.8	16	19.8
Vomiting NOS	1-4	9	20.9	5	13.2	14	17.3
Constipation	1-4	6	14.0	5	13.2	11	13.6
	3-4	-	-	1	2.6	1	1.2
Hypertension NOS	1-4	7	16.3	4	10.5	11	13.6
	3-4	2	4.7	-	-	2	2.5
Phlebitis NOS	1-4	9	20.9	1	2.6	10	12.3
Abdominal pain NOS	1-4	3	7.0	4	10.5	7	8.6
Oedema peripheral	1-4	2	4.7	5	13.2	7	8.6
Anaemia NOS	1-4	4	9.3	2	5.3	6	7.4
Haemoglobin decreased	1-4	2	4.7	4	10.5	6	7.4
	3-4	1	2.3	-	-	1	1.2
Dizziness	1-4	3	7.0	2	5.3	5	6.2
Headache	1-4	1	2.3	4	10.5	5	6.2
Leukopenia NOS	1-4	4	9.3	1	2.6	5	6.2
	3-4	1	2.3	1	2.6	2	2.5
Alanine aminotransferase increased	1-4	3	7.0	1	2.6	4	4.9
	3-4	-	-	1	2.6	1	1.2
Dry mouth	1-4	3	7.0	1	2.6	4	4.9
Febrile neutropenia	1-4	2	4.7	2	5.3	4	4.9
	3-4	2	4.7	2	5.3	4	4.9
Mucosal inflammation NOS	1-4	2	4.7	2	5.3	4	4.9
Oral fungal infection NOS	1-4	2	4.7	2	5.3	4	4.9
Peripheral sensory neuropathy	1-4	3	7.0	1	2.6	4	4.9
Stomatitis	1-4	2	4.7	2	5.3	4	4.9
	3-4	1	2.3	-	-	1	1.2

Table 4. Drug related adverse events.

CTC: common toxicity criteria according to the NCI-CTCAE (Version 3.0); NOS: not otherwise specified.

(NEPC) or anaplastic prostate cancer demonstrates marked AURKA amplification compared with prostatic adenocarcinoma [20]. Aurora kinase A protein is also overexpressed in NEPC compared with adenocarcinoma. Both *in vitro* and *in vivo* models support the treatment of NEPC with danusertib. While clinical use of aurora kinase inhibitors has not demonstrated overwhelming success, it is possible that by selecting a more optimal population with NEPC would yield more promising results. It has been estimated that up to 25% of men with chemotherapy naive metastatic prostate cancer harbour clinical features of NEPC [21] and treatment with hormonal therapy may induce neuroendocrine differentiation [22,23]. While it would have been interesting to retrospectively assess responders and those with prolonged stable disease, we do not have data on NEPC features in this study.

There are a few limitations to the present study that should be considered. There was a discrepancy between the evaluable patients and the randomized patients. Exclusions from the evaluable patient population were in large part due to one or no PSA assessment on treatment, which reflected the high rates of early discontinuation of the study treatment due to rapid disease progression and clinical deterioration of patient's progression during or after docetaxel chemotherapy. The potential impact of these early terminations on the efficacy and safety end points should be considered.

Assessing activity of targeted therapies in patients with CRPC is challenging given the preponderance of bone metastases. In 2008, the Prostate Cancer Clinical Trials Working Group 2 consensus redefined consensus criteria for early-phase clinical trial end points which were only partly incorporated in this study. The application of the different criteria for progressive disease for patients without measurable disease could have lead to different outcomes in our study.

Another possible confounder is the use and reliance on PSA response as the primary endpoint of response [24]. Although an important and useful tumour marker, changes in PSA do not always correlate with regression of tumour and clinical benefit [25]. Interpretation of PSA data is even more obscured with the use of targeted agents, some of which result in discrepancy between PSA response and clinical benefit [26,27]. The limitations of PSA as the primary indicator of response have to be considered interpreting the results of our study.

In conclusion, in spite of minimal response in term of PSA decrease, the sizeable number of durable disease stabilizations observed may warrant further investigation in this patient population. Development of specific biomarkers predictive for response may enable patients to be selected with potential benefit of danusertib. Further assessment of this aurora kinase inhibitor in CRPC is not indicated from the results of this study.

CONFLICT OF INTEREST

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Chapter 6

Tolerability, Safety and Pharmacokinetics of Ridaforolimus in combination with Bicalutamide in patients with asymptomatic, metastatic Castration Resistant Prostate Cancer (CRPC)

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Article submitted

ABSTRACT

Background: The mammalian target of rapamycin (mTOR) pathway is activated in the majority of clinical prostate cancers. Recent data indicate that there is a significant cross-talk between the PI3K/Akt/mTOR and androgen receptor signalling pathways.
Objective: Evaluate the safety, tolerability and potential drug-drug interaction of ridaforolimus, an mTOR inhibitor, when combined with the androgen receptor inhibitor bicalutamide in patients with asymptomatic, metastatic castration resistant prostate cancer.
Design, setting and participants: Non randomized open-label safety lead-in phase, to a multi-centre, randomized Phase II trial. Twelve patients were enrolled including one screen failure.
Intervention: Treatment with the combination of ridaforolimus 30 mg daily for 5 consecutive days each week and bicalutamide 50 mg daily.
Outcome measurements and statistical analysis: Ridaforolimus pharmacokinetics were assessed with and without bicalutamide. Adverse events and efficacy analyses were analyzed using SAS version 9.1.
Results and limitations: Dose reductions were required in seven patients. Three of the 11 patients experienced a dose limited toxicity (DLT), one with Grade 3 hyperglycemia, and two with Grade 2 stomatitis leading to less than 75% of planned ridaforolimus dose during the first 35 days of treatment. Four patients had ≥ 30% PSA decline from baseline within the first 12 weeks of study treatment. The pharmacokinetic results showed no differences in exposures to ridaforolimus with and without concomitant bicalutamide administration. The major limitation of the study was that no definitive answer on the potential efficacy of mTOR inhibitors in CRPC can be given.
Conclusions: Although there was no evidence of a clinically relevant pharmacological drug-drug interaction, the occurrence of dose-limiting toxicities in 3 of 11 evaluable patients at a reduced dose of ridaforolimus of 30 mg daily suggests that this combination may not be well-suited for asymptomatic or minimally symptomatic prostate cancer patients. NCT00777959.

Keywords: androgen receptor inhibitor; bicalutamide; castration resistant prostate cancer; mTOR inhibitor; pharmacokinetics; ridaforolimus.

INTRODUCTION

Patients with advanced prostate cancer (PC) who undergo androgen deprivation therapy (ADT) invariably relapse and develop castration resistant prostate cancer (CRPC) [1]. Since ADT is monitored by frequent testing of the prostate specific antigen (PSA), there is a relatively large population of patients with asymptomatic CRPC and a rising PSA as the only evidence of disease progression. Many secondary endocrine therapies have been evaluated, but none have been shown to prolong survival [2]. The lack of effective secondary hormonal treatments contributes to a large unmet medical need for patients with asymptomatic chemonaive metastatic CRPC.

The mammalian target of rapamycin (mTOR) is a critical molecule for controlling proliferation of tumor cells and can be activated by mutation or activation of signalling molecules such as PI3K or Akt (Fig 1.). Alterations in the PI3K/Akt/mTOR pathway plays a prominent role in PC, it is estimated that upregulation occurs in 30-50% of PCs [3]. PTEN is a negative regulator of the PI3K/Akt/mTOR pathway and is frequently mutated in PC leading to increased activity of the PI3K/Akt/mTOR pathway [4]. Ridaforolimus is an analog of rapamycin that demonstrated antiproliferative activity in prostate cancer cells [5]. Recent data indicate that there is significant cross-talk between the androgen receptor (AR) and PI3K/Akt/mTOR pathways. The combination of anti-androgens and mTOR inhibitors has additive or synergistic antiproliferative activity in models of androgen independent PC in vitro [6,7]. The combination of ridaforolimus and bicalutamide had synergistic anti-tumor activity in both androgen-dependent LNCaP and in androgen-independent C4-2 cells [8].

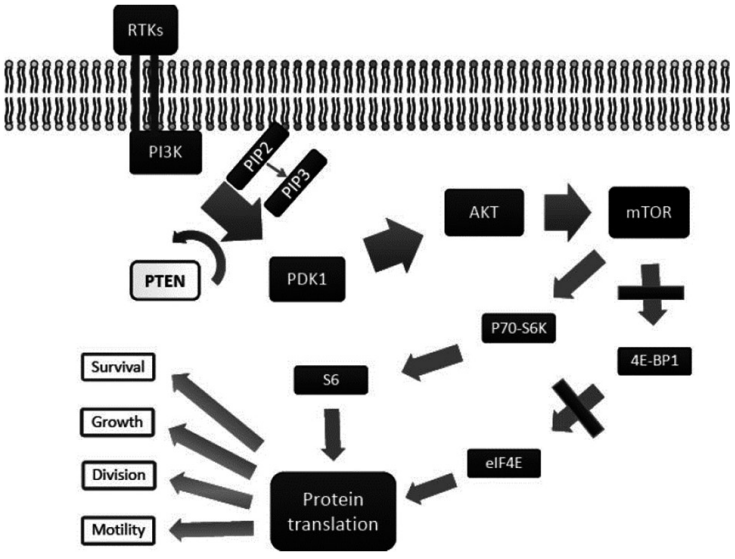

Figure 1. PIK3/Akt/mTOR pathway. Adapted with permission from Morgan TM, et al [3].

To determine the efficacy of mTOR inhibition in combination with ADT a Phase II multi-centre, randomized, double- blind, placebo controlled trial of ridaforolimus (40 mg/day for 5 days per week) combined with bicalutamide, an anti-androgen, compared with placebo plus bicalutamide in patients with asymptomatic, metastatic CRPC was designed. Among the first 11 randomized patients, there were four cases of Grade 3 stomatitis. A standing internal Data Monitoring Committee (DMC) at the Sponsor reviewed the interim safety data, and advised unblinding the study and amending it to address the higher than expected frequency of severe stomatitis. The unblinded safety data showed that among four patients randomized to ridaforolimus plus bicalutamide, all four patients had grade 3 stomatitis or related adverse events (AEs), while none of the seven patients on placebo plus bicalutamide had stomatitis. In previous studies, stomatitis has been the dose-limiting toxicity (DLT) for mTOR inhibitors. Adverse events related to stomatitis have been reported in over 70% of patients enrolled in prior Phase I trials of ridaforolimus, but are usually grade 1-2 events, and grade 3 stomatitis has generally been reported in fewer than 10% of patients [9]. Because of this unexpected severe ridaforolimus toxicity and hence a conceivable drug-drug interaction between ridaforolimus and bicalutamide, the protocol was amended to proceed with a non-randomized safety lead-in study to test an open label and reduced dose of ridaforolimus 30 mg daily on a five day each week schedule in combination with bicalutamide and collect pharmacokinetic (PK) samples to determine if a drug-drug interaction leading to higher exposure of ridaforolimus contributed to the unexpectedly high frequency of severe stomatitis initially reported with the combination. The results of the open label safety lead-in of the ridaforolimus-bicalutamide combination are reported here.

MATERIALS AND METHODS

Patients

Asymptomatic patients with metastatic adenocarcinoma of the prostate and disease progression despite castration level of testosterone were eligible. Progressive disease (PD) was defined as PSA progression, appearance of ≥ two new lesions on bone scan, or objective progression according to Response Evaluation Criteria in Solid Tumours (RECIST) [10].

Other inclusion criteria were ongoing androgen deprivation, a baseline PSA of ≥7 ng/dL; Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 1; age ≥ 18 years; adequate bone marrow, hepatic, and renal functions.

Exclusion criteria included use of anti-androgens within the past 4 weeks; previous use of rapamycin analogs or chemotherapy; prior surgery or radiotherapy within the past 4

weeks; pain requiring opioid or narcotic analgesics; central nervous system localisation; malignant effusions; clinically significant cardiac disease; concurrent use of cytochrome P450 3A inducers or inhibitors; known history of human immunodeficiency virus or viral hepatitis.

The study was conducted according to the Declaration of Helsinki and in compliance with Good Clinical Practice and local ethical and legal requirements. The protocol was approved by the appropriate institutional review board or ethics committee. Written informed consent was obtained from all participants.

Study design

This prospective, open-label, international, multicenter safety lead-in trial, was undertaken from February 2010 to May 2010. The primary objective was to determine the safety and tolerability of ridaforolimus when combined with bicalutamide. Additional objectives included the percent of patients achieving 30% PSA decline within the first 3 months [11,12], and characterization of the PK profile. Toxicity was evaluated according to Common Terminology Criteria for Adverse Events (CTCAE version 3.0). The safety lead-in targeted an observed rate of DLTs of ≤two in ten DLT-evaluable patients. DLTs were defined as any of the following events related to the ridaforolimus/bicalutamide combination and which occurred during the first 35 days of treatment: Grade 3 non-hematologic toxicity lasting > 3 days, with the exception of self-limiting or medically controllable toxicities; Grade 4 non-hematological toxicity; Grade 4 neutropenia lasting > 5 days or neutropenic fever; Grade 4 thrombocytopenia; inability due to drug-related toxicity to complete the first 28 days of study treatment; delay in treatment of >2 weeks due to drug-related toxicity; inability to receive at least 75% of the dose due to drug-related toxicity during the first 35 days.

