Phase I Pharmacokinetic and Pharmacodynamic Study of 17-dimethylaminoethylamino-17-demethoxygeldanamycin, an Inhibitor of Heat-Shock Protein 90, in Patients With Advanced Solid Tumors

Ramesh K. Ramanathan, Merrill J. Egorin, Charles Erlichman, Scot C. Remick, Suresh S. Ramalingam, Cynthia Naret, Julianne L. Holleran, Cynthia J. TenEyck, S. Percy Ivy, and Chandra P. Belani

ABSTRACT

Purpose

To define the maximum tolerated dose, toxicities, pharmacokinetics, and pharmacodynamics of 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17DMAG).

Methods

17DMAG was given intravenously over 1 hour daily for 5 days (schedule A) or daily for 3 days (schedule B) every 3 weeks. Plasma 17DMAG concentrations were measured by liquid chromatography/mass spectrometry. Heat-shock proteins (HSPs) and client proteins were evaluated at baseline and after treatment on day 1 in peripheral blood mononuclear cells (PBMCs) and in pre- and post-treatment (24 hours) biopsies done during cycle 1 at the recommended phase II dose ($n = 7$).

Results

Fifty-six patients were entered: 26 on schedule A; 30 on schedule B. The recommended phase II doses for schedules A and B were 16 mg/m² and 25 mg/m², respectively. Grade 3/4 toxicities included liver function test elevation (14%), pneumonitis (9%), diarrhea (4%), nausea (4%), fatigue (4%) and thrombocytopenia (4%). There were no objective responses. Four patients had stable disease. 17DMAG half-life was 24 ± 15 hours. 17DMAG area under the curve (range, 0.7 to 14.7 mg/mL $\times$ h) increased linearly with dose. The median HSP90, HSP70, and integrin-linked kinase levels were 87.5% ($n = 14$), 124% ($n = 20$), and 99.5% ($n = 20$) of baseline. Changes in HSPs and client proteins in tumor biopsies were not consistent between baseline and 24 hours nor did they change in the same direction as those in PBMCs collected at the time of biopsy.

Conclusion

The recommended phase II doses of 17DMAG (16 mg/m² $\times$ 5 days or 25 mg/m² $\times$ 3 days, every 3 weeks) are well tolerated and suitable for further evaluation.

J Clin Oncol 28:1520-1526. © 2010 by American Society of Clinical Oncology

INTRODUCTION

Heat-shock protein 90 (HSP90) chaperones multiple client proteins such as mutant p53, HER-2/*neu*, raf-1, and Bcr-Abl that are involved in cell signaling, proliferation and survival. HSP90 inhibition thus is a rational target for drug development.¹⁻³ The geldanamycin analog, 17-(allylamino)-17-demethoxygeldanamycin (17AAG, NSC 330507) was the first HSP90 inhibitor to enter clinical evaluation. Phase I studies of 17AAG (tanespimycin) given to adults and children weekly, twice-a-week, daily for 3 days or daily for 5 days have been completed,⁴⁻¹¹ and this agent is now in phase II/III evaluation.¹

17-dimethylaminoethylamino-17-demethoxygeldanamycin, (17DMAG, NSC 707545) is a water-soluble analog of 17AAG.^{12,13} In addition to having superior preclinical activity compared to 17AAG, 17DMAG has higher solubility, higher oral bioavailability in mice, lower binding to plasma proteins, and does not undergo extensive metabolism as does 17AAG.^{12,13} Both 17AAG and 17DMAG are excreted primarily through the hepatobiliary system.¹⁴ The primary objective of this study was to define the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) of 17DMAG. Secondary objectives included characterizing the pharmacokinetics (PK) of 17DMAG and evaluating the pharmacodynamics (PD) of 17DMAG, as reflected by changes in HSPs

From the Division of Hematology/Oncology, Departments of Medicine and Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine; Molecular Therapeutics/Drug Discovery Program, University of Pittsburgh Cancer Institute, Pittsburgh, PA; Department of Oncology, Mayo Clinic, Rochester, MN; Developmental Therapeutics Program, Case Comprehensive Cancer Center, University Hospitals Case Medical Center, Cleveland, OH; and the Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment and Centers, National Cancer Institute, Bethesda, MD.

Submitted July 7, 2009; accepted November 16, 2009; published online ahead of print at www.jco.org on February 22, 2010.

