# 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

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**Abstract**

**Purpose:** 17-(Allylamino)-17-demethoxygeldanamycin (17AAG), a benzoquinone antibiotic, down-regulates oncoproteins by binding specifically to heat shock protein 90 (HSP90). We did a phase I study of 17AAG to establish the dose-limiting toxicity and maximum tolerated dose and to characterize 17AAG pharmacokinetics and pharmacodynamics.

**Experimental Design:** Escalating doses of 17AAG were given i.v. over 1 or 2 hours on a weekly × 3 schedule every 4 weeks to cohorts of three to six patients. Plasma pharmacokinetics of 17AAG and 17-(amino)-17-demethoxygeldanamycin (17AG) were assessed by high-performance liquid chromatography. Expression of HSP70 and HSP90 in peripheral blood mononuclear cells was measured by Western blot.

**Results:** Forty-five patients were enrolled to 11 dose levels between 10 and 395 mg/m$^2$. The maximum tolerated dose was 295 mg/m$^2$. Dose-limiting toxicity occurred in both patients (grade 3 pancreatitis and grade 3 fatigue) treated with 395 mg/m$^2$. Common drug-related toxicities (grade 1 and 2) were fatigue, anorexia, diarrhea, nausea, and vomiting. Reversible elevations of liver enzymes occurred in 29.5% of patients. Hematologic toxicity was minimal. No objective responses were observed. 17AAG pharmacokinetics was linear. Peak plasma concentration and area under the curve of 17AG, the active major metabolite of 17AAG, increased with 17AAG dose, but the relationships were more variable than with 17AAG. 17AAG and 17AG in plasma were >90% protein bound. There were no consistent changes in peripheral blood mononuclear cell HSP90 or HSP70 content.

**Conclusions:** 17AAG doses between 10 and 295 mg/m$^2$ are well tolerated. 17AAG pharmacokinetics is linear. Peripheral blood mononuclear cell HSP90 and HSP70 are uninformative pharmacodynamic markers. The dose recommended for future studies is 295 mg/m$^2$ weekly × 3, repeated every 4 weeks.

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17-(Allylamino)-17-demethoxygeldanamycin (17AAG) is an analogue of geldanamycin (1, 2) and is the first compound of its class to enter clinical trial (3–7). 17AAG specifically binds to and inhibits the cytosolic chaperone protein, heat shock protein 90 (HSP90), which constitutes 1% to 2% of all cytosolic proteins. Among the ~100 proteins known to be chaperoned by HSP90 are the receptors for progesterone, estrogens, androgens, and glucocorticoids (2, 8–10). In addition, 17AAG also inhibits HSP90-mediated conformational folding and promotes degradation of oncoproteins, such as mutant p53, HER-2/neu, raf-1, and Bcr-Abl (2, 8–14). Preclinical studies with 17AAG have shown inhibition of tumor cell growth in a number of cell lines (8, 11, 14–18). Because of its unique mechanism of action and preclinical activity in cell lines, 17AAG was selected for clinical development (2–7).

Preclinical toxicology studies of 17AAG showed hepatic and gallbladder toxicity to be dose limiting (19). Hepatotoxicity was characterized by increases in transaminases and bile acids, and

drug-related histopathologic lesions were documented in the gallbladder, common bile duct, and gastrointestinal tract. Preclinical pharmacokinetic (1) studies in mice showed 17AAG to widely distribute (20) and undergo extensive hepatic metabolism (21). One of its major metabolites, 17-(amino)-17-demethoxygeldanamycin (17AG), is known to be as active as 17AAG in depleting cellular HER-2/neu (22, 23) and was shown to be generated by CYP3A metabolism of 17AAG (21).

Based on these preclinical data, we conducted a phase I study of 17AAG. Our study used a weekly × 3 schedule, repeated every 4 weeks. The starting dose was 10 mg/m$^2$, which was 1/10 of the maximal tolerated dose in dogs treated on a daily × 5 schedule, repeated every 3 weeks (19). The principal objectives of the study were to define the dose-limiting toxicity and maximal tolerated dose of 17AAG, recommend a dose for use in future phase II studies, and characterize the pharmacokinetics and pharmacodynamics of 17AAG and its major metabolite, 17AG.

