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CLINICAL TRIAL REPORT

# A phase I trial of docetaxel and pulse-dose 17-allylamino-17-demethoxygeldanamycin in adult patients with solid tumors

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**Abstract**
*Purpose* To define maximum tolerated dose (MTD), clinical toxicities, and pharmacokinetics of 17-allylamino-17-demethoxygeldanamycin (17-AAG) when administered in combination with docetaxel once every 21 days in patients with advanced solid tumor malignancies.
*Experimental design* Docetaxel was administered over 1 h at doses of 55, 70, and 75 mg/m$^2$. 17-AAG was administered over 1–2 h, following the completion of the docetaxel infusion, at escalating doses ranging from 80 to 650 mg/m$^2$ in 12 patient cohorts. Serum was collected for pharmacokinetic and pharmacodynamic studies during cycle 1. Docetaxel, 17-AAG, and 17-AG levels were determined by high-performance liquid chromatography. Biologic effects of 17-AAG were monitored in peripheral blood mononuclear cells by immunoblot.
*Results* Forty-nine patients received docetaxel and 17-AAG. The most common all-cause grade 3 and 4 toxicities were leukopenia, lymphopenia, and neutropenia. An MTD was not defined; however, three dose-limiting toxicities were observed, including 2 incidences of neutropenic fever and 1 of junctional bradycardia. Dose escalation was halted at docetaxel 75 mg/m$^2$-17-AAG 650 mg/m$^2$ due to delayed toxicities attributed to patient intolerance of the DMSO-based 17-AAG formulation. Of 46 evaluable patients, 1 patient with lung cancer experienced a partial response. Minor responses were observed in patients with lung, prostate, melanoma, and bladder cancers. A correlation between reduced docetaxel clearance and 17-AAG dose level was observed.
*Conclusions* The combination of docetaxel and 17-AAG was well tolerated in adult patients with solid tumors, although patient intolerance to the DMSO formulation precluded further dose escalation. The recommended phase II dose is docetaxel 70 mg/m$^2$ and 17-AAG 500 mg/m$^2$.

**Keywords** 17-AAG · Geldanamycin · Hsp90 · Docetaxel · Phase I

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## Introduction

Heat shock protein 90 (Hsp90) is an abundant cellular chaperone that is required for refolding of unfolded proteins, cellular survival under stress conditions, and the conformational maturation of a variety of proteins that play key roles in transducing proliferative and anti-apoptotic signals [1, 2]. 17-allylamino-17-demethoxygeldanamycin (17-AAG), a derivative of the natural product geldanamycin, was the first Hsp90 inhibitor to enter clinical testing [3–7]. Geldanamycin and its derivatives bind to a regulatory ATP/ADP pocket in the aminoterminal portion of Hsp90 that is conserved across species [8]. Occupancy of the pocket by drug leads to the degradation of Hsp90 client proteins, many of which play central roles in tumor initiation, maintenance of the transformed phenotype, and tumor progression [9, 10]. These Hsp90 clients include steroid receptors such as the androgen and estrogen receptors and a subset of serine/threonine and tyrosine kinases, including Raf-1, Akt, HER2, and insulin-like growth factor-1 receptor [11–15].

In cell culture models, 17-AAG synergistically enhances the activity of chemotherapeutics including paclitaxel and doxorubicin [16–18]. 17-AAG induces a potent G1 arrest in tumors with intact retinoblastoma protein 1 (RB1) function, and thus, sensitization to cell cycle-specific cytotoxics is schedule dependent in most systems [16]. For example, in tumors with intact RB1 function, 17-AAG enhances the antiproliferative and pro-apoptotic effects of paclitaxel when the 2 agents are administered either simultaneously or when the taxane precedes 17-AAG [16, 19]. In contrast, pretreatment with 17-AAG in such systems induces G1 cell cycle arrest and antagonizes the pro-apoptotic effects of the taxane. In human xenograft models, maximal enhancement occurs when the taxane and 17-AAG are administered on the same day, suggesting that a mechanistic interaction between these agents is responsible for the observed synergy [13]. Further exploration of the mechanisms responsible for synergy in HER2-driven breast cell lines suggested that inactivation of PI3 kinase/Akt signaling following Hsp90 inhibition was required for synergy [13]. Notably, a pulsatile 17-AAG dosing regimen with no single-agent activity that inhibits Akt activity for 24–48 h was sufficient to sensitize tumors to paclitaxel [13].

On the basis of these preclinical data, we initiated a phase I clinical trial of docetaxel in combination with pulse-dosed 17-AAG in patients with advanced solid tumor malignancies. The primary objectives of the study were to define the maximum tolerated dose (MTD) of 17-AAG as well as clinical toxicities of the combination. Secondary endpoints included pharmacokinetic studies and the effects of treatment on the expression of Hsp90 clients in peripheral blood mononuclear cells (PBMCs).

## Materials and methods

### Patient eligibility

The trial was approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center on January 23, 2003 (clinicaltrials.gov identifier: NCT00058253). All patients signed written informed consent. Patients were required to have a histologically confirmed malignancy that was metastatic or unresectable and for which curative or palliative measures did not exist or were no longer effective. Progressive disease could be documented either by new lesions or an increase in preexisting lesions on bone scintigraphy, computed tomography (CT), magnetic resonance imaging (MRI), or by physical examination. For patients with prostate cancer, a rising prostate-specific antigen (PSA) alone was sufficient to document progression.

Patients were required to be over 18 years of age, have a Karnofsky Performance Status $\geq$70%, and a life expectancy >6 months. Intact hepatic, renal, and bone marrow function as defined by specific laboratory ranges was a prerequisite for enrollment.