Study treatments and PK assessments

The dosing of ridaforolimus and bicalutamide were staggered to enable evaluation of the PK profile of ridaforolimus with and without bicalutamide. Patients were treated with a single oral dose of ridaforolimus of 30 mg on day 1 and then daily for five consecutive days each week starting on day 8. Bicalutamide 50 mg/day was administered orally from day 2 onwards. The PK evaluations were performed on day 1 and 8, at pre-dose and 30 minutes and 1, 2, 4, 6-8 and 24 hours postdose. The effect of bicalutamide co-administration on the PK of ridaforolimus was evaluated by comparing PK parameters from day 1 with those of day 8 within each patient. Ridaforolimus concentration time data were analyzed using WinNonlin version 5.2.1 (Pharsight, St. Louis, MO). Area under the concentration versus time curve ($AUC_{0\text{-}24h}$ and $AUC_{0\text{-}last}$), maximum plasma concentra-

tion (C_{max}), time to maximum concentration (T_{max}), $AUC_{0\text{-last}}$ for ridaforolimus following a single dose of ridaforolimus alone or following concomitant administration of multiple doses of bicalutamide with a single dose of ridaforolimus were calculated. The ratio of geometric least-squares means (GMR) and the corresponding 90% confidence intervals (90% CI) for blood $AUC_{0\text{-}24}$ and C_{max} were calculated using a linear mixed-effects model. There were no dose modifications permitted with bicalutamide. The dose of ridaforolimus could be briefly reduced or interrupted to manage treatment-related AEs ≥ Grade 2 according to prescribed dose modifications. Dose modifications for stomatitis were specifically defined per protocol.

Patient evaluation and follow-up

Efficacy assessments included radiographic imaging, PSA concentration, and pain level on the Brief Pain Inventory (BPI) [13]. Clinical evaluations included medical history, ECOG PS, physical examination, weight, vital signs, concomitant medications, and review of AEs. Serum lipids, hematology and clinical biochemistry were performed at baseline, weekly for the first 5 weeks, and every 4 weeks thereafter. Response evaluation was performed in accordance with the Prostate Cancer Working Group 2 (PCWG2) guidelines [14]. AE and efficacy analyses were analyzed using SAS version 9.1 (SAS Institute, Inc., Cary, NC).

RESULTS

Twelve patients were enrolled in the safety lead-in including 1 screen failure. The median age was 63 years (range 58 to 72 years).

Safety and dose reductions

The most common drug-related AEs were fatigue (n=8), rash (n=6), stomatitis (n=5), hypercholesterolaemia (n=5), dysgeusia (n=5), anorexia (n=5), and pneumonitis (n=5) (Table 1). Four patients (36.4%) had at least one Grade 3 or higher drug-related treatment-emergent adverse event (TEAE).

One patient developed Grade 3 and one patient Grade 4 pneumonitis that improved with steroid treatment. There were no treatment-related deaths. The median duration of treatment was 14.8 weeks (range 3.4 to 56.0 weeks). Three patients discontinued treatment due to drug-related AEs, seven patients due to PD and one patient due to physician decision. A total of seven patients required ≥ one ridaforolimus dose reductions. The actual median administered dose of ridaforolimus was 21.6 mg/day (range 17 to 28 mg/day) on the daily x 5 schedule, 72% of the planned 30 mg dose. Reasons for dose

Toxicity	Toxicity Grade (No. of Patients)				Total No. of Patients With Adverse Events
	1	2	3	4	
Blood & lymphatic system disorders					
Anaemia	1	1			2
Neutropenia			1		1
Thrombocytopenia	1				1
Gastro-intestinal disorders					
Cheilitis		1			1
Diarrhoea	3				3
Nausea	2				2
Stomatitis	2	3			5
Lip pain	1				1
Lip ulceration	1				1
Tongue ulceration		1			1
General disorders & administration site conditions					
Fatigue	5	3			8
Mucosal inflammation	1	3			4
Oedema	1				1
Oedema peripheral		1	1		2
Infections & infestations					
Paronychia	1				1
Investigations					
ASAT† increased	1				1
γ-GT‡ increased		1			1
Blood glucose increased			1		1
Haemoglobin decreased		1			1
Platelet count decreased	1				1
White blood cell count decreased		2			2
Metabolism & nutrition disorders					
Decreased appetite	5				5
Dehydration	1		1		2
Hypercholesterolaemia	4	1			5
Hyperglycaemia			2		
Hypertriglyceridaemia	2	1			3
Hypokalaemia		1			1
Hypophosphataemia	1		1		2
Musculoskeletal & connective tissue disorders					
Arthralgia	1				1
Joint swelling	1				1
Myalgia	1				1
Pain extremity	1				1
Nervous system disorders					
Headache	1				1
Dysgeusia	4	1			5
Respiratory, thoracic & mediastinal disorders					
Dyspnoea	1				1
Pneumonitis	1	2	1	1	5

Toxicity	Toxicity Grade (No. of Patients)				Total No. of Patients
	1	2	3	4	With Adverse Events
Epistaxis	2				2
Nasal inflammation	1				1
Oropharyngeal pain	1				1
Pharyngeal erythema	1				1
Skin & subcutaneous tissue disorders					
Dry skin	1				1
Erythema	1				1
Nail disorder		1			1
Onychoclasis	1				1
Palmar-plantar erythrodysaesthesia syndrome		1			1
Pruritus	1				1
Rash	5	1			6
Skin fissures	1				1
Skin ulcer	1				1
Vascular disorders					
Hot Flush		1			1

† Aspartate aminotransferase

‡ Gamma-glutamyltransferase

Table 1. Patients with drug related adverse events by maximum toxicity grade. Every patient was counted a single time for each applicable specific adverse event with the highest reported grade. Grades according to CTCAE version 3.0. No.: number.

reductions included ≥ Grade 2 stomatitis (n=6), Grade 2 cheilitis (n=1), Grade 2 palmar-plantar erythrodysaesthesia syndrome (n=1), and Grade 2 dysgeusia (n=1).
Four patients required dose interruption of ridaforolimus; one patient due to an intercurrent anal abscess not related to the study drug, one patient because of dehydration possibly related to the study drug and two patients due to pneumonitis related to the study drug. One patient was a protocol violator due to a dosing error and was replaced for PK purposes but remained evaluable for the safety analysis.

Dose Limiting Toxicities

Three patients experienced a DLT according to the pre-specified definitions. One patient developed Grade 3 hyperglycemia lasting > 5 days. Two patients had recurring Grade 2 stomatitis considered a DLT because of dose reduction leading to < 75% of planned ridaforolimus dose being given. In addition, although they occurred after the first cycle when DLTs were assessed, two additional patients required discontinuation of ridaforolimus due to a grade 3 and 4 pneumonitis.
The number of DLTs exceeded the protocol-specified target of ≤two DLTs in at least ten DLT evaluable patients. As such, the study was terminated after the safety lead-in was completed, and did not proceed to the randomized, double-blind phase.

PK results

Ridaforolimus levels were similar following a single dose of ridaforolimus alone or following concomitant administration of multiple doses of bicalutamide (Fig. 2). Considerable inter-patient variability in AUC_{0-24} (geometric CV: 68% and 68% on day 1 and 8 respectively) and C_{max} (geometric CV: 69% and 93% on day 1 and 8 respectively) was observed. The GMR of intra-individual for AUC_{0-24} (ridaforolimus with bicalutamide / ridaforolimus alone) was 0.86 (90%CI; 0.61-1.22). The GMR for C_{max} (ridaforolimus with bicalutamide / ridaforolimus alone) was 0.82 (90%CI; 0.53-1.27) (Table 2).

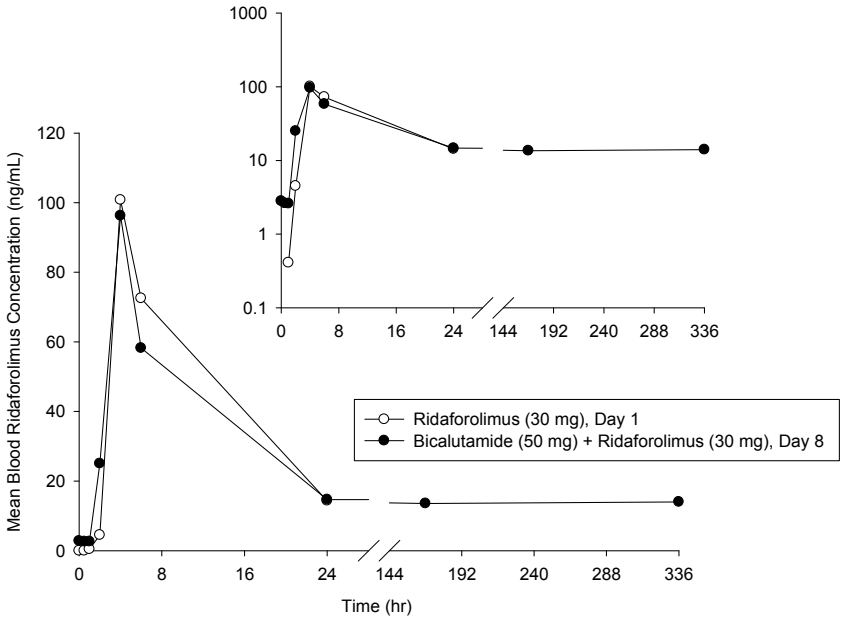

Figure 2. Arithmetic mean blood concentration profiles for ridaforolimus following a single 30 mg oral dose of ridaforolimus alone or following concomitant administration of multiple doses of 50 mg bicalutamide with a single 30 mg dose of ridaforolimus to male patients.
Insert: semi-log scale.

Efficacy

The primary efficacy endpoint was a 30% PSA decline from baseline within the first 12 weeks of study treatment. The decline was determined by the lowest post-baseline PSA value within the first 12 weeks. Ten out of 11 patients had both baseline PSA and post-baseline PSA measurements within 12 weeks. Four patients (36%) had ≥30% PSA decline within 12 weeks, while 5 patients (45%) had persistently increasing PSA within 12 weeks of treatment (Fig 3).

Pharmacokinetic	Bicalutamide 50-mg QD, D2-35 + Rida 30-mg QDx5, Day 8			Ridaforolimus 30-mg, Day 1			[Bicalutamide 50-mg QD, D2-35 + Rida 30-mg QDx5, DS] / Ridaforolimus 30-mg, Day 1		
Parameter	N	GM	95%CI	N	GM	95% CI	GMR	90% CI	rMSE‡
AUC_{0-24} (ng/ml*hr)†	9	735.42	(476.58, 1134.85)	9	853.68	(553.22, 1317.32)	0.86	(0.61, 1.22)	0.3762
AUC_{last} (ng/ml*hr)†	10	694.79	(460.35, 1048.62)	9	856.91	(559.26, 1312.95)	0.81	(0.57, 1.14)	0.3980
C_{max} (ng/ml)†	10	82.66	(50.95, 134.11)	9	101.19	(61.06, 167.69)	0.82	(0.53, 1.27)	0.5123
$T_{max (hr)}$§	10	4.2	(2.2, 4.8)	9	4.2	(4.0, 6.1)			

† Back-transformed least squares mean and confidence interval from mixed effects model performed on natural log-transformed values.

§Median; minimum, maximum.

‡ rMSE: Square root of conditional mean squared error (residual error) from the linear mixed effect model. rMSE*100% approximates the within-subject %CV on the raw scale

B003800003 Cycle 1 Day 1 data are not included in summary statistics since this patient received bicalutamide with ridaforolimus for this treatment

Storage conditions for B003900001 cannot be confirmed and data cannot be considered valid. Accordingly, these data are not included in summary statistics

AUC_{last} estimates are provided for completeness since there were instances where insufficient data were available for calculation of AUC_{0-24}. Please see individual PK parameter tables for details.

Table 2. Summary statistics and statistical comparisons of blood pharmacokinetic parameters following a single dose of 30 mg oral dose of ridaforolimus alone or following concomitant administration of multiple doses of 50 mg bicalutamide with single 30 mg dose of ridaforolimus.

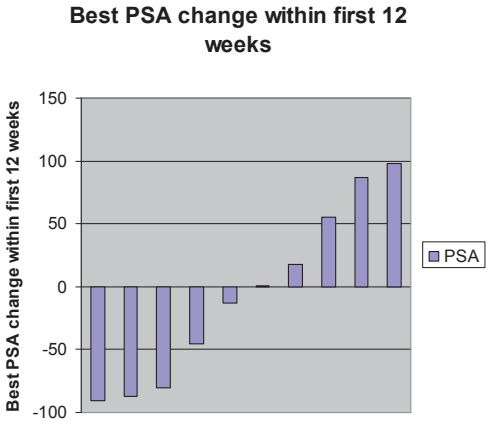

Figure 3. Best PSA change within 12 weeks.

DISCUSSION

There is a large population of asymptomatic patients with prostate cancer that progress during initial ADT with a rising PSA as the only evidence of PD. The dilemma of how to treat these patients represents an unmet medical need. There is a strong rationale for combining an anti-androgen with an mTOR inhibitor [6,7]. However, there is a potential for drug-drug interactions between ridaforolimus and bicalutamide. The safety lead-in reported here was designed to assess the safety profile and pharmacokinetics of combining ridaforolimus with bicalutamide.

Three out of the 11 patients experienced a DLT, as defined per protocol. Two patients because of recurring Grade 2 stomatitis with required dose reduction and one patient due to Grade 3 hyperglycaemia.

