

## A Phase II Trial of 17-Allylamino-17-Demethoxygeldanamycin in Patients with Hormone-Refractory Metastatic Prostate Cancer

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**Abstract** **Purpose:** 17-Allylamino-17-demethoxygeldanamycin (17-AAG) is a benzoquinone ansamycin antibiotic with antiproliferative activity in several mouse xenograft models, including prostate cancer models. A two-stage phase II study was conducted to assess the activity and toxicity profile of 17-AAG administered to patients with metastatic, hormone-refractory prostate cancer. **Experimental Design:** Patients with at least one prior systemic therapy and a rising prostate-specific antigen (PSA) were eligible. Patients received 17-AAG at a dose of 300 mg/m<sup>2</sup> i.v. weekly for 3 of 4 weeks. The primary objective was to assess the PSA response. Secondary objectives were to determine overall survival, to assess toxicity, and to measure interleukin-6, interleukin-8, and maspin levels and quality of life. **Results:** Fifteen eligible patients were enrolled. The median age was 68 years and the median PSA was 261 ng/mL. Patients received 17-AAG for a median number of two cycles. Severe adverse events included grade 3 fatigue (four patients), grade 3 lymphopenia (two patients), and grade 3 back pain (two patients). The median PSA progression-free survival was 1.8 months (95% confidence interval, 1.3–3.4 months). The 6-month overall survival was 71% (95% confidence interval, 52–100%). **Conclusions:** 17-AAG did not show any activity with regard to PSA response. Due to insufficient PSA response, enrollment was stopped at the end of first stage per study design. The most significant severe toxicity was grade 3 fatigue. Further evaluation of 17-AAG at a dose of 300 mg/m<sup>2</sup> i.v. weekly as a single agent in patients with metastatic, hormone-refractory prostate cancer who received at least one prior systemic therapy is not warranted.

The androgen receptor (AR) is a member of the steroid receptor family that binds to testosterone and dihydrotestosterone on cellular entry (1). AR is also important for the growth of male urogenital structures and for spermatogenesis. In

hormone-refractory prostate cancer, increased AR activity may result from mutations, increased AR phosphorylation by upstream signaling pathways, or by increased transcription of AR. The AR function may be further regulated through conformational changes due to its dynamic partnership with heat shock proteins. In its inactive state, AR is bound to at least three heat shock proteins (Hsp90, Hsp70, and Hsp56; ref. 2). On activation, AR is released from heat shock proteins, interacts with other cellular proteins, and ultimately activates target genes.

Docetaxel-based chemotherapy regimens are now considered the standard of care for the treatment of men with metastatic, hormone-refractory prostate cancer (3, 4). Treatment options for those patients who fail docetaxel-based chemotherapy are limited. We postulate that targeting multiple mitogenic signaling pathways may delay or block the progression of hormone-refractory metastatic prostate cancer. To this end, multiple mitogenic signaling pathways (including the AR pathway) depend on the chaperoning activity of heat shock protein, especially Hsp90. Predominantly a cytoplasmic protein during normal conditions, Hsp90 may be accumulated and continue to act as a chaperon in the nuclei in response to stressful cellular environment (5, 6). In addition to AR, Hsp90 client proteins include Akt kinase, Raf-1 kinase, Bcr-Abl kinase, HER2, and hypoxia-inducible factor-1 $\alpha$ . The activity of Hsp90 can be regulated through its association with different sets of interacting molecules. Interestingly, tumor-suppressive protein

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**Note:** This phase II trial is being conducted within the phase II consortium. Participating institutions include Karmanos Cancer Institute at Wayne State University (Detroit, MI); principal investigator: Elisabeth I. Heath, M.D., Mayo Clinic Cancer Center (Rochester, MN), University of Wisconsin Carbone Comprehensive Cancer Center (Madison, WI), Howard University College of Medicine (Washington, DC), The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins (Baltimore, MD), Mayo Clinic Jacksonville (Jacksonville, FL), and Mayo Clinic Scottsdale (Scottsdale, AZ).

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maspin is recently shown to interact with Hsp90 (7). Furthermore, maspin expression in prostate cancer is inversely correlated with tumor grade and AR but positively correlated with disease-free survival of patients who received hormonal ablation therapies (8, 9).

