A Phase I Trial of Twice-Weekly 17-Allylamino-Demethoxy-Geldanamycin in Patients with Advanced Cancer

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Abstract

Purpose: To determine the maximum tolerated dose (MTD), dose-limiting toxicity, and pharmacokinetics of 17-allylamino-demethoxy-geldanamycin (17-AAG) administered on days 1, 4, 8, and 11 every 21 days and to examine the effect of 17-AAG on the levels of chaperone and client proteins.

Experimental Design: A phase I dose escalating trial in patients with advanced solid tumors was done. Toxicity and tumor responses were evaluated by standard criteria. Pharmacokinetics were done and level of target proteins was measured at various points during cycle one.

Results: Thirteen patients were enrolled in the study. MTD was defined as 220 mg/m^2. Dose-limiting toxicities were as follows: dehydration, diarrhea, hyperglycemia, and liver toxicity. At the MTD, the mean clearance of 17-AAG was 18.7 L/h/m^2. There was a significant decrease in integrin-linked kinase at 6 hours after infusion on day 1 but not at 25 hours in peripheral blood mononuclear cells. Treatment with 17-AAG on day 1 significantly increased pretreatment levels of heat shock protein (HSP) 70 on day 4, which is consistent with the induction of a stress response. *In vitro* induction of a stress response and up-regulation of HSP70 resulted in an increased resistance to HSP90-targeted therapy in A549 cells.

Conclusions: The MTD of 17-AAG on a twice-weekly schedule was 220 mg/m^2. Treatment at this dose level resulted in significant changes of target proteins and also resulted in a prolonged increase in HSP70. This raises the possibility that HSP70 induction as part of the stress response may contribute to resistance to 17-AAG.

Heat shock protein (HSP) 90 is part of a chaperone complex for multiple client proteins involved in cell signaling, proliferation, and survival (1–5). HSP90 function can be disrupted by geldanamycin (6), which results in the dissociation and degradation of client proteins, such as HER-2, RAF, mutant p53, cyclin-dependent kinase 4, Src, focal adhesion kinase, AKT, nuclear factor-κB, and insulin-like growth factor receptor 1 (7–9). The binding of geldanamycin and its analogues to HSP90 also induces a stress response, which is manifested in part by increased levels of cochaperone and other stress proteins, such as HSP70 (10). In fact, cells deficient in HSF1 do not induce a stress response and are more sensitive to geldanamycin (11). Geldanamycin caused hepatotoxicity in dogs in preclinical studies, so further development was terminated (12). The geldanamycin analogue 17-allylamino-demethoxy-geldanamycin (17-AAG, NSC 330507) was shown to be less toxic than geldanamycin and shown activity in mouse xenograft models (13–17). 17-AAG is metabolized to 17-aminogeldanamycin (17-AG) by cytochrome *P*450 3A4/5 and is widely distributed in body tissues but not in the central nervous system (18, 19). The 17-AAG metabolite, 17-AG, also binds to HSP90, disrupting its ability to chaperone client proteins.

Goetz et al. (20) reported recently results of the phase I study of 17-AAG administered on a weekly schedule to patients with advanced cancer. The maximum tolerated dose (MTD) of weekly 17-AAG was determined to be 308 mg/m^2. Dose-limiting toxicities (DLT) were related to liver toxicity and anemia, nausea, vomiting, and myalgias. 17-AAG consistently increased HSP70 levels in the peripheral blood mononuclear cells (PBMC), indicating that there was a target effect. At the MTD, the half-life ($t_{1/2}$) of 17-AAG was 4.15 hours, whereas the active metabolite 17-AG had a $t_{1/2}$ of 7.63 hours. Although 17-AAG induced HSP70 in PBMCs, no reproducible changes in levels of client proteins could be detected. We had found previously that integrin-linked kinase (ILK) was a HSP90 client protein, and its degradation after geldanamycin or 17-AAG treatment was associated with the inhibition of Akt phosphorylation on Ser^{473} (21). ILK is also involved in tumor cell migration, so its degradation along with that of Src and focal adhesion kinase could contribute to 17-AAG anti-invasive effects.

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Based on the relatively short $t_{1/2}$ of the parent drug and the estimated $t_{1/2}$ of client proteins, we reasoned that a more frequent administration would be potentially more biologically effective. Other studies had already attempted a daily ×5 schedule every 3 weeks (22) and found that the DLT of hepatotoxicity occurred at low dose levels. Hence, we tested a twice-weekly schedule of 17-AAG administration. The goals of the current study were to determine the MTD, DLT, and pharmacokinetics of 17-AAG for a twice-weekly schedule (days 1, 4, 8, and 11 schedule repeated every 21 days). We also undertook to examine the effect of 17-AAG on the levels of HSP70 and ILK administered in this schedule and relate them to the drug pharmacokinetics.

