Phase I Pharmacokinetic and Pharmacodynamic Study of 17-Allylamino, 17-Demethoxygeldanamycin in Patients With Advanced Malignancies

Udai Banerji, Anne O’Donnell, Michelle Seurr, Simon Pacey, Sarah Stapleton, Yasmin Asad, Laura Simmons, Alison Maloney, Florence Raynaud, Maeli Campbell, Michael Walton, Sunil Lakhani, Stanley Kaye, Paul Workman, and Ian Judson

ABSTRACT

Purpose
To study the toxicity and pharmacokinetic-pharmacodynamic profile of 17-allylamino, 17-demethoxygeldanamycin (17-AAG) and to recommend a dose for phase II trials.

Patients and Methods
This was a phase I study examining a once-weekly dosing schedule of 17-AAG. Thirty patients with advanced malignancies were treated.

Results
The highest dose level reached was 450 mg/m²/week. The dose-limiting toxicities (DLTs) encountered were grade 3 diarrhea in three patients (one at 320 mg/m²/week and two at 450 mg/m²/week) and grade 3 to 4 hepatotoxicity (AST/ALT) in one patient at 450 mg/m²/week. Two of nine DLTs were at the highest dose level. Two patients with metastatic melanoma had stable disease and were treated for 15 and 41 months, respectively. The dose versus area under the curve–relationship for 17-AAG was linear ($r^2 = 0.71$) over the dose range 10 to 450 mg/m²/week, with peak plasma concentrations of 8,998 μg/L (standard deviation, 2,881) at the highest dose level. After the demonstration of pharmacodynamic changes in peripheral blood leukocytes, pre- and 24 hours post-treatment, tumor biopsies were performed and demonstrated target inhibition (c-RAF-1 inhibition in four of six patients, CDK4 depletion in eight of nine patients and HSP70 induction in eight of nine patients) at the dose levels 320 and 450 mg/m²/week. It was not possible to reproducibly demonstrate these changes in biopsies taken 5 days after treatment.

Conclusion
It has been possible to demonstrate that 17-AAG exhibits a tolerable toxicity profile with therapeutic plasma concentrations and target inhibition for 24 hours after treatment and some indications of clinical activity at the dose level 450 mg/m²/week. We recommend this dose for phase II clinical trials.

J Clin Oncol 23:4152-4161. © 2005 by American Society of Clinical Oncology

INTRODUCTION

Heat shock protein 90 (HSP90) is a highly conserved molecular chaperone that plays an important role in regulating post-translational folding, stability, and function of its client proteins.¹ Client proteins important to cancer include steroid hormone receptors (progesterone receptor,² androgen receptor³), protein kinases (ERB-B2,⁴ BCR-ABL,⁵ c-RAF-1,⁶ AKT/PKB,⁷ and CDK4⁸), mutant p53⁹ and telomerase h-TERT.¹⁰ Stabilization and regulation of client proteins by HSP90 is dependent on binding and hydrolysis of ATP at the N-terminus,¹¹ and inhibition of this activity leads to degradation of client proteins by the ubiquitin-proteasome pathway.¹²
Phase I Trial of the HSP90 Inhibitor 17-AAG

Benzoquinone ansamycins, such as geldanamycin and 17-allylaminio, 17-demethoxygeldanamycin (17-AAG), compete with ATP for binding to the N-terminus of HSP90.13 Geldanamycin showed promising preclinical activity in vitro, but was found to be hepatotoxic in animal models and could not be tested in humans.14 17-AAG is a structurally similar analog with a better toxicity profile and is the first HSP90 inhibitor to enter clinical trials.15 17-AAG has shown a promising preclinical profile with a mean growth inhibitory IC50 of approximately 120 nmol/L in the National Cancer Institute (NCI) 60 human tumor cell line panel. It induces apoptosis and cell cycle arrest in cancer cells16 and has shown activity in a variety of xenograft models.17-19

The primary aims of this phase I study were to determine the toxicity, pharmacokinetic profile, and pharmacodynamic effects of 17-AAG. The secondary end point was to assess disease response. The phase I trial was conducted with the support of the Cancer Research UK Drug Development Office (London, United Kingdom), and 17-AAG (NSC330507) was supplied by the NCI (National Institutes of Health, Bethesda, MD).