Exclusion criteria included prior chemotherapy, radiotherapy, or other investigational therapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C); active brain metastases or epidural disease; symptomatic peripheral neuropathy $\geq$Grade 2; a history of severe hypersensitivity reaction to paclitaxel, docetaxel, or polysorbate 80; and allergy to egg or egg products (because of its inclusion in the EPL diluent vehicle).

Cardiac-specific exclusion criteria were altered during the course of the trial. The initial 43 patients enrolled required screening radionuclide angiography or echocardiogram for suspected or documented congestive heart failure with a depressed ejection fraction, coronary artery disease, or arrhythmia other than atrial fibrillation. Following reports of prolonged QTc intervals on other 17-AAG trials, we also excluded from this study patients with significant cardiac disease including heart failure that met New York Heart Association (NYHA) class III or IV definitions, a history of myocardial infarction or active ischemic heart disease within 12 months of study entry, uncontrolled dysrhythmias, and individuals requiring antiarrhythmic drugs. Patients with the following were also excluded: history of serious ventricular arrhythmia (VT or VF lasting $\geq$ 3 consecutive beats); QTc > 450 ms for men and >470 ms for women; congenital long QT syndrome; left bundle branch block; history of prior radiation that potentially included the heart in the field (e.g., mantle); or LVEF $\leq$ 40% by multi-gated acquisition scan or echocardiography. Medications previously shown to prolong the QTc interval were restricted.

### Treatment

On a 21-day cycle, patients were treated intravenously with docetaxel over 1 h followed immediately by 17-AAG. The infusion duration of 17-AAG at doses of 450 mg/m$^2$ or higher was 2 h, while doses of 375 mg/m$^2$ or less were infused over 1 h. All patients received dexamethasone premedication to reduce the incidence and severity of fluid retention or hypersensitivity reactions.

### Patient assessment

Pharmacokinetic samples were drawn at specific time points on day 1 (pretreatment, 30 and 55 min after initiation of docetaxel infusion, and 30 min, 1, 2, 3, 4, and 6–8 h after initiation of 17-AAG infusion), and also on days 2 and 3 of cycle 1.

PBMCs were assessed for changes in Hsp70, Raf-1, and Akt by immunoblot. Blood samples were drawn into heparin-containing tubes and PBMCs were isolated by centrifugation. Up to 6 samples were drawn during cycle 1 only. Cells were lysed in NP40 lysis buffer (50 mM Tris [pH 7.4], 1% NP40, 150 mM NaCl, 40 mM NaF, 1 mM $Na_3VO_4$, 1 mM phenylmethylsulphonylfluoride, and 10 mg/ml each of leupeptin, aprotinin, and soybean trypsin inhibitor) for 30 min on ice. Lysates were centrifuged at approximately 13,000×*g* for 10 min, and the protein concentration of the supernatant was determined by bicinchoninic acid (BCA) assay (Pierce, Rockford, IL). Equal amounts of total protein were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and transferred onto nitrocellulose membranes. Blots were probed overnight at 4°C with the primary antibody. After incubation with horseradish peroxidase-conjugated secondary antibodies, proteins were detected using chemiluminescence. The following primary antibodies were used: Akt (Cell Signaling, Beverly, MA), Hsp70 (StressGen, Victoria, BC, Canada), p85 subunit of PI3 kinase (Upstate Biotechnology, Lake Placid, NY), and Raf-1 (Santa Cruz Biotechnology, Santa Cruz, CA). The p85 subunit of PI3 kinase was used as a loading control since its expression is unaffected by 17-AAG.

Although response was not the primary endpoint of this trial, patients with measurable disease were assessed by the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0.

### Toxicity and MTD

Toxicities and adverse events were assessed using the National Cancer Institute (NCI) Common Toxicity Criteria (CTC) version 2.0. Dose-limiting toxicity (DLT) was defined as the occurrence of any of the following events during cycle 1 of treatment:

1. Grade 4 neutropenia lasting greater than 7 days (defined as 2 or more consecutive CBC with differentials documenting grade 4 neutropenia drawn ≥8 days apart)
2. Febrile neutropenia (ANC < 1,000/mm$^3$ and temperature ≥38.5°C)
3. Grade 3 or greater thrombocytopenia, or other grade 3 or higher neurologic, gastrointestinal, or cardiovascular toxicities attributable to the study treatment
4. Grade 3 or greater QTc interval prolongation (QTc > 0.50 s)

The MTD was defined as the highest dose level with an observed incidence of DLT in no more than 1 out of 6 patients treated at a particular dose level. The dose escalation scheme was as follows: if none of an initial 3 patients at a given dose level experienced a DLT, the next dose level was studied in another cohort of 3 patients. Escalation then continued if none or one of the additional patients experienced a DLT. If 1 of the initial 3 patients at a given dose level experienced a DLT, up to 3 additional patients were treated at that same dose level. Escalation then continued if none of the additional patients experienced a DLT. If 2 or 3 of the first 3 patients experienced a DLT, or 2 or more patients out of a cohort of 6 experienced a DLT at a given dose level, the MTD was defined as the preceding dose level. If 3 or fewer patients were treated at a dose under consideration as the MTD, additional patients to total 6 were to be treated at that level to confirm the MTD.

### Analytical chemical methods and pharmacokinetic data analysis

Concentrations of 17-AAG and its metabolite 17-AG were measured using a high-performance liquid chromatography (HPLC) assay with absorbance detection [20], while docetaxel plasma concentrations were quantitated with a liquid chromatography-mass spectrometry assay, both of which were developed and validated in the Egorin laboratory [21]. Plasma concentration was correlated with time data for 17-AAG and 17-AG non-compartmentally using the LaGrange function [22] as implemented by the Lagran computer program [23].