Patients and Methods

Patients. Patients with histologic or cytologic confirmed measurable or evaluable metastatic or locally advanced cancer, for which no established life-prolonging therapy was available, were eligible for this study. Patients had to be older than 18 years, have an estimated life expectancy of >12 weeks, an Eastern Cooperative Oncology Group performance status of ≤2, and had to be willing to provide all biological specimens as required by the protocol. Adequate organ function defined as neutrophil count >1,500/mm^3; platelet count >100,000/mm^3; hemoglobin >9.0 g/dL; serum creatinine <1.25 × upper limit of normal (ULN) or actual or estimated creatinine clearance >60 mL/min (utilizing the Cockcroft-Gault formula); and total bilirubin <2 × ULN and aspartate aminotransferase <2.5 × ULN and alkaline phosphatase <2 × ULN in the absence of demonstrable liver metastases or <5 × ULN in the presence of liver metastases was required. Patients were excluded if they had received chemotherapy, radiation therapy, biological therapy, or immunotherapy ≤4 weeks before study registration (≤6 weeks in patients treated with mitomycin C or nitrosoureas), if they had failed to recover from any toxic effects of prior treatment, or if they received radiation therapy to >25% of bone marrow. The exclusion criteria also included active or uncontrolled infection, concomitant pregnancy, lactation or unwillingness to use adequate contraception, known central nervous system metastases or uncontrolled seizure disorder, uncontrolled intercurrent illness, including but not limited to symptomatic congestive heart failure (New York Heart Association classification III or IV), unstable angina pectoris, or cardiac arrhythmia, and a history of serious allergic reaction to eggs. Patients receiving known cytochrome *P*450 3A4 inhibitors were excluded. All patients gave written informed consent according to institutional and federal guidelines.

Dosage and administration. 17-AAG was supplied by the National Cancer Institute (Bethesda, MD) as a sterile single-use amber vial containing 50 mg 17-AAG in 2 mL frozen DMSO. Egg phospholipid diluent, NSC 704057, was supplied in a 50 mL flint vial containing 48 mL of 2% egg phospholipids and 5% dextrose in water for injection, USP. After thawing the drug, the diluent was added to give a final concentration of 1 mg/mL, which was dispensed in a glass bottle.

Patients received 17-AAG i.v. as a 60-minute infusion on days 1, 4, 8, and 11 of a 21-day cycle. An accelerated titration design was used wherein one patient was accrued at successive dose levels until moderate toxicity (MT) was observed (23). The accelerated phase ended when one patient experienced a DLT or two patients experienced MT during the first course of treatment. Subsequently, a standard cohort of three designs was used and three to six patients were treated at each dose level. If zero of three experienced first course DLT, three additional patients were treated at the next dose level (1.4 dose factor). If one of three experienced DLT, up to three more patients were entered at that same dose level. If two or more experienced DLT, no further patients were started at that dose, and three more patients were treated at the next lower dose level to more fully assess the toxicities associated with the MTD.

DLT and MT. All toxicities were graded according to the National Cancer Institute common toxicity criteria (version 2). The MTD was defined as one dose level below the dose that induced DLTs in one third or more of the patients (at least two of a maximum of six new patients). MTD was defined based on toxicities documented in the first cycle of treatment only. Grade 3 or 4 nonhematologic toxicity (with the exception of nausea, vomiting, and diarrhea) was considered dose limiting. Grade 3 or 4 nausea, vomiting, or diarrhea in patients who had received prophylaxis and treatment with an optimal antiemetic or antidiarrheal regimen was considered dose limiting. Grade 4 neutropenia lasting ≥5 days or associated with fever or infection and grade 4 thrombocytopenia or anemia of any duration were also DLTs. Any omission of either day 4, 8, or 11 dose during cycle 1 due to toxicity was a DLT. The MT was defined as grade 2 hematologic or nonhematologic toxicity. Alopecia was not considered dose limiting.