PATIENTS AND METHODS

Trial Conduct
The trial was conducted under the auspices of Cancer Research UK at the Royal Marsden Hospital (Sutton). The protocol was reviewed by the Cancer Research UK protocol review committee and the clinical research and ethics committees of the Royal Marsden Hospital. Patients signed an informed consent for entry into the study. Separate consent forms were signed before pre- and post-treatment biopsies at higher dose levels.

Inclusion and Exclusion Criteria
The trial included patients with histologically proven malignancy refractory to conventional treatment who had not had chemotherapy or hormonal therapy in the last 4 weeks. Patients needed to be 18 years of age or older, have a WHO performance status of 0 to 2, and a life expectancy of at least 12 weeks. Adequate organ function included a hemoglobin ≥ 10 mg/dL, absolute neutrophil count ≥ 1.5 × 109/L, platelets ≥ 100 × 109/L, plasma creatinine < 130 mmols/L or glomerular filtration rate assessed by ethylene diamine tetraacetic acid clearance > 60 mL/min, plasma bilirubin < 17 umol/L and a normal AST/ALT or an AST/ALT 2.5 × upper limit of normal if there were known liver metastases. Exclusion criteria included pregnancy or lactation in women, a myocardial infarction in the last 6 months, abnormal ECG with a left ventricular ejection fraction less than 45%, and history of an egg allergy.

Toxicity
Toxicity was monitored by NCI Common Toxicity Criteria version 2. Definitions of dose-limiting toxicities (DLTs) included a grade 4 neutropenia and/or febrile neutropenia, grade 4 thrombocytopenia or grade 3 thrombocytopenia with fever, grade 3 or 4 nonhematologic toxicity excluding grade 3 nausea and/or vomiting, or grade 3 asymptomatic reversible rise in liver transaminases (AST/ALT).

Dose Expansion
The starting dose, 10 mg/m2, was determined by one tenth of the dose lethal to 10% of animals (LD10) in mice. Three patients would be included in each cohort and the dose doubled until there was a drug-related grade 1 renal or cardiac toxicity, or two episodes of other grade 2 nonhematologic toxicities following which 40% dose increments would be employed. If a DLT was observed, the cohort would be expanded to six patients. The maximum-tolerated dose (MTD) would be defined when two or more of six patients at a dose level experienced DLTs.

Characterization of Response
Tumor assessments were performed before treatment and every 8 weeks thereafter. Responses in target lesions were assessed by the WHO criteria.

Drug Administration
17-AAG and the egg phospholipid diluent were supplied by the NCI. The final concentration for intravenous use was 1 mg/mL 17-AAG in 4% dimethyl sulfoxide (DMSO) and 5% dextrose, and the drug was administered over 15 to 200 minutes. 17-AAG was administered once a week without a break. One cycle of chemotherapy was defined as 4 weeks.

Pharmacokinetic Sampling and Analysis
Blood samples were drawn predose, at infusion end, and then at predetermined intervals for 24 hours at the starting dose and up to 48 hours at the dose level 160 mg/m2/week and above. 17-AAG, its metabolite 17-AG (17-amino, 17-demethoxygeldanamycin) and the internal standard α-naphthoflavone were extracted from 500 µL human plasma using solid phase extraction. Extracts were dried and redissolved in 150 µL 50% acetonitrile/water. Analysis was carried out by high-performance liquid chromatography (HPLC) with UV detection at 330 nm on a Thermosynchronizations system (Thermo Electron, San Jose, CA). HPLC was performed using a Supelcosil μmum 150 × 4.6 mm internal diam- eter LC-18 column with a Supelguard LC-18 (Supelco; Sigma-Aldrich, Dorset, United Kingdom) and a gradient mobile phase containing water and acetonitrile. The assay was linear over the range 10 to 2,400 ng/mL with quality controls (n = 2) analyzed at 20, 200, 800, and 1,600 ng/mL. Acceptance criteria were estab- lished according to published guidelines.20