A very high fraction of patients (45%) had any grade of pneumonitis in our study, of which four patients had a ≥ Grade 2 pneumonitis; of whom one patient had grade 3 and one patient had Grade 4 pneumonitis. All patients with ≥Grade 2 pneumonitis were managed successfully with dose interruption and glucocorticoids. Pneumonitis is a known class effect of mTOR inhibitors and incidences have been reported ranging from 5% to 36% [15,16]. Pathogenesis of pneumonitis is uncertain, but various hypotheses have been suggested, including cell-mediated immune response to the drug [15]. The reason why the incidence of pneumonitis in our study is higher then expected has not yet been fully identified. Possible explanations are statistical aberration, or that castration increases the risk of developing pneumonitis.

Several Phase I trials have reported PK results of ridaforolimus and showed a non-linear increase in the AUC, C_{max}, and total body clearance [17,18]. Prior studies established that rapamycin analog pharmacokinetic are affected by CYP3A4 mediated drug-drug interactions [19,20,21]. Co-administration of ridaforolimus with ketoconazol, a potent CYP3A4 inhibitor, resulted in an 8.5- and 1.9-fold increase in $AUC_{0\text{-}inf}$ and C_{max}, respectively (unpublished data). In vitro studies indicated that bicalutamide is an inhibitor of CYP 3A4 [22]. Collectively there is a potential interaction between ridaforolimus and bicalutamide. However, our PK data did not point to an appreciable inhibition of ridaforolimus metabolism following concomitant administration of bicalutamide.

PI3K/Akt/mTOR pathway inhibition activates AR relieving feedback inhibition of HER kinases. Androgen ablation can activate the PI3K/Akt/mTOR pathway via tumor suppressor INPP4B and PHLPP downregulation [23,24,25], therefore patients undergoing ADT may benefit from Akt-targeting therapies. In PTEN loss CRPC mTOR kinases and Akt are preferred targets since PHLLP1 and INPP4B loss can activate the PI3K/Akt/mTOR pathway too.

To evaluate safety and tolerability a randomized Phase II study of the recommended single agent dose of ridaforolimus (40 mg/day five days each week) combined with

bicalutamide (50 mg/day) was initiated. The first four patients randomized to ridaforolimus and bicalutamide arm experienced Grade 3 stomatitis. While stomatitis is an expected toxicity for the mTOR inhibitor class, because of the unexpectedly high rate of high grade stomatitis, the protocol was amended to test a reduced dose of ridaforolimus at 30 mg/day for five days each week combined with bicalutamide in the safety lead-in study reported here. Although there were no unexpected toxicities noted, the number of protocol-defined DLTs and AEs was not well-suited to a basically asymptomatic CRPC patient population. Therefore, the study did not proceed to the Phase 2 part of the study. The impact of AR blockade on the toxicity of mTOR inhibitors is not fully elucidated, but was in our study not affected by PK interaction. Whether castration is associated with enhanced pharmacodynamic effects is not clear. However, it was decided not to pursue the lower dose further, as we reasoned lower dose might not be effective. Since we expected no additional benefit or reduced toxicity of other treatment designs we determined that it was not worthwhile to evaluate other schedules.
In conclusion, despite the sound rationale for combining an anti-androgen with an mTOR inhibitor with different but potentially complementary mechanisms of action, the tolerability of ridaforolimus combined with bicalutamide, in spite of a reduced ridaforolimus dose, was not well-suited to generally asymptomatic patients with CRPC, and led to the decision not to enroll additional patients on this study.

ACKNOWLEDGMENTS

We thank Scott Vuocolo, PhD, of Merck for his critical review of the paper and editorial assistance.

TAKE HOME MESSAGE:

Since the bidirectional cross talk between AR and PI3K/Akt/mTOR pathways and frequent PTEN mutations in prostate cancer, there is a strong rationale for combining ridaforolimus with bicalutamide, however the tolerability of the combination was not well-suited to asymptomatic CRPC patients.

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Chapter 7

Can human prostate xenograft models predict negative results in large phase 3 trials? Our experience with the combination of Docetaxel plus Zibotentan or Lenalidomide in the orthotopic PC346C human prostate cancer xenograft

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Article submitted

ABSTRACT

Background

To determine if human prostate xenograft models are able to predict results in large phase 3 trials, we initiated a preclinical study in parallel to ongoing clinical trials investigating the added value of zibotentan (formerly ZD4054) or lenalidomide to enhance the potency of docetaxel in the orthotopic human prostate cancer xenograft model PC346C.

Materials and Methods

Athymic NMRI nude mice were inoculated orthotopically with PC346C cells and tumor-bearing mice were randomized and treated with docetaxel (33 mg/kg i.p., day 0-21-42) alone or in combination with zibotentan (25 mg/kg/day p.o.). In a separate experiment, PC346C tumor-bearing mice were randomized to receive lenalidomide (100 mg/kg/day, p.o.) prior to or after docetaxel (33 mg/kg, i.p.). Tumor volume (TV) was monitored by transrectal ultrasonographic (TRUS) and plasma prostate specific antigen (PSA) was analysed every 2 weeks.

Results

Single-agent treatment with zibotentan or lenalidomide did not affect tumor growth and PSA release of PC346C tumors. While docetaxel showed a significant antitumor effect, reflected by increased log cell kill, zibotentan treatment did not improve the docetaxel response. The combination adversely affected animal welfare. Daily oral treatment of lenalidomide given prior to docetaxel injection did not improve docetaxel-induced growth inhibition, but the antitumor effect was enhanced when lenalidomide was given post-docetaxel injection. The larger inhibitory effect of the latter sequence could not be confirmed by the treated over control (%T/C) value 14 days post-docetaxel treatment. The %T/C value for both sequences did not reach a level of significant antitumor effectiveness. For all treatment strategies, tumor growth responses were reflected by changes in PSA release.

Conclusions

Combination therapy of zibotentan or lenalidomide in addition to docetaxel in orthotopic PC346C tumor-bearing mice did not enhance antitumor activity of docetaxel. The observed tumor growth delay in mice treated with lenalidomide post-docetaxel, however, suggest that the sequence of the lenalidomide plus docetaxel treatment is essential for

obtaining optimal antitumor efficacy. Circulating PSA was a good biomarker for treatment response and was directly related to TV changes in this model. The results reflect the lack of clinically relevant activity in large clinical randomized phase III trials in patients with castration resistant prostate cancer (CRPC) to test combination therapies of docetaxel and zibotentan or lenalidomide. The orthotopic human prostate cancer xenograft model PC346C is a representative and valuable asset to identify novel agents and combination therapies and their optimal sequence schedules for further clinical development.

Keywords: castration resistant prostate cancer, docetaxel, lenalidomide, PSA, xenograft, zibotentan

INTRODUCTION

Prostate cancer is a heterogeneous disease whose underlying pathogenic mechanisms are being increasingly elucidated. In patients with metastatic disease androgen-deprivation therapies is usually initially effective, but nearly all patients eventually progress from androgen-sensitive prostate cancer to castration-resistant prostate cancer (CRPC). Chemotherapy with docetaxel represents the standard first-line treatment in patients with CRPC[1,2]. Recently new systemic approaches, cabazitaxel[3], abiraterone acetate[4], enzalutamide[5] and radium-223 chloride[6] have been shown to prolong survival in patients with CRPC progressing after first line treatment with docetaxel. However, despite these improvements in therapy, overall survival in patients with CRPC remains poor. Since novel agents in combination with docetaxel may provide further avenues through which CRPC can be treated more effectively, the development of docetaxel-based combination therapies is of great interest.

Zibotentan is an oral endothelin antagonist that selectively inhibits the endothelin A (ET_A) receptor without inhibiting the endothelin B (ET_B) receptor. Endothelin-1 (ET-1) is a potent vasoconstricting peptide that exerts his effects by binding to ET_A and ET_B receptor. ET_A receptor is expressed primarily in endothelial cells of the stromal compartment[7]. ET-1 and the ET_A receptor have been reported to play a key role in the development and progression of prostate cancer by modulating cell proliferation, angiogenesis, and anti-apoptosis[8,9,10]. In contrast to the ET_A receptor, the ET_B receptor is endowed with some protective functions, by promoting apoptosis and endothelin clearance[11]. In prostate cancer, key components of the ET-1 clearance pathway, ET_B and neutral endopeptidease (NEP), are diminished, resulting in an increase in local ET-1 concentrations. Increased ET_A receptor expression is also seen with advancing tumor stage and grade in both primary and metastatic prostate cancer[9]. Therefore ET-1 and its receptors could be a therapeutic target in CRPC.

Lenalidomide is an immunomodulatory analogue of thalidomide with significant T cell stimulatory and antiangiogenic properties. Lenalidomide is FDA approved for multiple myeloma (MM) and in del 5q myelodysplastic syndrome[12,13]. Besides the effects on immune function, lenalidomide has been reported to affect a multitude of signalling pathways related to proliferation and cell survival, which may constitute a mechanism of action in solid tumors. Lenalidomide has been linked to modulation of cellular pathways such as down-regulation of pSTAT3 and inhibition of VEGF-induced PI3K/Akt signalling pathway[14,15,16]. Alterations in the PI3K/Akt pathway have been described extensively in prostatic tissues in several studies and have shown to play a prominent role in the development and progression of prostate cancer[17]. The PTEN tumor suppressor gene is a negative regulator of activity of the PI3K/Akt pathway and loss of PTEN leads to increased activity of this pathway. PTEN deletions and mutations are common events in prostate cancer, with studies showing loss of heterozygosity at the PTEN locus in up to 60% of prostate cancer samples[18].

Multiple studies have demonstrated that mean proliferation indices, as measured by Ki-67 staining, are associated with progression of prostate cancer[19,20]. Ki-67 is a nuclear antigen associated with cell proliferation, and is present throughout the active cell cycle but absent in resting cells[21]. The fraction of Ki-67-positive tumor cells (the Ki-67 labeling index) is often correlated with the clinical course of the disease[22].

With the multitude of options that are available to try to improve and/or prolong the efficacy of docetaxel in CRPC, a relevant preclinical model system is needed to test and validate the potential benefits of the suggested combination treatments. The orthotopic PC346C model is an attractive alternative for the traditional subcutaneous xenograft model, which may be especially relevant in evaluating targeted therapies where expression of the tissue-specific target is essential and may be influenced by the implantation environment[23]. Orthotopic PC346C is a well-characterized xenograft model that is responsive to androgen manipulation and docetaxel treatment. PC346C cells express wild type AR and secrete PSA. ET_A expression was observed predominantly in (murine) endothelial and stromal cells, but not in the epithelial cells, of PC346C xenografts. The tumor-inhibiting effect of zibotentan is considered to work through blockade of epithelial ET-1-activated ET_A receptors in the tumor stroma. Like the majority of prostate tumors, PC346C cells have a mutated, inactive PTEN gene, which will unleash the PI3K/Akt signaling pathway from its negative regulator and be continuously activated[24].

The xenograft model allows monitoring tumor inhibition and testing PSA as potential response marker, and enabling to determine the mechanism of action providing potential pharmacodynamic response parameters. The present preclinical study was initiated to evaluate whether zibotentan or lenalidomide treatment in addition to docetaxel is able to extent the duration and/or enhance the effect of docetaxel and if such preclinical studies could predict clinical outcome of parallel phase III clinical trials.

MATERIALS AND METHODS

Reagents

Zibotentan was obtained from AstraZeneca (Nether Alderley, Cheshire, UK), lenalidomide was provided by Celgene Corporation (Summit, NJ). Zibotentan was prepared fresh weekly at a dose of 25 mg/kg BW in 1% Tween-80 solution and stored at 4°C. Lenalidomide was prepared at a concentration of 50 mg/kg BW in 1% carboxymethylcellulose fresh weekly and stored at 4°C. Docetaxel was used in a clinical formulation of 33 mg/kg BW and injected intraperitoneal (i.p.).

PC346C cell line

The human prostate cancer cell line PC346C was developed from the PC346 xenograft originating from tissue from a non-progressive prostate cancer patient. PC346C is an androgen responsive xenograft, which after androgen ablation progresses towards castration-resistant disease mimicking the clinical situation of progressive disease. It harbours a non-mutated, wild type androgen receptor (AR), a major characteristic of most clinical CRPC and produces high levels of PSA.

Cell Culture

PC346C cells were routinely cultured in a complex Prostate Growth Medium (PGM) based on DMEM/F12 (Cambrex BioWhittaker, Verviers, Belgium) supplemented with 2% fetal calf serum (FCS), 0.01% BSA bovine serum albumin (BSA), 1% insulin-transferrin-selenite (ITS), 10 ng/ml EGF, 10^{-10}M synthetic androgen R1881, 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were grown at 37°C in a humidified atmosphere containing 5% CO_2. For preparation of cell suspensions, semi-confluent cultures were harvested by trypsin digestion and cells were re-suspended at a density of 1.10^6 cells in 20 µl of PBS.

Animals

Athymic NMRI nu/nu male mice were purchased from Taconic Europe (Ry, Denmark) and were housed in accordance with the code of practice for animals in cancer research. The experiments were approved by the National Dutch Animal Ethic Committee and performed in agreement with The Netherlands Experiments on Animal Act 1977, and the European Convention for protection of Vertebrate Animals used for Experimental Purposes (Strasbourg, 18 march 1986).