## Patients and Methods

***Patient selection.*** Eligible patients had histologically confirmed advanced cancer not curable by standard therapies. Other requirements were Eastern Cooperative Oncology Group performance status 0, 1, or 2; no chemotherapy or radiation therapy for at least 4 weeks before study entry; and a negative pregnancy test for women of child-bearing potential. Adequate organ function was required and defined by: absolute neutrophil count >1,500/μL, platelets >100,000/μL, serum creatinine <1.5 mg/dL, or measured creatinine clearance >60 mL/min if serum creatinine was between 1.5 and 2.0 mg/dL, bilirubin ≤1.5 mg/dL, and serum aspartate aminotransferase ≤2× the upper limit of normal. Women of child-bearing potential were required to have a negative pregnancy test before study entry and to use an effective means of contraception.

The following were grounds for exclusion from the trial: pregnancy or lactation; untreated brain metastasis; active infections; or serious concomitant conditions. Because 17AAG is formulated in a diluent containing egg phospholipids, patients with a past history of serious allergic reactions to eggs were excluded. All patients gave written consent according to institutional and Federal guidelines.

***Drug administration.*** 17AAG (NSC 330507) and EPL diluent (NSC 704057) were supplied by the Pharmaceutical Resources Branch of the National Cancer Institute (Rockville, MD). 17AAG was supplied in sterile vials that contained 50 mg of 17AAG in 2.0 mL of DMSO. EPL diluent was supplied in sterile, 50-mL flint-glass vials that contained 48 mL of 2% egg phospholipids and 5% dextrose in water for injection. 17AAG was prepared for infusion by adding 2 mL of 17AAG to 48 mL of EPL diluent to produce a 17AAG concentration of 1 mg/mL. For the initial, low doses of 17AAG, the 1 mg/mL solution was diluted further with 5% dextrose in water or with 0.9% NaCl to a concentration between 0.2 and 0.5 mg/mL. The final dosing solution of 17AAG was transferred to a glass bottle and administered within 6 hours of preparation. Most 17AAG infusions were given over 1 hour; however, if the volume of the infusion exceeded 500 mL, the infusion could be given over 2 hours. The starting 17AAG dose was 10 mg/m$^2$ given weekly × 3, repeated every 4 weeks. Subsequent dose levels were 20 and 30 mg/m$^2$. On completion of the 30 mg/m$^2$ cohort, doses were escalated by increments approximating 30%, which resulted in cohorts being treated with 40, 55, 75, 97.5, and 127 mg/m$^2$. After the study had completed accrual to the 127 mg/m$^2$ cohort, safety information for the dose of 220 mg/m$^2$ became available from a parallel study using 17AAG on a similar schedule (4). Therefore, a protocol amendment was made to omit the planned doses of 165 and 214 mg/m$^2$ and pursue further dose escalation from 220 mg/m$^2$. A modified Fibonacci schema, with three to six patients per cohort, was used. No intrapatient dose escalation was allowed. At least three patients were to be enrolled at each dose level, assuming dose-limiting toxicity did not occur in the first two patients enrolled at that level. The first three patients enrolled at a given dose level were observed for at least 4 weeks after initiation of 17AAG before patients were enrolled at the next dose level. If none of the patients treated at a given dose level had dose-limiting toxicity as defined below, patients were enrolled at the next dose level. If one of three patients experienced a dose-limiting toxicity, up to three additional patients were accrued at the same dose level, and only if none of those additional three patients had dose-limiting toxicity was the next cohort of three patients accrued to the next higher dose. If two or more patients treated at any dose level experienced dose-limiting toxicity, that level was considered the excessively toxic dose, and accrual to that dose level ceased. If only three patients had been treated at the dose level immediately below the excessively toxic dose, that dose level was expanded to six patients, assuming fewer than two of those six patients experienced a dose-limiting toxicity. The highest dose level at which zero or one of six patients experienced a dose-limiting toxicity was considered the maximal tolerated dose or the dose recommended for future phase II studies.

***Dose-limiting toxicities.*** Toxicity was graded according to National Cancer Institute-Common Toxicity Criteria, version 2.0. Dose-limiting toxicity was defined as any drug-related (possible, probable, or definite) grade 3 or greater nonhematologic toxicity, except alopecia, occurring in cycle 1. In addition, nausea or vomiting ≥grade 3 despite maximal antiemetic therapy, persistent decrease of creatinine clearance to <50% of baseline, or increase of serum creatinine to >2 times baseline was also considered a dose-limiting toxicity. Hematologic criteria for a dose-limiting toxicity were thrombocytopenia <25,000/μL or leukopenia <500/μL.

