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A phase II trial of ganetespib, a heat shock protein 90 Hsp90) inhibitor, in patients with docetaxel-pretreated metastatic castrate-resistant prostate cancer (CRPC)-a prostate cancer clinical trials consortium (PCCTC) study

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Summary

Introduction—Heat shock protein 90 (Hsp90) has been studied as a therapeutic target in many cancers. In pre-clinical trials, the Hsp90 ATPase inhibitor ganetespib demonstrated potent inhibition of solid tumor growth, with superior potency than prior Hsp90 inhibitors. Given the promising pre-clinical outcome and favorable pharmacologic properties of ganetespib, we conducted a phase II trial of single-agent ganetespib in patients with metastatic, castrate-resistant prostate cancer (mCRPC). The primary objective of the study was to determine the 6-month progression-free survival (PFS) rate.

Methods—Patients with mCRPC who had been previously treated with docetaxel were enrolled after meeting eligibility criteria. All patients received ganetespib at 200 mg/m^2 on days 1, 8, and 15 of every 28 days (one cycle). Subjects who tolerated therapy were continued on ganetespib until disease progression. Considering that Hsp90 acetylation may confer insensitivity to Hsp90 inhibitors and maspin inhibits protein deacetylation, maspin-associated molecular markers were evaluated.

Results—Eighteen patients were recruited into the trial; most were Caucasian, had performance status 1, had received prior docetaxel, and were heavily pretreated. Of the 17 patients who were treated, none attained 6-month PFS. Only 2 patients achieved PFS > 4 months. The median PFS was 1.9 months. As per the study design, the trial was terminated after the interim analysis. The most frequent types of Grade 3 toxicity were dehydration, diarrhea, and fatigue. Molecular markers provided little additional insight regarding drug activity.

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Conclusions—Ganetespib demonstrated minimal clinical activity in men with mCRPC. The true 6-month PFS rate was, at most, 0.20. Possible reasons for this include selection of a heavily pretreated patient population and lack of agent potency in patients with mCRPC.

Keywords

Prostate cancer; Heat shock protein; Ganetespib

Introduction

Advancements in the mechanistic studies of cancer progression and treatment resistance have fueled the search for novel therapeutic agents for metastatic castrate-resistant prostate cancer (mCRPC) [1]. Some of the mechanisms of progression involve dysregulation of the androgen receptor (AR) and changes in AR function, including mutations, increased phosphorylation, or increased transcription of AR [2, 3]. These changes allow the AR to respond to lower levels of androgens and steroids. Thus, inhibiting the AR and reducing the level of androgen are both desired in order to block AR-dependent prostate tumor progression. Heat shock protein (Hsp)-based chaperone mechanisms have been shown to regulate the AR by affecting the levels of Hsp stability and activity [4-6]. Although several heat shock proteins in the chaperone complex, such as Hsp90, Hsp70, and Hsp56, contribute to the overall regulation of AR, Hsp90, the most abundant Hsp in mammalian cells, is upregulated in prostate cancer [7]. Hsp90 regulates AR by forming a complex with unliganded AR along with co-chaperones to create a foldosome complex [8]. This AR-Hsp90 interaction complex then regulates the activation, maturation, and stability of AR and maintains AR in a conformation that potentiates high-affinity ligand binding but can also result in proteasome-mediated AR turnover if the ligand is not available [5].

Although AR is a bona fide drug target for human prostate tumors, prostate cancer metastasis is typically characterized by a variety of genetic alterations that collectively contribute to the transformed state. A subset of prostate cancer appears to depend on a single oncoprotein for its genesis, sustained proliferation, and survival. This phenomenon has been termed "oncogene addiction" [9]. Thus, we reasoned that inhibiting Hsp90-mediated chaperone activity, which is propelled by Hsp90 ATPase may have extensive anti-tumor effects due to Hsp90 regulation of the folding, stability, and function of many oncogenic clients, such as AKT, B-Raf, c-KIT, c-MET, and EGFR [10].

