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Phase I study of 17-allylamino-17 demethoxygeldanamycin, gemcitabine and/or cisplatin in patients with refractory solid tumors

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Summary

Purpose—To determine the maximum tolerated dose (MTD) and characterize the dose-limiting toxicities (DLT) of 17-AAG, gemcitabine and/or cisplatin. Levels of the proteins Hsp90, Hsp70 and ILK were measured in peripheral blood mononuclear cell (PMBC) lysates to assess the effects of 17-AAG.

Conflicts of interest The authors have nothing to disclose.

Experimental design—Phase I dose-escalating trial using a "3+3" design performed in patients with advanced solid tumors. Once the MTD of gemcitabine + 17-AAG + cisplatin was determined, dose escalation of 17-AAG with constant doses of gemcitabine and cisplatin was attempted. After significant hematologic toxicity occurred, the protocol was amended to evaluate three cohorts: gemcitabine and 17-AAG; 17-AAG and cisplatin; and gemcitabine, 17-AAG and cisplatin with modified dosing.

Results—The 39 patients enrolled were evaluable for toxicity and response. The MTD for cohort A was 154 mg/m^2 of 17-AAG, 750 mg/m^2 of gemcitabine, and 40 mg/m^2 of cisplatin. In cohort A, DLTs were observed at the higher dose level and included neutropenia, hyperbilirubinemia, dehydration, GGT elevation, hyponatremia, nausea, vomiting, and thrombocytopenia. The MTD for cohort C was 154 mg/m^2 of 17-AAG and 750 mg/m^2 of gemcitabine, with one DLT observed (alkaline phosphatase elevation) observed. In cohort C, DLTs of thrombocytopenia, fever and dyspnea were seen at the higher dose level. The remaining cohorts were closed to accrual due to toxicity. Six patients experienced partial responses. Mean Hsp90 levels were decreased and levels of Hsp70 were increased compared to baseline.

Conclusions—17-AAG in combination with gemcitabine and cisplatin demonstrated antitumor activity, but significant hematologic toxicities were encountered. 17-AAG combined with gemcitabine is tolerable and has demonstrated evidence of activity at the MTD. The recommended phase II dose is defined as 154 mg/m^2 of 17-AAG and 750 mg/m^2 of gemcitabine, and is currently being investigated in phase II studies in ovarian and pancreatic cancers. There is no recommended phase II dose for the cisplatin-containing combinations.

Keywords

17-allyaminogeldanamycin; Phase I; Heat shock protein 90; Cisplatin; Gemcitabine; Heat shock protein 70; ILK

Introduction

The molecular chaperone Hsp90 is an excellent target for anticancer therapy. Many of its client proteins are transcription factors or protein kinases involved in multiple oncogenic processes including proliferation, differentiation and apoptosis [1,2]. Examples of client proteins of Hsp90 include EGFR, RAF, MEK, p53, Akt and Bcr-Abl, as well as steroid hormone receptors [3]. Geldanamycin (GA), a member of the benzoquinone ansamycin antibiotic family, binds to the Hsp90 complex and inhibits the chaperone function, resulting in the degradation of these client proteins [4,5].

While GA was determined to be too toxic for administration to humans [6], 17-allylamino-17-demethoxygeldanamycin (17-AAG) appears to have similar mechanism of action with a more tolerable toxicity profile [7]. A prior phase I clinical trial determined the MTD of single agent 17-AAG administered weekly which achieved biologically relevant plasma concentrations was 308 mg/m^2 [8]. The main DLTs were nausea, vomiting diarrhea, fatigue whereas hematologic toxicity was rare. Weekly administration of 17-AAG was better tolerated than daily administration for 5 days repeated every 3 weeks [8,9]. In this latter schedule DLT was hepatotoxicity. 17-AAG has shown little activity as a single agent [10,11] prompting studies of agents in combination with 17-AAG.