Pharmacodynamic Analysis
Procedures were based on validated molecular markers.19,21 Peripheral blood leukocytes (PBLs) were sampled for varying pe- riods (24 to 96 hours) at dose levels of 10 to 450 mg/m2/week. Tumor biopsies were performed before and 1 to 5 days after treatment in 12 patients at dose levels of 320 to 450 mg/m2/week. Tumor samples were obtained by excision biopsy in 6 patients who had superficial skin tumors and by ultrasound-guided core biopsies (16 G Tru-Cut needle; UK Medical, Sheffield, UK) in five patients who had visceral metastasis. Tumor cells were obtained in one patient with ascites by centrifugation (5,000 g for 5 minutes). The samples were arbitrarily divided in two sections (approximately 20% and 80%) within 1 minute of performing the biopsies or pipetting the supernatant in the case of the ascitic fluid sample. The larger section was immediately flash frozen in liquid nitrogen while the smaller section fixed in buffered 10% formalin. Whole blood was collected in heparinized tubes (Vacutainer, BD, Franklin Lakes, NJ), diluted 1:1 with 0.9% saline, after which it was layered over ficoll (Lymphoprep, Neegeard, Norway) and centrifuged at 400 xg for 30 minutes. The
lymphocytes were then isolated from the plasma-ficol interface and resuspended in 0.9% NaCl. Cell pellets were obtained by centrifugation (600 xg) and flash frozen in liquid nitrogen.

**Western Blotting**

Cell pellets were lysed in lysis buffer (0.1% Nonidet P-40), 50 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (pH, 7.4), 250 mmol/L NaCl, 1 mmol/L phenylmethylsulfonyl fluoride, 10 µg/mL aprotinin, 20µg/mL leupeptin, 1 mmol/L dithiothreitol, 1 mmol/L EDTA, 1 mmol/L NaF, 10 mmol/L β-glycerophosphate, and 0.1 mmol/L sodium orthovanadate. Samples (50 µg protein) were denatured in Laemmli loading buffer (10% glycerol, 5% beta mercaptoethanol, 2% sodium dodecyl sulfate, 62.5 mmol/L Tris [pH, 6.8] and 0.05% bromophenol blue), resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and transferred onto 0.45 µm polyvinylidene fluoride membranes (Millipore Corp, Billerica, MA). All products in lysis and loading buffer were obtained from Sigma, Poole, United Kingdom. Membranes were blocked in casein blocking buffer (0.5% casein, 150 mmol/L NaCl, 10 mmol/L Tris base, 0.25 mmol/L thimerosal). They were then probed with primary antibodies c-RAF-1 (SC-133, 1:500; Santa Cruz Biotechnology, Santa Cruz, CA), HSP70 (SPA-810, 1:2,000; Stressgen, Biotechnologies, Victoria, Canada), LCK (06-583, 1:5,000; Upstate Biotechnology, Lake Placid, NY), CDK4 (SC-260, 1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; MAB-374, 1:5,000; Chemicon International Inc, Temecula, CA). Visualization of the bound primary antibody was performed by probing with horseradish peroxidase conjugate secondary antibodies (1:1,000; Amersham Biosciences, Buckinghamshire, United Kingdom). A semi-quantitative analysis of the protein expression in Western blots was carried out using Image Quant 5 (Molecular Dynamics, Amersham Biosciences, Sunny Vale, CA). Levels of protein expression were normalized to GAPDH and a 1.5-fold change in treatment over control was arbitrarily considered as a significant change. Statistical analysis was carried out using Graph Pad Prism version 3 (San Diego, CA).

**Immunohistochemistry**

After tumor biopsy, approximately 20% of the tissue was fixed in 10% buffered formalin. It was possible to detect tumor tissue in 22 of 26 samples on hematoxylin and eosin stained sections. For immunohistologic analysis, paraffin-embedded sections were deparaffinized with HistoClear (National Diagnostics, Hull, United Kingdom) and rehydrated through graded alcohols. Endogenous peroxidase was quenched with 3% hydrogen peroxide–methodanol mixture following which sections were rinsed in phosphate buffered saline-Tween (PBST) and preincubated with 2% blocking serum. Sections were then incubated with HSP70 antibody at 1:500 dilution (SPA-810, Stressgen, Biotechnologies, Victoria, Canada) for 1 hour, rinsed in PBST, and then incubated at a 1:100 ratio with secondary biotinylated horse antimouse antibody (Vector Laboratories, Burlingame, CA). Tissue sections were rinsed in PBST and then incubated with avidin-biotin complex. Sections were further rinsed in PBST and incubated with diamobenzene substrate (Sigma). Tissues were finally rinsed in PBST and water and counterstained with Mayer’s hematoxylin (Sigma) before dehydrating in graded ethanol, clearing in HistoClear (National Diagnostics) and mounting with glass cover slips using DePEx (BDH; Poole).

