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PHASE II STUDIES

Phase I/II study evaluating the safety and clinical efficacy of temsirolimus and bevacizumab in patients with chemotherapy refractory metastatic castration-resistant prostate cancer

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Summary

Background Mammalian target of rapamycin (mTOR) pathway and angiogenesis through vascular endothelial growth factor (VEGF) have been shown to play important roles in prostate cancer progression. Preclinical data in prostate cancer has suggested the potential additive effect dual inhibition of VEGF and mTOR pathways. In this phase I/II trial we assessed the safety and efficacy of bevacizumab in combination with temsirolimus for the treatment of men with metastatic castration-resistant prostate cancer (mCRPC). *Methods* In the phase I portion, eligible patients received temsirolimus (20 mg or 25 mg IV weekly) in combination with a fixed dose of IV bevacizumab (10 mg/kg every 2 weeks). The primary endpoint for the phase II portion was objective response measured by either PSA or RECIST criteria. Exploratory endpoints included changes in circulating tumor cells (CTC) and their correlation with PSA response to treatment. *Results* Twenty-one patients, median age 64 (53–82), with pre-treatment PSA of 205.3 (11.1–1801.0), previously treated with a median of 2 (0–5) lines of therapy for mCRPC received the combination of temsirolimus weekly at 20 mg ($n = 4$) or 25 mg ($n = 17$) with bevacizumab 10 mg/kg every 2 weeks ($n = 21$). Median time to progression was 2.6 months (95% CI, 1.2–3.9) and the median best PSA change from baseline to 12 weeks was a 32% increase (−40–632%) which met the predefined futility rule and led to early termination of the study. Nine patients (43%) had ≥ grade 3 toxicity that included fatigue (24%), anorexia (10%), nausea/vomiting (5%) and lymphopenia (5%). In exploratory analysis, a decrease in CTC levels was observed in 9 out of 11 patients. No association between PSA levels and CTC levels was detected. *Conclusions* The combination of temsirolimus and bevacizumab showed limited clinical activity in mCRPC patients previously treated with chemotherapy and was associated with significant adverse events (AEs). Transient decrease in CTC levels was independent from PSA response. NCT01083368.

Keywords Phase I/II · Castration-resistant prostate cancer · Temsirolimus · Bevacizumab

Introduction

Prostate cancer is the most common cancer among men in the United States and the second highest leading cause of death with an estimated 29,430 deaths in the year 2018 [1]. While most men with advanced disease respond to androgen deprivation therapy (ADT), over time it becomes resistant to castration which is evidenced by a rising PSA, clinical and radiological evidence of disease progression, ultimately leading to mortality [2].

The treatment of metastatic castration resistant prostate cancer (mCRPC) has evolved significantly in the last decade. Prior to the regulatory approval of docetaxel in 2004 for mCRPC [3, 4], the most commonly used cytotoxic agent was mitoxantrone and prednisone, providing palliation but no survival benefit [5, 6]. In addition, limited data existed for those with disease progression after docetaxel-based chemotherapy. Over the past several years a number of new agents (novel hormonal therapies, chemotherapy,

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immunotherapy) have shown significant survival advantage and revolutionized the treatment landscape of mCRPC [7–10].

Angiogenesis, through vascular endothelial growth factor (VEGF) has shown to play an important role in prostate cancer progression. VEGF is one of the most potent and specific proangiogenic factors and its biological effects include endothelial cell mitogenesis and migration, increased vascular permeability and suppression of dendritic cell maturation. In prostate cancer, several studies demonstrated an association of elevated levels of VEGF and higher Gleason scores, PSA levels, clinical progression and inferior survival [11–14]. Bevacizumab is a humanized immunoglobulin G1 monoclonal antibody that binds and inhibits the biologically active isoforms of human VEGF. This agent has shown to be effective in several malignancies such as colorectal, breast, non-small cell lung and kidney cancer [15–19].

The phosphatidylinositol 3-kinase (PI3K)/Akt pathway plays a key role in oncogenesis and deregulation of this pathway through phosphatase and tensin homolog (PTEN) suppression was observed in aggressive prostate cancers [20, 21]. Mammalian target of rapamycin (mTOR) is a downstream signaling kinase of this pathway, regulating cell growth and proliferation [22]. The inhibition of mTOR pathway have shown clinical efficacy in other malignancies such as renal cell [23], breast [24] and neuroendocrine tumors [25]. Nonetheless, the complex regulatory process of the PI3K/Akt pathway makes this pathway a challenging therapeutic target with a number of clinical studies exploring its inhibition with modest efficacy in prostate cancer. Temsirolimus, an mTOR inhibitor, targets essential regulatory functions in the tumor as well as the tumor microenvironment with anti-angiogenic properties via interfering with hypoxia inducible factor HIF1-alpha and VEGF production [26, 27].