***Dose modifications.*** If any grade 3 or greater nonhematologic toxicity occurred, 17AAG administration was withheld until resolution to grade ≤1 or baseline, at which time 17AAG dosing could be resumed at a dose one level below that which produced the toxicity. Drug dosing was also withheld, if serum creatinine was elevated to ≥2 times baseline. On recovery of the creatinine to ≤1.5 times baseline, reinitiation of treatment was allowed at a dose one level below that associated with the elevated creatinine. If grade 2 neuropathy developed, treatment was withheld until normalization of signs and symptoms. If absolute neutrophil count nadir was <500/μL or platelet nadir was <100,000/μL, subsequent 17AAG treatment could be resumed after hematologic recovery to an absolute neutrophil count ≥1,500/μL or platelet count ≥100,000/μL, but at a 17AAG dose one level below that which produced the hematologic toxicity. A dose delay of up to 4 weeks was permitted before treatment discontinuation.

***Study requirements and assessments.*** History and physical examinations were done before the study and before every cycle. Complete blood count and electrolyte and chemistries were done before the study and weekly during treatment. Serum cortisol, testosterone, estradiol, progesterone, follicle-stimulating hormone, and luteinizing hormone were measured before the study and before every cycle. Radiographs to follow response were done before the study and after every two cycles. The WHO response criteria were used (24).

***Pharmacokinetic sampling and quantitation of 17AAG and 17AG.*** Serial blood samples were collected in heparinized tubes before the first dose of 17AAG, 30 minutes into the infusion, at the end of the infusion, and at 5, 10, 15, and 30 minutes and 1, 2, 4, 8, 12, 16, 18, and 24 hours after completion of the infusion. Plasma was prepared by centrifuging blood samples at 1,000 × *g* for 10 minutes and was stored at −70°C until analyzed. Urine was collected on ice as 6-hour aliquots for the first 24 hours after 17AAG administration. Concentrations of 17AAG and 17AG in plasma were quantitated with a previously described high-performance liquid chromatography assay that was developed and validated in our laboratory (20). The lower limit of quantitation of the assay was 0.1 μmol/L for both 17AAG and 17AG, and the assay was linear between 0.1 and 25.6 μmol/L.

***Assessment of unbound 17AAG and 17AG.*** Plasma ultrafiltrates were prepared by placing 1 mL of plasma into Amicon Centrifree YM-30 regenerated cellulose, 30,000 MW cutoff centrifugal filter devices (Millipore Corporation, Billerica, MA), which were then centrifuged for 20 minutes at 1,500 × *g* at 4°C. In a similar fashion, ultrafiltrates were prepared from 0.85 and 8.5 μmol/L solutions of 17AAG and 17AG prepared in RPMI 1640 (BioWhittaker, Walkersville, MD) containing 10% fetal bovine serum (Biofluids, Rockville, MD). Concentrations of 17AAG and 17AG in the ultrafiltrates were determined by high-performance liquid chromatography (20).

***Pharmacokinetic analyses.*** The time courses of 17AAG and 17AG in plasma were analyzed noncompartmentally. The area under the curve (AUC) from zero to infinity and the terminal half-life ($t_{1/2}$) were estimated using the LaGrange function (25), as implemented by the computer program LAGRAN (26). Total body clearance (CLtb) for 17AAG was calculated using the following equation: CLtb = dose / AUC. Urinary excretion of 17AAG and 17AG was calculated from urinary volume and concentrations of 17AAG and 17AG.

***Assessment of heat shock proteins 90 and 70 in peripheral blood mononuclear cells.*** Blood samples (10 mL) were collected in heparinized tubes before administration of the first dose of 17AAG and at 4, 8, and 24 hours after completion of the 17AAG infusion. Cells were separated from plasma by centrifugation for 15 minutes at 1,400 × *g* at 4°C. Cells were then diluted with 3 volumes of PBS, underlayered with 10 mL of Histopaque (density 1.077; Sigma-Aldrich, St. Louis, MO), and centrifuged for 15 minutes at 400 × *g* at room temperature. The resulting peripheral blood mononuclear cell band was transferred to a 50-mL conical centrifuge tube, mixed with ~23 mL of PBS, and centrifuged again for 15 minutes at 1,250 × *g* and room temperature. The resulting cell pellet was resuspended in 1 mL PBS, transferred to a 1.5-mL microcentrifuge tube, and centrifuged at 12,000 × *g* for 5 minutes at room temperature. The final supernatant was decanted and the microcentrifuge tubes were carefully dried before final cell pellets were stored at −70°C until analysis.