Pretreatment and follow-up patient evaluations. A clinical evaluation, including history, physical examination, complete blood count, electrolytes, and chemistries, was done at baseline and before each course of treatment. Complete blood counts were done weekly during the study. Response evaluation by radiographic studies was done at baseline and after every two courses of therapy. A partial response was defined as a ≥50% reduction in the sum of the largest perpendicular diameters of indicator lesion(s) chosen before therapy. A complete response was the disappearance of all evidence of the tumor. Tumor progression was defined as the appearance of new lesion(s) or a 25% increase in size of indicator lesion(s). Stable disease was documented when there was a failure to meet the criteria for complete response, partial response, or progression. All objective responses were required to be confirmed no <4 weeks from the initial documentation.

Pharmacokinetic studies. Blood samples (5 mL) were drawn into heparin-containing tubes at the following times during cycle 1: day 1—before treatment, 0.5, 0.9, 1.08, 1.25, 1.5, 2, 3, 5, 9, and 25 hours after beginning the infusion; day 4—6 and 24 hours after beginning the infusion. Blood samples were collected from a site remote from the 17-AAG infusion line and immediately cooled in ice water. All blood samples were handled and processed in a standard manner in the Mayo Clinical General Clinical Research Center. Hemolysis was not seen in any of the samples. Plasma was separated by centrifugation (1,000-1,200 × *g* for 10 minutes) at 4°C and stored at −70°C. Plasma concentrations of 17-AAG and 17-AG were determined by the reverse-phase high performance liquid chromatography procedure of Egorin et al. (19) as described previously (20).

Biomarkers. Blood samples (35 mL) were drawn into heparin-containing tubes (Vacutainer CPT, Becton Dickinson, Franklin Lakes, NJ) on days 1 and 4 of cycle 1 before treatment and 6 and 25 hours after treatment. PBMCs were isolated, and target protein concentration was measured as described previously (20, 24). The proteins detected and antibodies used were as follows: HSP70, SPA 810 (Stressgen, San Diego, CA), and ILK (BD Biosciences, San Jose, CA).

***The effect of heat stress response on sensitivity to geldanamycin and on the level of HSP70* in vitro.** A549 cells, a human lung cancer cell line, were selected for assessment of the stress-induced response because we have found that resistance to 17-AAG was partially mediated by overexpression of HSPs.[5] A549 cells were cultured in RPMI 1640 with 5% fetal bovine serum, 100 μg/mL streptomycin, and 100 units/mL penicillin. Cells were seeded in 96-well plates at a density of 1 × 10^3 per well and allowed to adhere for 24 hours. The plates were then heat shocked at 42°C for 1 hour and allowed to recover for 6 hours at 37°C. Geldanamycin at concentration of 0.1, 0.3, 1, 3, or 10 μmol/L (from Dr. V.L. Narayanan, Drug Synthesis and Chemistry Branch, National Cancer Institute) or DMSO alone was added to the wells and allowed to incubate at 37°C for 24 hours. Cells were subsequently washed twice

[5] A. McCollum et al. Cancer Res (in press).

Table 1. Baseline characteristics

Characteristics, *n* (%)	*N* = 13
Median age, y, (range)	59.0 (40.0-73.0)
Gender	
Female	4 (30.8)
Male	9 (69.2)
Race	
White	12 (92.3)
Black	1 (7.7)
Performance score	
0	8 (61.5)
1	5 (38.5)
Tumor type	
Melanoma	2 (15.4)
Sarcoma	2 (15.4)
Renal	1 (7.7)
Gastrointestinal	7 (53.8)
Skin	1 (7.7)

with serum-free medium. Cells were then incubated with fresh medium for an additional 72 hours at 37°C. Cell survival was determined using the CellTiter 96 AQueous Cell Proliferation Assay from Promega (Madison, WI) according to the manufacturer's instructions.

The changes of HSP70 in the response to heat shock were assessed by immunoblotting. After 1 hour of heat shock at 42°C and 6 hours of recovery period ended, nonadherent cells were removed, and adherent cells were lifted from flasks, washed with ice-cold PBS, then sedimented, and lysed on ice with lysis buffer for 15 minutes. Cells were pelleted at 1,000 rpm for 5 minutes at 4°C, rinsed once with ice-cold PBS, and lysed in lysis buffer containing 10 mmol/L HEPES (pH 7.4), 150 mmol/L KCl, 10 mmol/L $MgCl_2$, 0.1% NP40, 1 mmol/L Na_3VO_4, and 1 μg/mL each of aprotinin, leupeptin, and pepstatin. After 10 minutes of incubation on ice, the detergent insoluble fractions were pelleted at 14,000 rpm for 2 minutes at 4°C. Total protein concentration of cell lysates was estimated by the bicinchoninic acid method as described (25). Samples containing 50 μg total protein were separated by one-dimensional SDS-PAGE on a 10% acrylamide gel. The separated polypeptides were transferred to nitrocellulose, probed with antibodies [mouse monoclonal anti-HSP70 from Stressgen and mouse monoclonal anti-actin from Sigma-Aldrich (St. Louis, MO)], and visualized by enhanced chemiluminescence as described previously (26).