While both temsirolimus and bevacizumab have modest clinical activity when used as monotherapy in prostate cancer [28, 29], both preclinical and clinical data suggested that this combination is least additive in a variety of tumors [30–32]. Of note, a phase I/II study investigating a fixed dose of temsirolimus with escalating doses of bevacizumab was safe and showed clinical antitumor activity in metastatic renal cell cancer [33].

To assess the potential synergistic activity of the dual inhibition of mTOR and VEGF pathways, we conducted a phase I/II trial of temsirolimus in combination with bevacizumab in men with chemotherapy refractory mCRPC.

Materials and methods

Eligibility

Eligible patients were > 18 years old, Eastern Cooperative Oncology Group (ECOG) 0–2 with histological confirmed adenocarcinoma of the prostate, with mCRPC and progressed after either one or two prior chemotherapy regimens (docetaxel based or mitoxantrone based regimens). All patients were required to have a castrated testosterone level of ≤50 ng/dL and to remain on testosterone suppression therapy for the duration of the study. Prior chemotherapy had to be completed at least 4 weeks prior to study entry. Similarly, the use of a prior radiopharmaceutical agent (strontium, samarium) was permitted provided it was completed at least 8 weeks prior to screening with no residual adverse events (AEs) > grade (G)1 at the time of screening. Other inclusion criteria included adequate bone marrow, kidney and hepatic function. Patients were excluded if they had significant cardiovascular disease and had received prior treatment with a VEGF or mTOR inhibitor. The use of strong CYP450 inducers or inhibitors was also not allowed.

This phase I/II study was conducted at the Cleveland Clinic and University Hospitals. The protocol (CASE7808 / NCI2010–00308) was reviewed and approved by the Case Comprehensive Cancer Center Institutional Review Board and was conducted in accordance with the Declaration of Helsinki for human subject protection. All patients provided written informed consent prior to study entry. ClinicalTrials.gov Identifier: NCT01083368.

Study design and treatment

Eligible patients received weekly intravenous infusions of temsirolimus 20 mg (dose level 1) or 25 mg (dose level 2) in combination with intravenous infusions of bevacizumab 10 mg/kg every 2 weeks. This study featured a staged-cohort design to study primarily safety of the combination of temsirolimus and bevacizumab (phase I) and a main study cohort of up to 28 patients to evaluate the safety and efficacy of the combination (phase II).

The phase I portion of the study intended to establish a maximum tolerated dose (MTD) of temsirolimus in combination with bevacizumab and employed a "3 + 3" statistical design model. In this portion of the study, three patients were initially treated at dose level 1 (temsirolimus 20 mg weekly plus bevacizumab 10 mg/kg every 2 weeks). If no unacceptable toxicities were observed, a new cohort of 3 patients would be treated at dose level 2 (temsirolimus 25 mg weekly plus bevacizumab 10 mg/kg every 2 weeks), which was the expected recommended dose for the phase II portion of the study. Two other levels (dose 0 and − 1 were planned in the event of a dose-limiting toxicity - DLT). The phase II portion consisted of treatment for up to 6 cycles of combination therapy. Each cycle consisted of temsirolimus dosed weekly and bevacizumab every 2 weeks (1 cycle = 4 weeks).

Efficacy and safety assessment

Baseline evaluations included a medical history and physical examination, laboratory work, imaging studies included whole body bone scans and CT scans. PSA levels were obtained at baseline and prior to each cycle of treatment. Restaging scans were performed every 12 weeks. Measurable disease/target lesions and non-measurable disease/non-target lesions were evaluated according to the standardized RECIST criteria v1.0 [34]. PSA response was evaluated according to the recommendations from National Cancer Institute PSA Working Group 2 (PCWG 2) [35] and defined by any change in PSA from baseline to 12 weeks or earlier for patients who discontinued therapy, as well as the maximum decline in PSA that occurs at any point after treatment for each patient.