## Results

### Patients

A total of 49 patients were treated, with 46 evaluable for response. Baseline demographics are shown in Table 1. Three patients were not evaluable for response due to removal from the study for toxicity during cycle 1. Nine patients were removed before the first scheduled restaging

**Table 1** Baseline demographics and characteristics

| Characteristics | |
|---|---|
| Patients treated | 49 |
| Age, years | |
| Median | 61 |
| Range | 32–78 |
| Sex | |
| Male | 42 |
| Female | 7 |
| Race | |
| White non-Hispanic | 44 |
| White Hispanic | 1 |
| Black non-Hispanic | 3 |
| Black Hispanic | 1 |
| KPS* | |
| Median | 90 |
| Range | 70–90 |
| No. prior chemotherapy regimens | |
| Median | 2 |
| Range | 0–8 |
| Primary tumor site | |
| Prostate | 16 |
| Melanoma | 12 |
| Head/Neck | 7 |
| Renal | 4 |
| Bladder | 3 |
| Lung | 3 |
| Breast | 2 |
| Other | 2 |

* Karnofsky Performance Status; available on 48 patients

at 9 weeks due to rapid progression of disease. The dose escalation scheme as well as the number of patients treated at each dose level is shown in Table 2. The mean number of cycles was 4 (range 1–13).

## Safety and tolerability

Toxicities observed during cycle 1 that were probably or definitely attributable to the study drugs are listed in Table 3. The most common grade 3 and 4 drug-related toxicities in cycle 1 included leukopenia (22 and 8%, respectively) and neutropenia (20 and 16%, respectively). Three patients developed dose-limiting toxicities in cycle 1, including 2 episodes of neutropenic fever (1 patient each on dose levels 6 and 9) and 1 episode of junctional bradycardia (dose level 11). These dose levels were thus expanded to 6 patients each. One patient enrolled in dose level 11 (docetaxel 75 mg/m$^2$ and 17-AAG 650 mg/m$^2$) developed third-degree AV block associated with asymptomatic bradycardia (HR of 43 bpm) immediately posttreatment on day 1 of cycle 1. The

**Table 2** Dose escalation scheme

| Dose level | Docetaxel (mg/m$^2$) | 17-AAG (mg/m$^2$) | No. of patients | No. of cycles | |
|---|---|---|---|---|---|
| | | | | Median | Range |
| 1 | 55 | 80 | 3 | 3 | 3 |
| 2 | 70 | 80 | 3 | 6 | 2–6 |
| 3 | 70 | 110 | 3 | 5 | 2–5 |
| 4 | 70 | 160 | 3 | 3 | 3 |
| 5 | 70 | 220 | 3 | 2 | 2–3 |
| 6 | 70 | 300 | 6 | 3 | 1–9 |
| 7 | 70 | 375 | 3 | 6 | 2–11 |
| 8* | 70 | 450 | 4 | 9 | 3–9 |
| 9 | 75 | 450 | 6 | 2.5 | 1–6 |
| 10 | 75 | 540 | 3 | 3 | 3–6 |
| 11 | 75 | 650 | 6 | 2 | 1–4 |
| 12 | 70 | 500 | 6 | 3.5 | 3–13 |

* One patient enrolled on dose level 8 was replaced as the patient was non-evaluable for toxicity, as described in the text

heart block and bradycardia resolved spontaneously. This event was classified as a DLT and the patient was not rechallenged with 17-AAG; however, subsequent treatment with single-agent docetaxel occurred without incident. One patient enrolled in cohort 8 was not evaluable for liver toxicity. This patient had a normal AST and grade 1 ALT elevation at the time of screening evaluation 10 days prior to first treatment, but on day 1 had asymptomatic Grade 4 AST and ALT elevations pretreatment. As these abnormalities were present pretreatment, they were not drug related. This patient tolerated treatment well and the liver function abnormalities resolved by day 40. The patient was later rechallenged with the study drugs and in total received 9 cycles of 17-AAG and docetaxel without further Grade 2 or greater transaminitis. During cycle 1, 6 patients experienced grade 3 hyperglycemia, 3 patients experienced grade 3 hyponatremia, and 1 patient grade 4 hypophosphatemia. These events were not associated with clinical symptoms and were not thought to be related to 17-AAG therapy.

Table 4 lists the grade-specific frequency of any toxicities that occurred with grade 3 or 4 severity as well as any grade 1 and 2 toxicities which occurred at a frequency of 25% or greater, regardless of causality. As expected with a docetaxel-based combination regimen, cytopenias were frequently observed, including grade 3 leukopenia and lymphopenia (41% each), as well as grade 4 neutropenia (47%). Two patients developed delayed grade 3 or greater transaminitis. In one instance, grade 4 elevation of bilirubin, AST, and ALT occurred following cycle 4 in a patient with colon cancer treated at the highest dose level (dose level 11: 17-AAG 650 mg/m$^2$). These liver abnormalities were associated with biliary duct obstruction secondary to disease progression and thus were unlikely to be drug