The ability of Hsp90 to chaperone protein kinases or transcription factors depends on the binding and hydrolysis of ATP at its binding domain (10). Accordingly, multiple mitogenic pathways may be blocked simultaneously by synthetic inhibitors of the Hsp90 ATPase activity, such as 17-allylamino-17-demethoxygeldanamycin (17-AAG; refs. 11–13). 17-AAG is a benzoquinone ansamycin antibiotic with antiproliferative activity. Its parent compound, geldanamycin, showed promising antitumor properties in preclinical studies. 17-AAG proved to be less hepatotoxic than its parent compound. Both compounds are believed to act biologically similar by binding to the hydrophobic ATP/ADP-binding site on Hsp90.

In preclinical studies, 17-AAG was found to be active in several mouse xenograft models, including breast cancer, melanoma, ovarian cancer, and prostate cancer. Solit et al. (14) reported growth inhibition of both androgen-sensitive and androgen-insensitive tumors in prostate cancer xenografts treated with 17-AAG. In addition, 17-AAG caused the down-regulation and reduction in HER2, HER3, and wild-type and mutant AR expression.

Phase I clinical trials of 17-AAG were conducted in patients with advanced solid tumors (15–18). In a phase I trial of 17-AAG involving patients with advanced prostate cancer, one patient treated with twice weekly 17-AAG treatment achieved a prostate-specific antigen (PSA) response (25% decline; ref. 18). Based on promising preclinical and clinical data and its unique mechanism of action, 17-AAG was evaluated in a multicenter, phase II trial in poor-prognosis, metastatic, hormone-refractory prostate cancer patients.

## Materials and Methods

**Eligibility criteria.** Men with histologically confirmed prostate adenocarcinoma with metastasis were eligible if they met the following criteria: objective disease progression or rising PSA despite androgen deprivation therapy and antiandrogen withdrawal, patients with rising PSA must show a rising trend with two successive elevations at a minimum interval of 1 wk, a minimum PSA of 5 ng/mL or new areas of bony metastases on bone scan are required for patients with no measurable disease, baseline imaging for disease assessment must be done  $\leq 28$  d before registration, all patients must have had at least one prior regimen of chemotherapy for metastatic disease, and all patients must be documented to be castrate with a testosterone level of  $< 50$  ng/mL. Luteinizing hormone-releasing hormone agonist therapy must be continued if required to maintain castrate levels of testosterone. Patients must be off antiandrogens for  $\geq 4$  wk for flutamide and 6 wk for bicalutamide or nilutamide; prior radiation therapy completed  $\geq 28$  d before registration; life expectancy of  $\geq 12$  wk;  $\geq 18$  y of age; absolute neutrophil count  $\geq 1,500/\text{mm}^3$ ; platelets  $\geq 100,000/\text{mm}^3$ ; hemoglobin  $\geq 8.0$  g/dL; total bilirubin  $\leq 1.5 \times$  upper normal limit (UNL); aspartate aminotransferase (SGOT) and/or alanine aminotransferase (SGPT)  $\leq 2.5 \times$  UNL if alkaline phosphatase  $\leq$  UNL or alkaline phosphatase  $\leq 4 \times$  UNL if SGOT and/or SGPT are  $\leq$  UNL; calculated creatinine clearance of  $\geq 60$  mL/min or serum creatinine  $\leq$  UNL; and Eastern Cooperative Oncology Group Performance Status of 0, 1, or 2.

Contraindications to enrollment into the study included the following: men of childbearing potential or their sexual partners who are unwilling to use adequate contraception (condoms, diaphragm, birth control pills, injections, intrauterine device, surgical sterilization, s.c. implants, or abstinence), use of investigational agents for treatment of prostate cancer  $\leq 28$  d before registration, and current treatment with any other antineoplastic agent. Patients may continue to receive zoledronic acid for bone metastases or hypercalcemia; known brain metastatic disease requiring active therapy; any of the following conditions  $\leq 6$  mo before registration: myocardial infarction, severe/unstable angina, symptomatic congestive heart failure, cerebrovascular accident or transient ischemic attack, coronary/peripheral artery bypass grafting, or patients who have experienced a pulmonary embolus, deep venous thrombosis, or other clinically significant