Peripheral blood mononuclear cells were thawed, incubated on ice for 15 minutes with 50 to 100 μL of ice-cold lysis extraction buffer (27), and then sonicated for 5 seconds using a Branson Sonifier 450 (Branson Ultrasonics, Danbury, CT) at energy setting 4. Protein concentrations were measured using the Bio-Rad protein assay system (Bio-Rad, Hercules, CA) with bovine serum albumin as a standard. Western blot analyses were done using a standard method (27). Polyvinylidene diflouride membranes (Bio-Rad) were stained with 1:1,000 dilutions of anti-HSP90 (SPA-830) and anti-HSP70 (SPA-810) monoclonal antibodies (StressGen Biotechnologies Corp., Victoria, BC, Canada). All polyvinylidene diflouride membranes were also stained with a 1:4,000 dilution of anti–β-actin (Sigma-Aldrich) to control for protein loading. Enhanced chemiluminescence Western blotting kits (NEN Life Science, Inc., Boston, MA) were used to visualize immunoreactive bands. Immunoreactivity signals were quantitated by densitometry using UN-Scan-It digitizing software (Silk Scientific, Inc., Orem, UT). Densities within similarly sized areas of bands for HSP90, HSP70, β-actin, and selected background were digitized. A ratio was then calculated by dividing the density of each band of interest by the density of the actin band from the same sample. Ratios were then further normalized by comparing them with the corresponding density ratio calculated for pretreatment samples.

## Results

***Patient characteristics.*** Forty-five patients were enrolled to 11 dose levels between 10 and 395 mg/m$^2$ and received 110 cycles (median 2 cycles/patient). Patient characteristics are given in Table 1. Forty-four patients were evaluable for toxicity because they received at least one dose of 17AAG. One inevaluable patient, at dose level 10 (295 mg/m$^2$), died of rapidly progressive disease before receiving any 17AAG treatment, and was replaced.

**Table 1.** Patient characteristics

| | |
|---|---|
| Patients | 45 |
| Male | 25 |
| Female | 20 |
| Age (y) | 24-83 |
| Median | 64 |
| Performance status (ECOG) | |
| 0 | 12 |
| 1 | 30 |
| 2 | 3 |
| Tumor types | |
| Colorectal | 14 |
| Lung | 8 |
| Head and neck | 7 |
| Genitourinary | 7 |
| Other* | 9 |

Abbreviation: ECOG, Eastern Cooperative Oncology Group.
*Cervix, breast, sarcoma, neuroendocrine, kidney, ureter, and esophagus.

***Toxicity.*** Dose-limiting toxicity was seen during cycle 1 at three dose levels: At dose level 1 (10 mg/m$^2$), one patient developed a pericardial effusion. At dose level 5 (55 mg/m$^2$), one patient exhibited grade 3 elevation of γ glutamyl transpeptidase. Both patients treated at dose level 11 (395 mg/m$^2$) developed dose-limiting toxicity. One patient treated at 395 mg/m$^2$ developed severe abdominal pain with increased pancreatic enzymes, within 1 day of receiving the first dose of 17AAG. The other patient at 395 mg/m$^2$ had grade 3 fatigue in the first cycle following administration of 17AAG. After six patients had been treated with at least one cycle at a dose of 295 mg/m$^2$ and experienced no dose-limiting toxicity, that dose was determined to be the maximal tolerated dose (Table 2). The most common drug-related toxicities were grades 1 and 2 fatigue, anorexia, diarrhea, nausea, and vomiting. At higher doses and at the maximal tolerated dose, most patients had nausea, and prophylactic antiemetic therapy was necessary. Reversible elevations of liver enzymes, mainly aspartate aminotransferase and γ glutamyl transpeptidase, were seen in 13 (29.5%) patients. Hematologic toxicity was minimal. Anemia, of any degree, was the most common hematologic toxicity, and was seen in 10 (22.7%) of subjects (Table 3). A mildly unpleasant odor that usually lasted for a few days after each dose of 17AAG was consistently noticed by family members and nursing staff.

***Changes in hormone levels.*** There were no consistent changes from baseline levels of serum cortisol, testosterone, estradiol, progesterone, follicle-stimulating hormone, or luteinizing hormone.