**Table 3** Drug-related toxicities during cycle 1 of therapy, in 49 evaluable patients

| Toxicity, *n* (%) | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|---|
| Hematologic | | | | |
| Lymphopenia | 0 | 0 | 7 (14) | 0 |
| Leukocytes | 11 (22) | 16 (33) | 11 (22) | 4 (8) |
| Neutrophils/granulocytes | 5 (10) | 10 (20) | 10 (20) | 8 (16) |
| Platelets | 2 (4) | 0 | 0 | 0 |
| Gastrointestinal | | | | |
| Nausea | 2 (4) | 0 | 0 | 0 |
| Vomiting | 1 (2) | 0 | 0 | 0 |
| Constipation | 1 (2) | 0 | 0 | 0 |
| Anorexia | 1 (2) | 0 | 0 | 0 |
| Hepatic | | | | |
| SGOT (AST) | 12 (24) | 3 (6) | 0 | 0 |
| SGPT (ALT) | 9 (18) | 0 | 0 | 0 |
| Alkaline phosphatase | 1 (2) | 0 | 0 | 0 |
| Cardiovascular | | | | |
| Nodal/junctional arrhythmia* | 0 | 1 (2) | 0 | 0 |
| Infection | | | | |
| Febrile neutropenia* | 0 | 0 | 2 (4) | 0 |
| Neurologic | | | | |
| Insomnia | 1 (2) | 0 | 0 | 0 |
| Constitutional symptoms | | | | |
| Fatigue | 2 (4) | 0 | 0 | 0 |
| Fever | 1 (2) | 0 | 0 | 0 |
| Other | 2 (4) | 0 | 0 | 0 |

* Dose-limiting toxicity

related. The second patient (dose level 9: 17-AAG 450 mg/m$^2$) developed grade 3 nausea, dehydration, and AST elevation following cycle 4. This patient also developed grade 3 neutropenic fever. As a result of these adverse events, the patient was dose-reduced and rechallenged with the combination and went on to receive 2 additional cycles of treatment which were tolerated well.

As preclinical studies suggested a role for Hsp90 in the maturation of the hERG cardiac potassium channel [24], QTc was assessed pre and posttreatment. The mean QTc interval for all patients was 425 ms; mean posttreatment QTc interval was 428 ms. No patient had a QTc interval posttreatment of greater than 500 ms. PR interval prolongation was observed in several patients. The mean pretreatment PR interval was 158 ms; mean posttreatment PR interval was 174 ms. Six patients on the study developed first-degree AV block posttreatment. Two additional patients had preexisting first-degree AV block. Further, as described previously, 1 patient enrolled on dose level 11 (docetaxel 75 mg/m$^2$ and 17-AAG 650 mg/m$^2$) developed asymptomatic junctional bradycardia immediately posttreatment on day 1 of cycle 1 which resolved spontaneously.

An intolerance of the odor related to the DMSO-based formulation of 17-AAG, with associated nausea and vomiting at higher dose levels, ultimately led to discontinuation of this trial in favor of alternative formulations of 17-AAG currently in development.

## Antitumor activity

Of 46 evaluable patients, 1 patient with non-small cell lung cancer had a partial response to therapy. Nineteen patients (41%) experienced stable disease lasting 3 cycles: 11 (24%) experienced stable disease for at least 6 cycles, and 4 (9%, all melanoma and prostate cancer) having stable disease lasting 9 cycles or greater.

In patients with prostate cancer ($n = 16$), 4 patients exhibited a PSA decline of 20% or greater, and 2 of these patients had received prior docetaxel treatment. The patient who experienced the greatest PSA response had received 1 dose of the study drugs, with subsequent removal from the study due to febrile neutropenia. At 3 weeks postdrug administration, the patient had a 69.5% decline in PSA (baseline 13.73, nadir 3.19). This patient was taxane-naïve.

## Pharmacokinetics

The pharmacokinetics of 17-AAG, its active metabolite 17-AG, and docetaxel were examined during cycle 1. Estimates of pharmacologic parameters grouped by dose level are listed in

**Table 4** All grade 3/4 toxicities, and any grade 1/2 toxicity present in >25% patients, regardless of causality, during all cycles