Disease progression (PD) was defined as new sites of metastatic disease on radiographic imaging (RECIST v1.0), PSA progression or clinical deterioration according to treating physician. PSA progression was defined as a 25% or greater increase and an absolute increase of 2 ng/mL or more from the nadir, which was confirmed by a second value 4 weeks later (PCWG2).

Adverse effects were reported according to National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 [36]. DLT was defined as any of the following that occurred in the first 4 weeks of treatment that were attributed to treatment and meeting the following criteria: grade (G)4 absolute neutrophil count for >5 days; G4 anemia or thrombocytopenia; serum creatinine >2 times baseline or > 2 times upper limit normal (ULN) if baseline levels < ULN; G3 hypertriglyceridemia despite appropriate lipid lowering drug therapy; G3 nausea, vomiting or diarrhea with maximal supportive treatment; other, non-hematologic toxicity > G3 with the only exceptions being fatigue, and hypertension that were controlled with oral medication. Dose adjustments were permitted for both drugs depending on DLT. Patients experiencing a DLT had therapy held until toxicity resolved to a G1 or less. No intra-patient dose escalation was allowed. For the phase II portion of the study, treatment could be delayed until toxicity recovered to < G1, except for nausea/vomiting, fatigue, anorexia, alopecia or anemia. Dose reductions were implemented for patients who experienced recurrent or specific severe toxicities. Treatment delays were not allowed to last longer than 4 weeks for bevacizumab and 2 weeks for temsirolimus.

Correlative studies

For CTC measurement, blood samples were collected in 10-mL EDTA Cell Saver tubed (full drawn) of peripheral blood at baseline (pre-treatment), on day one of each cycle prior to therapy, and at discontinuation of treatment. All evaluations were performed without knowledge of the clinical status of the patients and the controls at the Cleveland Clinic laboratory. The CellSearch System (Veridex), consisting of a semiautomated system, was used for the isolation and enumeration of CTC.

Statistical methods

The primary end points of this study were MTD (phase I portion) and objective response defined by either by PSA or objective disease assessed by RECIST v1.0 for the phase II portion of the study (phase II). Exploratory endpoints included changes in circulating tumor cells (CTCs) and their correlation with PSA response to treatment. For the phase 1 safety portion of this study, the MTD was determined using a standard "3 + 3" design with starting dose at dose level 1. Once the MTD had been established a Phase II trial was implemented. Patients treated at the MTD during the Phase I portion of the study were "folded" into the phase II trial. The study aimed to enroll up to 28 eligible patients allowing for a 90% confidence interval to detect ≥20% overall response rate. A two-staged accrual design allowed the study to be terminated early if a response rate ≤ 5% was observed indicating a relatively lack of efficacy for the combination regimen. Patients coming off study secondary to adverse events/toxicities or treating physician discretion will be considered to be "non-responders", as benefit from therapy was not observed. Progressive disease was defined by Exploratory endpoints included changes in circulating tumor cells (CTCs) and their correlation with PSA response to treatment. Statistical comparisons were performed using the Chi-square and Fisher exact test and Spearman rank correlations were used to assess associations, such as the association between PSA and CTC's. $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

Between March 2009 and July 2011, a total of twenty-two patients were enrolled however, one patient withdrew consent and did not receive any study treatment. Of the 21 patients, 4 were treated with a at dose level 1 (temsirolimus 20 mg plus bevacizumab 10 mg/kg) and four patients at dose level 2 (temsirolimus 25 mg plus bevacizumab 10 mg/kg). In the phase II portion, a total of 13 patients received temsirolimus 25 mg combined with bevacizumab 10 mg/kg. Table 1 summarizes baseline demographics and disease characteristics of eligible patients. All patients had metastatic disease in the bones (100%), and one third of them in the lymph nodes (33%). Soft tissue (10%) and visceral (5%) disease was uncommon. Patients were treated with a median of 2 (0–5) lines