Supported in part by National Institutes of Health Grants No. U01-CA099168, U01-CA69855, and P30CA47904, and NIH/GCRC #5M01 RR 00056 to the University of Pittsburgh Cancer Institute and Medical Center; NCI U01 CA 62502 and NIH/GCRC M01-RR00080 to University Hospitals Case Medical Center; and U01CA69912, R01CA90390, CA15083, and UL1RR24150 to the Mayo Clinic.

Presented in part at the 42nd Annual Meeting of the American Society of Clinical Oncology, Atlanta, GA, June 2-6, 2006 and the Molecular Targets and Cancer Therapeutics, American Association for Cancer Research/National Cancer Institute/European Organisation for the Research and Treatment of Cancer Conference, San Francisco, CA, October 22-26, 2007.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

Corresponding author: Ramesh K. Ramanathan, MD, Scottsdale Clinical Research Institute, 13208 East Shea Blvd, Ste 100, Scottsdale, AZ 85259; e-mail: rramanathan@tgen.org.

© 2010 by American Society of Clinical Oncology

0732-183X/10/2809-1520/\$20.00

DOI: 10.1200/JCO.2009.25.0415

and client proteins in peripheral blood mononuclear cells (PBMC) and paired tumor biopsies. The starting dose for human trials was 1.5 mg/m²/d for 5 days, which was one sixth of the well-tolerated dose in dogs, the most sensitive species.¹⁵

METHODS

Patient Selection

Pertinent requirements were: histologically confirmed advanced cancer not curable by standard therapies; Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 ; Adequate hematologic and renal function; total bilirubin $\leq$ upper limit of normal (ULN) and serum AST and ALT $\leq 1.5 \times$ the ULN. Before entering the study, all patients gave written consent according to institutional and federal guidelines. Two major protocol amendments were made during the course of the study. Due to concerns about cardiac toxicity in ongoing studies of 17AAG, that were subsequently not validated in a central ECG review,¹⁶ additional exclusion criteria were added in August 2005. These were: a history of congenital long QT syndrome; use of concomitant medications that could prolong the QTc interval;¹⁷ heart failure (New York Heart Association, class III and IV); history of myocardial infarction within 1 year of study entry; uncontrolled dysrhythmias; or poorly controlled angina. In addition, patients with a history of serious ventricular arrhythmia (ventricular tachycardia or ventricular fibrillation, ≥ 3 premature ventricular contractions in a row), QTc ≥ 450 milliseconds for men and 470 milliseconds for women, left ventricular ejection fraction $\leq 40\%$ by multiple-gated acquisition, prior cardiac radiation, uncontrolled dysrhythmias or requiring antiarrhythmic drugs, or left bundle branch block were excluded. In June 2006, due to DLT of pulmonary toxicity in this study, patients with symptomatic pulmonary disease, such as those requiring medications for pulmonary disease or those that met Medicare criteria for receiving home oxygen, were excluded.

Drug Administration

17DMAG was supplied by the Division of Cancer Treatment and Diagnosis (Rockville, MD) under a cooperative research and development agreement with Kosan Biosciences (Hayward, CA), in sterile, single-use vials containing either 10 mg or 50 mg of lyophilized 17DMAG with citrate buffer and mannitol. Vial components were reconstituted with sterile water to yield a 5 mg/mL (free base) clear, dark purple solution of 17DMAG. The required dose of drug was further diluted in 40 to 200 mL of 0.9% NaCl to a concentration between 0.1 mg/mL and 1 mg/mL and infused over 1 hour. Prophylactic antiemetic therapy with oral or intravenous prochlorperazine or metoclopramide before each dose was recommended for all patients.

Patient Accrual

Patients were entered onto schedule A or B independently. The starting dose for Schedule A was 1.5 mg/m²/d for 5 days, and the starting dose for schedule B was 2.5 mg/m²/d for 3 days. Initially, an accelerated titration schema with one to two patients/dose level was followed with dose-doubling in sequential cohorts of patients until grade 2, or higher, hematologic or nonhematologic toxicity (except nausea, vomiting, and alopecia) was observed. At that point, dose doubling was to be terminated, and patients were to be accrued to dose levels of approximately 35% dose increments, with three to six patients in each cohort until the MTD was reached.¹⁸ Inpatient dose escalation was allowed if higher dose levels had been evaluated and had been determined to be safe in other patients. The highest dose level at which at least one of six patients experienced a DLT was considered the MTD or the dose recommended for future phase II studies. The MTD cohort could be expanded to 12 patients.

DLTs

Toxicity was graded according to National Cancer Institute Common Toxicity Criteria, version 2.0. DLT was defined as any drug-related (possible, probable, or definite) grade ≥ 3 nonhematologic toxicity (except alopecia), thrombocytopenia, febrile neutropenia or grade 4 neutropenia occurring in cycle 1. A grade ≥ 3 QTc prolongation or a delay in starting cycle 2 by longer than 2 weeks due to toxicity also constituted a DLT.