***Antitumor activity.*** There were no objective responses observed.

***Pharmacokinetics.*** A representative concentration versus time profile for 17AAG and 17AG in plasma of a patient treated with a 1-hour infusion of 295 mg/m$^2$ is presented in Fig. 1. Over the range of doses delivered, 17AAG pharmacokinetics was linear. Specifically, maximum plasma concentrations of 17AAG ($C_{max}$), which ranged between 0.148 and 17.34 μmol/L, and 17AAG AUC, which ranged between

**Table 2.** Dose levels

| Dose level | Dose ($mg/m^2$) | Number treated | DLT |
|---|---|---|---|
| 1 | 10 | 8 | Pericardial effusion |
| 2 | 20 | 3 | |
| 3 | 30 | 3 | |
| 4 | 40 | 3 | |
| 5 | 55 | 6 | Grade 3 GGT |
| 6 | 75 | 3 | |
| 7 | 97.5 | 3 | |
| 8 | 127 | 3 | |
| 9 | 220 | 3 | |
| 10 | 295 (MTD) | 8* | |
| 11 | 395 | 2 | Grade 3 pancreatitis and fatigue |

Abbreviations: MTD, maximal tolerated dose; DLT, dose limiting toxicity; GGT, γ glutaryl transpeptidase.
*One patient did not receive any drug.

0.39 and 35.37 μmol/L × h, both increased linearly with 17AAG dose (Fig. 2A and B). There was significant interpatient variability in both 17AAG $C_{max}$ at all doses, with coefficient of variation values ranging from 17.6% at 127 $mg/m^2$ to 55% at 10 $mg/m^2$. There was also significant interpatient variability in 17AAG AUC, with coefficient of variation values ranging from 17.1% at 295 $mg/m^2$ to 50% at 30 $mg/m^2$. In view of the linear relationships between 17AAG doses and 17AAG $C_{max}$ and AUC, it was not surprising that there was also a linear relationship between 17AAG AUC and $C_{max}$, which was described by the equation:

$$17AAG\ AUC = 2.09 \times 17AAG\ C_{max} + 0.713 (r^2 = 0.913).$$

Furthermore, the linear relationship between 17AAG dose and AUC indicated that 17AAG clearance (CLtb), which averaged 22.6 ± 11.6 $l/h/m^2$ (mean ± SD) and ranged between 8.7 and 76.6 $L/h/m^2$, did not vary systematically across the doses studied. The coefficient of variation values for 17AAG CLtb ranged from 16% at 127 $mg/m^2$ to 69% at 30 $mg/m^2$. There was no obvious relationship between interpatient variability of any 17AAG pharmacokinetic variable and 17AAG dose.

Although 17AG $C_{max}$ and AUC also increased with increasing 17AAG dose (Fig. 3A and B), these relationships were more variable than those relating 17AAG dose to 17AAG $C_{max}$ and AUC. Consequently, 17AAG $C_{max}$ and AUC were poor predictors of 17AG $C_{max}$ and AUC, respectively. 17AG $C_{max}$ ranged between 0.074 and 5.92 μmol/L and was observed between 0.5 and 2 hours after the initiation of the 17AAG infusion. 17AG AUC ranged between 0.11 and 55.03 μmol/L × h.

Both 17AAG and 17AG were highly protein bound and there was no systematic change in protein binding across the doses administered in this study. 17AAG was 2.1% to 11.9% unbound, with a mean ± SD free fraction of 5.7 ± 2.2%. 17AG was 2.8% to 15.4% unbound, with a mean ± SD free fraction of 7.8 ± 3.4%. In comparison, the unbound percentages of 0.085 and 8.5 μmol/L 17AAG in RPMI 1640 with 13% fetal bovine serum were 45% and 50%, respectively. The unbound percentages of 0.085 and 8.5 μmol/L 17AG in RPMI 1640 with 13% fetal bovine serum were 95% and 84%, respectively.

In the first 24 hours after 17AAG administration, urinary excretion accounted for 6.8 ± 3.1% (range 1.9-14.5%) of the administered dose. Of this, 17AAG accounted for 4.2 ± 2.1% (range 0.7-9.7%) and 17AG accounted for 2.6 ± 1.9% (0.5-11.2%). There was no relationship between 17AAG dose and urinary excretion of 17AAG, 17AG, or the sum of the two.

***Assessment of heat shock proteins 90 and 70 in peripheral blood mononuclear cells.*** There was no consistent alteration in HSP90 or HSP70 in peripheral blood mononuclear cells isolated from patients at 4, 8, and 24 hours after 17AAG administration when compared with the amounts of those proteins in peripheral blood mononuclear cells isolated before 17AAG administration. In some patients, concentrations of HSP90 and HSP70 were increased in comparison with pretreatment concentrations, but there was no clear relationship between the degree of increase and the dose of 17AAG administered. When increases in HSP70 were observed, they were observed by 4 hours and remained elevated at 24 hours after 17AAG administration.