Although preclinical studies showed that several pharmacologic inhibitors of Hsp90 ATPase have anti-tumor activities [11-14], clinical trials of single-agent Hsp90 inhibitors in prostate cancer have not shown promising results. We were the first to report the results of a Phase II study of 17-allylamino-17-demethoxygeldanamycin (17-AAG) in men with mCRPC in which 17-AAG showed negligible activity with regard to PSA response [15]. Another Hsp90 inhibitor, IPI-504 (retaspimycin hydrochloride), had minimal effect on PSA or tumor burden and was associated with unacceptable toxicities in several patients [16]. It is thought that the previous pharmacological Hsp90 inhibitors are ineffective, at least in part, because they have insufficient potency.

However, ganetespib (formerly known as STA-9090 [*5-[2,4-Dihydroxy-5-(1-methylethyl)phenyl]-4-(1-methyl-1 H-indol-5-yl)-2,4-dihydro-[1, 2, 4]triazol-3-one*], a novel, synthetic small molecule with a unique triazolone-containing chemical structure [17] acts as a potent Hsp90 inhibitor. By inhibiting Hsp90, it in turn downregulates Hsp90 client protein levels. It binds to the ATP-binding pocket at the N-terminus of Hsp90 and was more potent than 17-AAG [18, 19] and other first-generation agents. In initial Phase 1 clinical trials, ganetespib was well tolerated and safe [20, 21]. Given the scientific rationale for Hsp90 inhibition in advanced prostate cancer and ganetespib's favorable pharmacologic properties with significant preclinical activity, we conducted a phase II trial of single-agent ganetespib in patients with mCRPC.

In the current study, in addition to monitoring cancer growth using prostate-specific antigen (PSA) and other clinical parameters, we examined whether the tumor suppressor maspin and maspin-associated molecules could serve as surrogate predictors of prostate cancer response to ganetespib. We have shown previously that maspin acts as a deacetylase inhibitor and binds to Hsp90 [22]. Maspin also sensitizes prostate tumor cells to drug-induced apoptosis [23-25] and controls the expression of a set of genes that might regulate tumor cell differentiation and drug sensitivity [26].

Materials and methods

Study design

This was a single-arm, multicenter Phase 2 study of ganetespib in patients with mCRPC who previously were treated with docetaxel. The primary objective of the study was to determine the 6-month progression-free survival (PFS) rate. The secondary objectives were to assess overall safety and tolerability of ganetespib, to evaluate the overall survival, and to investigate the association of PFS with primary and secondary target markers, including maspin, PSA, cytokeratin 18 (CK18), interleukin (IL)-6, urokinase plasminogen activator (uPA) and receptor activator of nuclear factor κB (RANK) that could evaluate the effect of Hsp90 inhibition. The study was approved by the institutional review board at each clinical center, and written informed consent was obtained from all patients before registration.

Patient selection

Patients who had metastatic adenocarcinoma of the prostate and who received at least one prior docetaxel-based regimen for metastatic disease were included. All patients had to be castrate with a testosterone level   50 ng/dl, and Luteinizing Hormone Releasing Hormone (LHRH) agonist therapy must be continued. Patients had to discontinue antiandrogens, including flutamide for a minimum of 4 weeks and bicalutamide or nilutamide for 6 weeks, before ganetespib treatment. Patients must have had adequate performance status of 0–2 and a life expectancy of at least 3 months. Patients were required to have functioning bone marrow as defined by an absolute neutrophil count   1500 cell/μL, platelets   100,000/μL, hemoglobin   9.0 g/dL, serum creatinine   1.5 x Upper Limit of Normal (ULN) or calculated creatinine clearance   50 mL/min, and total bilirubin   1. 5 x ULN. Patients without documented bone me-tastases or patients with liver metastases had to have aspartate transaminase (SGOT) and/or alanine transaminase (SGPT)   2.5 x ULN if alkaline

phosphatase was ULN; alternatively, alkaline phosphatase could have been up to 4 x ULN if SGOT and/or SGPT were ULN. For patients with documented bone metastases, SGOT and/or SGPT had to be <2.5 x ULN, without regard to the alkaline phosphatase level.