Dose Modifications

A 2-week delay was permitted until recovery from toxicity or for logistical reasons. A maximum of two dose reductions was allowed, with reductions being to the next lower dose level or, in the case of dose level 1, a 25% dose reduction. Dose reductions were made if treatment was delayed by 1 week for toxicity-related failure to meet prestudy requirements. In the case of grade ≥ 3 neutrophil, platelet, or nonhematologic toxicity, treatment was held until recovery to $\leq$ grade 1, and treatment was resumed with a dose reduction. If left ventricular ejection fraction decreased by $\geq 25\%$ from baseline or was $\leq 40\%$, patients were removed from study. New onset arrhythmia, cardiac ischemia or QTc prolongation by ≥ 50 milliseconds also necessitated removal from study.

Study Requirements and Assessments

A history and physical examination were done prestudy and before every cycle. A CBC, serum electrolytes, and chemistries were evaluated prestudy and then weekly. Radiographs to follow response were done prestudy and after every two cycles. Response Evaluation Criteria in Solid Tumors were used to assess response.¹⁹

PK Assessment

On day 1, blood samples (5 mL) were collected in heparinized tubes at the following times: predose, 30 minutes into and 5 minutes before the end of the 1-hour infusion, and at 5, 10, 15, 30 minutes, 1, 2, 4, 8, 12, 16, and 24 hours after the end of the infusion. A predose sample was drawn on all subsequent days of therapy. On day 5 for schedule A and on day 3 for schedule B, sampling similar to that of day 1 was performed until 4 hours after the end of the 17DMAG infusion. Blood samples were centrifuged at $1,000 \times g$ for 10 minutes, and the resulting plasma supernatants were stored at -70°C until analyzed. On day 1, urine was collected from 0 to 24 hours as 6-hour aliquots. The volume of each 6-hour aliquot was measured, and a portion was stored at -70°C until analyzed. 17DMAG concentrations in blood and urine were measured by an liquid chromatography/mass spectrometry assay developed and validated at the University of Pittsburgh.¹⁵ 17DMAG concentration versus time data were modeled noncompartmentally using the LaGrange function²⁰ as implemented by the LAGRAN computer program.²¹

Assessment of HSP90 and Client Proteins in PBMCs and Tumor Biopsies

Blood samples (25 mL) for PBMCs were collected from patients predose, 4 hours postinfusion, and before each subsequent dose at 24 and 48 hours during cycle 1. PBMCs were isolated and protein extracted as previously reported.^{5,6} At the phase II dose, tumor biopsies were obtained predose and at 24 ± 3 hours after dosing on day 1. Tumor samples were snap-frozen in liquid nitrogen and stored at -80°C until analysis. Changes in selected marker proteins were measured by Western blotting. HSP90 and HSP70 were determined in PBMCs and HSP70 and HSP27 were assessed in tumor biopsies as indicators that 17DMAG had bound HSP90. CDK4, RAF-1, AKT, and ILK were used as markers of HSP90 client protein degradation in tumor biopsies. Only ILK was measured in PBMCs to assess client protein degradation. PBMC and tumor biopsy samples were analyzed at Mayo Clinic by methods previously described.⁶ Results were normalized for actin loading and expressed as a fraction of the pretreatment sample.⁶ Descriptive statistics and vertical scatter plots were used to present protein levels. Levels of these proteins were expressed as a percentage change in their levels relative to baseline and analyzed for significance using a Wilcoxon signed rank test. A *P* value lower than .05 was considered as statistically significant. Due to the exploratory nature of the analysis, the significance level was not adjusted for multiple comparisons.

RESULTS

Patient Characteristics

Between July 2004 and January 2007, 56 patients (26 on schedule A and 30 on schedule B) were enrolled in the study at three participating institutions. Patient characteristics are described in Table 1. Dose levels evaluated on schedule A were 1.5, 3, 6, 9, 12, 16, and 22 mg/m² (Table 2). Dose levels evaluated on schedule B started at 2.5 mg/m². A

Table 1. Patient Demographic and Clinical Characteristics

Characteristic	No.
Total	56
Male	32
Female	24
White race	56
%	100
Median age, years	62
Range	28-32
Tumor type	
Colorectal	17
Ovarian	4
Esophageal	5
Head and neck	4
Sarcoma	4
Neuroendocrine	3
Breast	3
Melanoma	3
Salivary gland	3
Lung	2
Prostate	2
Other*	6
ECOG performance status	
0	25
1	27
2	4