## Discussion

Administration of 17AAG at doses between 10 and 295 $mg/m^2$ was found to be safe and associated with minimal toxicity. The maximal tolerated dose or the dose for future single-agent studies is 295 $mg/m^2$ given weekly × 3, every 4 weeks. Dose-limiting toxicities occurred in both patients treated at the highest dose level delivered (395 $mg/m^2$). We believe that the pancreatitis observed in one patient could

**Table 3.** Drug-related toxicity

| Toxicity | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|---|
| Hematologic | | | | |
| Anemia | 3 (6.8%) | 6 (13.6%) | 1 (2.3%) | 0 |
| Platelets | 4 (9.1%) | 1 (2.3%) | 1 (2.3%) | 0 |
| Leukopenia | 2 (4.6%) | 1 (2.3%) | 0 | 0 |
| Liver | | | | |
| AP | 0 | 0 | 1 (2.3%) | 0 |
| Bilirubin (total) | 0 | 0 | 1 (2.3%) | 0 |
| AST | 4 (9.1%) | 0 | 1 (2.3%) | 0 |
| GGT | 2 (4.6%) | 1 (2.3%) | 2 (4.6%) | 0 |
| ALT | 0 | 0 | 0 | 1 (2.3%) |
| Other | | | | |
| Anorexia | 11 (25%) | 1 (2.3%) | 0 | 0 |
| Fatigue | 15 (34.1%) | 4 (9.1%) | 1 (2.3%) | 0 |
| Diarrhea | 11 (25%) | 1 (2.3%) | 0 | 0 |
| Nausea | 8 (18.1%) | 1 (2.3%) | 1 (2.3%) | 0 |
| Vomiting | 7 (15.9%) | 0 | 0 | 0 |
| Stomatitis | 4 (9.1%) | 0 | 0 | 0 |
| Pericardial effusion | 0 | 0 | 1 (2.3%) | 0 |
| Pancreatitis | 0 | 0 | 1 (2.3%) | 0 |

NOTE: Selected toxicities; worst grade per patient.
Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, γ glutamyl transpeptidase.

have been due to the EPL diluent, which contains egg phospholipids. Although fatigue is common in cancer patients and attribution to drug therapy alone can be difficult, the abrupt onset of grade 3 fatigue with administration of the first dose of 17AAG to the other patient treated at 395 mg/m$^2$ and the resolution of that fatigue within a few days led us to attribute that event to administration of 17AAG. The pericardial effusion with tamponade observed in a patient with lung cancer treated at dose level 1 was initially attributed to drug administration, so that the 10 mg/m$^2$ cohort was expanded to six patients. However, on subsequent evaluation, disease progression was felt to be a more likely cause of the pericardial effusion. Although hepatic toxicity was dose limiting in animal studies with 17AAG, hepatic toxicity was not dose limiting in this study. Reversible elevations of liver enzymes, mainly aspartate aminotransferase and γ glutamyl transpeptidase, were often seen at higher dose levels but were not dose limiting. Hyperbilirubinemia was only seen in one patient. Hematologic and other toxicities were mild and predominantly grades 1 to 2 in nature. At the maximal tolerated dose (295 mg/m$^2$), therapy was well tolerated, pancreatitis was absent, and fatigue (grade 2) was seen in only two patients.

The unpleasant odor associated with 17AAG administration was probably related to DMSO, in which 17AAG was formulated.

Other phase I studies of 17AAG have been recently completed or are ongoing. In a phase I study conducted by Mayo Clinical investigators using a dosing schedule similar to that used in our study, the maximal tolerated dose was 308 mg/m$^2$ (4). Dose-limiting toxicities were nausea, vomiting, fatigue, anemia, and liver toxicity. Investigators at the Institute of Cancer Research in England have described patients receiving 17AAG doses up to 450 mg/m$^2$ in a study using a weekly schedule of 17AAG administration (5). Daily × 5 and daily × 3 schedules of 17AAG administration were evaluated by investigators at the Memorial Sloan Kettering Cancer Center (7). On the daily × 5 schedule the maximal tolerated dose was 80 mg/m$^2$/d, and on the daily × 3 schedule the maximal tolerated dose was 112 mg/m$^2$/d. With both schedules, hepatitis and diarrhea were the dose-limiting toxicities. A phase I study using the daily × 5 schedule of 17AAG administration was done at the National Cancer Institute and found the maximal tolerated dose to be 40 mg/m$^2$/d × 5 days, with hepatotoxicity being dose limiting (6). No objective responses have been documented in any of these phase I studies.