Experimental design

For both experiments, PC-346C cells (10^6 cells/20 µl) were inoculated orthotopically into the dorsolateral prostate of male NMRI nude mice. Mice bearing orthotopic PC346C xenografts were randomized into different experimental groups. Animals were sacrificed when tumor volume (TV) was below 50 mm^3, exceeded 1000 mm^3 or at 60 days of treatment. In the lenalidomide treatment groups additionally 2 animals were sacrificed for intermediate analyses at day 7 and 14 of start of treatment. At sacrifice, plasma and tumor tissue were sampled. Animal welfare was monitored by changes in body weight (BW).

Zibotentan
To assess the effectiveness of zibotentan to docetaxel, mice were randomized into 4 experimental groups: (A) placebo oral daily plus placebo bolus injection i.p.; (B) zibotentan (25 mg/kg) oral daily plus placebo bolus injection i.p.; (C) placebo oral daily plus docetaxel (33 mg/kg) bolus injection i.p. on day 0 and 28; (D) zibotentan (25 mg/kg) oral daily plus docetaxel (33 mg/kg) bolus injection i.p. on day 0 and 28. Treatment was initiated at TV 200-300 mm^3 with daily oral medication of zibotentan (or placebo) followed one day later with the bolus injection i.p. of docetaxel (or placebo).

Lenalidomide
To determine the potency of lenalidomide to enhance the effect of docetaxel, mice were randomized into 5 treatment groups: (A) placebo oral daily starting at TV 50-100 mm^3 plus placebo bolus injection i.p. at TV 400-500 mm^3; (B) lenalidomide (100 mg/kg) oral daily starting at TV 50-100 mm^3; (C) docetaxel (33mg/kg) bolus injection i.p. at TV 400-500 mm^3; (D) lenalidomide (100 mg/kg) oral daily starting at TV 50-100 mm^3 plus docetaxel (33mg/kg) bolus-injection i.p. at TV 400-500 mm^3; and (E) docetaxel (33mg/kg) bolus-injection i.p. at TV 400-500 mm^3 plus lenalidomide (100 mg/kg) oral daily starting one week after docetaxel bolus injection (Fig.2A).

Tumor growth and PSA analysis

Tumor volume of the intraprostatically growing tumors was monitored weekly by transrectal ultrasonography (TRUS), using an intravascular ultrasound probe adapted for use in mice[25]. PC346C tumor growth was also monitored by measurement of circulating levels of PSA[26]. Plasma samples were collected and stored at –20°C for PSA analysis every 2 weeks. PSA concentration was analyzed by an automated enzyme-linked immunosorbent assay (ELISA) on an Elecsys total PSA Immunoassay; lower detection limit 0.0002 ng/ml (Roche Diagnostics GmbH, Mannheim, Germany).

Non-quantitative assessment of antitumor activity

To assess the additional value of lenalidomide to the docetaxel response, the median TV response at day 14 was calculated (TV day 0 start of docetaxel treatment/ TV 14 days post-docetaxel). The treated over control (T/C) value is an indication of antitumor effectiveness. The treatment is considered effective by the NCI if T/C< 42% while T/C<10% indicate high effectivity.

Quantitative assessment of antitumor activity

The tumor growth delay of treated versus control (T-C) was calculated from the median time to reach TV 1000 mm^3 of tumors in the control group A versus treated groups. The tumor doubling time (TD) of PC346C was calculated from the control group in exponential growth. The log_{10} cell kill is calculated from T-C (days)/3.32*TD. For comparison of activity log_{10} cell kill values were converted into an arbitrary rating[27] (table 1). The Drug Evaluation Branch of the Division of Cancer Treatment of the NCI considered log_{10} cell kill values of <0.7 to reflect no antitumor activity.

Antitumor activity	log_{10} tumor cell kill
Highly active ++++	>2.8
+++	2.0-2.8
++	1.3-1.9
+	0.7-1.2
Inactive	<0.7

Table 1. Arbitrary antitumor activity rating by log_{10} cell kill values according to NCI.

Immunohistochemistry

To determine the degree of cell proliferation and tumor angiogenesis, immunostainings were performed using a mouse anti ki-67 monoclonal antibody clone 7b11 (Invitrogen, USA, 180192z, 1:100 dilutions) and CD31 monoclonal rat anti-mouse antibody (Dianova, Hamburg, Germany), respectively. First 5-µm paraffin embedded sections were cut, paraffin embedded sections of formalin-fixed tissues were deparaffinized in xylene and rehydrated in alcohol. Endogenous peroxidase was inhibited using 3% H_2O_2 in methanol. The sections were washed in distilled water and heated in a microwave oven (in citrate buffer 10 mM, pH 6), 15 min for epitope retrieval. Then, slides were incubated for 1 h at room temperature in 1% nonfat dry milk in TBS-Tween, and incubated overnight at 4°C with primary antibody (1:100). Sections were rinse with TBS-Tween and incubated with polyclonal Goat Anti-Mouse IgG HRP (1:400,DAKO, Denmark) for Ki-67 and polyclonal rabbit anti-rat Biotin conjugate secondary antibody (1:400,abcam Cambridge, UK) (for

CD31) for 60 min at room temperature. After rinsing with TBST wash buffer, CD31 slides were incubated with streptavidine /HRP complex (DAKO, Denmark) for 60 min. further the complex incubated in 3, 3'- diaminobenzidine (DAB) (K3468, DAKO, Denmark) for 5 min, and counter-stained with hematoxylin.

Statistical analysis

Survival estimates were determined by the method of Kaplan and Meier. Survival data were compared by the nonparametric log-rank test. Survival time was defined as the day at death or euthanasia due to a TV exceeds 1000 mm^3 or at 60 days of treatment. Graphpad Prism version 4.1 software was used to analyze the survival data and compose all figures.

RESULTS

Effect on orthotopic tumor growth and survival

Tumor take rate, as defined by TV >50 mm^3, was 100% and 95% for the zibotentan and lenalidomide study, respectively. Mice were randomized based on TV at time of start of treatment and BW. For zibotentan 15 mice per group were included, for lenalidomide 14 mice were included. In the latter study, 3 mice per group were sacrificed at 7 and at 14 days of treatment for intermediate analyses leaving 8 mice per group for tumor growth monitoring.

Zibotentan

A single dose of docetaxel in mice with established PC346C (TV 245±35 mm^3) resulted in pronounced and significant reduction in TV: TV 968±118 mm^3 and 167±23 mm^3 at 14 days post-treatment in placebo-placebo and placebo-docetaxel treated mice, respectively. Zibotentan as monotherapy did not significantly affect tumor growth rate. The calculated median time to reach a TV of 1000 mm^3 was used as surrogate for survival, which was not affected by Zibotentan alone (Fig. 1). Daily oral administration of Zibotentan starting 1 day prior to the single docetaxel injection did not change the efficacy of docetaxel; TV at 14 days: 167±23 mm^3 and 165± 21 mm^3, respectively, nor did it extend docetaxel-induced tumor growth delay (T-C) as reflected by the surrogate survival curve (Fig. 1). The combination of zibotentan and docetaxel adversely affected animal welfare causing increased respiration frequency and loss of BW (32.3±2.9 and 26.5±5.4 g for placebo and zibotentan plus docetaxel-treated animals, respectively). Due to this significant toxicity, 9/15 animals did not reach the end point of TV of 1000 mm^3 and had to be sacrificed. The study had to be terminated at day 50.

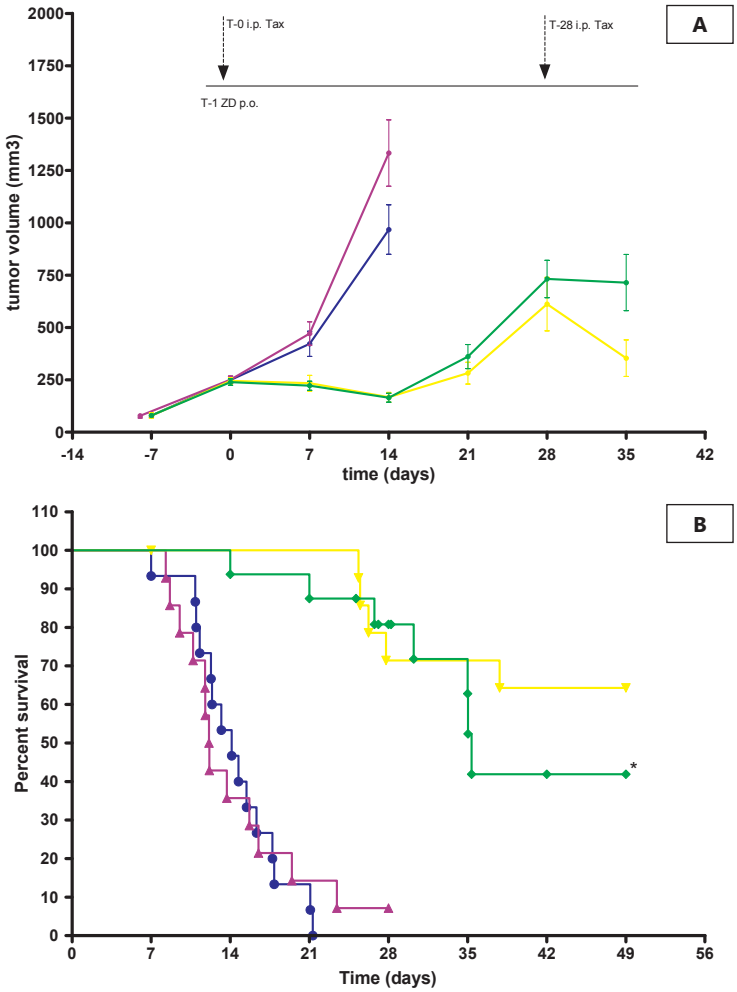

Figure 1. Zibotentan therapy alone or in combination to docetaxel did not affect PC346C tumor growth or docetaxel response of orthotopic PC346C. Mean change in PC tumor volume (A) and survival of animals (B) after treatment with Zibotentan. * Remaining mice in group D (green) had to be sacrificed due to illness.

tax = docetaxel; i.p: intraperitoneal; p.o.: orally.

Group A (blue): placebo p.o + placebo i.p.; group B (purple): Zibotentan p.o. + placebo i.p; group C (yellow): placebo p.o + docetaxel i.p.; group D (green): Zibotentan p.o. followed by docetaxel i.p.

Lenalidomide

Daily oral treatment of PC346C tumor-bearing mice with lenalidomide alone starting at TV 77±20 mm3 did not affect PC346C tumor growth; with TV of 276±29 mm3 in placebo-placebo treated mice (group A) versus 342±53 mm3 lenalidomide-treated mice (group B) at 14 days post-lenalidomide, respectively. The calculated median time

A

group	Median time to reach TV 1000mm^3 (days)	Growth delay T-C (days)	Log_{10} cell kill*	Antitumor activity**
A	43.4	-	-	
B	48.4	5.0	0.18	–
C	63.7	20.3	0.73	+
D	71.2	27.8	1.00	+
E	84.8	41.4	1.49	++

B

group	median change %TV	%T/C	% Ki-67	CD31
C	92.5		27.4	+
D	62.3	67.4	24.7	+
E	56.5	61.1	19.1	+

Table 2. (A) Overall tumor growth response characteristics of orthotopic PC346C after treatment with docetaxel in combination to lenalidomide. Tumor doubling time of orthotopic PC346C tumors was calculated from the average of all mice from group A (8.4 days). * Log_{10} cell kill= T-C (days)/3.32*TD; **Antitumor activity as defined by arbitrary activation rating: – not active, + poorly active, ++ active (see Materials & Methods and Table 1).
(B) PC346C tumor response at 14 days post-treatment with docetaxel and/or lenalidomide.
TV= tumor volume; T-C= tumor growth delay in treated mice relative to placebo-treated mice.
Group A: placebo p.o; Group B: lenalidomide p.o.; Group C: placebo p.o + docetaxel i.p.; group D: lenalidomide p.o. followed by docetaxel i.p. at TV 400-500 mm^3; group E: docetaxel i.p followed by lenalidomide p.o.

to reach a TV of 1000 mm^3 was used as surrogate for survival (Table 2A and Fig 2B). Log_{10} cell kill is a quantitative determinant of tumor growth response. The extent of antitumor activity of docetaxel alone (group C) was effective with a log_{10} cell kill of 0.73 as compared to 0.18 for the control treated tumors (group A). The combination treatment with lenalidomide pre-docetaxel (group D) did not increase antitumor activity of docetaxel alone (log_{10} cell kill 1.00), but lenalidomide treatment post-docetaxel (group E; log_{10} cell kill 1.49) was slightly more effective. Further assessment of the added value of lenalidomide to the docetaxel-induced antitumor effect was performed using non-quantitative parameters such as median %TV and treated (lenalidomide) over control (docetaxel) (%T/C) at 14 days after docetaxel injection. The tumor growth inhibition at 14 days was increased in both lenalidomide groups (%TV 62.3 and 56.5) as compared to the docetaxel treatment alone (%TV 92.5). The %T/C values for the treatment of lenalidomide pre- or post-docetaxel were not different (67.4 and 61.1, respectively) (Table 2B and Fig. 2C). These %T/C values are not considered significant antitumor activity according to NCI standards (see Table 1). Finally, in contrast to zibotentan, lenalidomide was well tolerated and did not alter mean BW change (data not shown).