**Table 1.** Patient characteristics at baseline

Characteristic	n (range), n = 15	%
Median age	68 (52-78)	
Median PSA	261 (46-1705)	
Median Gleason score	8 (5-10)	
Median hemoglobin	11.6 (9.3-14.3)	
Median alkaline phosphate	154 (52-323)	
Median days since last chemotherapy	91 (28-925)	
Performance status		
0	4	27
1	10	67
2	1	6
Receiving concurrent zoledronic acid		
Yes	9	60
No	6	40
Race		
White	12	80
Black	3	20
Hypercalcemia		
No	15	100
Prostatectomy		
Yes	5	33
No	10	67
Chemotherapy		
Taxane based	13	87
Other	2	13
Radiation therapy		
Yes	12	80
No	3	20
Androgen ablation		
Yes	14	93
No	1	7
Other treatment		
Yes	9	60
No	6	40
Site of disease (visceral)		
Yes	6	40
No	9	60
Site of disease (bone)		
Yes	11	73
No	4	27
Site of disease (soft tissue)		
Yes	4	27
No	11	73
Other site		
Yes	9	60
No	6	40
Disease status		
Measurable	2	13
Measurable and evaluable	9	60
Evaluable, not measurable	4	27

**Table 2.** 17-AAG dose administered

Cycle	n	17-AAG		
		Median total dose (mg/m <sup>2</sup> ) administered	Median dose level (mg/m <sup>2</sup> ) administered	% Receiving full dose
1	15	1,710	300	93.3*
2	10	1,795	300	100
3	1	1,872	300	100
4	1	1,881	300	100
5	1	1,254	300	100

\*One patient reduced for SGOT/SGPT.

thromboembolic event  $\leq 6$  mo before registration are eligible if they are clinically stable on anticoagulation therapy; significant cardiac disease including heart failure that meets New York Heart Association classification III or IV, history of myocardial infarction  $\leq 1$  y of study entry, uncontrolled dysrhythmias, or poorly controlled angina; and current active infection, including known HIV positivity. For HIV patients on highly active antiretroviral therapy, the pharmacokinetics of 17-AAG may be seriously affected. When appropriate, 17-AAG will be studied in patients with HIV on highly active antiretroviral therapy and serious allergy to eggs (i.e., hypotension, dyspnea, anaphylaxis, and edema).

This study was approved by the local institutional review board at all clinical centers and written informed consents were obtained from all patients before registration.

Patients underwent a complete medical examination before registration and every other cycle. The medical examination included measurement of PSA, indicator lesion(s) (every other cycle if done by radiologic method), hematologic and chemistry laboratory tests (hemoglobin, absolute neutrophil count, platelets, WBC, sodium, potassium, calcium, blood urea nitrogen, creatinine, alkaline phosphatase, SGOT, and SGPT), exam, history, weight, performance status assessment, and digital rectal examination.

**Treatment plan.** Patients were treated with 17-AAG at 300 mg/m<sup>2</sup> i.v. on days 1, 8, and 15 every 28 d. Every 28 d was considered one cycle. Patients underwent laboratory testing (including PSA and testosterone) and imaging studies (computed tomography scan of abdomen/pelvis, bone scan, and magnetic resonance imaging of brain) at baseline and after every two cycles.

**Dose reductions.** No dose modifications were made for grade  $< 1$  toxicity. For grade 3 neutropenia, the dose of 17-AAG was reduced to 240 mg/m<sup>2</sup> i.v. (dose level -1). For grade 4 neutropenia, the regular dose was held and readministered at dose level -1 when toxicity has resolved to grade  $\leq 1$ . For grade 2 thrombocytopenia, the dose was reduced to dose level -1, but for grades 3 and 4 thrombocytopenia, the regular dose was held and readministered at dose level -1 when toxicity has resolved to grade  $\leq 1$ . For grade 2 diarrhea and/or transaminitis, the dose was reduced to dose level -1. For grade 3 diarrhea and/or transaminitis, the dose was held and readministered at dose level -1 when toxicity has resolved to grade  $\leq 1$ . For grade 4 diarrhea and/or transaminitis, 17-AAG therapy was discontinued. For other grade 3 toxicities, the regular dose was held and readministered at dose level -1 on resolution of toxicity. For other grade 4 toxicities, treatment with 17-AAG was discontinued. For any cardiac toxicities, specific guidelines about QTc prolongation, atrial/ventricular dysrhythmia, and left ventricular ejection fraction were followed.