**RESULTS**

**Demographics**

Thirty-one patients were recruited into the study, of whom 30 received treatment (one patient experienced disease progression between registration and planned first treatment dose). The male-female ratio was 13:17 and the median age was 51 years (range, 27 to 66). Patients with a wide variety of solid tumors were accrued. Patients had a performance status of 0 to 2 (Table 1).

**Dose Escalation**

The starting dose was 10 mg/m² and was doubled to 20, 40 and 80, 160, and 320 mg/m²/week (three patients per cohort). At the dose level 320 mg/m²/week, after three patients had been recruited, there were pharmacodynamic (PD) changes in PBLs and plasma concentrations of 17-AAG consistent with antiproliferative activity in preclinical models. It was therefore decided to expand the cohort to six patients with a view to performing pre- and post-treatment biopsies. There was one DLT (grade 3 diarrhea) in the expanded cohort. Because of a DLT in one of six patients at the dose level 320 mg/m²/week, the next dose was escalated by 40% to 450 mg/m²/week. One patient in the first cohort of three patients at 450 mg/m²/week experienced DLTs (grade 3 diarrhea, grade 3 ALT elevation and grade 4 AST elevation) and the dose level was expanded to 6 patients in whom no further DLTs were observed. Three additional patients were recruited at the dose level 450 mg/m²/week to further characterize pharmacodynamic changes. One
additional DLT (grade 3 diarrhea) occurred in this cohort, taking the number of patients experiencing DLTs at 450 mg/m²/week to two of nine. Although the MTD was not reached, further dose escalation was not attempted for the following reasons: pharmacokinetic (PK) and PD targets had been met; the volume of infusate reached 660 to 990 mL with infusion times ranging from 15 to 200 minutes/week; and the volume of DMSO delivered each week was 25 to 40 mL.

**Toxicity**

Toxicities during the first cycle at all dose levels are summarized in Table 2. Grade 3 vomiting was first noted at the dose level 80 mg/m²/week; however, because reversible grade 3 nausea and vomiting were not considered DLTs in the protocol, this did not necessitate dose expansion. Patients were treated with prophylactic granisetron (1 mg administered intravenously) above this dose level; however, one patient each at dose levels 320 and 450 mg/m²/week developed grade 3 vomiting. Among other significant GI toxicities, grade 3 diarrhea was noted in three patients (one patient at 320 mg/m²/week and two patients at 450 mg/m²/week) and were classed as DLTs. Grade 3 or 4 hepatotoxicity (elevated AST and/or ALT) was noted in one patient at 450 mg/m²/week during the first cycle of chemotherapy. One additional patient developed grade 3 hepatotoxicity, but this was not considered a DLT because it was asymptomatic and occurred after 1 year of continuous dosing (it is accepted practice in phase I trials to consider toxicities occurring in the first cycle, in this case 4 weeks, as DLTs). Both patients received further doses of 17-AAG at reduced doses after the transaminitis had recovered. Among other significant toxicities, grade 3 and 2 hypersensitivity reactions were noted in three patients (two at 320 mg/m²/week and one at 450 mg/m²/week) during the first cycle of treatment. It was possible to control the reactions by the administration of parenteral antihistamine and steroids in all patients. It was possible to retreat the patients at slower infusion rates of 90 to 200 minutes. Grade 3 hypersensitivity reactions were not classified as DLTs following review by the institutional review board of Cancer Research UK because they were considered to be due to the egg phospholipid vehicle used to reconstitute the 17-AAG.

**Clinical Efficacy**

There were no documented complete or partial responses. Two patients with metastatic malignant melanoma achieved stable disease for prolonged periods of 15 and 35 months (confirmed by independent external review). One patient continues to receive treatment at the time of submission (total treatment duration to date, 41 months) and toxicity and radiologic assessment will continue.