Statistical methods. Toxicity was defined as any adverse event that was deemed at least possibly, probably, or definitely related to study treatment. Descriptive statistics and frequency distributions were used to summarize the patient characteristics, toxicity patterns, tumor response, pharmacokinetics, and client protein data. Spearman rank correlations were used to examine the 17-AAG pharmacokinetics patterns over time and to explore the relationship between the client protein and 17-AAG pharmacokinetics. Changes in client proteins from baseline were analyzed using a Wilcoxon signed-rank test.

Results

Patients and toxicity. A total of 13 patients (11 dose escalation and 2 additional patients at the MTD) were enrolled in the study. Baseline characteristics for all patients are shown in Table 1. There was a predominance of males (69.2%), and 54% had gastrointestinal primaries. The dose levels studied and the number of patients treated at each dose level are included in Table 2. The starting dose of 57 mg/m^2 administered twice-weekly was selected because it was one third of the weekly dose being administered at the time this schedule was initiated. No MT or DLT was seen at dose levels 1 to 4. Of the two patients treated at dose level 5, one patient was reported as having MT (grade 2 diarrhea and grade 2 skin irritation), and the decision to escalate to the dose level 6 was made. The second patient on dose level 5 was later reevaluated and reported to have a grade 2 blurred vision (MT). Two patients at dose level 6 experienced a DLT; both patients experienced grade 3 dehydration and liver toxicity (grade 3 aspartate aminotransferase, alkaline phosphatase elevation; grade 4 aspartate aminotransferase, grade 3 alanine aminotransferase). One patient on dose level 6 also had grade 3 diarrhea and grade 3 hyperglycemia. Due to two DLTs at this level, the accelerated titration phase ended, and three patients (following the cohorts of three design) were treated at the next lower dose level (dose level 5). No DLTs were observed in these three patients and dose level 5 was, therefore, declared as the MTD and the recommended phase II dose. Two additional patients were enrolled at the MTD. One patient died shortly after enrollment (before cycle 1 completion) due to complications related to rapidly progressive disease (non-Hodgkin's lymphoma). Therefore, 12 treated

Table 2. Dose escalation and hematologic values for cycle 1 and all cycles

Dose level	17-AAG (mg/m^2/dose)	No. patients	Median ANC nadir x10^9/L (range)	Median WBC nadir x10^9/L (range)	Median platelet nadir x10^9/L (range)
Cycle 1					
1	57	1	4.4	5.8	326
2	80	1	4.1	7.0	254
3	112	1	3.0	4.5	159
4	157	1	4.4	8.0	250
5	220	6*	3.7 (3.1-4.5)	5.8 (4.2-7.0)	226 (139-386)
6	308	2	3.8 (3.6-4.1)	5.7 (5.2-6.2)	431 (297-565)
All cycles					
1	57	1	4.2	5.8	297
2	80	1	4.1	7.0	236
3	112	1	3.0	4.1	135
4	157	1	3.5	5.1	147
5	220	6*	3.1 (2.6-4.3)	5.0 (3.8-6.9)	231 (84-362)
6	308	2	3.8 (3.6-4.1)	5.7 (5.2-6.2)	427 (297-557)

Abbreviation: ANC, absolute neutrophil count.
*Seven patients were treated at 220 mg/m^2, however, data from only six patients are available.