**Statistical methods.** The primary objective of this study was to assess the PSA response as defined by the Prostate-Specific Antigen Working Group (19) to 17-AAG in patients with metastatic, hormone-refractory prostate cancer who have failed front-line chemotherapy. The secondary objectives were to determine overall survival, disease-free survival, and response of 17-AAG on measurable disease and

evaluate correlative serum markers. Quality of life (QOL) assessments were obtained only in patients treated at the Karmanos Cancer Institute using the QLQ-30 European Organization for Research and Treatment of Cancer questionnaire. The emotional functioning (EF) and physical functioning (PF) scales are scored with a valid score between 0 and 100 points.

The study used a Simon MinMax two-stage design where a maximum of 25 evaluable patients would be accrued unless undue toxicity was encountered (20). An interim analysis was to be conducted after 16 patients were accrued and observed for 6 mo. If at most one success was observed in the first 16 patients (stage 1), this was considered early evidence of an ineffective treatment regimen in this patient population, leading to termination of accrual. If at least two successes were observed, the study was able to proceed to the next stage (stage 2) to accrue nine additional evaluable patients. Success was defined as a PSA response, which was confirmed by a second value at least 4 wk later.

The distribution of overall survival and disease-free survival is estimated using the method of Kaplan-Meier. Overall survival time is defined as the time from registration to death due to any cause. Disease-free survival is defined as the time from registration to documentation of disease progression. Disease progression was defined as a 25% or greater PSA increase over the nadir (or baseline if PSA never decreased) and an increase in PSA at least 5 ng/mL, which was confirmed by a second value obtained  $\sim 1$  wk later. If the PSA did not show progression, but a bone scan did show progressive disease (defined as development of at least two new lesions), the patient was also considered to have progression of disease.

Interleukin (IL)-6, IL-8, and maspin were obtained at baseline, on day 15, and at the end of treatment. Changes from pretreatment levels were assessed using the Wilcoxon signed rank test. The relationship of each marker with PSA progression was also evaluated. The percent change in each marker at treatment failure was compared with the % change in PSA at treatment failure using the Spearman rank correlation coefficient.

**Correlative markers.** The correlative markers evaluated in this study included serum levels of IL-6, IL-8 in peripheral blood lymphocytes, and serum levels of maspin. The correlative markers were selected to help determine potential effects of 17-AAG on the mechanism of hormone-refractory prostate cancer.

**Immunoassay for quantitation of IL-6/IL-8.** For the determination of IL-6/IL-8 in human serum samples, the blood samples were centrifuged for 15 min at 1,000  $\times g$ . The serum was collected and analyzed for IL-6 or IL-8 using ELISA kit (R&D Systems) following standard procedures supplied by the manufacturer. Briefly, for ELISA, 100  $\mu$ L of assay diluents RD1W or RD1-85 were added to 96-well plate coated with mouse monoclonal antibodies against IL-6 or IL-8. About 50 to 100  $\mu$ L of either standard or sample were added to each well and incubated for 2 h at room temperature. After washing four times with washing buffer, conjugate solution was added to each well and incubated for 2 h at room temperature. Wells were washed four times with washing buffer,

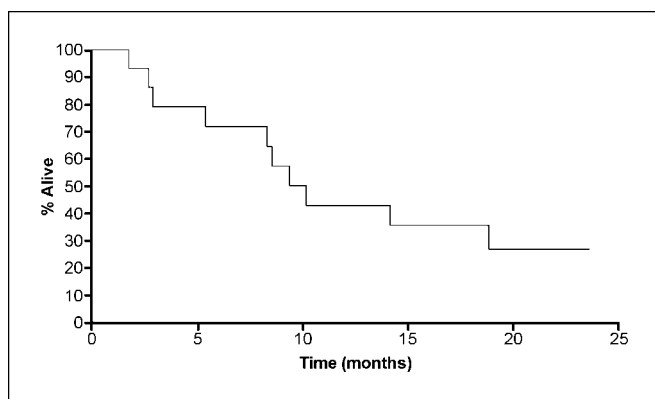


Fig. 1. Overall survival in patients with metastatic, hormone-refractory prostate cancer.

and 200  $\mu$ L of substrate solution containing a mixture of color reagent A and B were added and plate was incubated at room temperature for 20 min. About 50  $\mu$ L of stop solution were added to each well and absorbance was measured at 450 nm within 30 min. Standard curve was generated and concentration of IL-6 or IL-8 was calculated from the standard curve. Appropriate assay blanks and control well were also included during the assay.