Pre-clinical studies suggest 17-AAG may sensitize tumor cells to some cytotoxic chemotherapeutic agents. When cells undergo DNA damage and replication stress after exposure to chemotherapy, cell replication checkpoints are activated promoting DNA repair and cell survival [12,13]. Chk1 enhances DNA repair by arresting cells in the G2 and S phases of cellular replication [14,15]. Arlander et al. [16] identified Chk1 as an Hsp90 client

and demonstrated that exposure of cells to 17-AAG led to degradation of Chk1, abrogating a G1/S arrest induced by gemcitabine. This resulted in enhanced cytotoxicity of gemcitabine when 17AAG exposure followed gemcitabine treatment.

In vitro evaluation of 17-AAG and cisplatin showed the combination of the two agents was synergistic [17]. This was due at least in part to inhibition the heat shock response that 17-AAG induces. HSF1, a transcription factor, is complexed with Hsp90 under non-stress conditions but is released when 17-AAG binds HSP90 thereby causing a heat shock response [18] and overcoming the effect of targeting HSP90 [19-21]. McCollum et al. [17] demonstrated that cisplatin could block the HSF1 mediated heat shock response by blocking HSF binding to the promoter region for this transcription factor. This is supported by Bagatell et al. [22] who showed that $HSF1^{-/-}$cells were significantly more sensitive to 17-AAG than wild-type controls.

Based on these preclinical studies of 17AAG with gemcitabine and cisplatin we undertook a phase I trial of these drugs to determine the MTD of 17-AAG when given in combination with gemcitabine and cisplatin, gemcitabine alone or cisplatin alone. We also investigated the effect of each regimen on the levels of chaperone protein Hsp90, Hsp70 and the client protein integrin-linked kinase (ILK). Previously it has been shown that induction of a stress response by the 17-AAG binding of Hsp90 leads to elevations of the stress protein HSP70 [8,9,23,24]. ILK is a Hsp90 client protein involved in cell proliferation and survival [25], and inhibition of Hsp90/ILK interaction leads to its degradation [26]. If Hsp90 is effectively targeted by 17-AAG, we would predict an increase in levels of HSP70 and a decrease in levels of ILK. The levels of these proteins in peripheral blood mononuclear cells (PMBCs) were measured at different time points to evaluate whether HSP90 had been targeted in patients normal tissue.

Patients and methods

Patients with a histologically confirmed malignancy considered unresectable and for which no other curative or life-extending therapy was available were eligible for the trial. Patients were ≥18 years of age, had a life expectancy of ≥12 weeks, an Eastern Cooperative Oncology Group performance status ≤2, and were willing to provide all biologic specimens required by the protocol.

Exclusion criteria included any chemotherapy, immunotherapy, biologic therapy, or radiation therapy ≤4 weeks prior to study registration (≤6 weeks with mitomycin or nitrosoureas). Prior treatment with cisplatin and gemcitabine was allowed. Patients who failed to recover from toxic effects of prior treatment, received radiopharmaceuticals, or who received radiation therapy to the chest, potentially the heart or >25% of the bone marrow were also excluded. Patients were required to have the following laboratory values: hemoglobin ≥9.0 g/dL, absolute neutrophil count ≥1,500/μL, platelet count ≥100,000/μL, total bilirubin ≤2 × the upper limit of normal (ULN), AST≤2.5×ULN, alkaline phosphatase ≤2×ULN or ≤5×ULN if liver involvement, creatinine ≤1.5×ULN. Patients who received prior anthracycline therapy had to have a normal ejection fraction on MUGA.

Other exclusion criteria included uncontrolled infection; pregnancy, lactation or unwillingness to use adequate contraception; significant cardiac disease; CNS metastases or seizure disorder; ≥ grade 2 peripheral neuropathy, as defined by the NCI Common Toxicity Criteria Version 2.0; history of serious allergic reactions to eggs; and concurrent use of drugs that inhibit the CYP450 3A4 enzyme or that may prolong the QTc interval.

Dosage and administration

17-AAG supplied by the National Cancer Institute as a sterile single-use amber vial containing 50 mg of 17-AAG in 2 mL of dimethylsulfoxide was diluted in an egg phospholipids diluent as previously described [8] and dispensed in a glass bottle. Vials of commercial cisplatin and gemcitabine were administered within 24 h of reconstitution.