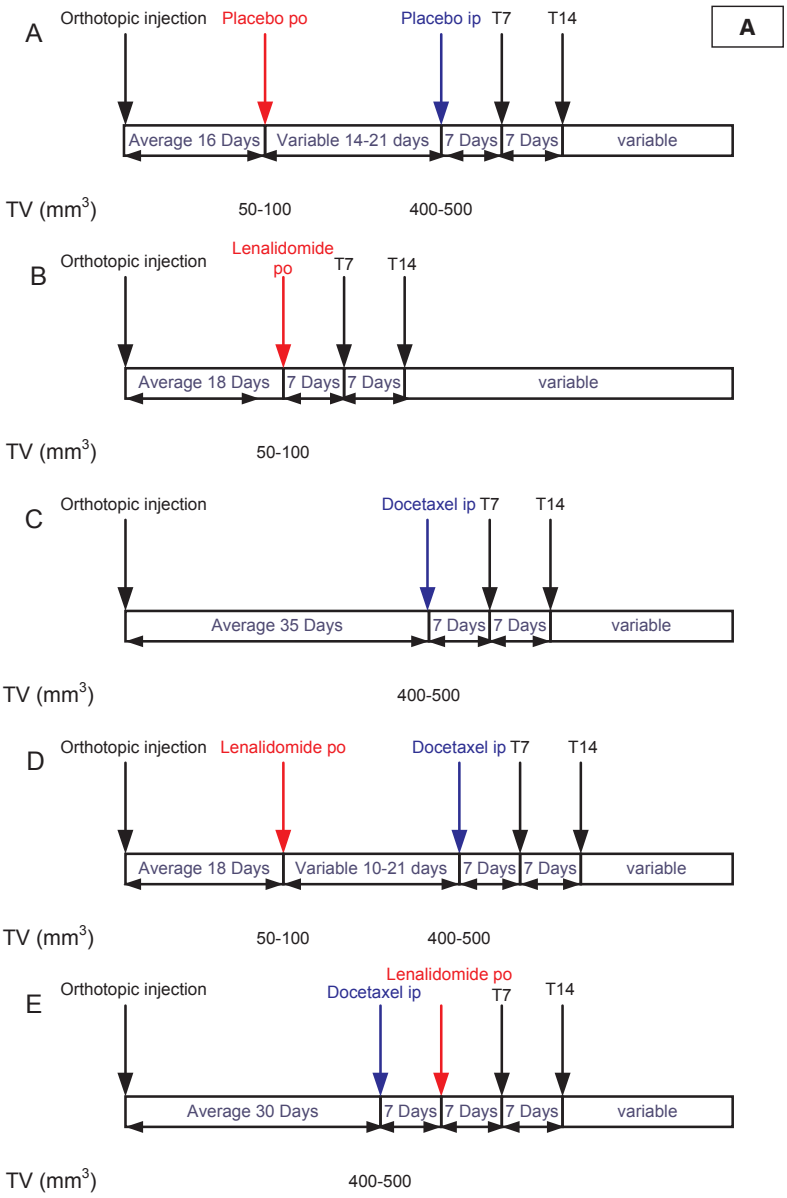

Figure 2. (A) Treatment schedule to assess the anti-proliferative activity of lenalidomide with or without docetaxel on PC346C tumor growth.

TV: tumor volume; i.p: intraperitoneal; p.o.: orally; T7: 7 days after starting last new agents; T14: 14 days after starting last new agent.

Group A: placebo p.o + placebo i.p.; group B: lenalidomide p.o. + placebo i.p; group C: placebo p.o + docetaxel i.p.; group D: lenalidomide p.o. followed by docetaxel i.p. at TV 400-500 mm^3; group E: docetaxel i.p one week later followed by lenalidomide p.o.

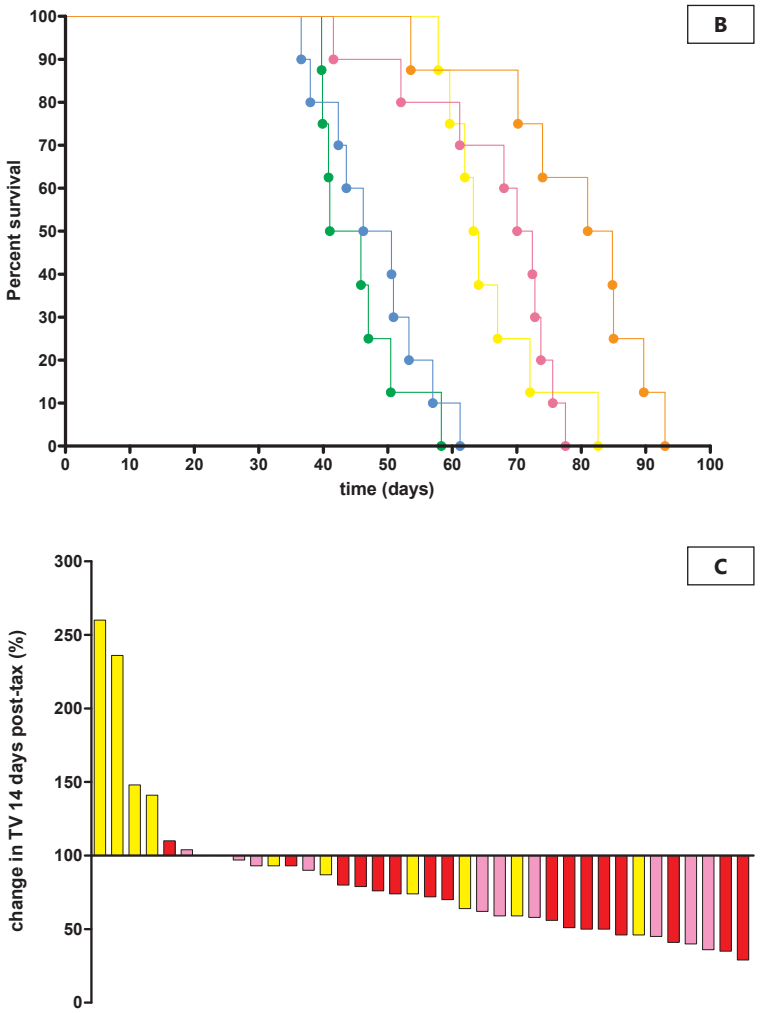

Figure 2. (B) Survival of mice in days after transplantation (sacrificed when TV>1000 mm3). Group A: green; group B: blue; group C: yellow; group D: pink; group E: purple (C) Relative tumor volume (TV T14/T0) 14 days after docetaxel injection (day 0) of individual mice. Group C: yellow; group D; pink; group E: red.

Effect on plasma PSA levels

Zibotentan

PSA is released from the tumor into the blood of PC346C tumor-bearing mice. Circulating levels of PSA increased proportionally with tumor burden. This was true for in all treatment groups indicating that PSA is not differentially affected by the treatments

and that PSA may be considered as a parameter for tumor growth response (Fig. 3A). In line with the TV data, plasma PSA levels were not significantly different between mice treated with docetaxel alone or in combination with zibotentan.

Lenalidomide
PSA plasma levels related well to tumor burden for all treatment groups indicating PSA as a useful parameter for tumor growth response (Fig. 3B). In support of the TV data, also plasma PSA was reduced by the combination therapies of docetaxel and lenalidomide, although without significance (Fig. 3B). The mean PSA release at 14 days after docetaxel injection was 869.6±120.8 vs 610.3±60.9 vs 547.7±72.5 ng/l for placebo and lenalidomide pre- or post docetaxel, respectively.

Immunohistochemical estimation of cell proliferation by Ki-67 and angiogenesis by CD31

Lenalidomide
Further evaluation of the effect of the combination of docetaxel plus lenalidomide in PC346C tumor tissues with cell proliferation by Ki-67 immunohistochemistry and CD31 staining did not reveal an added value of the combination for either sequence (Table 2 and Figure 4).

DISCUSSION

Although treatment options for patients with CRPC have improved, clinical benefit with systemic therapies is transient and survival times remain short. To improve outcomes, the development of novel agents and combination therapies for further clinical development is of great interest. In order to test the multitude of potential options for combination studies that have come available more recently and to determine if the treatment sequence is of relevance to the tumor response, relevant preclinical models are needed that are able to predict clinical response. The present study was undertaken to determine if the well-characterized orthotopic human prostate cancer in PC346C xenograft model is able to fulfill this need. In parallel to two large ongoing phase III clinical trials (Enthuse M1c[28] and MAINSAIL[29] for combination of docetaxel with ZD4054 or lenalidomide, respectively), we evaluated whether zibotentan or lenalidomide treatment was able to extend the duration and/or enhance the effect of the docetaxel-induced antitumor response of PC346C xenografts in nude mice.
Our results show that PC346C xenografts responded to docetaxel treatment, but that daily oral dosing with zibotentan starting 1 day prior to administration of docetaxel did

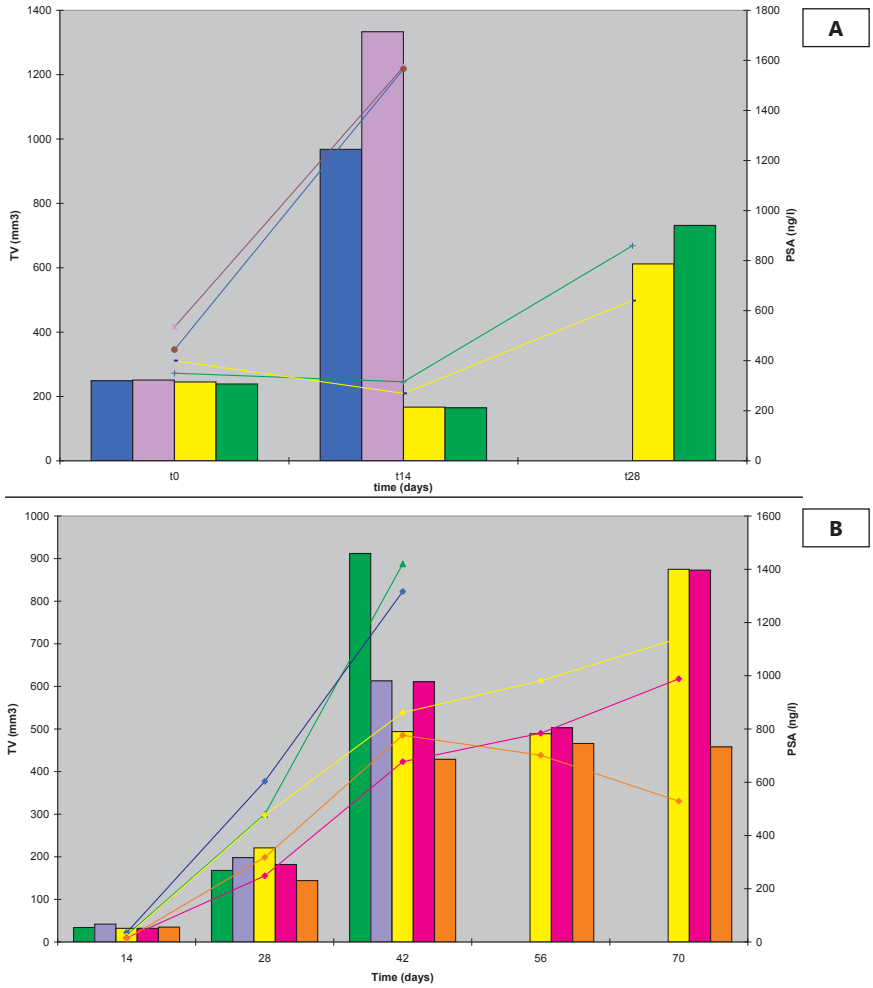

Figure 3. Relationship between TV change and PSA release in PC346C tumor-bearing mice treated with zibotentan or lenalidomide in combination with docetaxel.

(A) TV and PSA responses over time (t0 is docetaxel injection) to zibotentan and docetaxel treatment. Group A (blue): placebo p.o. plus placebo i.p.; group B (purple): placebo i.p. + zibotentan p.o.; group C (yellow): placebo p.o. + docetaxel i.p.; group C (green): zibotentan p.o. + docetaxel i.p. **(B)** TV and PSA release over time (day 0 is orthotopic tumor cell inoculation) after combination treatment of lenalidomide and docetaxel (day 28 is docetaxel injection). Group A: placebo p.o + placebo i.p.; group B: lenalidomide p.o. + placebo i.p; group C: placebo p.o + docetaxel i.p.; group D: lenalidomide p.o. followed by docetaxel i.p. at TV 400-500 mm^3; group E: docetaxel i.p one week later followed by lenalidomide p.o.

not show an additive effect to the docetaxel-induced inhibition of tumor growth and PSA release, nor did the combination show an effect on log_{10} cell kill reflected by an extended survival induced by docetaxel treatment in these animals.

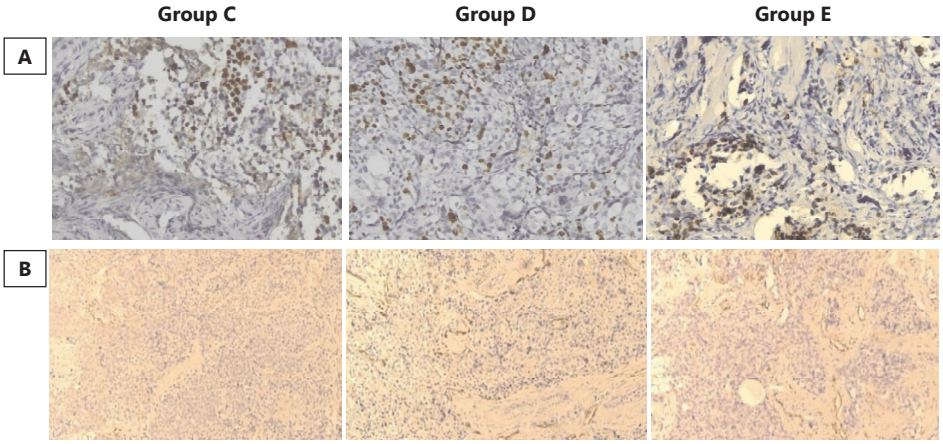

Figure 4. Immunohistochemical staining for A: cell proliferation (Ki-67) and B: angiogenesis (CD31) in PC346C tumors after combination therapy with lenalidomide plus docetaxel 14 days post-docetaxel.