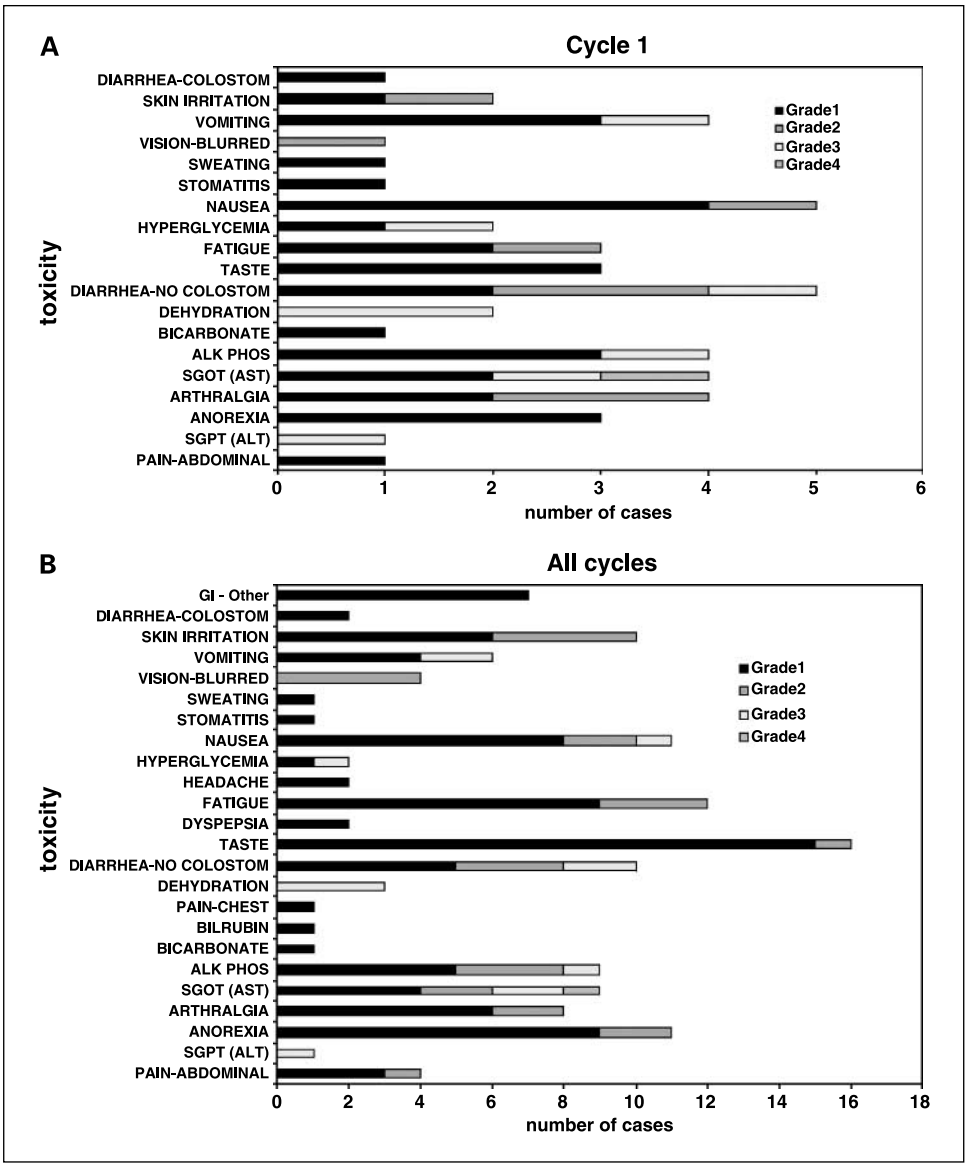

Fig. 1. Frequency of nonhematologic toxicities for cycle 1 (*A*) and all cycles (*B*).

patients were evaluable for toxicity, response, and pharmacologic analyses.

The laboratory hematologic data for cycle 1 and all cycles are summarized in Table 2. The only hematologic toxicities were grade 1 neutropenia and thrombocytopenia. The grade 3 nonhematologic toxicities over all cycles of treatment were alanine aminotransferase (1), aspartate aminotransferase (2), alkaline phosphatase elevation (1), dehydration (3), diarrhea (2), hyperglycemia (1), nausea (1), and vomiting (2). Nonhematologic toxicities for cycle 1 and all cycles are summarized in Fig. 1.

Antitumor activity. No tumor responses were seen. Two patients (one with pancreatic cancer on dose level 5 and one with colon cancer on dose level 5) had stable disease for 2 cycles, and one patient (with melanoma) on dose level 4 had stable disease for 10 cycles.

Pharmacokinetics. The pharmacokinetics of 17-AAG and 17-AG are summarized in Table 3. At the MTD, the mean clearance of 17-AAG was 18.7 L/h/m^2. The mean peak plasma concentration of 10.2 μmol/L 17-AAG was achieved at the end of the infusion, and 2.69 μmol/L 17-AG was achieved 15 to 30 minutes after the end of the infusion at the 220 mg/m^2 dose level. The decline in the plasma concentration of 17-AG (mean $t_{1/2}$, 4.92 hours) was slower than that of 17-AAG (mean $t_{1/2}$, 3.13 hours). The sum of the 17-AAG and 17-AG plasma concentrations remained above 1 μmol/L for ~8 hours after the end of the infusion.