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

*Includes (one of each): bladder, gallbladder, mesothelioma, kidney, cervix, and uterus cancer.

grade 2 elevation of AST was noted in the first patient treated on schedule B, and, following protocol guidelines, this dose level was expanded to three patients. By the time the first dose level in schedule B had completed accrual of three patients, schedule A had completed

accrual of patients at the 12 mg/m² dose level without experiencing a DLT. A protocol amendment to begin accrual on schedule B at 14 mg/m² was submitted and approved. The doses subsequently evaluated in schedule B were 14, 19, 25, 34, and 46 mg/m² (Table 2). Patients received a median of two cycles (range, one to 14 cycles).

Toxicity

On schedule A, at the dose of 12 mg/m², one patient had renal failure initially thought to be a DLT, and the cohort was expanded to six patients. On subsequent review, the event was felt secondary to disease progression, and dose escalation to 16 mg/m² was initiated. The maximal dose evaluated in schedule A was 22 mg/m², with the first patient treated at that dose experiencing grade 3 dyspnea due to reversible pneumonitis. At that time, DLT was also noted in schedule B at the dose of 34 mg/m², where two patients had grade 3 dyspnea secondary to pneumonitis. These respiratory symptoms, which occurred after the second, third, and fourth doses, respectively, in the three patients, were acute events in cycle 1, required hospitalization with symptoms resolving rapidly in 1 to 2 days with steroid therapy and supportive care. Computed tomography scans of the chest revealed an interstitial pattern of injury compatible with pneumonitis. None of the three patients experiencing pulmonary toxicity were rechallenged with 17DMAG. Because pneumonitis occurred at similar cumulative doses in both schedules, accrual to schedule A was terminated after accrual of one patient at the 22 mg/m² dose level. The dose level of 16 mg/m² was then expanded to six patients and declared to be the recommended phase II dose. On schedule B, the highest dose evaluated was 46 mg/m². At this dose, two patients developed grade 3 fatigue in their first cycle of treatment. Therefore, the 34 mg/m² dose level was expanded by an additional three patients. Although the first three patients treated at this dose level did not have any DLTs, one of the additional three patients had grade 4 thrombocytopenia and the

Table 2. Study Schema and Dose Escalation

Level	Dose (mg/m ²)	No. of Patients	DLT	Comment
Schedule A*				
1	1.5	1	No	1-2 patients/cohort
2	3	1	No	1-2 patients/cohort
3	6	2	No	1-2 patients/cohort
4	9	3 (2 not evaluable)	No	1-2 patients/cohort
5	12	3	No	Start 3 patients/cohort (possible DLT)
		3 (1 not evaluable)	No	
6	16	3	No	Phase II dose
		5 (1 not evaluable)	Grade 3 ALT (n = 1)	2 patients had biopsies
7	22	1	Grade 3 dyspnea	Stop accrual expand level 6
Schedule B†				
1	2.5	3	No	Grade 2 AST in 1 patient, cohort expanded
2	14	3	No	Protocol amendment 3 patients/cohort
3	19	3	No	
4	25	5 (2 not evaluable)	No	Phase II dose
		8	N/V and Plt (n = 1)	5 patients had biopsies
5	34	3	No	Expand level 4
		3	Grade 3 dyspnea (n = 2)	
			Grade 4 Plt (n = 1)	
6	46	3	Grade 3 fatigue (n = 2)	Accrual stopped expand level 5

Abbreviations: DLT, dose-limiting toxicity; Plt, platelets; N/V, nausea and vomiting.

*Daily for 5 days every 3 weeks.

†Daily for 3 days every 3 weeks.

Table 3. Selected Toxicities (all cycles)

Toxicity	Grade			
	1/2		3/4	
	No.	%	No.	%
No. of patients	56			
Constitutional fatigue	23	41	2	4
Pulmonary dyspnea	4	8	5	9
Hepatic				
AST/ALT	7	13	3	6
AP	9	16	4	8
Total bilirubin	1	2	0	0
GI				
Diarrhea	12	21	2	4
Nausea	18	32	2	4
Vomiting	8	14	1	2
Hematologic				
Hemoglobin	13	23	1	2
Platelets	4	8	2	4
Neutrophils	0	0	0	0

NOTE. Scored by investigator as possibly, probably, or definitely related to therapy.