Group C: placebo p.o + docetaxel i.p.; group D: lenalidomide p.o. followed by docetaxel i.p. at TV 400-500 mm^3; group E: docetaxel i.p one week later followed by lenalidomide p.o.

Daily oral treatment of PC346C tumor-bearing mice with lenalidomide starting prior to docetaxel injection did not enhance the inhibitory effect of docetaxel. However, administration of lenalidomide post-docetaxel injection, moderately improved the docetaxel response duration resulting in an increased $\log_{10}$ cell kill, reflected by extended overall survival of the animals. The higher antitumor effect was not supported by reduced cell proliferation ratio estimates by Ki-67 expression or by changes in CD31 expression indicative for impaired vascularisation. Although not supported by these tissue response biomarkers and thus potentially not physiologically significant, the $\log_{10}$ cell kill and corresponding difference in antitumor activity score suggest that the sequence of administration of lenalidomide to docetaxel treatment may be relevant to achieve an optimal additive effect. Further efforts to clarify the observed difference between the sequence groups using western blot analyses of AKT and phosphorylated AKT (pAKT) revealed down-regulation in all docetaxel-treated tumors irrespective of the lenalidomide-docetaxel sequence (data not shown). We analysed tumor tissues retrieved at 14 days post-docetaxel treatment, which may have been too late to show an effect causing the observed growth delay in the lenalidomide post-docetaxel tumors.

So far this is the first report that took into consideration the therapy sequence of the compounds. A recent report by Henry et al.[30] tested the efficacy of bi-weekly docetaxel plus daily lenalidomide in the docetaxel-resistant the PC3 xenograft. Clearly, this set-up is very different from the present study where the tumor is still responsive to docetaxel. The mechanism of action of lenalidomide in docetaxel-resistant PC may be quite dif-

ferent to its effect in docetaxel-responsive PC. The present study used the orthotopic androgen responsive PC346C xenograft that reflects clinical CRPC. In contrast to PC3, docetaxel treatment of PC346C xenograft-bearing mice have shown notable anti-tumor efficacy that mimics the clinical situation of CRPC and thus provides a clinically relevant model to test new combinations for their efficacy to prolong and sustain the initial docetaxel effect in CRPC[23,31]. Although Henry et al.[30] also reported a reduction in IC50 values of docetaxel in prostate cancer cell lines when incubated simultaneously with lenalidomide in vitro, the lenalidomide effect on the docetaxel dose response curves were shown to be very small and the biological relevance of such small in vitro effects may be questioned. We were unable to show a significant shift in IC50 values using the PC346C cell line in vitro (data not shown).

The orthotopic model of human PC cells inoculated into the mouse prostate is an attractive alternative for the traditional subcutaneous xenograft model, which may have special relevance when evaluating targeted therapies that rely essentially on tissue-specific target expression and that may be regulated by the local micro-environment. Moreover, PC346C xenografts secrete PSA and allow for validation of PSA as potential therapy response marker. The use of PSA as biomarker for treatment efficacy has been used in former studies using orthotopic PC346C model[26]. The confirmation that PSA response follows tumor volume changes in the present study is an important validation for the use of PSA as for monitoring tumor responses. PSA responses indicate that zibotentan and lenalidomide do not affect PSA secretion differentially and that changes in circulating PSA reflect changes in tumor burden.

The orthotopic PC346C xenograft model provides a powerful tool to validate the most optimal sequence of administration of novel agents to reach the highest clinical efficacy. The negative results of zibotentan and the modest efficacy of lenalidomide post-docetaxel in the present study reflect the lack of clinically relevant activity seen in the recent large clinical phase III trials with docetaxel +/- zibotentan, and docetaxel+/- lenalidomide. The randomized double blind phase III trial combining docetaxel with lenalidomide administered simultaneously (MAINSAIL study) in patients with CRPC, was terminated early because of failure to meet study goals[29]. Zibotentan was tested in three prospective, randomized, double-blind phase III trials. The first study (Enthuse M0) to test zibotentan monotherapy in patients with rising PSA after hormonal therapy and no metastasis was stopped because the primary efficacy end points were unlikely to be met[32]. The second study (Enthuse M1) in patients with asymptomatic or mildly symptomatic CRPC with bone metastasis zibotentan monotherapy did not lead to a significant improvement in OS or any secondary end points[28].The results of the third study (Enthuse M1c) evaluating the efficacy of zibotentan in combination with docetaxel in patients with metastatic CRPC are awaited[33]. Taken together, zibotentan and lenalidomide are currently not implemented in the treatment of prostate cancer.

Representative xenograft models, such as the orthotopic, androgen responsive PC346C system, are valuable asset to select combination therapies and novel agents for potential clinical efficacy in the treatment of CRPC. The model allows identifying novel agents and combination therapies for further clinical development and provides an efficient predictive selection model for the most promising options. This is especially good news in the light of the increasing number of new potential anti-cancer compounds for (CR) PC in a background of reducing resources to conduct large phase III clinical trials.

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Chapter 8

General discussion

Although most men who develop prostate cancer will not die from their disease, those who develop castration-resistant prostate cancer (CRPC) have high rates of morbidity and mortality, and treatment of patients with CRPC remains a considerable therapeutic challenge. Until 2004, clinical management for advanced prostate cancer has been primarily focused on controlling symptoms. In 2004, docetaxel based chemotherapy was established as the standard first-line treatment in patients with CRPC based on two phase III trials that showed for the first time a survival benefit, with a median survival time of 19 months[1,2]. Since then molecular, basic, and translational research has given us a better understanding on the mechanisms of CRPC, and in the past three years additional treatment options have been approved by the US Food and Drug Administration (FDA) for the treatment of CRPC. The cytotoxic cabazitaxel[3], the androgen biosynthesis inhibitor abiraterone acetate[4], the immunotherapy sipuleucel-T[5], the radioisotope alpharadin[6] and the anti-androgen enzalutamide[7] have all been shown to improve overall survival in randomized phase III studies for patients with metastatic CRPC. Also there is an armentarium of other promising novel agents, which are currently being tested in the clinical setting[8]. These include tasquinimod[9], orteronel[10], PROSTVAC-VF[11], and cabozantinib[12], each with a different mechanism of action.

However, all these novel agents do not confer long-term benefit and are accompanied by deleterious side effects. Moreover, optimal methods of treatment selection, combination and sequencing have yet to be determined. The challenge will be to position the current established and expected novel agents in the new landscape of metastatic prostate cancer and to identify the ideal sequence of administration, best timing for initiation, combination strategies, discontinuation beyond progression and after commencement of subsequent therapies.

Approximately 70%- 80% of patients with prostate cancer will develop bone metastases that often lead to severe bone pain, hypercalcaemia and skeletal –related events (SREs)[13]. In the prostate cancer indication, zoledronic acid, a third generation bisphosphonate, is the only bisphosphonate that has demonstrated efficacy in terms of reduction in SREs and analgesic effects in a randomized, double-blind, phase III trial[14]. However, this study was done before docetaxel based chemotherapy in CRPC became the standard of care. To investigate whether bisphosphonates would provide an additional benefit at the time of initiation of effective chemotherapy the Dutch Nepro study group conducted a randomized phase II/III trial adding risedronate, a third generation bisphosphonate, to docetaxel in patients with CRPC with bone metastases. The addition of risedronate to docetaxel did not improve time to progression (TTP). Also, there was no statistically or clinically significant difference in pain response between patients who were treated with risedronate versus the docetaxel alone group. Therefore, starting bisphosphonates in combination with docetaxel based therapy in patients with CRPC should not be recommended routinely. Several new agents targeted towards the

bone are being investigated for the management of metastatic lesions. A new target is nuclear factor-κB (RANKL) which increases osteoclast differentiation and activity and may stimulate tumor cell growth. Denosumab, a recombinant human monoclonal IgG2 antibody directed to RANKL, recently demonstrated to be superior to zoledronic acid in preventing or delaying SREs in patients with bone metastases from CRPC in a large phase III trial[15]. Ongoing studies of emerging bone-targeted therapies in patients with CRPC will continue to define the role of these therapies in patients with prostate cancer.
The spectrum of prostate cancers that are progressing despite castrate levels of testosterone includes tumors that have shown varying degrees and durations of response to primary hormone treatment, and clinical manifestations that range from a rising prostate-specific antigen (PSA) alone, a rising PSA with bone metastases and/or soft-tissue metastases, or a predominantly visceral disease pattern[16]. Efforts are ongoing to develop drugs targeting mechanisms causing tumor progression across the entire spectrum of prostate cancer disease states. Novel therapies have been rationally designed to target molecular pathways involved in oncogenesis and disease progression although results from trials have been mixed. The biologic heterogeneity of CRPC, including potential involvement of androgen receptor (AR)-mediated or AR-independent pathways, is a probable cause of the variable responses seen with targeted therapies[17].
The mammalian target of rapamycin (mTOR) is a critical molecule for controlling the growth and division of tumor cells and can be activated by abnormal or inappropriate growth factor signaling, mutation or activation of signaling molecules such as PI3K or Akt. CRPC is characterized by its continuing dependence on signaling through the androgen receptor for growth and the frequent activation of the PI3K pathway, mostly through loss of PTEN. The PI3K/Akt/mTOR and androgen receptor signaling pathways have been implicated in prostate cancer progression and regulate each other by reciprocal negative feedback, such that inhibition of one activates the other[18]. Androgens regulate the Akt pathway by both genomic and non-genomic effects. This explains why prostate tumors subjected to androgen ablation experience an increase in Akt phosphorylation, and suggest that the tumor compensates for the loss of one pathway with another. Blockade of AR results in activation of Akt through reduced levels of FKBP5 impairing the stability of PHLPP. Conversely, inhibition of the PI3K pathway in PTEN negative prostate cancer results in feedback signaling to the receptor tyrosine kinase HER2/HER3 leading to activation of AR[18]. Because this significant bidirectional crosstalk between two critical survival pathways we evaluated, in an international study, simultaneously targeting both pathways with the combination of ridaforolimus, an mTOR inhibitor with bicalutamide, an androgen receptor inhibitor. Despite the sound rationale for combining an anti-androgen with an mTOR inhibitor, the toxicity of ridaforolimus combined with bicalutamide was not well-suited to generally asymptomatic patients with CRPC.

Another targeted drug we evaluated was danusertib, a pan-aurora kinase inhibitor. Aurora kinases play an essential role as key mitotic regulators and are frequently overexpressed in prostate cancer. Therefore these proteins are an attractive target for treatment in patients with CRPC[19]. Unfortunately, in our study danusertib monotherapy showed minimal efficacy in patients with CRPC.

In view of the other major advances in the treatment of patients with CRPC and the modest results of our studies with danusertib and ridaforolimus it is unlikely that these agents will be further explored for the treatment of patients with CRPC.

In 2008, the Prostate Cancer Working Group 2 (PCWG2) consensus redefined consensus criteria for early-phase clinical trial end points[20]. However, assessing activity of (targeted) therapies in patients with CRPC remains challenging given the preponderance of bone metastases. The current clinical biomarkers for prostate cancer are not ideal as there remains a lack of reliable biomarkers that can monitor the progression of the disease, and predicting the prognosis and survival after clinical intervention[21]. In the 1930s, human prostatic acid phosphatase (PAP) was reportedly the first serum biomarker for prostate cancer[22]. PSA was later discovered as a biomarker for prostate cancer following the discovery of serum PAP[21,23]. Although an important and useful tumor marker changes in PSA do not always correlate with regression of tumor and clinical benefit, especially in response to noncytotoxic agents[24]. Currently, due to new genomic technologies, several new prostate cancer biomarkers has emerged, introducing new assays in serum and urine, but it is beyond the scope of this discussion to cover all biomarkers under investigation.

One approach to identifying predictive biomarkers is to focus on genomic disease signatures, such as loss of the PTEN tumor suppressor gen. As previous mentioned PTEN loss activates the PI3K pathway, which inhibits AR signaling and causes resistance to AR-based therapies. Therefore, PTEN deletion may be both prognostic and predictive of response to therapy[25].

Circulating tumor cells (CTCs) represent a promising area of development and can predict survival benefit from treatment in metastatic CRPC[26]. CTC number was more predictive than post therapy changes in PSA, raising the likelihood that CTC number may be an intermediate end point of efficacy[26]. The comparison of CTCs before and after treatment constitutes a predictor of outcome. However, enumeration of CTCs and extracting molecular information is currently labor-intensive and expensive[25].

Prostate cancer is a complex and biologically heterogeneous disease that is not adequately assessed with conventional imaging alone. Imaging-based technologies have been developed as well, including transrectal ultrasound, computed tomography, magnetic resonance imaging, and positron emission tomography (PET). Multiple PET tracers are currently available, such as F18-Fluorodeoxyglucose (FDG), C11/F18-choline and sodium F18-fluoride, to aid in the detection and management of prostate cancer across

the clinical spectrum of the disease[27]. F18-fluorodihydrotestosterone is active in CRPC and is emerging as a valuable pharmacodynamic marker in the development of novel androgen receptor-targeted therapies[27].