**Quantitative real-time PCR detection of maspin.** Total RNA was extracted from the patient blood samples as described (21). The quality of the RNA was verified by agarose gel electrophoresis showing intact 18S and 28S rRNA and by UV spectrophotometry showing an  $A_{260\text{ nm}}/A_{280\text{ nm}}$  ratio between 1.8 and 2. One microgram of each RNA sample was reverse transcribed in a 20- $\mu$ L reaction as described (21). For real-time PCR, 1  $\mu$ L of the resulting cDNA was mixed with SYBR Green PCR Mastermix (Stratagene) and 300 nmol/L of the PCR primers. The primers for maspin coding sequence were 5'-CTA-CITTTGGCAAGTGGATGAA-3' and 5'-ACTGGTTGGTGTCTG-TCTTGTG-3'. The primers for glyceraldehyde-3-phosphate dehydrogenase were as previously described (22). The real-time PCR thermal profile was: 1 cycle of 95°C/10 min, 40 cycles of 95°C/30 s  $\rightarrow$  55°C/1 min  $\rightarrow$  72°C/30 s, 1 cycle of 95°C/1 min, and finally 41 cycles of 95°C  $\rightarrow$  (55°C + 1°C/cycle)/30 s. Critical threshold cycle numbers ( $C_t$ ) were obtained using the built-in software of the Stratagene Mx4000 Multiplex Quantitative PCR System. The measurement of maspin-specific cDNA species was normalized by the measurement of internal control glyceraldehyde-3-phosphate dehydrogenase. Data represent the average of three repeats.

**Quality of life.** Participants at Karmanos Cancer Institute were recruited independently from the larger study to provide QOL assessments in conjunction with their treatment. Patients were asked by physicians about their interest in participating in a companion QOL study. A member of the research team explained study procedures to any interested patients. Patients who agreed to participate provided consent in the form of a signature on a consent form, which described the nature of the study and explicitly stated that participants may discontinue their involvement with the study at any time. Refusal to participate in the QOL study did not affect their eligibility or participation in the larger clinical trial.

Participants in the QOL arm of the study completed the European Organization for Research and Treatment of Cancer Quality of Life questionnaire (QLQ-30-C30, version 3). For this study, the global PF and EF subscales were used in analyses. A baseline QOL assessment was conducted at the same time as the prestudy evaluation for the larger clinical trial. Subsequent assessments were conducted every 2 wk (corresponding to day 1 and day 15 of each treatment cycle) for the first two cycles of the trial. QOL assessments were completed using a computer-administered questionnaire via a laptop computer.

## Results

Seventeen patients were enrolled between January 11, 2005 and April 10, 2006. One patient never started treatment due to high potassium levels. Another patient was deemed ineligible because the patient was concurrently taking contraindicated medications. Six of the 15 evaluable patients completed QOL assessments. This study was permanently closed to patient enrollment per protocol design at the time of interim analysis due to insufficient PSA response.

The characteristics of the 15 evaluable patients are presented in Table 1. The median age of the cohort was 68 (range, 52-78). The median number of days from the last chemotherapy treatment was 91 (range, 28-925). Thirteen (87%) patients received prior taxane-based chemotherapy. The median PSA was 261 (range, 46-1705). A median of two cycles (range, 1-5) of treatment was given. Only one patient received more than two cycles of treatment. All 15 eligible patients have discontinued study treatment due to disease progression (13 patients, 87%), patient refusal (1 patient, 7%), and other medical problems (1 patient, 7%). See Table 2 for more details on 17-AAG dosage.

**Response.** No patient achieved a confirmed PSA response or an objective disease response as evaluated by radiologic imaging studies. One patient was able to maintain a stable PSA response for five cycles of treatment. This patient had a baseline PSA of 99 and maintained their PSA from 74 to 112 for the first four cycles before progressing after the fifth cycle of treatment.

**Survival and disease progression.** Patients were followed until death or a median of 10.1 months among living patients. At last contact, 5 (33%) of the 15 eligible patients were still alive with survival follow-up ranging from 1.7 to 23.5 months. Median time to disease progression was 1.8 months (95% confidence interval, 1.3-3.4 months). The 6-month overall survival was 71% (95% confidence interval, 52-100; see Figs. 1 and 2).