Abbreviation: AP, alkaline phosphatase.

other two developed the grade 3 dyspnea and pneumonitis described above. Therefore, the 25 mg/m² dose level was expanded, and after eight evaluable patients had been treated without experiencing DLTs, was declared to be the recommended phase II dose for schedule B. Common grade 3/4 toxicities observed in all cycles (Table 3) were liver function test elevations (14%), pneumonitis (9%), diarrhea (4%), nausea (4%), fatigue (4%), and thrombocytopenia (4%). Intensive EKG monitoring was done on the first day of cycle 1 in three patients treated at 34 mg/m² and three patients treated at 46 mg/m².

Antitumor Activity

There were no objective responses. Four patients had stable disease. These included patients with carcinoid (14 cycles on schedule A,

6-12 mg/m²), melanoma (12 cycles on schedule B, 19 mg/m²), non-small-cell lung cancer (10 cycles on schedule A, 12 mg/m²), and a salivary gland tumor (6 cycles on schedule A, 16 mg/m²).

17DMAG Pharmacokinetics

17DMAG PK on day 1 were linear over the dose range of 1.5 to 46 mg/m². The maximum plasma 17DMAG concentration (range, 0.071 to 1.7 µg/mL) and area under the curve (range, 0.7 to 14.7 µg/mL×h) increased linearly with dose, whereas clearance (79 ± 40 mL/min/m²) and half-life (24 ± 15 hours) did not vary systematically with dose (Table 4). Some patients had accumulation of 17DMAG with repeated dosing, whereas others did not (Fig 1). The 24-hour urinary excretion of 17DMAG accounted for 20% ± 9% of dose.

HSP and Client Proteins in PBMCs and Tumor

Fifty-five patients had PBMC samples collected at 24 hours, 16 patients had 4-hour samples, and 14 had 48-hour samples. At the recommended phase II doses, seven patients underwent simultaneous tumor biopsy and PBMC collection. On schedule A, at 16 mg/m², biopsies were done on two patients with parotid gland tumors. On schedule B, biopsies were done on one patient with head and neck cancer and four patients with colon cancer treated at 25 mg/m². However, the blot from one patient with a parotid gland tumor was not interpretable, which left six paired samples for analysis. There was wide variability in the changes seen in protein levels, particularly HSP90 and HSP70 in PBMCs (Fig 2A). The median HSP90, HSP70, and ILK levels were 87.5% (n = 14), 124% (n = 20), and 99.5% (n = 20) of baseline, respectively, in PBMCs obtained at 24 hours after 17DMAG administration. The change in HSP90 and ILK levels from baseline was not significant (*P* > .29), nor was the change in HSP70 levels was significantly different from baseline (*P* = .10). In tumor samples obtained before and at 24 hours after the first dose of 17DMAG, the mean HSP27 and HSP70 levels were 92% ± 18% (n = 6), and 74% ± 14% (n = 6) of baseline, respectively, which were no different from baseline. There was no consistent change from pretreatment levels of the client proteins AKT, RAF, ILK, or CDK4 in the tumor biopsies (Fig 2B). Furthermore, there were no consistent changes from pretreatment levels of the client proteins AKT, RAF,

Table 4. 17DMAG Pharmacokinetic Parameters

Dose (mg/m ²)	No.	C _{max} (µg/mL)		t _{1/2} (hour)		AUC _{0-inf} (µg/mL×h)		CL _{tb} (mL/h/m ²)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.5	1	0.042		65.9		1.1		23	
2.5	3	0.071	0.032	16.3	15.8	0.7	0.4	87	64
3	1	0.076		ND*		ND		ND	
6	1	0.132		18.9	7.4	1.0		96	
9	3	0.39	0.161	22.5	10.2	4.4	3.2	47	25
12	7	0.294	0.18	32.4	21.0	3.3	2.2	86	47
14	3	0.398	0.033	23.8	9.0	4.8	1.0	49	10
16	8	0.398	0.144	24.9	12.1	3.5	2.5	99	42
19	2	0.39	0.042	18.4	5.2	3.6	0.2	90	6
25	9	0.583	0.536	19.2	19.1	4.5	4.4	113	116
34	6	1.06	1.069	14.3	2.3	5.8	0.6	103	11
46	3	1.7	0.548	12.2	3.0	12.8	2.7	61	13

Abbreviations: 17DMAG, 17-imethylaminoethylamino-17-demethoxygeldanamycin; C_{max}, maximum concentration; t_{1/2}, terminal half-life; AUC_{0-inf}, area under the curve from zero to infinity; CL_{tb}, clearance; SD, standard deviation; ND, not done.

*ND due to inadequate sampling.