Patients must have had adequate cardiac function, defined as baseline QTc < 450 msec, ejection fraction >50 % at baseline, and no history of, or current, coronary artery disease, myocardial infarction, angina pectoris, angioplasty or coronary bypass surgery; history of, or current, uncontrolled dysrhythmias, or requirement for antiarrhythmic medications, or Grade 2 or greater left bundle branch block that occurred previously. Any other significant comorbidities, which in the investigator's judgment would rendered the subject inappropriate for entry into this study, were considered exclusion criteria.

Treatment plan

Ganetespib (200 mg/m^2 on days 1, 8, and 15 every 28 days) was administered to all subjects. Every 28 days was considered one cycle. Subjects who tolerated this therapeutic regimen were continued on ganetespib until disease progression. A medical history, physical examination, performance status assessment, measurement of PSA, hematologic and chemistry laboratory tests, and 12-lead electrocardiogram (ECG) were repeated before each cycle. Survival information was collected every 12 weeks (± 1 week) from the date of last dose of study drug until the subject's death or until the subject was lost to follow-up, or until study closure (approximately 6 months after the last subject terminated treatment).

Correlative markers

Blood specimens were collected from patients at baseline and at the conclusion of treatment. The level of CK18, maspin, PSA, IL-6, uPA, and RANK mRNA was determined by the RNA extraction from peripheral blood mononuclear cells (PBMCs) using the RNeasy Mini kit (Qiagen, Valencia, CA) and reverse-transcribed by iScript cDNA synthesis kit (Bio-Rad, Hercules, CA), according to the manufacturer's instructions. The q-RT-PCR analysis was performed as described where all data were normalized to the housekeeping gene GAPDH and baseline values [15]. List of all primers is provided in Table 1.

Statistical methods

Our primary objective was to determine the 6-month PFS rate by using a binary (yes/no) endpoint of 6 months of PFS. Treatment success was defined as achievement of at least 6 months of PFS. Patients who did not complete 6 months of ganetespib therapy for any reason (including death from any cause) were considered treatment failures and were recorded as not achieving the primary endpoint. Progression after docetaxel treatment was determined by radiography or rising PSA level consistent with the Prostate Cancer Working Group 2 guidelines.

This multi-institution phase 2 trial was planned with a Simon two-stage near-optimal design [27] resulting from the Simon algorithm modifications of Hintze [28]. The statistical goal was to determine whether the true 6-month PFS rate was >20 % or not. The study was powered for an alternative rate of 35 %. We assumed alpha =0.15 and power = 0.85. Under those assumptions, the design called for a maximum of 51 patients, 18 in Stage 1 and 33 in

Stage 2 (if needed). If, among the first 18 patients, 3 or fewer successes are observed, the study was to be stopped, with the conclusion that ganetespib was not effective enough for further evaluation. The probability of early termination with this design was 0.501 when the true success rate was 20 %, and the average total sample size for this design was 34.47 patients.

Ninety percent confidence intervals (CIs) for response and toxicity rates were calculated using Wilson's method as implemented in Stata 12 software. PFS was measured from treatment start date to the first date of documented PSA progression, discontinuation of ganetespib therapy, or death from any cause, whichever occurred first. Patients not experiencing any of those 3 terminating events were censored for PFS as of the date of their last PSA determination. Overall survival (OS) was measured from treatment start date to the date of death from any cause. Patients still alive were censored for OS as of the last date on which they were confirmed to be still alive. Standard Kaplan-Meier (K-M) estimates of the censored PFS and OS distributions were computed. Due to the small sample size, survival statistics (e.g., median) were estimated more conservatively using linear interpolation among successive event times on the K-M curves [29].