Client protein changes. The changes of protein concentrations of HSP70 and ILK in the PBMC of all treated patients are summarized in Table 4. There was a significant increase in levels of HSP70 6 and 25 hours following treatment on days 1 and 4 relative to pretreatment for that respective day. When the HSP70 levels before treatment on days 1 and 4 were compared, we found that day 4 levels were between 2- and 4-fold greater than day 1 levels ($P < 0.0001$; Fig. 2A). As an increase in HSP70 is an indicator of a stress response, we sought to determine if inducing such a response could make cells less sensitive to HSP90-directed therapy. A549 cells were heat shocked to

Table 3. Pharmacokinetics variables

Dose level (mg/m²)	n	17-AAG					17-AG		
		C_{max} (μmol/L)	$t_{1/2}$ (h)	AUC (μmol/L*h)	Cl (L/h/m²)	Vss (L)	C_{max} (μmol/L)	$t_{1/2}$ (h)	AUC (μmol/L*h)
57	1	1.4	4.84	3.0	32.2	120.2	0.57	10.53	3.60
80	1	3.0	5.43	6.8	20.2	91.1	0.69	8.96	3.26
112	1	1.9	4.06	5.9	32.5	119.3	0.50	8.47	2.55
157	1	6.8	4.05	15.0	17.9	59.6	1.02	6.09	7.29
220	6*								
mean		10.2	3.13	21.8	18.7	48.6	2.69	4.92	16.01
SD		2.8	0.88	5.8	5.1	17.0	1.66	1.07	11.11
308	2								
mean		16.8	2.75	53.2	10.1	29.9	5.87	4.93	59.29
SD		4.5	0.09	10.6	2.0	1.3	1.51	0.41	13.22

Abbreviations: Vss, steady-state volume of distribution; Cl, clearance.
*Seven patients were treated; however, data for only six patients are available.

induce HSP70 (Fig. 2C) and then treated with increasing concentrations of geldanamycin for 24 hours. Heat-shocked cells were more resistant to geldanamycin than unshocked cells with an increase in IC_{50} from 146 nmol/L to 1,910 nmol/L (Fig. 2D). The effect of treatment on ILK levels was variable (Fig. 2B). In some cases, ILK levels declined at 6 hours but not 25 hours; in others, ILK levels declined at both time points. Furthermore, there was variability in the amount of degradation seen from patient to patient. Despite this variability, there was a significant decrease in the level of ILK at 6 hours ($P < 0.01$) after infusion on day 1; however, the change of ILK level at 25 hours after infusion on day 1 and at 6 and 25 hours on day 4 did not reach statistical significance (Table 4).

We explored the relationship between pharmacokinetic variables and change in ILK and HSP70. There was no correlation between C_{max} and area under the time-concentration curve (AUC) of 17-AAG or AG and change in ILK or HSP70.

Discussion

17-AAG interferes with HSP90 function as a chaperone for multiple client proteins, some of which are of critical importance for tumor cells survival. Therefore, the biological effect of 17-AAG is dependent not only on its pharmacokinetics and its ability to disrupt HSP90 function but also on the $t_{1/2}$ of client proteins and the ability of neoplastic cells to alter protein expression in response to HSP90 inhibition. The short $t_{1/2}$ of 17-AAG, along with the short $t_{1/2}$ of most of the intracellular proteins, provides the rationale for twice-weekly dosing rather than weekly, as reported in the initial study by Goetz et al. (20).

In the current study, the MTD and recommended phase II dose of 17-AAG for days 1, 4, 8, and 11 of a 21-day cycle schedule was established to be 220 mg/m^2. Hepatic toxicity with transient transaminitis, nausea, vomiting, anorexia, and dehydration were the DLT, which is similar to the DLT observed in other trials of 17-AAG (20, 27). In this study, we limited the infusion time to 60 minutes. It is conceivable that higher doses of the drug could be administered if the infusion duration was increased. However, at these doses, we saw a target effect in PBMCs, suggesting that higher doses may not be necessary, provided that the target effects in PBMCs reflect target changes in the tumor. The established MTD resulted in a significant increase in HSP70 and decrease in ILK on day 1 providing evidence of target effect. We did not see a correlation between 17-AAG, 17-AG, C_{max}, or AUC and changes in client protein concentration. This may be due to the small number of patients in this study and the variability in the changes in HSP70 and ILK between patients.