**Adverse events.** Adverse event data were available on 15 patients. Overall, 60% (9 of 15) of patients experienced a grade 3 adverse event. There have been no grade 4 or 5 adverse events reported. The most common severe (grade 3) adverse event reported in the study was fatigue with four (27%) events reported (see Table 3).

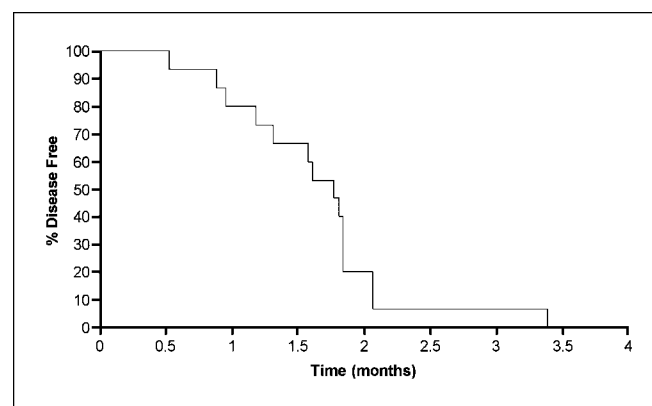


Fig. 2. Disease-free survival in patients with metastatic, hormone-refractory prostate cancer.

**Table 3.** Severe hematologic/nonhematologic adverse event (regardless of attribution)

Toxicity	Grade 3	
	n	%
Pain (abdominal)	1	7
Anorexia	1	7
SGOT	1	7
Pain (back)	2	13
QTc internal prolong	1	7
Fatigue	4	27
Anemia	1	7
Hypophosphatemia	1	7
Leukopenia	1	7
Lymphopenia	2	13
Neuromotor	1	7
Thrombosis	1	7

**IL-6 and IL-8 and maspin.** No significant changes in IL-6, IL-8, and maspin were noted at day 15. A borderline significant ( $P = 0.09$ ) up-regulation of maspin was observed at treatment failure (see Table 4A and B). No associations between IL-6, IL-8, and maspin with PSA at treatment failure were observed (data not shown).

**Quality of life.** Of the patients who completed QOL assessments, six patients completed questionnaires at baseline and end of study treatment and five patients completed questionnaires through 28 and 56 days (end of cycles 1 and 2) after registration. The median EF and PF scores at baseline were 83.3 and 76.7 points, respectively. At 28 and 56 days after registration, no clear trends in EF scores were observed. The PF

scores declined a median of 13.3 points from baseline at both 28 and 56 days.

EF and PF scores declined or remained unchanged for most patients at the end of study treatment. At the end of treatment, EF scores decreased a median of 4.2 points with declines in three (50%) patients and no change in one (17%) patient. The median PF score dropped 20 at the end of treatment (range, 60-point decline to 6.7-point increase) with five of six (83%) patients having a declining PF score.

Three of the four patients who experienced grade 3 fatigue completed QOL questionnaires during the adverse event. Both EF and PF scores had decreased from baseline values. The PF scores declined from 60 to 40, 93.3 to 67.7, and 60 to 0 points from baseline for three patients. Similarly, EF scores declined from 91.7 to 83.3 for two patients and from 58.3 to 33.3 points from baseline for one patient at the time of the event.

## Discussion

Patients with metastatic, hormone-refractory prostate cancer who fail front-line docetaxel-based chemotherapy have no standard treatment options available to them. Newer treatment for this poor-prognosis patient population is urgently needed. Concern surrounding the dysregulation of the AR in patients with metastatic, hormone-refractory disease is substantiated by many preclinical studies. Novel agents that affect the AR have the potential to affect the biology of the disease.

17-AAG, a benzoquinone ansamycin antibiotic with anti-proliferative activity, acts by binding to the hydrophobic ATP/ADP-binding site on Hsp90. Hsp90, along with other client proteins, is integral in activating downstream target genes.