| Toxicity, *n* (%) | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|---|
| Hematologic | | | | |
| Hemoglobin (Hgb) | 23 (47) | 13 (27) | 9 (18) | 0 |
| Transfusion pRBCs | 0 | 0 | 2 (4) | 0 |
| Leukocytes | 6 (12) | 11 (22) | 20 (41) | 8 (16) |
| Lymphopenia | 0 | 0 | 20 (41) | 0 |
| Neutrophils/granulocytes | 5 (10) | 9 (18) | 9 (18) | 23 (47) |
| Coagulation | | | | |
| PT | 24 (49) | 0 | 1 (2) | 0 |
| PTT | 19 (39) | 2 (4) | 0 | 0 |
| Endocrine | | | | |
| Hyperglycemia | 13 (27) | 22 (45) | 11 (22) | 2 (4) |
| Hypoglycemia | 3 (6) | 0 | 0 | 1 (2) |
| Infection | | | | |
| Febrile neutropenia | 0 | 0 | 2 (4) | 0 |
| Infection without neutropenia | 2 (4) | 2 (4) | 2 (4) | 0 |
| Infection with Grade 3/4 neutropenia | 0 | 0 | 4 (8) | 1 (2) |
| Gastrointestinal | | | | |
| Anorexia | 22 (45) | 0 | 0 | 0 |
| Dehydration | 0 | 0 | 1 (2) | 0 |
| Dysphagia | 5 (10) | 1 (2) | 1 (2) | 0 |
| Nausea | 19 (39) | 2 (4) | 1 (2) | 0 |
| Vomiting | 13 (27) | 0 | 1 (2) | 0 |
| Diarrhea | 21 (43) | 3 (6) | 0 | 0 |
| Constipation | 17 (35) | 6 (12) | 0 | 0 |
| Abdominal pain | 11 (22) | 1 (2) | 1 (2) | 0 |
| Other | 4 (8) | 0 | 2 (4) | 0 |
| Cardiovascular | | | | |
| Chest pain | 1 (2) | 0 | 1 (2) | 0 |
| Supraventricular tachycardia | 0 | 1 (2) | 1 (2) | 0 |
| Thrombosis | 0 | 0 | 1 (2) | 0 |
| Metabolic/Laboratory | | | | |
| Hyperkalemia | 8 (16) | 4 (8) | 4 (8) | 0 |
| Hypokalemia | 4 (8) | 0 | 1 (2) | 0 |
| Hyponatremia | 24 (49) | 0 | 3 (6) | 0 |
| Hypocalcemia | 17 (35) | 4 (8) | 1 (2) | 0 |
| Hypophosphatemia | 1 (2) | 9 (18) | 7 (14) | 2 (4) |
| Hyperuricemia | 0 | 0 | 0 | 1 (2) |
| Alkaline phosphatase | 23 (47) | 4 (8) | 2 (4) | 0 |
| Bilirubin | 10 (20) | 4 (8) | 1 (2) | 1 (2) |
| AST | 15 (31) | 4 (8) | 2 (4) | 1 (2) |
| ALT | 19 (39) | 3 (6) | 1 (2) | 1 (2) |
| Hypoalbuminemia | 27 (55) | 4 (8) | 0 | 0 |
| Renal/Genitourinary | | | | |
| Creatinine | 16 (33) | 0 | 0 | 1 (2) |
| Urinary frequency | 24 (49) | 0 | 0 | 0 |
| Pulmonary | | | | |
| Dyspnea | 0 | 18 (37) | 1 (2) | 1 (2) |
| Pneumonitis | 0 | 0 | 0 | 1 (2) |
| Musculoskeletal | | | | |
| Myalgias | 18 (37) | 0 | 0 | 0 |
| Arthralgia | 9 (18) | 1 (2) | 1 (2) | 0 |
| Bone pain | 6 (12) | 0 | 1 (2) | 0 |
| Neurologic | | | | |
| Neuropathy–sensory | 16 (33) | 5 (10) | 1 (2) | 0 |
| Other | 0 | 0 | 1 (2) | 0 |
| Constitutional symptoms | | | | |
| Fatigue | 33 (67) | 13 (26) | 0 | 0 |
| Weight loss | 23 (47) | 4 (8) | 0 | 0 |
| Other | 5 (10) | 1 (2) | 0 | 1 (2) |
| Pain | | | | |
| Headache | 9 (18) | 1 (2) | 1 (2) | 0 |
| Tumor pain | 11 (22) | 3 (6) | 2 (4) | 0 |
| Other | 6 (12) | 2 (4) | 1 (2) | 0 |

Table 5. Peak plasma concentrations of both 17-AAG and 17-AG were greater than those required for antitumor effects in preclinical models. At the 450 mg/m$^2$ dose level ($n = 9$), the mean Cmax was 15.9 ± 0.5 μmol/L, and the $t_{1/2}$ and clearance values for 17-AAG were 2.9 ± 0.1 h and 13.3 ± 0.4 L/h/m$^2$, respectively. A linear increase in 17-AAG Cmax was observed with increasing dose (Table 5), while clearance of docetaxel was inversely proportional with the 17-AAG dose (Fig. 1). 17-AG was detected at all dose levels. At the 220 mg/m$^2$ 17-AAG dose level, a mean peak 17-AG level of 1.4 ± 0.3 μmol/L was observed 1.7 h after initiation of the 17-AAG infusion, and the mean $t_{1/2}$ was 5.9 ± 0.6 h (Table 5).

### Lymphocyte studies

To assess for inhibition of Hsp90, PBMCs were collected pretreatment and following drug administration. As has been reported with single-agent administration of 17-AAG [3], increased expression of Hsp70 was observed in the majority of patients treated at or above the 110 mg/m$^2$ dose level. Representative immunoblots from 4 patients treated at the docetaxel 75 mg/m$^2$ and 17-AAG 450 mg/m$^2$ dose level are shown in Fig. 2. A consistent pattern of changes in Raf1 or Akt expression was not observed.

## Discussion

This phase I trial was initiated to determine the maximal tolerated dose of the Hsp90 inhibitor 17-AAG that could be