The study was an open-label, multiple dose, phase I clinical trial. The original protocol consisted of cohort A only, where patients received gemcitabine IV over 30 min followed by 17-AAG IV over 1 h followed by cisplatin IV over 2 h given on days 1 and 8. Escalation of doses of each of the three drugs was planned in cohort A. When it became apparent dose escalation in this cohort was limited by the hematologic cytotoxicity of cisplatin with gemcitabine and did not allow for dose escalation of 17-AAG to pharmacodynamically optimal levels, the protocol was amended. As a result of the amendment, cohort B was established to start dose escalation at a lower dose of gemcitabine and higher dose of 17-AAG. After dose escalation was not possible due to toxicity on cohort B, the protocol was amended to simultaneously evaluate three additional cohorts: 17-AAG with gemcitabine (cohort C), 17-AAG with cisplatin (cohort D) and all three agents at a lower starting dose of cisplatin (cohort E). As preclinical data had demonstrated synergy with 17-AAG and gemcitabine as a simultaneous or sequential combination, cohort C patients received gemcitabine IV on days 1 and 8 and 17-AAG was on days 2 and 9. All cohorts were treated on 21-day cycles.

Doses were escalated using a “3+3” design, with observation for a minimum of 3 weeks before new patients were treated. Doses were not escalated within individual patients. If dose-limiting toxicity (DLT) was not seen in any of the three patients, three new patients were accrued and treated at the next higher dose level. When DLT was seen in ≥2 patients treated at a given dose level, then the next three patients were treated at the next lower dose level. DLT was defined as grade 4 neutropenia or grade 3 thrombocytopenia; serum creatinine ≥2 times baseline or ULN; ≥ grade 3 nonhematologic toxicity; if patients had grade 2 alkaline phosphatase at baseline then grade 4 alkaline phosphatase was considered dose limiting; and any omission of day 8 dose during the first cycle. Grade 3 nausea, vomiting or diarrhea was only considered dose limiting if patients were receiving maximal supportive treatment. MTD was defined as the dose level below the lowest dose that induces dose-limiting toxicity in at least one-third of patients (at least two of a maximum six new patients). If only three patients were treated at the lower dose level, three additional patients were treated at the MTD, such that a total of six patients were treated at the MTD to assess the associated toxicities.

Patient evaluation

All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0). Each patient underwent a complete history and physical exam, CBC, electrolyte panel, and pulmonary function tests (PFTs) with diffusing capacity of the lung for carbon monoxide (DLCO) within 1 week of registration. EKGs were performed at baseline and pre-, during, and postinfusion during the first cycle and subsequently only if patients were symptomatic. ECGs and PFTs were performed as there has been reports of possible cardiac and pulmonary toxicities related to 17-AAG. For patients with measurable disease, radiographic imaging was performed at baseline and after every two cycles of therapy to assess tumor response. Complete response (CR), partial response (PR) stable disease (SD) and progressive disease (PD) were defined according to RECIST criteria.

Correlative studies

To assess the effect of 17-AAG on biomarkers, PBMCs were collected at 0, 6 and 25 h after the start of infusion on days 1 and 8 of cycle 1 in cohorts A, B, D and E. For patients in cohort C, PBMCs were collected immediately prior to gemcitabine therapy on day 1, prior to 17-AAG on day 2, then at 6 and 25 h after the start of the 17-AAG infusion on day 2.

The levels of Hsp90, Hsp70 and ILK were measured in cell lysates of PBMCs, by gel electrophoresis and western blotting with appropriate monoclonal antibodies as previously described [8]. Samples for each cohort were batched and assayed in the same experiment. The blots were assessed by scanning densitometry and compared to purified protein standards, as previously described [8].

Statistical methods

Per NCI CTCAE version 2.0 guidelines adverse event attributions were defined as possibly, probably, or definitely related to study treatment. The number and severity of adverse events were tabulated and summarized within each cohort. Responses were summarized by simple descriptive statistics delineating complete and partial responses as well as stable and progressive disease within each cohort.

Analysis of covariance was used to compare measurements of Hsp90, Hsp70 and ILK at various time points with respect to clinical data of DLT, objective response, and dose of 17-AAG. A compound-symmetry structure is assumed for the correlation of measurements at various time points. The *p*-values for multiple comparisons are adjusted by the Tukey-Kramer method [27]. A *p*-value of <0.05 was considered statistically significant.