For statistical analysis of the molecular marker measurements, 1-tailed paired Student's t-tests were used. P values <0.05 are considered significant.

Results

Between January 2011 and September 2012, 18 eligible patients were recruited into Stage 1 of the trial. One patient never started ganetespib treatment due to rapid disease progression. Patient follow-up continued through April 2013.

Patient characteristics

The baseline patient characteristics are summarized in Table 2. Seventeen patients completed the trial. Thirteen patients had received 1 prior (docetaxel-based) chemotherapy regimen. Fifty percent of the patients were treated with at least 4 prior regimens. Seventy two percent of the patients were Caucasian and 72 % had performance status 1.

Treatment and adverse events

Five patients received 1 cycle of ganetespib, 5 received 2 cycles, 6 received 3 cycles, and 1 received 5 cycles. There were 22 unique types of Grade 3 toxicity observed (Table 3). The 4 most frequent types of Grade 3 toxicity were dehydration, diarrhea, and fatigue. Hypocalcemia in one patient was the only Grade 4 toxicity observed.

Clinical efficacy

None of the 17 treated patients achieved the primary endpoint of remaining on ganetespib and progression free for at least 6 months (90 % CI: 0.00–0.14). However, the true 6-month PFS rate was at most 0.20, which was not considered adequate. Thus, as per the statistical design, the trial was terminated early after the interim analysis at the end of Stage 1. Only 2 patients achieved PFS > 4 months: one progressed at 4.1 months, and the other at 4.4 months

after start of treatment with ganetespib. The median PFS was 1.9 months. The median OS was 12.5 months, and 7 patients were still alive as of the data cutoff in April 2013. Detailed summary statistics of PFS and OS are given in Table 4. Their Kaplan-Meier graphs are shown in Figs. 1 and 2, respectively.

Correlative biomarkers

To evaluate the expression of tumor suppressor maspin and its associated proteins at the baseline and at the end of the treatment we analyzed the mRNA levels of these markers in patients' PBMCs. As shown in Fig. 3, there was no statistically significant difference in mRNA expression before and after treatment of any of the markers studied.

Discussion

Despite the potent inhibition of Hsp90 in preclinical studies, ganetespib did not show clinical efficacy in men with mCRPC. The primary objective of this clinical trial was to evaluate the impact of ganetespib on the 6-month PFS rate. No patients achieved 6 months of PFS, and only 2 achieved PFS > 4 months. This group of patients had fairly advanced disease with half of the patients receiving at least 4 prior regimens, and their results are comparable to those of patients who were treated with 17-AAG in our previous trial [15]. Although these results are disappointing, they seem to support an emerging consensus that the ineffectiveness of Hsp90 inhibition may not be due to failure of the Hsp90 inhibition, but rather that Hsp90 inhibition alone may not be mechanistically sufficient to yield cytotoxic effects in men with advanced prostate cancer. Centenera et al. reported evidence of efficacy of new Hsp90 inhibitors, NVP-AUY922 and NVP-HSP990, used to treat *ex vivo* culture of human prostate tumors [30]. They showed higher antiproliferative and proapoptotic activity than 17-AAG. Although similar results were seen with ganetespib in animal models, it is possible that in humans, treatment targeted at Hsp90 may lead to stasis or autophagy. Hsp90-dependent stability and the activity of Hsp90 client molecules, such as AR and other signaling molecules, might further depend on co-chaperone molecules, which may help preserve tumor cell viability by essential Hsp90-dependent chaperone mechanism even when Hsp90 ATPase is significantly inhibited.

The correlative markers, which were available both before and after treatment in only 6 patients, did not show any appreciable changes, suggesting that the current dose and schedule of treatment did not impact the expression of these specific markers. It remains unclear at this time of the significance of these results as the trial did not yield any patients with PFS 6 months.