**Table 5** Pharmacokinetic parameters

| Dose level | *n* | 17-AAG Dose (mg/m$^2$) | Docetaxel Dose (mg/m$^2$) | 17-AAG $C_{max}$ (μmol/L) | 17-AG $C_{max}$ (μmol/L) | 17-AAG AUC (μmol/L h) | 17-AG AUC (μmol/L h) | 17-AAG $t_{1/2}$ (h) | 17-AG $t_{1/2}$ (h) | 17-AAG CL (L/h/m$^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| *A. 17-AAG and 17-AG pharmacokinetic parameters as a function of 17-AAG dose (n = 41)* | | | | | | | | | | |
| 1, 2 | 5 | 80 | 55–70 | 3.1 ± 0.5 | 0.4 ± 0.0 | 7.5 ± 0.8 | 2.1 ± 0.3 | 2.3 ± 0.1 | 5.0 ± 0.6 | 22.5 ± 2.1 |
| 3 | 3 | 110 | 70 | 5.6 ± 1.6 | 1.5 ± 0.4 | 9.4 ± 1.0 | 3.5 ± 0.4 | 3.6 ± 0.5 | 3.8 ± 0.4 | 21.5 ± 2.6 |
| 4 | 2 | 160 | 70 | 2.9 ± 0.3 | 0.7 ± 0.1 | 9.6 ± 1.6 | 3.3 ± 0.6 | 2.2 ± 0.4 | 3.4 ± 1.0 | 30.1 ± 4.9 |
| 5 | 3 | 220 | 70 | 7.2 ± 0.7 | 1.4 ± 0.3 | 19.3 ± 0.9 | 10.1 ± 2.6 | 3.7 ± 0.4 | 5.9 ± 0.6 | 19.7 ± 1.0 |
| 6 | 6 | 300 | 70 | 12.9 ± 0.6 | 2.9 ± 0.2 | 36.3 ± 1.4 | 24.1 ± 1.4 | 7.7 ± 1.9 | 4.4 ± 0.2 | 14.9 ± 0.6 |
| 7 | 3 | 375 | 70 | 19.1 ± 0.7 | 7.0 ± 0.6 | 47.9 ± 2.7 | 69.8 ± 6.5 | 4.2 ± 0.3 | 6.9 ± 1.4 | 13.6 ± 0.7 |
| 8, 9 | 9 | 450 | 70–75 | 15.9 ± 0.5 | 5.3 ± 0.4 | 60.9 ± 2.4 | 53.9 ± 4.7 | 2.9 ± 0.1 | 5.6 ± 0.3 | 13.3 ± 0.4 |
| 10 | 2 | 540 | 75 | 20.6 ± 3.5 | 7.1 ± 1.1 | 68.3 ± 19.5 | 53.2 ± 15.4 | 2.1 ± 0.2 | 5.5 ± 1.9 | 16.2 ± 4.6 |
| 11 | 6 | 650 | 75 | 29.8 ± 1.2 | 12.3 ± 0.9 | 139.9 ± 11.2 | 172.5 ± 17.9 | 4.6 ± 0.9 | 5.6 ± 0.3 | 9.6 ± 0.7 |
| 12 | 2 | 500 | 70 | 15.1 ± 1.6 | 7.6 ± 3.1 | 62.3 ± 3.0 | 48.6 ± 15.8 | 3.1 ± 0.9 | 3.9 ± 0.4 | 13.8 ± 0.7 |

| Dosage (mg/m$^2$) | Patients (*n*) | $C_{max}$ (μmol/L) | $T_{1/2}$ (h) | AUC (μmol/L h) | CLtb (L/h/m$^2$) | CLtb (L/h) |
|---|---|---|---|---|---|---|
| *B. Docetaxel pharmacokinetic parameters as a function of docetaxel dose (n = 37)* | | | | | | |
| 70 | 23 | 2.5 | 24.2 | 3.3 | 28.1 | 55.2 |
| 75 | 14 | 3.3 | 22.5 | 4.4 | 24.1 | 51.1 |

Pharmacokinetic parameters are shown as value ± SD

*CLtb* Total Body Clearance

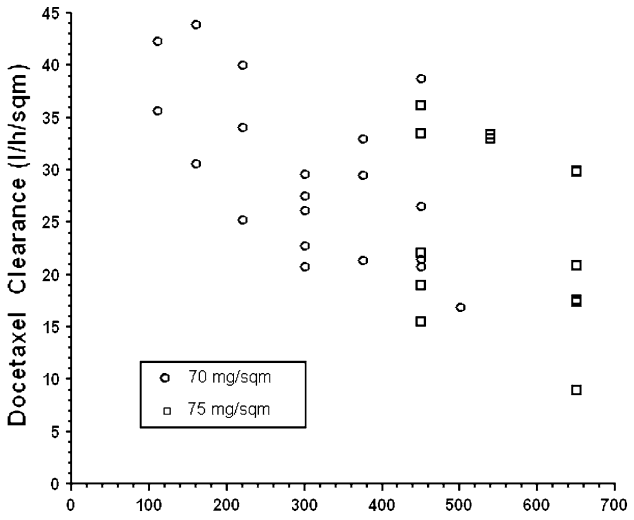


**Fig. 1** Effect of 17-AAG dose on docetaxel clearance (*n* = 33). Docetaxel pharmacokinetic data were not collected from patients receiving 80 mg/m$^2$ 17-AAG

coadministered with docetaxel when each agent was pulse dosed on an every 3 week schedule. Hsp90 is overexpressed in cancer cells, and Hsp90 inhibitors have been shown to be selectively toxic to tumor cells [25, 26]. These observations and the antitumor activity of 17-AAG in preclinical models prompted several groups, including our own, to initiate phase I clinical trials of 17-AAG in patients with advanced cancer [3–7]. Demonstrable single-agent clinical activity with Hsp90 inhibitors has been limited, however. In patients with solid tumors, the most compelling clinical data to date with Hsp90 inhibitor monotherapy have been observed in patients with HER2-amplified breast cancer and non-small cell lung cancer [27–30]. Due to evidence of synergy between 17-AAG and a broad range of cytotoxic agents, a logical iterative approach in the clinical development of this compound was to combine it with standard cytotoxics.

A 3-week interval dosing schedule was chosen because every 3 week docetaxel administration has been shown to be optimal for many solid tumors, and our preclinical data suggested that concurrent administration of 17-AAG only on those days on which the taxane was administered would maximize synergy between the two agents [13]. Using this schedule, we were able to safely and with minimal toxicity deliver doses of 17-AAG as high as 650 mg/m$^2$ in combination with the full standard dose of docetaxel to a population of heavily pretreated cancer patients. The antitumor responses noted with 17-AAG in breast cancer when administered weekly at a dose of 450 mg/m$^2$ [31] suggest that the dose levels achieved in this trial were likely sufficient to induce the degradation of at least some Hsp90 clients, such as HER2. As pre and posttreatment tumor biopsies were not incorporated into this study, however, the complement of Hsp90 clients degraded at the dose levels achieved remains unknown.