Current advances in molecular technique facilitating the discovery of new biomarkers and novel imaging-based technologies for prostate cancer can help further drug development, evaluation of new therapies and can be used as criteria for deciding on lead compounds for the third phase of clinical trials[21].

With the rapid development and increase of new (targeted) agents that have the potential to show clinical activity against CRPC, preclinical testing should be a basis for selecting compounds for further clinical development in the limited number of expensive clinical trials that can be carried out. The orthotopic human prostate cancer xenograft model PC346C seems a powerful tool which fulfils most of the desirable criteria of preclinical models selecting agents or combination therapies, with potential clinical efficacy, for further clinical testing. The PC346C xenograft model provides an efficient predictive selection model for the most promising options.

Currently, we try to develop docetaxel resistant prostate cancer xenograft models to identify mechanism of resistance to docetaxel and to investigate cabazitaxel sensitivity. Hopefully, these models may allow development of potential predictive biomarkers, based on gene expression, for docetaxel resistance and sensitivity to cabazitaxel in prostate cancer.

Because taxanes impair AR signaling through significant AR translocation[28], we also try to determine with our xenograft models if taxanes are as active following treatment with agents targeting AR signaling (e.g. abiraterone or enzalutamide), or if these agents will negatively impact taxanes benefit.

Besides preclinical studies we are currently enrolling patients with CRPC in a phase II clinical study to investigate the effects of budesonide on the grade of diarrhea, the most common non-haematological toxicity of cabazitaxel. A translational side study of this study is to enumerate, isolate and subsequent characterize CTCs to enable there use as a predictive marker of cabazitaxel response and to gain insight into the mechanisms of sensitivity and resistance to cabazitaxel.

For many years, docetaxel was the only agent showing a survival benefit for patients with CRPC. Over the last few years the therapeutic spectrum changed dramatically reflecting a change in the understanding of the biology of CRPC which is now far more sophisticated. There has been considerable progress in understanding biologic events in CRPC, from the mechanisms driving continued androgen receptor signaling, the engagement of bone remodeling mechanisms, and immunobiology including the role of immune checkpoints[29]. However, many challenges remain for prostate cancer researchers like the urgent need to develop analytically validated and clinically qualified intermediate end points (surrogate biomarkers) for overall survival[29]. Another challenge is

how to sequence and combine the multiple novel agents, including the additive toxicity. Another major challenge is moving toward predictive medicine where specific tumor biomarkers can select subsets of patients for a given therapy in order to maximize benefit to those patients who are likely to respond to a therapy.

Taken together, there is notable improvement made through clinical trials and the treatment paradigm for patients with CRPC is rapidly evolving. However, the prognosis of patients with advanced CRPC remains poor and more preclinical as well as clinical research is necessary, including defining the optimal sequence and potential combinations of new agents.

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Chapter 9

Summary/Samenvatting

Summary

Prostate cancer is, besides non-melanoma skin cancer, the most common cancer among men in the Western world. Epidemiological studies have demonstrated that prostate cancer frequently runs an indolent course and many patients may never develop disease related symptoms. There is however a sub-group of patients with a more aggressive type of prostate cancer resulting in a greater risk of prostate cancer induced mortality.
In patients with metastatic prostate cancer, androgen derivation therapy (ADT) is universally accepted as the initial treatment of choice, with a response rate ranging from 80% to 90%. However, these tumors, after a median of 18 months, become androgen-independent and grow despite androgen ablation. Prostate cancer progressing despite castrate levels of testosterone is classified Castration Resistant Prostate Cancer (CRPC). Although treatment options for patients with advanced CRPC have recently increased, patients still have a poor prognosis and novel therapeutic options are needed.
This thesis describes both clinical and pre-clinical studies aiming to investigate several treatment methods for patients suffering from CRPC in different stages of progression.
Chapter 2 provides a general introduction and an overview of chemotherapy developments for patients suffering from CRPC. In 2004, two landmark trials, demonstrated a survival benefit in patients with advanced CRPC by docetaxel-based chemotherapy as compared to mitoxantrone plus prednisone. Docetaxel based therapy has become the standard treatment for patients with CRPC. Considerations when to start chemotherapy especially in asymptomatic patients is comprehensively discussed and may depend on prognostic factors including symptomatic disease, two or more new lesions on the bone scan, visceral metastases and presence of anaemia.
Chapter 3 presents the results of a randomized phase 3 study which aimed to investigate the potential benefit of adding risedronate to docetaxel based chemotherapy in patients with CRPC with bone metastases. Bisphosphonates are potent inhibitors of osteoclast- mediated bone resorption. An emerging body of preclinical and clinical evidence indicate that bisphosphonates might also exhibit direct antitumor activity and have shown synergistic action of zoledronate and taxanes. There are preclinical data suggesting that bisphosphonates have osteoclast-independent effects that can be associated with an antitumor effect. In CRPC zoledronic acid, a third generation bisphosphonate has shown to delay the onset of skeletal related events (SREs). This study was however completed before docetaxel became standard chemotherapy, by demonstrating improved overall survival. The objective of this study was to evaluate the effect of adding a bisphosphonate to the effective first line treatment with docetaxel-based chemotherapy on the time to progression, overall survival and pain.
Adding risedronate to docetaxel-based chemotherapy did not have an effect on the time to progression and overall survival. Furthermore, there was no evidence of different

pain response between patients who were treated with risedronate versus the docetaxel alone group. Our study demonstrated that the addition of risedronate to docetaxel had no impact on disease progression and overall survival. We found neither reduction in pain scores with the addition of risedronate to docetaxel in our study. The results of this study demonstrate that the addition of risedronate to effective first line treatment with docetaxel does not have added value.

Chapter 4 provides an overview of studies conducted with the aurora kinases inhibitor danusertib (formerly PHA-739358) in different types of solid and haematological malignancies. Aurora-kinases play an essential role as key mitotic regulators and are frequently overexpressed in prostate cancer.

In the subsegment **chapter 5**, a phase 2 study is reported, investigating danusertib for the treatment of patients with CPRC. Danusertib is a small ATP competitive molecule that specifically inhibits aurora A, B and C kinases. This study aimed to investigate the efficacy and toxicity of danusertib administered intravenously (i.v.) in two different dosing schedules in patients with metastatic castration resistant prostate cancer with progressive disease after docetaxel-based treatment. Danusertib was generally well tolerated; the most common grade 3 and 4 drug related adverse events were uncomplicated neutropenia. Danusertib monotherapy demonstrated only modest efficacy in patients with CRPC. Further studies are required to establish specific biomarkers predictive for either response or prolonged disease stabilization, to enable selecting patients who may benefit from danusertib.

Chapter 6 describes the results of a study with bicalutamide, an anti-androgen, combined with ridaforolimus, an mTOR inhibitor in patients with asymptomatic metastatic CRPC and disease progression despite castration level of testosterone. There is a relatively large population of patients with asymptomatic CRPC and rising PSA as the only evidence of disease progression. In these patients there may be no urgency to commence chemotherapy. The dilemma of how to treat these patients represents an unmet medical need. Several secondary endocrine therapies, including anti-androgens, such as bicalutamide, have been evaluated in this patient group. Although bicalutamide may result in PSA responses, clinical benefit is modest and as single agent has not shown to prolong survival. The mTOR kinase plays an important role in the phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) signalling pathway. The PI3K/Akt/mTOR pathway plays a prominent role in the development and progression of prostate cancer. PTEN is a tumor suppressor gene and a negative regulator of activity of the PI3K/Akt/mTOR pathway. Mutations in the PTEN are common events in prostate cancer and mutations that result in expression of inactive protein leads to increased activity of the PI3K/Akt/mTOR pathway. Recent data indicate that there is a significant cross-talk between the PI3K/Akt/mTOR and androgen receptor signalling pathways.

To determine the efficacy of bicalutamide in combination with ridaforolimus in patients with asymptomatic, metastatic CRPC a phase II randomized trial was designed. A safety analysis performed after the first eleven patients identified four cases of grade 3 oral ulcerations. The unblinded safety data showed that all 4 patients had been allocated to the ridaforolimus plus bicalutamide group. Because of this unexpected severe ridaforolimus toxicity with the standard dose of 40 mg and hence a conceivable drug-drug interaction between ridaforolimus and bicalutamide, the protocol was amended to proceed with a non-randomized safety lead-in study to test an open label and reduced dose of ridaforolimus (30 mg) in combination with bicalutamide. Eleven patients were treated. Three of the eleven patients experienced a dose limited toxicity (DLT), one with grade 3 hyperglycemia, and 2 with grade 2 stomatitis. The pharmacokinetic results showed no differences in exposures to ridaforolimus with and without concomitant bicalutamide administration. This frequency and severity of adverse events did not warrant further investigation in a basically asymptomatic CRPC patient population. Therefore, the study was halted.

Chapter 7 presents the results of an *in vivo* study with zibotentan or lenalidomide combined with docetaxel in the orthotopic PC346C human prostate cancer xenograft. PC346C is an androgen responsive xenograft, which after androgen ablation progresses towards castration-resistant disease mimicking the clinical situation of progressive disease. Zibotentan is an endothelin-A (ET_A) receptor antagonist. Endotheline-1 and the ET_A receptor play a key role in the development and progression of prostate cancer by modulating cell proliferation, angiogenesis, and anti-apoptosis. Therefore ET-1 and its receptors may be a therapeutic target in CRPC. In our study however, zibotentan in combination with docetaxel did not augment the efficacy of docetaxel on tumor growth nor did it prolong the extended survival induced by docetaxel treatment.

Lenalidomide is an immunomodulatory analogue of thalidomide with an inhibiting effect on the PI3K/Akt signalling pathway and antiangiogenic properties. Lenalidomide given prior to docetaxel injection did not enhance docetaxel response. Administration of lenalidomide post-docetaxel injection, though, moderately improved the response duration of a single dose of docetaxel.

The negative results of zibotentan and the modest efficacy of lenalidomide given after docetaxel largely correlated with the lack of clinically relevant activity seen with docetaxel +/− zibotentan, and docetaxel +/− lenalidomide in phase 3 trials. Therefore we think our model is representative and a valuable asset to identify novel agents and combination therapies for further clinical development, especially in the light of the large variety of new potential anti-cancer compounds and in a background of reducing resources to conduct large phase III clinical trials.

The results of this thesis described in chapter 2-7 are discussed in **Chapter 8** and suggestions for further research are mentioned.

Despite recent improvements in treatment of patients with CRPC, with new agents becoming available such as cabazitaxel, abiraterone and enzalutamide, the disease remains a serious health problem with high morbidity and mortality. It is therefore essential to continue translational and clinical studies into different (new) treatment opportunities for different stages of disease, in order to develop new treatment options with prolonged overall survival.

Samenvatting

Prostaatkanker is, na huidkanker, de meest voorkomende vorm van kanker bij mannen in de Westerse landen en de tweede aan kanker gerelateerde doodsoorzaak. Epidemiologische studies hebben aangetoond dat prostaatkanker vaak een betrekkelijk indolent verlopende aandoening is, waarbij de patiënt bijna nooit ziekte gerelateerde symptomen zal ontwikkelen. Echter, er bestaat een subgroep van patiënten met een meer agressieve vorm van prostaatkanker waarbij een grotere kans bestaat om te overlijden ten gevolge van de kanker. In het geval van gemetastaseerde ziekte is androgeen deprivatie de eerste keus van behandeling, waarop in 80 tot 90% een goede respons wordt gezien. Het effect van castratie is echter maar beperkt tot gemiddeld anderhalf jaar. Op het moment dat er sprake is van progressie van de ziekte onder testosteronspiegels tot castratieniveau spreekt men van castratie resistent prostaatcarcinoom (CRPC). De prognose van patiënten met CRPC blijft, ondanks recente nieuwe ontwikkelingen in de behandelingsmogelijkheden met een toegenomen levensverwachting, beperkt en het ontwikkelen van nieuwe behandelingsmogelijkheden is noodzakelijk. In het huidige proefschrift zijn verschillende behandelingsmogelijkheden voor patiënten met CRPC in verschillende stadia van de ziekte, zowel klinisch als preklinisch geanalyseerd.

Hoofdstuk 2 is een algemene inleiding en geeft een overzicht van de ontwikkelingen van chemotherapie bij patiënten met CRPC. In 2004 werden de resultaten van twee grote klinische studies bekend, waarin een overlevingswinst werd gevonden van chemotherapie met docetaxel ten opzichte van mitoxantrone in combinatie met prednison. Sindsdien is docetaxel de standaard 1^{e} lijns palliatieve behandeling bij patiënten met CRPC. Overwegingen over het beste moment om te starten met chemotherapie, met name bij asymptomatische patiënten, worden uitgebreid bediscussieerd en kunnen mede worden bepaald op basis van prognostische factoren, zoals het al dan niet hebben van symptomen, het ontstaan van twee of meer nieuwe laesies op een botscan, het hebben van viscerale metastasen en/of een anemie.