Table 1 Study baseline characteristics

Variable	N = 21 (100%)
Mean age, years (range)	64 (53–82)
Gleason Score	
< 7	7 (33)
≥ 8	9 (43)
Unknown	5 (24)
ECOG PS (%)	
0	4 (19)
1	13 (62)
2	3 (14)
Unknown	1 (5)
Castration	
Medical	20 (95)
Surgical	1(5)
Prior therapy for CRPC	2 (0–5)
Docetaxel	18 (86)
Mitoxantrone	6 (29)
Ketoconazole	5 (24)
Cabazitaxel	2 (10)
Gemcitabine	2 (10)
Abiraterone acetate	1 (5)
Sipuleucel-T	2 (10)
Unknown	1 (5)
PSA levels (ng/mL), median (range)	205.3 (11.1–1801.0)
Site of disease	
Bone	21 (100)
Soft tissue	2 (10)
Lymph nodes	7 (33)
Visceral	1 (5)
Unknown	1 (5)

of therapy for CRPC prior to study enrollment. Prior to study enrollment, the majority of patients had received docetaxel for CRPC, two of them in combination with gemcitabine. However, only one patient received abiraterone and none was treated with enzalutamide nor radium-223. On the other hand, ketoconazole and mitoxantrone were used in more than half of the study cohort, prior to enrollment. The baseline PSA at time of study initiation was 205.3 (11.1–1801.0) ng/mL.

Clinical activity

Patients received a median number of 4 cycles of treatment and only two patients completed the maximum number of treatments allowed [6]. All patients discontinued therapy due to disease progression (10/21, 48%), treatment-related adverse events (TRAEs) (4/21, 19%), physician's discretion (4/21, 19%) and patient's choice (1/21, 5%). Median time to progressive disease was 2.6 months (95% CI, 1.2–3.9).

For patients with available serial PSA data (at baseline and 12 weeks, primary endpoint, $n = 16$), the median PSA decline observed was a 32% increase (range − 40 - 632%). Five patients (31%) had at least some transient PSA declines (1–40%, median 26%), all on dose level 2. A total of five patients did not have repeated PSA for longitudinal evaluation at 12 weeks: one patient had a second PSA value at 4 months after therapy discontinuation; one patient had no baseline PSA; and three patients completed 1 cycle only and were lost to follow up.

Safety

The TRAEs were recorded by highest NCI-CTCAE v3.0 grade in each patient and are summarized in Table 2. No life-threatening AEs were reported. In the phase I portion of the study, the MTD for the combination was not reached at the highest dose level tested. Two patients discontinued therapy due to disease progression (one at each dose level). Both patients were replaced as per study protocol.

The most common (> 10% patients) TRAEs of any grade for all evaluable patients were anorexia (67%), nausea/vomiting (57%), weight loss (57%), fatigue (57%), lymphopenia (62%), thrombocytopenia and mucositis (53%). Significant TRAEs (G3) were reported in 43% and included fatigue (24%), anorexia (10%), nausea/vomiting (5%) and lymphopenia (5%). A total of four patients discontinue therapy due to toxicities.

Biomarker studies

A total of 11 patients had CTC data available at baseline (Fig. 1). The remaining patients on study did not have CTCs detected at baseline and thus no further collections were attempted. For patients with available CTC, there was a median of 80 cells (17–397) detected at baseline and no association with age ($p = 0.34$), Gleason score ($p = 0.54$) and PSA ($p = 0.33$) was found. For the 64% (7/11) of patients with CTC data at cycle 3 (12 weeks), CTC decreased in all patients with a median absolute decrease of 52 cells (14–296) and a median relative decrease of 82% (62–100%). In two cases, no CTC were detected at cycle 3. CTC at subsequent follow-ups remained below baseline levels in all patients; in two cases (patient #11, #16) CTC counts did began to rise after cycle 3. There was no correlation between changes in CTC and PSA changes.

Discussion

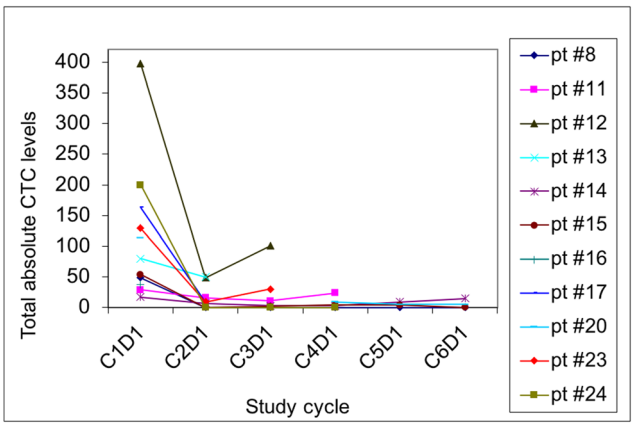
The objectives of this study were to determine the safety of combining temsirolimus and bevacizumab in patients with mCRPC and to identify preliminary signal of efficacy in patients previously treated with standard chemotherapy.