Fig 1. 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17DMAG) concentration versus time profiles from two patients, one of whom showed accumulation of 17DMAG with (A) daily dosing and the (B) other one of whom did not.

ILK, or CDK4, and when compared to the changes seen in PBMCs, there was no association (Appendix Fig A1, online only).

DISCUSSION

Based on our study, the recommended phase II doses for 17DMAG are $16 \text{ mg/m}^2 \times 5 \text{ days}$ or $25 \text{ mg/m}^2 \times 3 \text{ days}$ repeated every 3 weeks. Therapy was well tolerated at the phase II doses, and pharmacokinetics were linear. An unexpected DLT at the highest doses was reversible pneumonitis, which was not predicted by animal toxicology. Lung toxicity may be due to drug accumulation, in animal studies 17DMAG concentrations in liver, kidney, and lung were approximately 8- to 10-fold higher than concurrent plasma levels.¹³ Grade 1 to 3 dyspnea and pulmonary symptoms were seen in six other patients, but infection or disease progression were thought to be contributing factors. In this study, we evaluated the effect of drug on target modulation and client protein degradation at the blood levels achieved. The effect of DMAG on HSP90 and 70 levels in PBMCs was variable. This was, in part, due to the large variability in the levels of HSP90 among patients and probably because the samples were obtained from patients treated at different dose levels. The levels of ILK, a client protein, appeared to increase rather than decline, which may reflect rapid turnover of this

Fig 2. (A) Biomarker change in peripheral blood mononuclear cells (PBMCs) after a single dose of dimethylaminoethylamino-demethoxygeldanamycin (DMAG) in cycle 1 treated at different dose levels. PBMCs were collected as described in the Methods section. Protein lysates were then analyzed by western blotting for heat-shock protein (HSP) 90, HSP70, and ILK. Western blots were then quantitated by densitometry, and 24-hour samples were expressed as percentage of the pre-DMAG sample. (B) Western blots of paired patient tumor biopsies obtained before (B) and 24 hours after (A) the first dose of DMAG at the 16 mg/m^2 (patients 1 and 2) and 25 mg/m^2 (patients 3 to 7) dose levels.

protein and recovery at the 24-hour time point studied. This is consistent with the results we previously reported.¹⁰ Tumor biopsies also showed a difference between pre- and post-treatment biopsies (Figs 2B and 3) in individual patients, but the changes were not consistent between patients. Whether this was related to treatment or was between-day variation in expression is unclear. When samples were corrected for loading and expressed as change from baseline there was sufficient variability between patients that no definite conclusions can be drawn. The comparison of protein levels in PBMCs and biopsies taken at the same time suggests that at least for HSP70 and HSP27 an increase may have occurred in PBMCs but not the tumors. This raises the possibility that drug levels were sufficient to affect the target in one tissue and not the other or that simultaneous changes in 2 tissue compartments may not occur. Although our data are inconclusive with regard to this point, they do identify the issue that PBMCs may not be a good substitute for effects in solid tumors. These results indicate that, in future studies, it will be necessary to obtain paired PBMC and biopsy samples from more than six patients if data to assess target effect in tumor, as a potential surrogate and the relationship between them, is to be defined. Whereas the apparent lack of target modulation in biopsies taken at 24 hours raises the possibility that this schedule may not be optimal for further evaluation, the small sample size with two dose levels and only one time point to evaluate the effect on client proteins cannot preclude potential activity of the schedule.

Phase I studies of 17DMAG are evaluating a variety of schedules. When given on a twice-a-week schedule,²² the recommended phase II dose was 21 mg/m². Pacey et al²³ have reported results of 17DMAG given weekly, with the phase II recommended dose being 80 mg/m². Flaherty et al²⁴ have evaluated an oral formulation of 17 DMAG given daily or on alternate days on a 4-of-6 week schedule and established the recommended phase II doses of 20 mg and 40 mg, respectively. DLTs reported in these studies were varied and consisted of fatigue, diarrhea, dehydration, AST elevation, thrombocytopenia, hemorrhagic colitis, nephrotic syndrome, renal failure, and neuropathy.²²⁻²⁴ Phase I studies of 17 AAG have demonstrated that toxicity is schedule dependent. Weekly schedules (450 mg/m²) of 17 AAG are being utilized in ongoing phase II studies. A comprehensive evaluation of all 17DMAG trials will be required to determine the optimum schedule for future studies. Active investigation of HSP-targeted agents continues with second generation geldanamycins as well as a number of other small molecule inhibitors.²⁵⁻²⁹