Results

Thirty-nine patients (12 in cohort A, 7 in cohort B, 11 in cohort C, 6 in cohort D, and 3 in cohort E) were enrolled into the study from September 30, 2002 to April 6, 2007. Of these, two patients from cohort A, one patient from cohort B, and two patients from cohort C were replaced for inability to complete the first cycle due to progression of disease, and one patient from cohort A was ineligible. These six patients were evaluable for toxicity and response on an intention to treat basis.

Patients received a mean number of three cycles (median 2). Eighteen (46.2%) of patients discontinued treatment for progression of disease, 10 (25.6%) due to adverse events, 4 (10.3%) refused further treatment, and 7 (17.9%) came off trial for other reasons. Patients were followed for a maximum of 3 months after they went off-study.

Patient characteristics are presented in Table 1. The median age across all cohorts was 56 years (range, 25 to 80 years). All patients in the study were Caucasian. All patients had received surgery, 38 (97.4%) received prior chemotherapy and 17 (43.6%) had received prior radiation therapy. A variety of tumor types were treated, the most common were ovarian (n=7) and lung (n=5). Other tumor types (n=28) include bladder, breast, cholangiocarcinoma, colon, esophagus, kidney, melanoma, neuroendocrine, primary peritoneal, pancreas, prostate, sarcoma, thyroid and uterine.

Adverse events

All 39 patients were evaluable for DLT analysis. There was a total of 115 courses of treatment received (27 courses from cohort A, 24 courses from cohort B, 38 courses from cohort C, 16 courses from cohort D, and 10 courses form cohort E). Table 2 lists the dose escalation scheme and the number of DLTs for each cohort. In cohort A, DLT was seen in

four patients at dose level 2 (one patient had grade 3 neutropenia; one patient had grade 3 bilirubin, dehydration, GGT, hyponatremia, nausea, and vomiting; one patient had grade 3 nausea; and one patient had grade 3 thrombocytopenia). The MTD for cohort A therefore was 154 mg/m^2 of 17-AAG, 750 mg/m^2 of gemcitabine, and 40 mg/m^2 of cisplatin.

In cohort B, DLT was seen in two patients at dose level 1 (one grade 3 fatigue, hyponatremia, and thrombocytopenia; and one grade 4 neutropenia) and one patient at dose level 0 (one patient had grade 4 neutropenia and grade 3 thrombocytopenia). As a result, cohort B was closed to accrual due to excessive toxicity.

In cohort C, DLT was seen in one patient at dose level 1 (one patient had grade 3 alkaline phosphatase), expanding the cohort to 6. Two DLTs were seen at dose level 2 (one patient had grade 3 thrombocytopenia, one had fever and dyspnea). The MTD for cohort C has been determined at 154 mg/m^2 of 17-AAG and 750 mg/m^2 of gemcitabine (dose level 1). In cohort D, DLT was seen in two patients at dose level 1 (one patient had grade 3 abdominal pain and fatigue; and one patient had grade 3 anorexia). In cohort E, DLT was seen in two patients at dose level 1 (one patient had grade 3 thrombocytopenia; and one patient had grade 3 hypokalemia and thrombocytopenia). Both cohorts D and E were closed to accrual due to excessive toxicity, and a MTD was not defined.

Toxicities at least possibly related to treatment that were grade 2 or higher, as well as grade 1 toxicities occurring in ≥10% of the 39 evaluable patients are listed in Table 3. No grade 5 events were seen in this study. Among grade 3–4 toxicities, hematologic were most common: grade 3 neutropenia (*n*=11), grade 4 neutropenia (*n*=2), grade 3 leukopenia (*n*=13), and grade 3 thrombocytopenia (*n*=8). The most common grade 3–4 non-hematologic toxicities were dehydration (*n*=4), vomiting (*n*=4), nausea (*n*=3), abdominal pain (*n*=3), hyponatremia (*n*=3), hyperglycemia (*n*=3), and fatigue (*n*=3).