**PK Analysis**

At the highest dose level, 450 mg/m²/week, plasma concentration > 120 nmol/L (the mean IC₅₀ of 17-AAG in the NCI 60 human tumor cell line panel) was maintained for approximately 24 hours (Fig 1). At this dose level, the mean volume of distribution was 142.6 L (standard deviation [SD], 60.4), mean clearance was 32.2 L/h (SD, 15.3), and the mean peak plasma level was 8,998 µg/L (SD, 2,881). There was a linear correlation between the dose and area under the curve (AUC) for 17-AAG at the dose levels studied (r² = 0.71; Fig 2A). Furthermore, there was also a linear correlation between the AUCs of 17-AAG and 17-AG over the dose levels studied (r² = .83; Fig 2C). The PK parameters for 17-AAG and the active metabolite 17-AG are summarized in Table 3. In the limited number of patients (n = 14) in whom repeat PK sampling was performed a week apart, there was no significant difference in the AUC of either 17-AAG or its metabolite 17-AG (data not shown).

**PD Analysis**

PD markers were studied in both tumor tissue and PBLs. The earliest PD changes demonstrable were an induction of the cochaperone HSP70 in PBLs at 6 hours when patients were treated with 17-AAG at a dose of 80 mg/m²/week (data not shown). At the highest dose level, 450 mg/m²/week, PBLs were sampled in two patients for 96 hours (Fig 3). c-RAF-1 depletion, HSP70 induction, LCK and...
CDK4 depletion was seen in two of two, two of two, two of two and one of two patients, respectively. PBLs were also sampled in four patients for 48 hours at the dose level 320 mg/m²/week. c-RAF-1 depletion, HSP70 induction, and LCK and CDK4 depletion was seen in three of three informative cases (c-RAF-1 undetectable in one patient), three of four, zero of four, and zero of four cases, respectively.

Tumor biopsies were performed at dose levels of 320 to 450 mg/m²/week, because at these doses it was possible to achieve plasma concentrations of drug known to have activity in preclinical models and demonstrate reproducible changes in PD markers in PBLs. Twenty-four hours after infusion of 17-AAG at doses of 320 to 450 mg/m²/week, induction of the cochaperone HSP70 was demonstrated in eight of nine patients, as well as the depletion of the client proteins CDK4 from tumor biopsies in eight of nine patients. RAF-1 was detectable in the tumors of six patients biopsied at 24 hours, and it was possible to demonstrate depletion in four of six patients (Fig 4). The semi-quantitative analysis of Western blots of patients biopsied 24 hours after treatment is shown in Figure 5. In order to evaluate the duration of target inhibition, tumor biopsies were performed 5 days after treatment in four patients treated at a dose of 450 mg/m²/week. c-RAF-1 was expressed in the tumors of two of these patients and depletion was demonstrated in one. In addition CDK4 depletion and HSP70 induction were seen in two of four and zero of four patients respectively (Fig 4). In the one patient who had a tumor biopsy performed 3 days after treatment, c-RAF and CDK4 depletion was demonstrated.

In three patients (patients 20, 23, and 25) in whom PBLs and tumors were both sampled, c-RAF-1 depletion was seen in all PBLs and in two of two cases where it was expressed in the tumor. CDK4 depletion was seen in both PBLs and tumors in all three patients. LCK was not studied in tumors. HSP70 induction was seen in both PBLs and tumors in all three cases.
Immunohistochemical staining for HSP70 was performed in selected tumor samples. Figure 6 shows two cases in which increased intracellular expression of HSP70 was shown in melanoma tumor biopsies.

DISCUSSION

The present phase I trial of 17-AAG explored toxicity, PK and PD profiles of 17-AAG in a once-a-week schedule. Dose-limiting GI toxicities not amounting to a MTD were observed. Potentially therapeutic plasma drug concentrations were achieved. Molecular signatures indicative of target inhibition were demonstrable in tumor. Evidence of potential antitumor activity was seen. Thirty patients were treated at seven dose levels. Patients with a wide variety tumor of types were recruited. However, due to easy accessibility of tissue for biopsy, a significant number of melanoma patients were included (Table 2).