Ganetespib is currently evaluated in a large Phase 3 study in patients with metastatic lung cancer. The role of Hsp90 is also being investigated in a variety of other tumor types with Hsp90 inhibitor as a single agent or in combination with cytotoxic therapy. Since Hsp90 not only chaperones oncogenic proteins but also tumor suppressors, such as p53 [31], inhibition of Hsp90 could be a double-edged sword. Results from this study underscores the need for a better understanding of the underlying molecular mechanisms of Hsp90 in prostate cancer.

Acknowledgments

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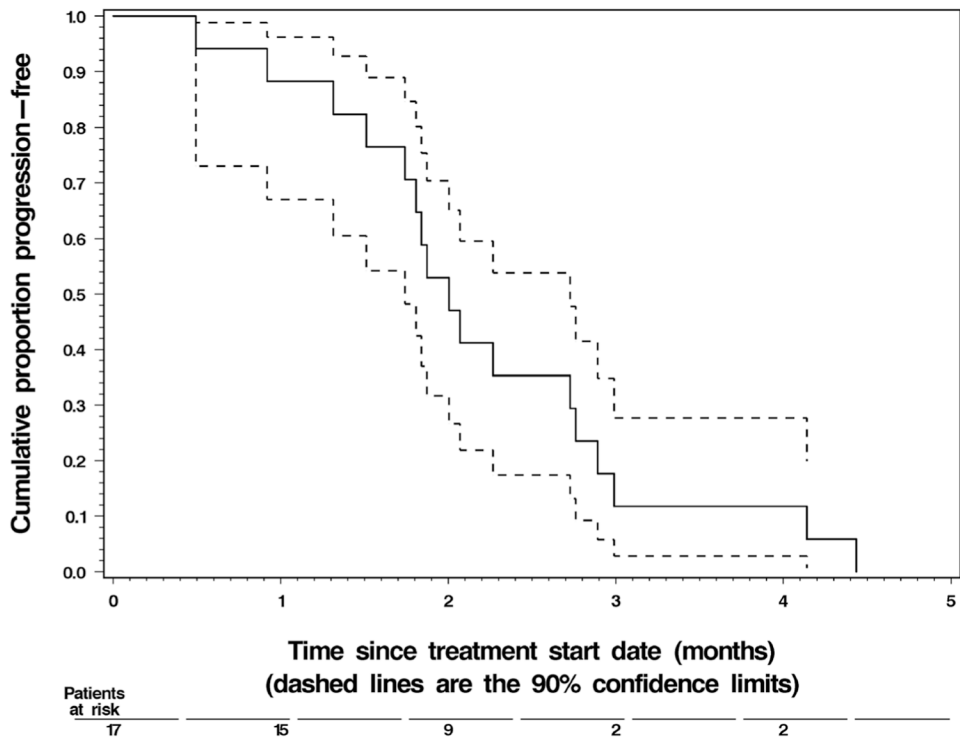
Fig. 1.