We decided to test a day 1, 4, 8, and 11 schedule of 17-AAG because there was concern that a weekly schedule would result in interval recovery of HSP90 protein stabilization, folding, and trafficking function. There was also a concern that newly synthesized client proteins would replenish those degraded with the day 1 treatment. We knew from previous work that a daily × 5 schedule was hepatotoxic to a degree that limited the ability to administer high doses of 17-AAG, which would be biologically effective in disrupting HSP90 folding and trafficking function. In this study, we found that at 220 mg/m^2, the MTD on this schedule, HSP70 levels increase at 6 and 25 hours after infusion by 25% to 100% above baseline. When we compared the HSP70 levels before treatment on days 1 and 4, we found that day 4 levels were between 2- and 4-fold greater than day 1 levels ($P < 0.0001$). This would suggest that the heat shock response induced by 17-AAG persists at least until day 4.

Table 4. Changes of HSP70 and ILK in PBMC following treatment with 17-AAG at 6 and 25 hours after infusion on days 1 and 4, cycle 1

Protein	Day 1, 6 h	P	Day 1, 25 h	P	Day 4, 6 h	P	Day 4, 25 h	P
HSP70	176 (102-271)	<0.01	193.5 (104-326)	<0.01	127 (97-181)	<0.01	137.5 (115-190)	<0.01
ILK	78.5 (50-98)	<0.01	91 (51-162)	0.13	92 (43-119)	0.08	96.5 (74-157)	0.91

NOTE: All data in table expressed as median % of baseline (range).