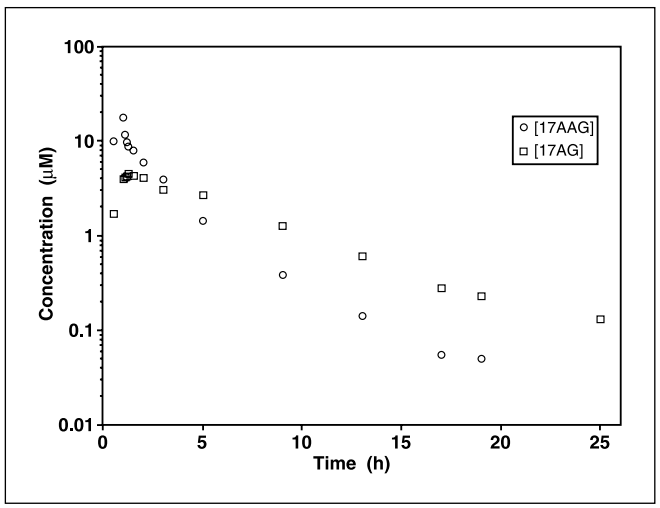


**Fig. 1.** Representative concentration versus time profile of 17AAG (○) and 17AG (□) in plasma of a patient treated with a 1-hour infusion of 295 mg/m$^2$ 17AAG.

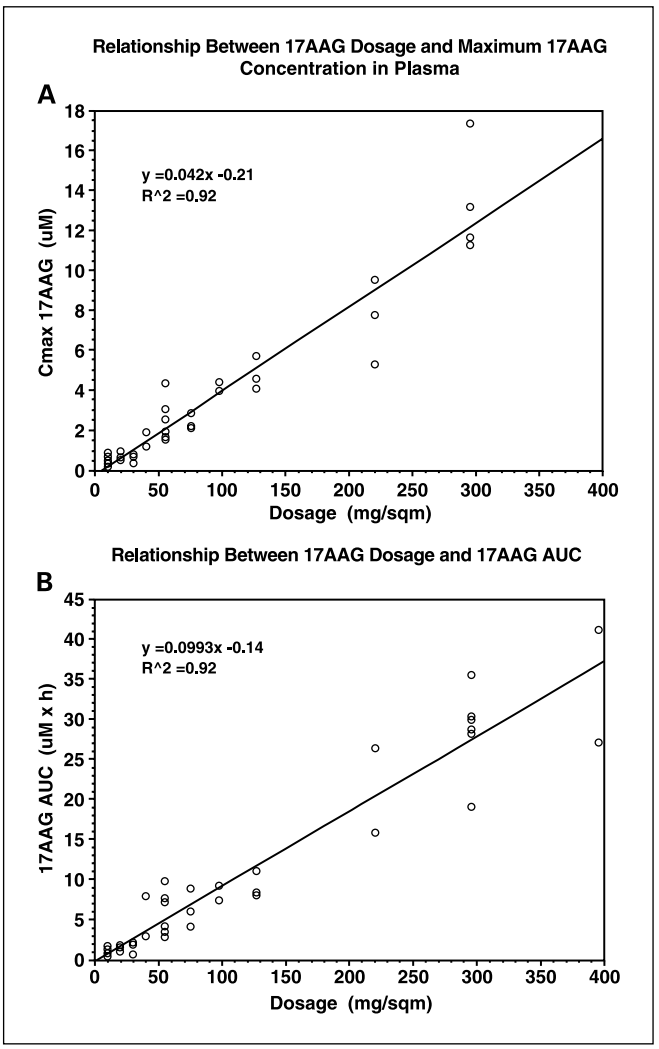


**Fig. 2.** Relationship between 17AAG dosage and (*A*) 17AAG $C_{max}$ and (*B*) AUC. Symbols represent individual patients.

The linearity of 17AAG pharmacokinetics in patients treated over a wide range of doses is consistent with preclinical data on 17AAG pharmacokinetics in mice. The $C_{max}$ and AUC associated with a 295 mg/m$^2$ dose are compatible with $C_{max}$ and AUC in mice treated with i.v. bolus doses of 40 and 60 mg/kg (20). The high correlation between end-of-level infusion 17AAG concentrations in plasma and 17AAG AUC could prove useful as a limited sampling strategy if 17AAG AUC proves to be a useful pharmacokinetic variable for correlating with a meaningful pharmacodynamic consequence of 17AAG treatment. The minor contribution of urinary excretion of 17AAG and 17AG to their overall disposition is also consistent with preclinical pharmacokinetic studies of 17AAG (20). The documentation of 17AG as a major metabolite of 17AAG is another aspect of this clinical study that is consistent with preclinical *in vivo* studies in mice (20).