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

REFERENCES

- Banerji U: Heat shock protein 90 as a drug target: Some like it hot. *Clin Cancer Res* 15:9-14, 2009
- Pearl LH, Prodromou C, Workman P: The Hsp90 molecular chaperone: An open and shut case for treatment. *Biochem J* 410:439-453, 2008
- Workman P, Burrows F, Neckers L, et al: Drugging the cancer chaperone HSP90: Combinatorial therapeutic exploitation of oncogene addiction and tumor stress. *Ann N Y Acad Sci* 1113:202-216, 2007
- Ramanathan RK, Trump DL, Eiseman JL, et al: Phase I pharmacokinetic-pharmacodynamic study of 17-(allylamino)-17-demethoxygeldanamycin (17AAG, NSC 330507), a novel inhibitor of heat shock protein 90, in patients with refractory advanced cancers. *Clin Cancer Res* 11:3385-3391, 2005
- Ramanathan RK, Egorin MJ, Eiseman JL, et al: Phase I and pharmacodynamic study of 17-(allylamino)-17-demethoxygeldanamycin in adult patients with refractory advanced cancers. *Clin Cancer Res* 13:1769-1774, 2007
- Goetz MP, Toft D, Reid J, et al: Phase I trial of 17-allylamino-17-demethoxygeldanamycin in patients with advanced cancer. *J Clin Oncol* 23:1078-1087, 2005
- Banerji U, O'Donnell A, Scurr M, et al: Phase I pharmacokinetic and pharmacodynamic study of 17-allylamino, 17-demethoxygeldanamycin in patients with advanced malignancies. *J Clin Oncol* 23:4152-4161, 2005
- Solit DB, Ivy SP, Kopil C, et al: Phase I trial of 17-allylamino-17-demethoxygeldanamycin in patients with advanced cancer. *Clin Cancer Res* 13:1775-1782, 2007
- Grem JL, Morrison G, Guo XD, et al: Phase I and pharmacologic study of 17-(allylamino)-17-demethoxygeldanamycin in adult patients with solid tumors. *J Clin Oncol* 23:1885-1893, 2005
- Nowakowski GS, McCollum AK, Ames MM, et al: A phase I trial of twice-weekly 17-allylamino-demethoxy-geldanamycin in patients with advanced cancer. *Clin Cancer Res* 12:6087-6093, 2006
- Bagatell R, Gore L, Egorin MJ, et al: Phase I pharmacokinetic and pharmacodynamic study of 17-N-allylamino-17-demethoxygeldanamycin in pediatric patients with recurrent or refractory solid tumors: A pediatric oncology experimental therapeutics investigators consortium study. *Clin Cancer Res* 13:1783-1788, 2007
- Egorin MJ, Lagattuta TF, Hamburger DR, et al: Pharmacokinetics, tissue distribution, and metabolism of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (NSC 707545) in CD2F1 mice and Fischer 344 rats. *Cancer Chemother Pharmacol* 49:7-19, 2002
- Eiseman JL, Lan J, Lagattuta TF, et al: 17-demethoxy 17-[(2-dimethylamino) ethyl]amino] geldanamycin (17DMAG, NSC 707545) in C.B-17 SCID mice bearing MDA-MB-231 human breast cancer xenografts. *Cancer Chemother Pharmacol* 55:21-32, 2005
- Musser SM, Egorin MJ, Zuhowski EG, et al: Biliary excretion of 17-(allylamino)-17-demethoxygeldanamycin (NSC 330507) and metabolites by Fischer 344 rats. *Cancer Chemother Pharmacol* 52:139-146, 2003
- Glaze ER, Lambert AL, Smith AC, et al: Pre-clinical toxicity of a geldanamycin analog, 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG), in rats and dogs: Potential clinical relevance. *Cancer Chemother Pharmacol* 56:637-647, 2005
- Cropp G, Solit D, Kersey K, et al: Tanespimycin (17-AAG, KOS-953): No effects on QTc interval using two formulations. Proceedings of Annual Meeting of the American Association for Cancer Research, Los Angeles, CA, April 14-17, 2007 (abstr 1544)
- Arizona CERT Center for Education and Research on Therapeutics: QT list by drug risk. <http://www.azcert.org/medical-pros/drug-lists/drug-lists.cfm>
- Simon R, Freidlin B, Rubinstein L, et al: Accelerated titration designs for phase I clinical trials in oncology. *J Natl Cancer Inst* 89:1138-1147, 1997
- Therasse P, Arbuck SG, Eisenhauer EA, et al: New guidelines to evaluate the response to treatment in solid tumors: European Organisation for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 92:205-216, 2000
- Yeh KC, Kwan KC: A comparison of numerical integrating algorithms by trapezoidal, Lagrange, and spline approximation. *J Pharmacokinet Biopharm* 6:79-98, 1978
- Rocci ML Jr, Jusko WJ: LAGRAN program for area and moments in pharmacokinetic analysis. *Comput Programs Biomed* 16:203-216, 1983
- Murgo AJ, Kummars S, Gardner ER, et al: Phase I trial of 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) administered twice weekly. *J Clin Oncol* 25:154s, 2007 (abstr 3566)
- Pacey S, Wilson R, Walton M, et al: A phase I of the heat shock protein 90 (HSP90) inhibitor 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG, alvespimycin) administered weekly. *J Clin Oncol* 25:154, 2007 (abstr 3568)
- Flaherty KT, Gore L, Avadhani AN, et al: First use of an oral Hsp90 inhibitor in patients (Pts) with solid tumors: Alvespimycin (A) administered QOD or QD. *J Clin Oncol* 26:112s, 2008 (abstr 2502)
- Sequist L, Janne P, Sweney J, et al: Phase 1/2 trial of the novel Hsp90 inhibitor, IPI-504, in patients with relapsed and/or refractory stage IIIb or stage IV non-small cell lung cancer (NSCLC) stratified by EGFR mutation status. Proceedings of the Molecular Targets and Cancer Therapeutics, American Association for Cancer Research/National Cancer Institute/European Organisation for the Research and Treatment of Cancer Conference, San Francisco, CA, October 22-26, 2007 (abstr B79)