PBMCs were collected to assess the effects of docetaxel/17-AAG treatment on the expression of Hsp70, Akt, and Raf-1. While the posttreatment rise in Hsp70 levels in most

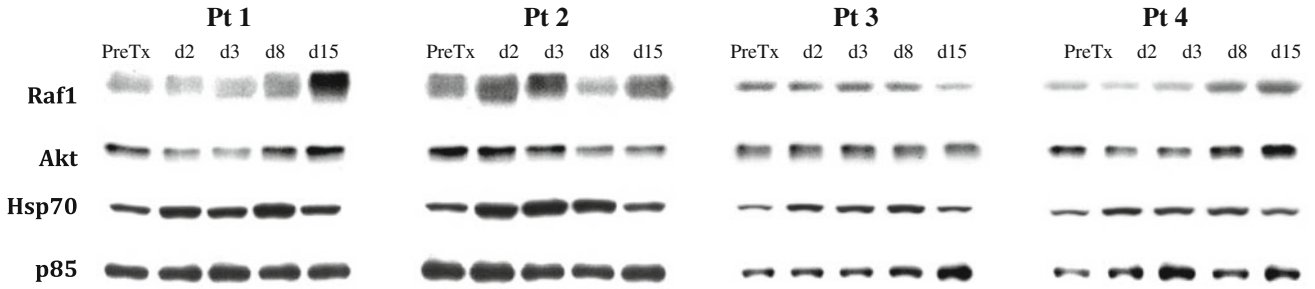


**Fig. 2** Immunoblot studies of peripheral blood mononuclear cells collected pretreatment and days 2, 3, 8, and 15 of cycle 1. The four representative patients shown were all treated at the docetaxel 75 mg/m$^2$ and 17-AAG 450 mg/m$^2$ dose level

patients suggests Hsp90 target modulation, a dose-dependent pattern of Akt and Raf-1 degradation was not observed. As recent studies have indicated that the affinity of 17-AAG for Hsp90 may vary between normal and tumor tissues [26], the utility of normal tissues for pharmacodynamic studies of Hsp90 inhibitors may be limited. Therefore, such studies do not prove that these clients were not degraded in the corresponding tumors and they should not be considered a substitute for the assessment of changes in the expression and activation of Hsp90 clients in tumor tissue.

An MTD was not defined for this study. Dose escalation was halted at the 650 mg/m$^2$ dose level because of cumulative toxicities that were attributable in part to the DMSO-based 17-AAG formulation. Specifically, for the majority of patients treated at this dose level, the odor associated with the DMSO formulation was the primary treatment-associated complaint. For this reason, and with the subsequent development of non-DMSO-based 17-AAG formulations, further dose escalation was not felt to be warranted.

Our results do suggest that docetaxel can be combined safely with an inhibitor of Hsp90 at doses above those sufficient to induce responses in patients with breast and lung cancers. On the basis of these results, a phase I trial of docetaxel and IPI-504 [32], an Hsp90 inhibitor that does not use a DMSO-based formulation, was proposed and is now ongoing.

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## References

1. Solit DB, Rosen N (2006) Hsp90: a novel target for cancer therapy. Curr Top Med Chem 6:1205–1214
2. Trepel J, Mollapour M, Giaccone G, Neckers L (2010) Targeting the dynamic HSP90 complex in cancer. Nat Rev Cancer 10:537–549
3. Banerji U, O'Donnell A, Scurr M, Pacey S, Stapleton S, Asad Y et al (2005) Phase I pharmacokinetic and pharmacodynamic study of 17-allylamino, 17-demethoxygeldanamycin in patients with advanced malignancies. J Clin Oncol 23:4152–4161
4. Goetz MP, Toft D, Reid J, Ames M, Stensgard B, Safgren S et al (2005) Phase I trial of 17-allylamino-17-demethoxygeldanamycin in patients with advanced cancer. J Clin Oncol 23:1078–1087
5. Grem JL, Morrison G, Guo XD, Agnew E, Takimoto CH, Thomas R et al (2005) Phase I and pharmacologic study of 17-(allylamino)-17-demethoxygeldanamycin in adult patients with solid tumors. J Clin Oncol 23:1885–1893
6. Ramanathan RK, Trump DL, Eiseman JL, Belani CP, Agarwala SS, Zuhowski EG et al (2005) Phase I pharmacokinetic-pharmacodynamic study of 17-(allylamino)-17-demethoxygeldanamycin (17AAG, NSC 330507), a novel inhibitor of heat shock protein 90, in patients with refractory advanced cancers. Clin Cancer Res 11:3385–3391
7. Solit DB, Ivy SP, Kopil C, Sikorski R, Morris MJ, Slovin SF et al (2007) Phase I trial of 17-allylamino-17-demethoxygeldanamycin in patients with advanced cancer. Clin Cancer Res 13:1775–1782
8. Stebbins CE, Russo AA, Schneider C, Rosen N, Hartl FU, Pavletich NP (1997) Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. Cell 89:239–250
9. Schulte TW, Neckers LM (1998) The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. Cancer Chemother Pharmacol 42:273–279
10. Mimnaugh EG, Chavany C, Neckers L (1996) Polyubiquitination and proteasomal degradation of the p185c-erbB-2 receptor protein-tyrosine kinase induced by geldanamycin. J Biol Chem 271:22796–22801
11. Sepp-Lorenzino L, Ma Z, Lebwohl DE, Vinitsky A, Rosen N (1995) Herbimycin A induces the 20 S proteasome- and ubiquitin-dependent degradation of receptor tyrosine kinases. J Biol Chem 270:16580–16587
12. Solit DB, Zheng FF, Drobnjak M, Munster PN, Higgins B, Verbel D et al (2002) 17-Allylamino-17-demethoxygeldanamycin induces the degradation of androgen receptor and HER-2/neu and inhibits the growth of prostate cancer xenografts. Clin Cancer Res 8:986–993
13. Solit DB, Basso AD, Olshen AB, Scher HI, Rosen N (2003) Inhibition of heat shock protein 90 function down-regulates Akt kinase and sensitizes tumors to Taxol. Cancer Res 63:2139–2144
14. Basso AD, Solit DB, Chiosis G, Giri B, Tsichlis P, Rosen N (2002) Akt forms an intracellular complex with heat shock protein 90 (Hsp90) and Cdc37 and is destabilized by inhibitors of Hsp90 function. J Biol Chem 277:39858–39866
15. Schulte TW, Blagosklonny MV, Ingui C, Neckers L (1995) Disruption of the Raf-1-Hsp90 molecular complex results in destabilization of Raf-1 and loss of Raf-1-Ras association. J Biol Chem 270:24585–24588