Progression-free survival of the 17 treated patients. Prostate cancer progressed in all patients by the data cutoff date of April 2013
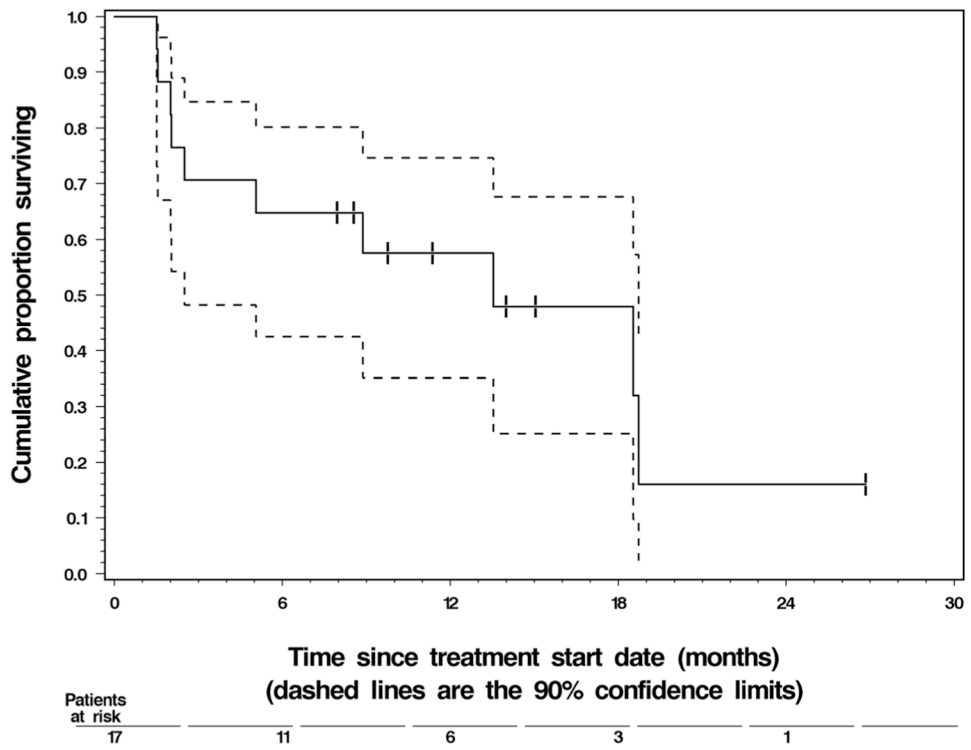
Fig. 2.
Overall survival of the 17 treated patients. Vertical tick marks on the Kaplan-Meier curve represent the 7 patients still alive at those respective points of follow-up
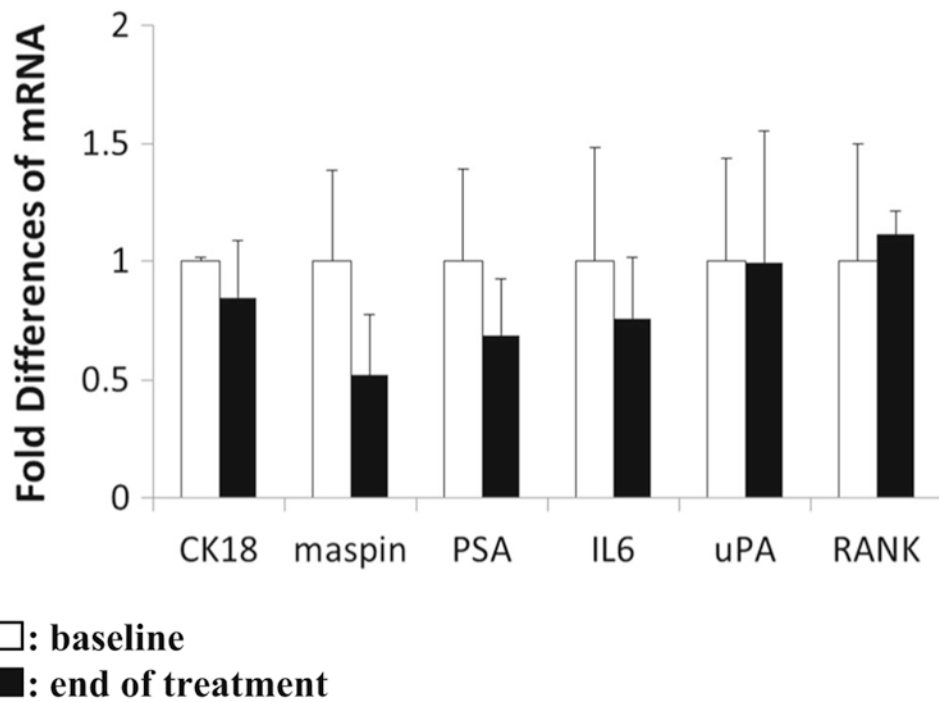
Fig. 3.
Detection of CK18, maspin, PSA, IL-6, uPA, and RANK mRNA by q-RTPCR in the patients' PBMC before ($n = 12$) and at the end of treatment ($n = 6$). The threshold cycle (CT) numbers obtained from q-RT-PCR were first normalized by the internal control of GAPDH, then by the baseline values and presented as the fold change in mRNA expression