**Table 4.** Correlative markers

A. Correlative marker values at 3 time points			
Marker	Median	Range	
IL-6 (pg/mL)			
Day 1	5.9	1.4-56.9	
Day 15	8.4	1.7-112.4	
End of treatment	11.9	1.7-62.7	
IL-8 (pg/mL)			
Day 1	13.8	6.9-162.6	
Day 15	14.3	9.0-217.6	
End of treatment	11.9	9.7-20.3	
Maspin* (fold change)			
Day 1	-3.0	-3.7-14.3	
Day 15	8.3	-7.2-11.5	
End of treatment	26.1	0.5-233.0	
B. Correlative marker change from baseline			
Marker	Median	Range	P
IL-6 (pg/mL)			
Day 15 change from baseline	0.4	-7.7-55.5	0.57
End of treatment change from baseline	4.5	0.3-16.0	0.03
IL-8 (pg/mL)			
Day 15 change from baseline	3.0	-49.5-55.0	0.73
End of treatment change from baseline	-1.8	-39.2-2.9	0.31
Maspin (fold change)			
Day 15 change from baseline	6.4	-8.5-15.3	0.44
End of treatment change from baseline	28.7	-13.8-236.0	0.09

\*Values represent a fold change from a normalized measurement of internal control glyceraldehyde-3-phosphate dehydrogenase.

In this phase II trial of 15 eligible patients, the patient population was typical of most studies in this setting: elderly males with good performance status with predominantly bony metastasis and on treatment with zoledronic acid. The median number of cycles given was 2 and only one patient required a dose reduction due to elevated transaminases. Unfortunately, there were no PSA responses seen. Indeed, this was a disappointing result. It is possible that a dose of 300 mg/m<sup>2</sup> i.v. weekly for two cycles of treatment was an insufficient amount of drug and treatment time to produce a meaningful PSA response at this highly elevated PSA level (median, 261 ng/mL). The median time from the last chemotherapy treatment was 91 days, suggesting that the prostate cancer was in an aggressive or advanced state. The median time to PSA progression was also rapid at 1.8 months and the 6-month overall survival of this group of patients was as expected at 71%.

The AR can also be activated by other factors, such as cytokines including IL-6. The IL-6 receptor is believed to interact with the HER2 receptor, promoting phosphorylation and activating downstream pathways, ultimately leading to increased AR activation (23). Increased serum levels of IL-6 have been reported in patients with hormone-refractory prostate cancer (24, 25). Preclinical data confirmed that androgen-sensitive prostate cancer cell lines do not constitutively express IL-6, whereas androgen-insensitive prostate cancer cell lines do (26). One reason IL-6 expression in prostate cancer cell is increased is attributed to deregulated IL-6 promoter activity. Although the number of samples available for correlative studies was small in this study, there was no apparent association of IL-6 or IL-8 activity with PSA.

Maspin is a Hsp90-associated tumor-suppressive protein (7). Maspin expression in prostate cancer is inversely correlated with tumor grade and AR but positively correlated with disease-free survival of patients who received hormonal ablation therapies (8, 9). Maspin protein has not been directly detected in patients' sera. However, real-time PCR detection of

maspin mRNA was previously shown to be specifically associated with circulating epithelial-derived cancer cells (27). Although 17-AAG treatment in our study did not lead to significant decrease of serum PSA levels, patients' tumor burden as judged by PSA was not further increased. Thus, the increase of maspin mRNA in circulation is likely a result of increased expression per circulating prostate tumor cell. Furthermore, because Hsp90 inhibition may lead to increased AR turnover (14, 28), 17-AAG may have increased maspin expression in prostate tumor cells by inhibiting AR, despite the fact that such inhibition on AR did not lower PSA in a clinically significant manner.

The most significant severe toxicity was grade 3 fatigue. In the four patients who experienced grade 3 fatigue, QOL measurements were obtained in three patients. The QOL measurements revealed declining PF and EF scores. Drops in PF scores were more dramatic than EF declines. In addition, these declines in PF were greater than any changes seen in the other three patients during cycles 1 and 2 with declines of 20, 26.7, and 60.0. These findings suggest that an increase in fatigue was correlated with patient subjective reports of declining QOL.

17-AAG in patients with metastatic, hormone-refractory prostate cancer who received at least one prior systemic therapy did not show a significant activity with regard to PSA response. However, correlative studies suggest that tumor-suppressive maspin might be up-regulated. Because maspin has been shown to further increase prostate tumor cell sensitivity to drug-induced apoptosis (29), it remains a possibility that hormone-refractory prostate cancer patients may respond more favorably to the combination of 17-AAG with another agent that can directly activate the apoptotic pathway. Single-agent activity at a weekly dose of 300 mg/m<sup>2</sup> was minimal and does not warrant further investigation.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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