Antitumor activity

Table 4 details the best response of patients throughout all cohorts. Six partial responses were seen on study, which occurred in ovarian cancer (*n*=2), primary peritoneal cancer (*n*=2), lung cancer (*n*=1), and bladder cancer (*n*=1). An additional 13 patients had stable disease as their best response on study.

Hsp90, Hsp70 and ILK analyses

Excluding three patients without assay measurements, data from 36 patients were available for determination of biomarker response to treatment during the first cycle (Table 5). Among all patients, the mean Hsp90 level at 6 h after 17-AAG administration was decreased, but not significantly changed from baseline (p=0.479). Mean Hsp90 levels at 25 h after 17-AAG administration were significantly decreased from baseline levels (p<0.016).

The mean Hsp70 levels were significantly increased from baseline at 6 h and 25 h after 17-AAG administration (p=0.003 and p<0.0001 respectively). No significant change from baseline was noted in the mean levels of ILK either 6 or 25 h after 17-AAG administration (p=0.993 and p=0.925 respectively).

Changes in Hsp90, Hsp70 or ILK levels did not correlate with dose or toxicity. However, there was a correlation between response and Hsp 90 levels. Patients with a PR or SD (*n*=17) had a significantly lower level of Hsp90 levels after treatment compared to those with progressive disease (*n*=19; p=0.013). There was no significant association between response and either Hsp70 or ILK levels (p=0.491 and p=0.880 respectively).

Discussion

The MTD of 17-AAG alone in a weekly schedule previously established by our group was 308 mg/m^2 [8]. When used in combination with gemcitabine and/or cisplatin, the dose of 17-AAG could not be increased to this level due to DLTs. In cohort A, which included the three drug regimen, the MTD was a dose of 154 mg/m^2 for 17-AAG, only half of the previously established MTD for 17-AAG alone. The MTD for cohort C also used a 17-AAG dose of 154 mg/m^2.

The ability to safely escalate the dose of 17-AAG was limited in combination with gemcitabine and/or cisplatin. Grade 3 hematologic toxicity occurred frequently in this study. Previous studies have documented the myelosuppressive effects of cisplatin and gemcitabine, alone and in combination [28-31]. In our study, we found that adding 17AAG to the combination of cisplatin and gemcitabine was not clinically tolerable. Due to the inability to escalate 17-AAG and cisplatin with or without gemcitabine to doses that would be anticipated to have activity, a clear recommended phase II dose for 17-AAG with cisplatin-containing combinations was not reached. The reason for the greater than expected hematologic toxicity in this study is not clear. Treatment with 17-AAG and cisplatin may have hindered normal marrow precursors' ability to tolerate the stress induced by treatment as proposed by in vitro studies of 17-AAG and cisplatin [17].

Despite only using one-half of the previously established MTD of 17-AAG, Hsp70 was found to be increased consistent with Hsp90 being targeted. Furthermore, objective tumor responses were seen when combined with chemotherapy. This is in contrast to our prior phase I study that established MTD of weekly 17-AAG at 308 mg/m^2, with no objective responses suggesting potential for combinations to be active. In the current trial, six patients had a documented partial response, five from cohorts involving the triple drug regimen and one from cohort C with 17-AAG and gemcitabine. This suggests that 17-AAG with cytotoxic chemotherapy may increase the effectiveness of the agent.

To date, several trials evaluating the combination of 17-AAG with other agents have been published. No partial or complete responses were seen when 17-AAG was combined with either paclitaxel or irinotecan [28,29]. In contrast to our study, combinations of 17-AAG and paclitaxel or irinotecan were tolerated within the range of the single agent MTD [8,23]. This may be due to the difference in the sequence of drug administration and mechanisms of resistance. Giving 17-AAG prior to the chemotherapeutic agent or twice-weekly administration of 17-AAG may have induced a heat shock response, which includes inducing p-glycoprotein, thus protecting cells from the toxicity of some cytotoxic agents such as paclitaxel [28].