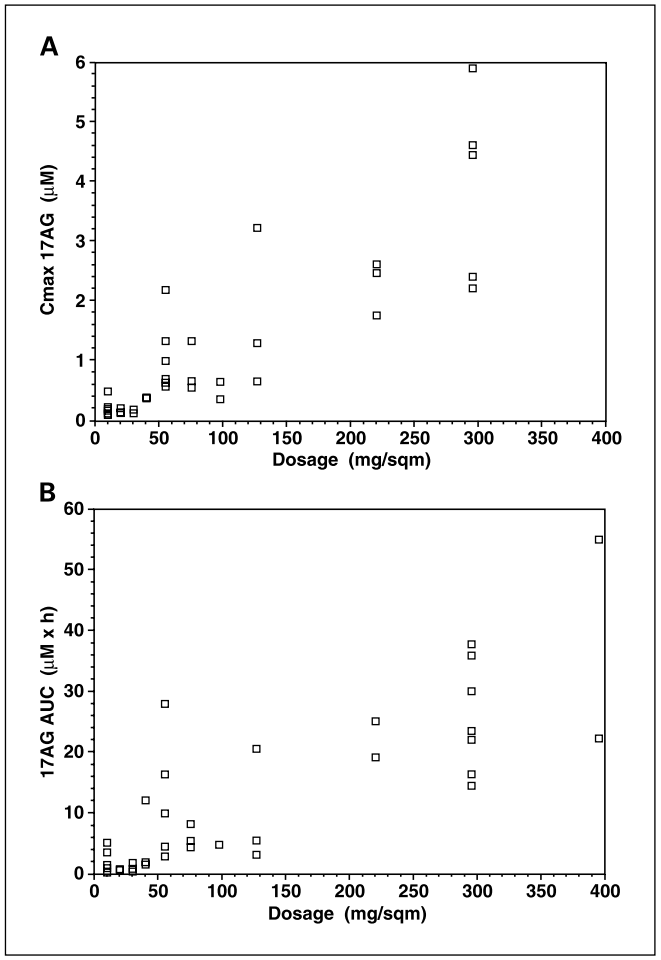


**Fig. 3.** Relationship between 17AAG dosage and (*A*) 17AG $C_{max}$ and (*B*) AUC. Symbols represent individual patients.

Furthermore, *in vitro* studies using mouse and human liver preparations have shown this metabolism to be due to CYP3A (21). This has implications regarding potential drug-drug interactions as 17AAG is evaluated in combination with other antineoplastic agents. It also has implications when 17AAG is administered to patients receiving CYP3A inducers, inhibitors, or substrates as treatment for other conditions. The documented high-protein binding of both 17AAG and its active metabolite 17AG needs to be considered when extrapolating data from *in vitro* studies done in systems in which the free fractions of 17AAG and 17AG are much greater than those fractions in plasma.

Our attempts to show various proteins as pharmacodynamic markers of 17AAG activity were not successful. Assessments of HSP90 and HSP70 in peripheral blood mononuclear cells were not useful as pharmacodynamic markers of 17AAG activity in our study. In other studies, preliminary analysis indicates that these biomarkers might correlate to therapy, and publication with full analysis is awaited (4–6). We also found no consistent, relevant changes in serum levels of cortisol, testosterone, estradiol, progesterone, follicle-stimulating hormone, or luteinizing hormone, despite the fact that 17AAG is known to interfere with the production of a number of sex- and steroid-hormone receptors. We did not observe a correlation between $C_{max}$, AUC of 17AAG, and observed toxicities. We are currently assessing the utility of plasma concentrations of insulin-like growth factor binding protein-2 and the extracellular domain of erb-B2 as pharmacodynamic markers in patients treated with 17AAG (28). Whether future clinical studies of 17AAG should include sequential tumor biopsies is another question to be considered carefully.

In summary, administration of 17AAG by a weekly schedule is well tolerated. In xenograft models, the effect of 17AAG on HSP90 client proteins lasted up to 72 hours, suggesting that more frequent dosing may be more effective than the weekly regimen used in our study (19). Therefore, we are now evaluating a twice-weekly schedule of 17AAG administration. The lack of significant toxicity with 17AAG should allow its combination with other agents. Preclinical studies have shown synergy when 17AAG has been combined with agents such as taxanes, doxorubicin, imatinib, gemcitabine, cisplatin, and bortezomib. Phase I studies of 17AAG in combination with these agents have been initiated (2, 29–31).

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