In **hoofdstuk 3** worden de resultaten van een gerandomiseerde fase 3 studie gepresenteerd waarin het effect van het toevoegen van een derde generatie bisfosfonaat, risedroninezuur, aan docetaxel chemotherapie wordt onderzocht bij patiënten met een ossaal gemetastaseerd CRPC. Bisfosfonaten hebben een remmend effect op de botresorptie door inactivatie van de osteoclasten. Preklinische studies suggereren een direct effect van bisfosfonaten op de tumorcel proliferatie en een synergistisch effect van bisfosfonaten in combinatie met taxanen. Uit onderzoek is gebleken dat zoledroninezuur, een derde generatie bisfosfonaat, het ontstaan van skeletcomplicaties ten gevolge van ossale metastasen bij patiënten met CRPC kan vertragen. Deze studie is echter uitgevoerd voordat docetaxel de standaard behandeling werd voor patiënten met CRPC, vanwege overlevingswinst. Het doel van de huidige studie was dan ook om te onderzoeken

wat het effect is op de tijd tot progressie, overleving en pijn van het toevoegen van een bisfosfonaat aan effectieve 1e lijns chemotherapie met docetaxel.
Het bleek dat de toevoeging van risedroninezuur aan docetaxel chemotherapie geen effect had op de tijd tot progressie en de totale overleving. Er werd ook geen verschil gevonden in de respons op pijn tussen de patiënten met en zonder het gebruik van risedroninezuur. De resultaten van deze studie tonen aan dat het toevoegen van risedroninezuur ten tijde van behandeling met effectieve chemotherapie met docetaxel geen meerwaarde heeft.
In **hoofdstuk 4** wordt een overzicht gegeven van studies gedaan met de aurora kinase remmer danusertib (voormalig PHA-739358) bij meerdere solide en hematologische maligniteiten.
Aurora kinases hebben een sleutelrol bij de regulering van verschillende stappen gedurende de celdeling en komen vaak tot overexpressie in prostaatkanker.
In aansluiting op hoofdstuk 4 wordt in **hoofdstuk 5** een fase 2 studie beschreven met danusertib voor de behandeling van patiënten met CRPC. Danusertib is een intracellulair werkende pan-aurora kinase remmer. Deze studie werd uitgevoerd om de veiligheid, verdraagzaamheid en de effectiviteit van twee verschillende dosering schema's van danusertib te onderzoeken bij patiënten met gemetastaseerd CRPC en progressieve ziekte na eerdere behandeling met docetaxel. Danusertib werd goed verdragen met ongecompliceerde neutropenie als meest voorkomende graad 3 en 4 toxiciteit. Danusertib monotherapie bleek slechts matig effectief te zijn bij patiënten met CRPC. Verdere analyses zijn vereist voor het vinden van predictieve biomarkers voor respons of ziekte stabilisatie om specifieke patiëntengroepen te kunnen selecteren die meer baat zouden kunnen hebben van behandeling met danusertib.
Hoofdstuk 6 beschrijft de resultaten van een studie met bicalutamide, een anti-androgeen, in combinatie met ridaforolimus, een mTOR remmer, bij asymptomatische patienten met gemetastaseerd CRPC en progressieve ziekte onder testosteronspiegels tot castratieniveau. Er is een relatief grote populatie patiënten met alleen asymptomatische PSA progressie waarbij er nog geen urgentie bestaat om te starten met chemotherapie. Het dilemma hoe deze patiënten te behandelen vormt een onbeantwoorde medische behoefte. Meerdere tweede lijns hormonale therapieën, inclusief anti-androgenen, zoals bicalutamide, zijn onderzocht in deze groep patiënten. Hoewel bicalutamide kan resulteren in een PSA respons zijn klinisch significante effecten beperkt en heeft het als monotherapie geen overlevingswinst.
Het mTOR-kinase is onderdeel van de phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin-cascade (PI3K/AKT/mTOR-cascade). Deze cascade speelt een belangrijke rol in de regulering van proliferatie en deling van tumor cellen en is vaak geactiveerd in prostaatkanker. PTEN is een tumor suppressor gen en een negatieve regulator van PI3K/AKT/mTOR signaaltransductie cascade. PTEN mutaties komen vaak voor

bij prostaatkanker en kunnen leiden in verhoogde activering van de PI3K/AKT/mTOR-cascade. Recente onderzoeken hebben aangetoond dat er een belangrijke interactie bestaat tussen de PI3K/AKT/mTOR-cascade en de androgeen receptor signaal transductie.
Om de effectiviteit van bicalutamide in combinatie met ridaforolimus te onderzoeken bij asymptomatische patiënten met progressie onder androgeen deprivatie therapie werd in eerste instantie gestart met een gerandomiseerde fase 2 studie. Bij de eerste 11 gerandomiseerde patiënten bleken 4 patiënten onverwacht ernstige (graad 3) bijwerkingen te hebben in de vorm van orale ulcera. Alle 4 bleken gerandomiseerd te zijn voor de arm met behandeling met bicalutamide in combinatie met ridaforolimus. Alhoewel mondzweertjes een bekende bijwerking betreft van mTOR remmers was deze ernst en frequentie bij de in de studie gebruikte standaard 40 mg dosis ongewoon. Daarop moest eerst een mogelijke interactie tussen de farmacologische blootstelling van de twee middelen worden onderzocht in een open label safety lead in studie met een gereduceerde dosis ridaforolimus (30 mg), om de verdraagbaarheid en de mogelijke interactie tussen ridaforolimus en bicalutamide te onderzoeken. Elf patiënten werden behandeld, waarvan 3 patiënten een dosis limiterende toxiciteit (DLT) ondervonden, 1 met een hyperglycaemie en 2 patiënten met een graad 2 stomatitis. Er werd geen klinisch significante pharmacokinetische interactie gevonden tussen ridaforolimus en bicalutamide. Het aantal en de ernst van de bijwerkingen, in een over het algemeen asymptomatische patiënten populatie, rechtvaardigde het continueren van verder onderzoek niet. Aldus werd besloten af te zien van verdere inclusie in de beoogde fase 2 studie.
In **hoofdstuk 7** worden de resultaten gepresenteerd van *in vivo* onderzoek in het orthotope humane prostaat kanker xenograft muismodel PC346C met docetaxel in combinatie met zibotentan of docetaxel in combinatie met lenalidomide. PC346C is een androgeen gevoelige xenograft die zich na androgeen deprivatie therapie ontwikkelt naar CRPC, gelijkend op de klinische situatie. Zibotentan is een endotheline-A (ET_A) receptor antagonist. Endotheline-1 (ET-1) en de ET_A receptor spelen een sleutelrol in de ontwikkeling en bij de progressie van prostaatkanker, door middel van het moduleren van proliferatie, angiogenese, en anti-apoptose. ET-1 en de ET_A receptor zijn daardoor een interessant therapeutische target voor de behandeling van CRPC. In onze studie bleek echter, dat zibotentan in combinatie met docetaxel ten opzichte van behandeling met docetaxel monotherapie, geen toegevoegde waarde heeft met betrekking tot tumor groei en overleving.
Lenalidomide is een analoog van thalidomide met een immunomodulerende werking en inhibitie van de PI3K/AKT-cascade en de angiogenese. Lenalidomide gegeven voorafgaand aan de bolusinjectie met docetaxel gaf geen versterking van het docetaxel effect.
Lenalidomide gegeven na de bolusinjectie met docetaxel gaf echter een beperkte verlenging van de respons duur van docetaxel alleen.

De negatieve uitkomsten van zibotentan en de geringe toegevoegde waarde van lenalidomide indien het wordt gegeven na docetaxel, komen grotendeels overeen met het gebrek aan klinisch relevante resultaten van klinische fase 3 studies met docetaxel +/− zibotentan en docetaxel +/− lenalidomide. Gezien deze overeenkomstige resultaten lijkt het xenograft PC346C een bruikbaar en representatief model om nieuwe middelen en combinaties van middelen te identificeren voor verdere klinische ontwikkeling, met name in het licht van de talrijke nieuwe potentiële antikanker middelen en het beperkt aantal patiënten en bronnen tot het doen van grote klinische studies.
De resultaten uit de hoofdstukken 2 t/m 7 worden besproken in **hoofdstuk 8** waarbij eveneens suggesties voor verder onderzoek in de toekomst worden gegeven.
Ondanks de recente verbeteringen in de behandeling van patiënten met uitgezaaide CRPC, met nieuwe beschikbare middelen, zoals cabazitaxel, abiraterone en enzalutamide, blijft het een ernstige progressieve en ongeneeslijke ziekte met hoge morbiditeit en mortaliteit. Daarom is het van belang translationeel en klinisch onderzoek naar verschillende (nieuwe) behandelingsmogelijkheden en combinaties van behandelingen voor verschillende stadia van de ziekte voort te zetten, zodat nieuwe medicijnen ontwikkeld kunnen worden en de behandeling verder verbeterd kan worden.

Appendix

Dankwoord

Wetende dat dit het best gelezen onderdeel van het proefschrift zal worden, onderstreept het belang van dit korte stukje tekst. En terecht, want het maken van een proefschrift is een werk waar zeer veel mensen bij betrokken zijn. Ik wil iedereen bedanken die een bijdrage heeft geleverd aan de totstandkoming van mijn proefschrift. Een aantal mensen wil ik hier in het bijzonder voor bedanken.

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Curriculum Vitae

Hielke Jodie Meulenbeld werd geboren op 19 maart 1978 te Almelo. Zij behaalde in 1996 haar VWO diploma aan Het Noordik te Almelo. Na het VWO ging zij, in verband met uitloting voor de studie geneeskunde, 1 jaar rechten studeren aan de Rijksuniversiteit Groningen. Hier haalde zij in 1997 haar propedeuse rechten.

Vervolgens ging zij geneeskunde studeren aan de Rijksuniversiteit Leiden, waar zij in november 2003, cum laude, de artsenbul kreeg uitgereikt. Aansluitend ging zij in opleiding tot internist in het Catharina ziekenhuis te Eindhoven (Opleider: Dr. B. Bravenboer). De opleiding tot internist-oncoloog werd in 2008 voortgezet in het Erasmus MC te Rotterdam (Opleiders: Prof. dr. J.L.C.M. van Saase en Prof. dr. J. Verweij).

Vanaf mei 2010 werkt zij als internist-oncoloog op de afdeling Interne Oncologie in het Erasmus MC.

In 2009 was zij reeds, onder leiding van Prof. dr. R. de Wit, gestart met de onderzoekswerkzaamheden die uiteindelijk hebben geresulteerd in dit proefschrift.

Sinds september 2012 is zij werkzaam als internist-oncoloog in Gelre ziekenhuizen te Zutphen.

List of publications

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Meulenbeld HJ, de Ridder CMA, Boer A, Stuurman D, Aghai A, de Wit R, van Weerden WM. Can human prostate xenograft models predict negative results in large phase 3 trials? Our experience with the combination of Docetaxel plus Zibotentan or Lenalidomide and Docetaxel in the orthotopic PC346C human prostate cancer xenograft.
Submitted.

PhD Portfolio

SUMMARY OF PHD TRAINING AND TEACHING

Name PhD student: Hielke Meulenbeld Erasmus MC Department: Medical Oncology	PhD period: 2009-2012 Promotor: Prof. dr R. de Wit Co-promotor: Dr. ir. W.M. van Weerden	
	Year	**Workload (Hours/ECTS)**
General courses		
- BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2011	1 ECTS
- Good Clinical Practice	2010	0.4 ECTS
Specific courses		
- English lessons	2011	2.4 ECTS
Seminars and workshops		
- OMBO cursus Daniel den Hoed	2009-2012	1.5 ECTS
- Refereerbijeenkomst dept. Medical Oncology	2009-2012	1.5 ECTS
- Urology Tour d'Europe	2011	0.5 ECTS
- Polikliniekbespreking	2009-2012	1.5 ECTS
Presentations		
- Scientific meeting Medical Oncology	2010	0.5 ECTS
- Multiple presentations Klinische Research Bespreking	2009-2012	1.5 ECTS
- IKNL urologische tumoren oral presentations	2010-2011	1.5 ECTS
- ASCO 2011 poster presentation	2011	1.2 ECTS
- ASCO 2011 oral presentation	2011	3.0 ECTS
- Urology Tour d'Europe oral presentation	2011	1.0 ECTS
- Dutch Uro-Oncology Studygroup (DUOS) oral presentation	2011	1.0 ECTS
(Inter)national conferences		
- Post-ASCO	2010-2012	0.6 ECTS
- Bossche Mamma congres	2010-2012	2.0 ECTS
- European Society for Medical Oncology (ESMO) congress	2010	1.2 ECTS
- Oncologiedagen voor Nederland en Vlaanderen, NVMO	2010	0.6 ECTS
- Jaarsymposium Continuüm Oncologie	2010-2012	0.8 ECTS
- Therapie op Maat	2011	0.4 ECTS
- Annual meetings American Society Clinical Oncology	2011	1.2 ECTS
- Targeted Therapy	2012	0.3 ECTS
Other		
- Wetenschapsdag Interne Oncologie	2010-2012	1.2 ECTS
- European Society of Urological Research, preparation poster	2011	0.4 ECTS
- Annual Meeting van ASCPT; preparation poster	2012	0.4 ECTS

Teaching		
Lecturing		
- Onderwijs AIOS oncologie EMC, Daniel den Hoed	2011-2012	1.2 ECTS
- Onderwijs AIOS/ANIOS interne geneeskunde	2011	0.5 ECTS
- Onderwijs oncologieverpleegkundigen	2011	0.5 ECTS
- OIO overleg	2011	0.4 ECTS