The addition of 17-AAG to targeted therapeutic agents has shown more promising activity. Modi et al. [29] reported evidence of activity of 17-AAG plus trastuzumab in trastuzumab refractory, human epidermal growth factor receptor 2 (HER-2)-positive metastatic breast cancer patients. The combination of 17-AAG and bortezomib also led to responses in patients with multiple myeloma in both bortezomib naïve and bortezomib refractory patients [30].

An interesting observation from this study was that mean Hsp90 levels in circulating PBMCs across all cohorts were significantly decreased at 25 h after the start of the 17-AAG infusion compared to baseline. This response was also significantly lower in the subset of patients with SD or PR compared to those with progressive disease. Four previous phase I trials involving single agent 17-AAG measured no significant change in Hsp90 levels of PBMCs after 17-AAG administration [8,9,23,24]. In two other trials that did show clinical responses with 17-AAG combined with another agent, Hsp90 levels were not reported

[29,31]. The decrease in HSP90 after treatment and its association with clinical benefit was unanticipated. It is conceivable that a decrease in the protein is a reflection of the chemotherapy inhibiting translation of HSP90 selectively. The relationship of this decrease to clinical benefit may be that in those cases where the stress response is not able to be induced by 17-AAG treatment is more effective. Since the antibody used to measure HSP90 detected total HSP90 and did not distinguish the inducible from the constitutively expressed forms it is not possible to dissect out whether the drop in total HSP90 was associated with more of the activated form of the protein (36) which would make tumors more sensitive to 17-AAG. Further studies in other trials to confirm this observation will be necessary.

Consistent with findings from several prior phase I clinical trials using single agent 17-AAG, levels of Hsp70 were significantly elevated both 6 and 25 h after 17-AAG administration. This biomarker response indicates that Hsp90 was effectively targeted despite lower doses of 17-AAG. However, we did not see a consistent decrease in ILK levels that was anticipated, suggesting the levels of ILK in normal tissue after 17-AAG treatment may not be a reliable indicator of Hsp90 degradation.

In conclusion, this phase I clinical trial established weekly administration of 17-AAG, gemcitabine and cisplatin can safely be given at doses of 154 mg/m^2, 750 mg/m^2, and 40 mg/m^2 respectively and has clinical activity. We also determined a MTD of 154 mg/m^2 of 17-AAG and 750 mg/m^2 of gemcitabine administered weekly, which had evidence of clinical activity. The results of this trial have led to ongoing phase II clinical trials with 17-AAG and gemcitabine in pancreas and ovarian cancers.

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Table 1

Patient characteristics

	Cohort A (*n*=12)	Cohort B (*n*=7)	Cohort C (*n*=11)	Cohort D (*n*=6)	Cohort E (*n*=3)	Total (*n*=39)
Age						
Median	51.0	59.0	63.0	64.5	48.0	56.0
Range	(25.0–74.0)	(46.0–78.0)	(38.0–76.0)	(28.0–80.0)	(35.0–56.0)	(25.0–80.0)
Gender						
Female	9	3	8	3	2	25
Male	3	4	3	3	1	14
Performance score						
0	6	4	4	3	1	18
1	6	3	7	3	2	21
Prior chemotherapy						
Yes	12	7	10	6	3	38
Prior surgery						
Yes	12	7	11	6	3	39
Prior radiation						
Yes	5	4	5	2	1	17
No	7	3	6	4	2	22
Primary site						
Ovarian	3	2	0	1	1	7
Lung	3	1	1	0	0	5
Other	6	4	10	5	2	27

Table 2

Dose escalation scheme. The starting dose level for all cohorts was dose level 1. Bold indicates the MTD, when determined. In cohort A, one of the patients experiencing DLT did not complete cycle 1 and was replaced

Cohort	Dose level	Dose (mg/m^2)			No. of patients	DLTs
		17-AAG	Gemcitabine	Cisplatin		
A	1	**154**	**750**	**40**	6	0
	2	154	1,000	40	3	3 (1)
B	0	154	750	40	3	1
	1	231	750	40	3	2
C	1	**154**	**750**		6	1
	2	231	750		3	2
D	0	154		30	3	0
	1	154		40	3	2
E	1	300	750	20	3	2