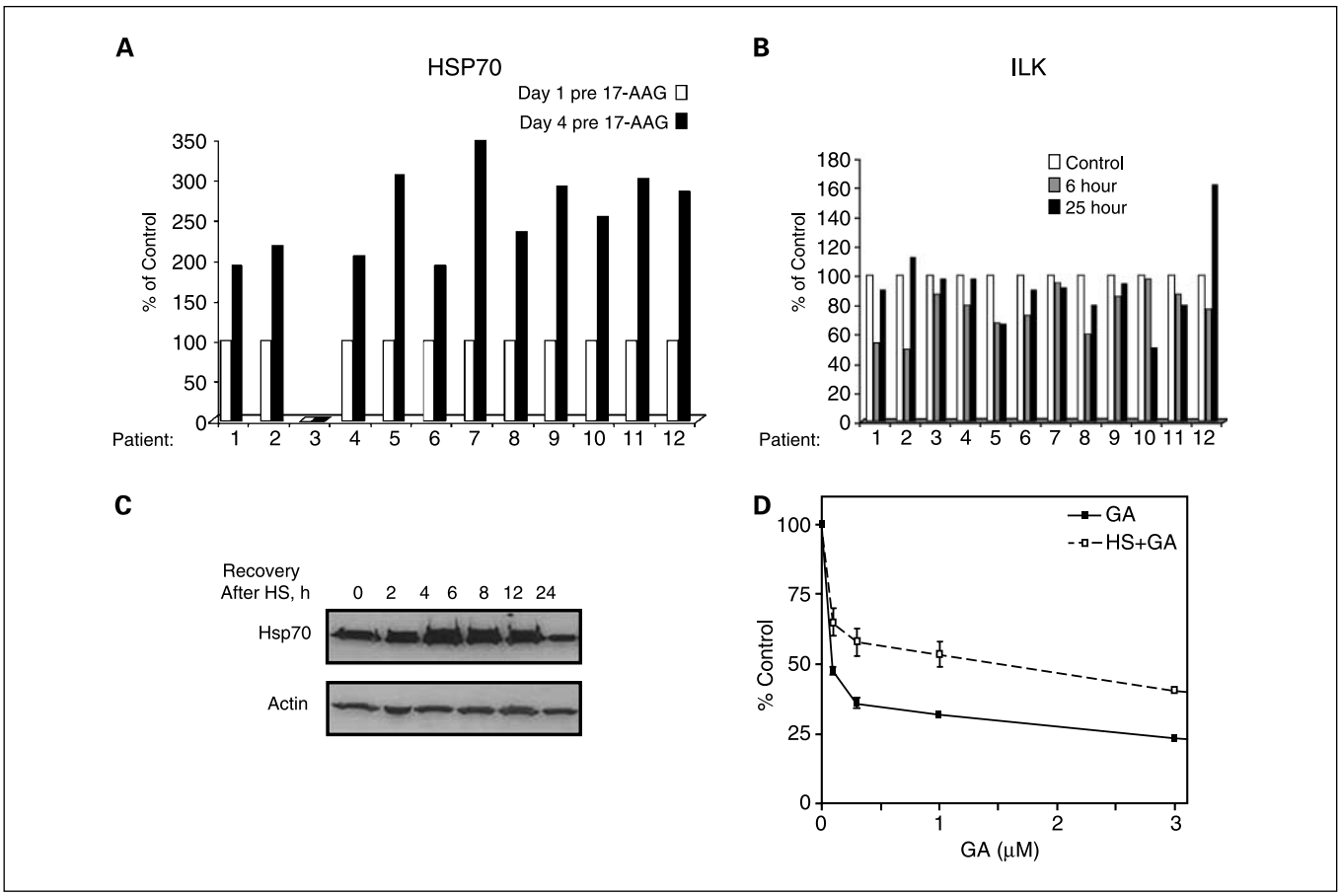

Fig. 2. Changes in HSP70 level before day 4 17-AAG infusion in PBMC *in vivo* and in A549 cells after heat shock *in vitro. A,* PBMC lysates were blotted for HSP70 and images were scanned. Pretreatment day 4 levels are expressed as a percentage of pretreatment levels on day 1. Sample of patient 3 could not be quantified despite multiple attempts. There was a significant increase in HSP70 on day 4 ($P < 0.001$). *B,* ILK levels before treatment and at 6 and 24 hours after treatment are expressed as a percentage of pretreatment levels. *C,* a 1 hour of heat shock of 42°C induces HSP70 in A549 cells between 6 and 24 hours. *D,* survival of A549 cells treated with increasing concentrations of geldanamycin without prior heat shock (■) and 6 hours after heat shock (□).

At the same time, we found that ILK levels decreased significantly at 6 hours but not 25 hours on day 1 and were not significantly decreased on day 4 after 6 or 25 hours after treatment. The lack of effect of ILK at 25 hours on day 1 may reflect the rapid resynthesis of ILK and possibly lesser dependence of it on chaperone function for stabilization than other clients. The lack of effect on ILK at all on day 4 would be consistent with the induced heat shock response "protecting" the protein from further degradation, although HSP90 folding and stabilizing function has been disrupted. To further support this premise, we have shown that when tumor cells are heat shocked and induce HSP70, they are less sensitive to geldanamycin. This is consistent with HSP90 targeting inducing its own resistance and is similar to the well-documented finding that many cells, which are heat shocked, become thermotolerant (28, 29). The release of HSF1 (30) from the chaperone complex when HSP90-directed therapies bind HSP90 will result in the increased transcription of several proteins whose genes contain heat shock elements in their promoter regions. HSP70, which is one such protein, may act to inhibit apoptosis through the mitochondrial pathway (31). Furthermore, the report by Bagatell et al. (11) that HSF-1$^{-/-}$ are more sensitive to geldanamycin than parent cells is consistent with the stress response contributing to the resistance of cells to HSP90-targeted agents. There are two implications of these findings. First, the use of client proteins as predictors of HSP90 therapeutic effect will be highly dependent on the rate of client protein turnover (i.e., resynthesis and degradation). Hence, the optimal time to sample patient tumors for client protein effects will differ depending on which client protein is being evaluated. The lack of an effect on one client protein would not preclude an effect on the chaperone complex. The second implication is that an agent that targets HSP90 and induces a heat shock response may protect normal tissue, such as PBMCs, from toxicity by increasing HSP70 levels and may result in tumors becoming resistant to further treatment with HSP90-directed agents. This may in part explain why the therapeutic benefit of single-agent 17-AAG has been limited despite its multitargeted kinase effect. We recognize that the results we obtained were in PBMCs, which are a normal tissue surrogate, and may not reflect changes in tumor tissue. Because repetitive biopsies in the phase I setting where dose escalation adds a confounding variable is not clinically practical and may be difficult to interpret, phase II trials in patients with hematologic and select solid tumors will need to be done to provide such a correlation.

HSP90 agents can cause inhibition of survival signaling through degradation of proteins, such as Akt and PDK1 (32),

or inhibit DNA repair through degradation of Chk1 and abrogation of the G_1-S checkpoint, making cells more susceptible to chemotherapy (33). This would make a HSP90 targeting strategy an excellent approach to combine with chemotherapeutic agents. This is the case when 17-AAG is combined with taxanes (34) or nucleoside antimetabolites, such as araC (35) and gemcitabine, respectively (33). Ongoing phase I trials of 17-AAG with taxotere, araC, or gemcitabine may result in an important role for HSP90-targeted therapy as part of a combination approach in the treatment of cancer.

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