GI toxicities including nausea, vomiting, and diarrhea were grade 3 toxicities observed during the trial. The formulation of 17-AAG contains 4% DMSO and at higher doses (ie, 160 to 450 mg/m²/week), the amount of DMSO administered varied from 10 to 40 mL in a single infusion. Although higher doses of DMSO have been administered safely, GI toxicities, mentioned above, are known adverse effects of DMSG22 and could have contributed to the nausea, vomiting, and possibly diarrhea seen in the trial. There was one DLT due to grade 3 to 4 hepatotoxicity at 450 mg/m²/week, and this patient had a plasma AUC higher than other patients and went on to receive 17-AAG at 320 mg/m²/week without further hepatotoxicity. There were 2 cases of grade 3 hypersensitivity reactions documented during the trial. All patients responded to parenteral antihistamines, steroids and reduction in the rate of infusion. The grade 3 toxicities were not classified as DLTs because the egg phospholipid diluent and not 17-AAG itself was thought to be the contributing factor. The problem of the diluent causing hypersensitivity reactions has been encountered in phase I trials before (eg, cremophor used in the formulation of taxanes23,24) and such reactions, if controllable, have not been clasied as DLTs. Finally, though not formally studied, the odor of DMSO persisted in patients’ secretions for variable lengths of time and had a negative impact on quality of life.

Table 3. Summary of Pharmacokinetic Parameters of 17-AAG and Its Metabolite 17-AG

<table>
<thead>
<tr>
<th>Drug/Metabolite</th>
<th>Dose (mg/m²/week)</th>
<th>T_max (Hr)</th>
<th>C_max (µg · L⁻¹)</th>
<th>AUC (µg · hr · L⁻¹)</th>
<th>Clearance (L · hr⁻¹)</th>
<th>VD_ss (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>17-AAG</td>
<td>10 0.4 0.1</td>
<td>315.7 115.1</td>
<td>311.0 72.6</td>
<td>51.1 5.3</td>
<td>173.7 40.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 0.4 0.1</td>
<td>713.4 273.0</td>
<td>718.4 165.7</td>
<td>51.9 5.9</td>
<td>162.5 36.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 0.3 0.1</td>
<td>1,363.2 347.3</td>
<td>1,741.2 442.3</td>
<td>43.0 17.0</td>
<td>120.9 33.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80 0.6 0.3</td>
<td>1,899.4 446.1</td>
<td>2,292.0 153.7</td>
<td>58.6 1.2</td>
<td>108.9 23.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>160 0.8 0.3</td>
<td>4,003.2 1,132</td>
<td>7,979.3 5,312.2</td>
<td>39.5 20.6</td>
<td>82.2 14.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>320 1.4 1.0</td>
<td>6,544.4 1,407.2</td>
<td>20,836.6 10,631.9</td>
<td>33.6 20.0</td>
<td>91.5 18.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>450 1.2 0.4</td>
<td>8,998.2 2,881.5</td>
<td>30,087.4 14,363.9</td>
<td>33.2 15.3</td>
<td>142.6 64.2</td>
<td></td>
</tr>
<tr>
<td>17-AG</td>
<td>10 0.4 0.1</td>
<td>49.6 16.3</td>
<td>160.7 121.7</td>
<td>211.9 282.1</td>
<td>442.2 47.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 0.7 0.3</td>
<td>106.1 72.2</td>
<td>820.3 711.1</td>
<td>70.2 70.8</td>
<td>753.4 590.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 0.7 0.2</td>
<td>220.4 74.1</td>
<td>1,726.9 964.8</td>
<td>42.6 21.9</td>
<td>567.7 214.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80 0.9 0.0</td>
<td>602.7 332.1</td>
<td>3,310.5 1,861.6</td>
<td>40.1 19.2</td>
<td>509.5 256.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>160 1.1 0.4</td>
<td>2,265.4 1,122.9</td>
<td>15,272.3 14,829.2</td>
<td>21.7 12.6</td>
<td>180.7 93.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>320 2.4 1.9</td>
<td>2,852.4 1,060.8</td>
<td>27,587.2 18,175.0</td>
<td>37.7 33.5</td>
<td>193.2 128.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>450 3.0 1.9</td>
<td>3,738.0 1,500.0</td>
<td>39,338.4 26,745.9</td>
<td>30.7 18.8</td>
<td>230.4 109.6</td>
<td></td>
</tr>
<tr>
<td>17-AG</td>
<td>10 0.4 0.1</td>
<td>49.6 16.3</td>
<td>160.7 121.7</td>
<td>211.9 282.1</td>
<td>442.2 47.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 0.7 0.3</td>
<td>106.1 72.2</td>
<td>820.3 711.1</td>
<td>70.2 70.8</td>
<td>753.4 590.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 0.7 0.2</td>
<td>220.4 74.1</td>
<td>1,726.9 964.8</td>
<td>42.6 21.9</td>
<td>567.7 214.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80 0.9 0.0</td>
<td>602.7 332.1</td>
<td>3,310.5 1,861.6</td>
<td>40.1 19.2</td>
<td>509.5 256.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>160 1.1 0.4</td>
<td>2,265.4 1,122.9</td>
<td>15,272.3 14,829.2</td>
<td>21.7 12.6</td>
<td>180.7 93.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>320 2.4 1.9</td>
<td>2,852.4 1,060.8</td>
<td>27,587.2 18,175.0</td>
<td>37.7 33.5</td>
<td>193.2 128.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>450 3.0 1.9</td>
<td>3,738.0 1,500.0</td>
<td>39,338.4 26,745.9</td>
<td>30.7 18.8</td>
<td>230.4 109.6</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: 17-AAG, 17-allylamino, 17-demethoxygeldanamycin; 17-AG, 17-amino, 17-demethoxygeldanamycin; T_max, time to maximum serum concentration; hr, hour; C_max, maximum serum concentration; AUC, area under the curve; VD_ss, steady-state volume of distribution; SD, standard deviation.