Table 3

Toxicities over all cohorts at least possibly related to treatment. Each unit represents an adverse event occurring on study that is possibly, likely or definitely related to study treatment and ≥ grade 2 in severity or grade 1 events occurring at least 10% of patients. Grade 1 toxicities occurring in <10% of patients include: dyspepsia, hiccups, SGPT (ALT) elevation, smell disturbances, cough, sinus tachycardia, weight loss, weight gain, creatinine elevation, rigors, low consciousness, hematemesis, rectal bleeding, headache, pneumonitis, rash, hypermagnesemia, arrhythmia and neurologic

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Anemia	12	14	4	0
Nausea	17	7	3	0
Fatigue	8	13	3	0
Thrombocytopenia	8	8	8	0
Leukopenia	2	6	13	0
Anorexia	9	7	1	0
Neutropenia	2	2	11	2
Vomiting	10	3	4	0
Neurosensory	12	2	0	0
Constipation	6	5	0	1
Alopecia	8	1	0	0
SGOT (AST)	8	0	0	0
Alkaline phosphatase	4	1	2	0
Diarrhea	6	1	0	0
Dehydration	1	1	4	0
Taste alteration	6	0	0	0
Hypokalemia	4	0	2	0
Hypomagnesemia	3	2	1	0
Stomatitis	5	1	0	0
GI - other	3	2	1	0
Abdominal pain	2	0	3	0
Dyspnea	0	5	0	0
Arthralgia	2	2	0	0
Hyperglycemia	1	0	2	1
Rash/desquamation	2	1	1	0
Skin irritation	2	2	0	0
Chest pain	1	2	0	0
Dizziness	2	1	0	0
Hyponatremia	0	0	3	0
Hyperbilirubinemia	0	1	1	0
Gastritis	0	2	0	0
Hypocalcemia	1	1	0	0
Lymphopenia	1	0	1	0
Myalgia	1	1	0	0
Palpitations	1	1	0	0

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Involuntary movement	0	1	0	0
Neutropenic fever	0	0	1	0
GGT elevation	0	0	1	0
Hypersensitivity	0	0	1	0
Hypoalbuminemia	0	1	0	0
Infection	0	0	1	0
Edema	0	1	0	0
Photopsia	0	1	0	0
Pruritus	0	1	0	0
Blurred vision	0	1	0	0

Table 4

Best response on study. The best antitumor response by RECIST criteria for each cohort. *PR* partial response, *SD* stable disease, *PD* progressive disease, *TE*/*NA* toxicity event/not assessed

Best response	Cohort A (*n*=12)	Cohort B (*n*=7)	Cohort C (*n*=11)	Cohort D (*n*=6)	Cohort E (*n*=3)	Total (*n*=39)
PR	2	2	1	0	1	6
SD	2	3	4	4	0	13
PD	4	0	4	0	1	9
TE/NA	4	2	2	2	1	11

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Table 5

Biomarker levels according to response. PBMCs were collected as described in the methods section at the time points indicated. Protein lysates were then analyzed for western blotting for hsp90, hsp70 and ILK. Western blots were then quantitated by densitometry and the 6 and 25 h samples were expressed as percent of the pre-17-AAG sample. *PR* partial response, *SD* stable disease, *PD* progressive disease, *TE*/*NA* toxicity event/not assessed

Factor	Level	# of patients	HSP90		HSP70		ILK	
			Mean	*p*-value	Mean	*p*-value	Mean	*p*-value
DLT	No	24	100.4		140.5		102.3	
	Yes	12	91.7	0.135	145.4	0.662	100.8	0.872
Response	PD+TE+NA	19	103.2		146.6		102.2	
	PR+SD	17	88.9	0.013*	139.3	0.491	100.9	0.880
17-AAG dose	154 mg/m^2	27	88.1		127.3		99.8	
	231 mg/m^2	6	100.0	0.190	149.2	0.232	99.9	1.000
	300 mg/m^2	3	100.1	0.318	152.3	0.278	104.9	0.905
Time	Pre-17AAG	36	103.2		110.5		100.8	
	6 h post	36	97.0	0.479	145.8	0.003*	99.9	0.993
	25 h post	36	88.0	0.016*	172.6	<.0001*	104.0	0.925

*$p<0.05$