16. Munster PN, Basso A, Solit D, Norton L, Rosen N (2001) Modulation of Hsp90 function by ansamycins sensitizes breast cancer cells to chemotherapy-induced apoptosis in an RB- and schedule-dependent manner. See: Sausville EA, Combining cytotoxics and 17-allylamino, 17-demethoxygeldanamycin: sequence and tumor biology matters. Clin Cancer Res 7:2155–2158, 2228–2236
17. Sain N, Krishnan B, Ormerod MG, De Rienzo A, Liu WM, Kaye SB et al (2006) Potentiation of paclitaxel activity by the HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin in human ovarian carcinoma cell lines with high levels of activated AKT. Mol Cancer Ther 5:1197–1208
18. Nguyen DM, Lorang D, Chen GA, Stewart JHt, Tabibi E, Schrump DS (2001) Enhancement of paclitaxel-mediated cytotoxicity in lung cancer cells by 17-allylamino geldanamycin: in vitro and in vivo analysis. Ann Thorac Surg 72:371–378; discussion 8–9
19. Nguyen DM, Chen A, Mixon A, Schrump DS (1999) Sequence-dependent enhancement of paclitaxel toxicity in non-small cell lung cancer by 17-allylamino 17-demethoxygeldanamycin. J Thorac Cardiovasc Surg 118:908–915
20. Egorin MJ, Zuhowski EG, Rosen DM, Sentz DL, Covey JM, Eiseman JL (2001) Plasma pharmacokinetics and tissue distribution of 17-(allylamino)-17-demethoxygeldanamycin (NSC 330507) in CD2F1 mice1. Cancer Chemother Pharmacol 47:291–302
21. Parise RA, Ramanathan RK, Zamboni WC, Egorin MJ (2003) Sensitive liquid chromatography-mass spectrometry assay for quantitation of docetaxel and paclitaxel in human plasma. J Chromatogr B Anal Technol Biomed Life Sci 783:231–236
22. Yeh KC, Kwan KC (1978) A comparison of numerical integrating algorithms by trapezoidal, Lagrange, and spline approximation. J Pharmacokinet Biopharm 6:79–98
23. Rocci ML Jr, Jusko WJ (1983) LAGRAN program for area and moments in pharmacokinetic analysis. Comput Programs Biomed 16:203–216
24. Ficker E, Dennis AT, Wang L, Brown AM (2003) Role of the cytosolic chaperones Hsp70 and Hsp90 in maturation of the cardiac potassium channel HERG. Circ Res 92:e87–e100
25. Vilenchik M, Solit D, Basso A, Huezo H, Lucas B, He H et al (2004) Targeting wide-range oncogenic transformation via PU24FCl, a specific inhibitor of tumor Hsp90. Chem Biol 11:787–797
26. Kamal A, Thao L, Sensintaffar J, Zhang L, Boehm MF, Fritz LC et al (2003) A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature 425:407–410
27. Xu W, Mimnaugh E, Rosser MF, Nicchitta C, Marcu M, Yarden Y et al (2001) Sensitivity of mature Erbb2 to geldanamycin is conferred by its kinase domain and is mediated by the chaperone protein Hsp90. J Biol Chem 276:3702–3708
28. Chandarlapaty S, Scaltriti M, Angelini P, Ye Q, Guzman M, Hudis CA et al (2010) Inhibitors of HSP90 block p95-HER2 signaling in Trastuzumab-resistant tumors and suppress their growth. Oncogene 29:325–334
29. Normant E, Paez G, West KA, Lim AR, Slocum KL, Tunkey C et al (2011) The Hsp90 inhibitor IPI-504 rapidly lowers EML4-ALK levels and induces tumor regression in ALK-driven NSCLC models. Oncogene 30:2581–2586
30. Sequist LV, Gettinger S, Senzer NN, Martins RG, Janne PA, Lilenbaum R et al (2010) Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. J Clin Oncol 28:4953–4960
31. Modi S, Stopeck A, Linden H, Solit D, Chandarlapaty S, Rosen N et al (2011) HSP90 Inhibition Is Effective in Breast Cancer: A Phase II Trial of Tanespimycin (17-AAG) Plus Trastuzumab in Patients with HER2-Positive Metastatic Breast Cancer Progressing on Trastuzumab. Clin Cancer Res 17:5132–5139
32. Sydor JR, Normant E, Pien CS, Porter JR, Ge J, Grenier L et al (2006) Development of 17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride (IPI-504), an anti-cancer agent directed against Hsp90. Proc Natl Acad Sci USA 103:17408–17413