Table 2 Commonly reported treatment-related toxicities (>10% frequency)

Toxicity	Grade 1-2 (%)	Grade 3 (%)	Overall (%)
Anorexia	12 (57)	2 (10)	14 (67)
Fatigue	7 (33)	5 (24)	12 (57)
Mucositis	11 (53)	0 (0)	11 (53)
Nausea/Vomiting	12 (57)	1 (5)	13 (62)
Weight loss	12 (57)	0 (0)	12 (57)
Lymphopenia	12 (57)	1 (5)	13 (62)
Thrombocytopenia	12 (57)	0 (0)	12 (57)

Our study was one of the first to investigate the combination of an mTOR with a VEGF inhibitor in mCRPC. Although we did not encounter a DLT in the phase I portion of the study, the combination regimen failed to show a clinically meaningful activity even at full doses with short-lasting PSA responses and a relatively short time to disease progression. This lack of clinical benefit met the predefined futility rule and led to early termination of the study. These findings compare similarly to the results of other studies evaluating mTOR inhibitors in the same disease setting, where transient PSA responses were seen in a fraction of patients without durable responses [28, 37, 38]. Likewise, none of the completed phase II/III trials evaluating VEGF inhibitors in CRPC has resulted in a meaningful survival improvement in the mCRPC setting [39–41].

Toxicity was an important drawback of our study with almost half of our patients suffering from significant toxicities, despite no DLTs were observed in the dose-finding portion of the study. Unsurprisingly, the same combination (at same dose levels) was tested in two other trials in metastatic renal cancer also leading to a high frequency of ≥ G3 TRAEs (77–80%) and high dropout rates secondary to treatment related AEs (20–42%) [42, 43]. Other studies evaluating similar combinations have also shown some safety signals of concerns. For example, in a three-arm phase II study of lenvatinib/everolimus regimen in renal cell carcinomas, significant TRAEs were reported in 45% of patients with 18% of them discontinuing therapy due to side effects [44].



Legend: C1 – cycle 1; CTC – circulating tumor cell; D1 – day 1; pt – study patient

Fig. 1 Absolute CTC counts change for patients with available CTC at baseline ($n = 11$)

At the time this study started enrolling patients, treatment options for patients who progressed on ADT were limited and offered modest clinical benefit. However, since this trial was launched, a number of therapies gained FDA approval, including novel androgen signaling inhibitors (abiraterone acetate [8], enzalutamide [7]), novel cytotoxic chemotherapy drugs (cabazitaxel [10]), and radiopharmaceuticals (radium-223 [9]). While only a few patients had received one of these novels agents, a significant proportion of patients enrolled on our study had received treatment in clinical trials using agents such as lenalidomide or gemcitabine [10, 45, 46]. Given the overall toxicities of this combination compared with available newer therapies and the short duration of response, the further development of this regimen in CRPC is not warranted.

The lack of correlation between changes in CTC and PSA levels in our study was somewhat surprising given the percentage of patients who had (transient) decreases in CTC levels. In other studies, CTC level has been shown to prognosticate better outcomes and predict overall survival better than PSA decrements, at least during treatment with chemotherapy or acetate abiraterone [47–49]. Our findings however were different but limited by the small number of patients with available CTC counts. A better understanding of how CTCs and PSA levels post treatment relate to survival will help us better predict how patients respond to therapy.

Limitations of this study included the small sample size, the lack of a control arm in the phase II portion and the limited valued of correlative data. Nevertheless, given the lack of activity observed with the temsirolimus/bevacizumab combination, the futility rule that was included in the study design was important to stop accrual early, particularly when a number of treatment options were becoming available to patients that were not available when this trial was planned in 2009.

Further research is needed to better understand the targets in the tumorigenesis pathways and how tumor cells overcome these road blocks causing disease progression.

Conclusion

The administration of temsirolimus and bevacizumab in chemotherapy refractory mCRPC did not result in meaningful clinical benefit and was associated with significant toxicities. While there were decreases in CTC levels, these did not correlate with PSA responses.

Authorship All authors were involved in this study. JG, MC, RD: conceptualization, review and editing; PB, RG, JG: formal analysis and writing, review and editing.

Compliance with ethical standards

Conflict of interest PB, MC, PM, RG and RD declare no conflict of interest for this study. JG has disclosed to be a consultant or advisor for Genentech, Pfizer; and received research funding from Genentech and Pfizer.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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