Fig 3. Representative Western blot analysis of serial peripheral blood leukocyte samples following a single dose of 17-allylamino, 17-demethoxygeldanamycin (17-AAG) at 450 mg/m²/week (Patient 28). The characteristic molecular signature of heat shock protein 90 (HSP90) inhibition (c-RAF-1, LCK, CDK4 depletion and HSP70 induction) was seen for varying periods of time after the infusion. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
This would need to be given serious consideration if patients were to receive DMSO more frequently than once a week.

There were no complete or partial responses recorded during the study. However, two patients with metastatic melanoma receiving 17-AAG achieved radiologically documented stable disease for prolonged periods (15 and 35 months), and assessment continues in one patient who has received treatment for 41 months to date. A cytostatic growth delay was the most commonly seen effect with 17-AAG in xenograft models. Disease stabilization is becoming an increasingly important end point during evaluation of novel agents that are often cytostatic rather than cytotoxic and will have to be studied in carefully designed phase II trials.

Since the dose-versus-AUC relationship for 17-AAG was linear and there was also a linear correlation between the AUC of 17-AAG and that of its metabolite 17-AG, this leads us to believe that, over the dose range studied (10 to 450 mg/m²/week), there was no saturation of the metabolism of 17-AAG. Previous studies have shown that the cytochrome P450 CYP3A4 is responsible for the metabolism of 17-AAG to 17-AG. The linear correlation between the AUC of 17-AAG and that of 17-AG (Fig 2C) suggests that CYP3A4 polymorphism is unlikely to explain variability in 17-AAG PK. At the highest dose level, 450 mg/m²/week, plasma 17-AAG concentrations exceeded 120 nmol/L, which is the IC₅₀ in the NCI cell line panel, for periods in excess of 24 hours. 17-AAG concentrations achieved were also in the range of drug concentrations shown to cause tumor growth.

Fig 4. Western blot analysis of tumor samples taken before treatment and 24 to 96 hours after treatment at dose levels 320 to 450 mg/m²/week of 17-allylamino, 17-demethoxygeldanamycin (17-AAG). Results show variable degrees of client protein suppression (c-RAF-1, CDK4) and co-chaperone induction (heat shock protein 70 [HSP70]). (A) 320 mg/m²/week; (B) and (C) 450 mg/m²/week.