Table 1

List of q-RT-PCR primers

Gene	Primer Sequence
Maspin	5′-cta ctt tgt tgg caa gtg gat gaa-3' 5′-act ggt ttg gtg tct gtc ttg ttg
GAPDH	5′-atc acc atc ttc cag gag cga-3' 5′-gcc agt gag ctt ccc gtt ca-3'
CK-18	5′-atc ttg gtg atg cct tgg aca-3' 5′-ctt tgc cat cca cta tcc gg-3'
uPA	5′-ggg ggc tct gtc acc tac g-3' 5′- ccc cag ctc aca att cca gtc-3'
IL-6	5′- ggt aca tcc tcg acg gca tc-3' 5′- cct tct ttg ctg ctt tca caa c-3'
RANK	5′- aag atg atg gca gcc ac-3' 5′- taa atg ctt gct gca taa ag-3'
PSA	5′-cct gag gaa tcg att ctt cag –3' 5′- gca tca gga aca aaa gcg tga-3

Table 2

Patient Characteristics (N = 18)

Age, median (range)	68 years (range 51–82 years)
Race	Patient number (%)
Caucasian	13 (72)
African American	4 (22)
Asian	1 (6)
Pretherapy PSA, median (range)	211 ng/ml (25.9–3489.2 ng/ml)
Performance status	Patient number (%)
0	2 (11)
1	13 (72)
2	3 (17)
Prior surgery	Patient number (%)
No	11 (61)
Yes	7 (39)
Prior radiation	Patient number (%)
No	5 (28)
Yes	13 (72)
Disease sites	Patient number (%)
Visceral and bone metastasis	4 (22)
Lymph node and bone metastasis	4 (22)
Bone metastasis only	10 (55)
Number of prior (medical) therapies[a]	Patient number (%)
1	4 (22)
2	3 (17)
3	2 (11)
4	6 (33)
5	3 (17)

PSA = prostate-specific antigen

[a] From among the following: docetaxel, cabazitaxel, abiraterone, Provenge, other. No patient received prior enzalutamide

Table 3

Grade 3 Toxicities [a] (N = 17 treated patients)

Type of toxicity	N	Rate (%)	Lower CL (%) [b]	Upper CL (%) [b]
Dehydration	3	18	7	37
Diarrhea	3	18	7	37
Fatigue	3	18	7	37
Abdominal pain	2	12	4	30
Aspartate aminotransferase increased	2	12	4	30
Nausea	2	12	4	30
Vomiting	2	12	4	30
Each of 15 different types of toxicities [c]	1	6	1	23

[a] Toxicity types are listed in decreasing order of frequency

[b] 90 % Wilson type confidence limit (CL)

[c] Anemia, hyperkalemia, hypoalbuminemia, lymphocyte count decreased, generalized muscle weakness, constipation, hyponatremia, hip fracture, blood bilirubin increased, dyspnea, enterocolitis, hypophosphatemia, platelet count decreased, non-cardiac chest pain, and elevated LDH

Table 4

Summary Statistics of Time-to-Event Endpoints (N = 17 treated patients)

Time-to-event endpoint	N	Events	Point estimate	90 % confidence interval	
Progression-free survival	17	17			
Median			1.9 months	1.7 months	2.7 months
1-month rate			87 %	72 %	100 %
2-month rate			47 %	27 %	67 %
3 -month rate			12 %	0 %	25 %
Overall survival	17	10			
Median			12.5 months	1.5 months	17.7 months
3-month rate			69 %	50 %	88 %
6-month rate			63 %	43 %	83 %
9-month rate			57 %	35 %	79 %
12-month rate			51 %	29 %	73 %