Fig 5. Semi-quantitative densitometry analysis of the data from Figure 4 in which expression levels of proteins have been normalized to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) signals in tumor biopsies before and 24 hours after treatment with 17-allylamino, 17-demethoxygeldanamycin (17-AAG). (A) c-RAF-1; (B) HSP70; and (C) CDK4.
delay in human tumor xenograft models. It has also been noted that the metabolite 17-AG is active and may contribute to the activity of 17-AAG. The high volume of distribution is consistent with preclinical experiments suggesting retention of both 17-AAG and its metabolite 17-AG in tumor when compared to plasma, possibly contributing to a viable therapeutic index. Thus, at the highest dose level studied, it has been possible to achieve adequate plasma concentrations of 17-AAG, without saturating its metabolism, leaving scope for further dose expansion in a once-a-week schedule if the formulation is improved.

One of the primary aims of this study was to determine whether evidence of HSP90 inhibition could be obtained in both healthy and tumor tissue in patients treated with 17-AAG. Preclinical models suggest that depletion of client proteins c-RAF-1 and CDK4, together with the induction of the co-chaperone HSP70, constitute a molecular signature of HSP90 inhibition which correlates with inhibition of growth of malignant cells both in vitro and in vivo. The demonstration of reproducible PD changes in PBLs (Fig 3) along with plasma levels of drug known to inhibit growth in preclinical models (Fig 1) led us to biopsy patients at the dose levels 320 to 450 mg/m²/week. PBLs were particularly helpful in deciding at which dose level tumor biopsies should be initiated. We were able to compare the molecular signature of HSP90 inhibition in tumor and PBLs of the same individual in two patients at 450 mg/m²/week and one patient at 320 mg/m²/week. c-RAF-1 depletion and HSP70 induction was seen in both PBLs and tumor in all three patients. CDK4 depletion was seen in the tumor biopsies in all three patients but in the PBLs of only one. The reasons for the differences are not known. It is well established, however, that depletion of particular client proteins by 17-AAG can occur at different rates in different cell lines possibly due to the varying rates of synthesis and ubiquitin-dependent proteasomal degradation. It was possible to demonstrate PD changes suggestive of HSP90 inhibition in tumor biopsies at 24 hours but not reproducibly at 96 hours after treatment (Fig 4). This leads us to believe that HSP90 is inhibited in tumor for somewhere between 1 and 5 days. Indeed in one patient in whom it was possible to obtain biopsies at 1 and 3 days, we demonstrated sustained c-RAF-1 and CDK4 depletion (patient 28, Fig 4). The PD data suggest that a schedule in which 17-AAG is administered more frequently than once a week may be needed to inhibit the target HSP90 continuously. In fact, a daily schedule (5 days of 7 every 3 weeks) has been assessed in a separate phase I trial. With this schedule the MTD was 40 mg/m²/week. Apart from much lower drug levels in plasma, this leaves the target uninhibited for 2 weeks at a time. Other phase I trials are ongoing in which the drug has been administered twice and/or three times a week; however, concerns remain about hepatotoxicity, time spent in hospital, DMSO-induced nausea, and persistent odor when the drug was administered more frequently than once a week.

In conclusion, this phase I trial of 17-AAG has demonstrated that it is feasible, in a once-a-week schedule, to deliver a dose resulting in a potentially therapeutic plasma concentration, target inhibition in tumor tissue, and possible antitumor activity (Fig 7). The present formulation of the drug does not allow further dose escalation to establish an MTD clearly attributable to the drug. Improvements in the formulation of
17-AAG would provide greater flexibility in scheduling and the discovery of new HSP90 inhibitor chemotypes with enhanced physicochemical properties is ongoing. A dose level of 450 mg/m²/week is recommended for the present formulation of 17-AAG in phase II clinical trials.

**Acknowledgment**

We thank Maureen Brampton and David Eaton for data management; Johann De Bono, Charlotte Benson, Cathryn Brock and Janet Hanwell for clinical management of the patients; and Simon Clark for laboratory support.

**Authors’ Disclosures of Potential Conflicts of Interest**

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Research Funding: Paul Workman, Vernalis. For a detailed description of this category, or for more information about ASCO’s conflict of interest policy, please refer to the Author Disclosure Declaration and Disclosures of Potential Conflicts of Interest found in Information for Contributors in the front of each issue.

REFERENCES