AUTHOR CONTRIBUTIONS

Conception and design: Ramesh K. Ramanathan, Merrill J. Egorin, Charles Erlichman, Suresh S. Ramalingam, Cynthia J. TenEyck, S. Percy Ivy, Chandra P. Belani
Administrative support: Merrill J. Egorin, Cynthia Naret, S. Percy Ivy, Chandra P. Belani
Provision of study materials or patients: Ramesh K. Ramanathan, Merrill J. Egorin, Charles Erlichman, Scot C. Remick, Suresh S. Ramalingam, Cynthia Naret, S. Percy Ivy, Chandra P. Belani
Collection and assembly of data: Ramesh K. Ramanathan, Merrill J. Egorin, Charles Erlichman, Cynthia Naret, Julianne L. Holleran, Cynthia J. TenEyck, Chandra P. Belani
Data analysis and interpretation: Ramesh K. Ramanathan, Merrill J. Egorin, Charles Erlichman, Suresh S. Ramalingam, Cynthia Naret, Julianne L. Holleran, Cynthia J. TenEyck, S. Percy Ivy, Chandra P. Belani
Manuscript writing: Ramesh K. Ramanathan, Merrill J. Egorin, Charles Erlichman, Suresh S. Ramalingam, Cynthia J. TenEyck, Chandra P. Belani
Final approval of manuscript: Ramesh K. Ramanathan, Merrill J. Egorin, Charles Erlichman, Scot C. Remick, Suresh S. Ramalingam, Julianne L. Holleran, Cynthia J. TenEyck, S. Percy Ivy, Chandra P. Belani

26. Bryson J, Infante J, Ramanathan RK, et al: A phase I dose-escalation study of the safety and pharmacokinetics (PK) of the oral Hsp90 inhibitor SNX-5422. *J Clin Oncol* 26:636s, 2008 (abstr 14613)

27. Wagner A, Morgan J, Chugh R, et al: Inhibition of heat shock protein 90 (Hsp90) with a novel agent

IPI-504 in metastatic GIST following failure of tyrosine kinase inhibitors (TKIs) or other sarcomas: Clinical results from a phase I trial. *J Clin Oncol* 26:553s, 2008 (abstr 10503)

28. Elfiky A, Saif MW, Beeram M, et al: BIB021, an oral, synthetic non-ansamycin Hsp90 inhibitor:

Phase I experience. *J Clin Oncol* 26:112s, 2008 (abstr 2503)

29. Ide S, Motwani M, Jensen MR, et al: Pharmacodynamics and pharmacokinetics of AUY922 in a phase I study of solid tumor patients. *J Clin Oncol* 27:15s, 2009 (abstr 3533)

EVERY 6 MINUTES Research Published in *JCO* Is Cited in Other Peer-Reviewed Journals

As reported by Thomson Reuters in its 2008 Journal Citation Report®, *Journal of Clinical Oncology's* Impact Factor has increased to 17.157 from 15.484. This is *JCO's* fourth straight year-on-year increase.

In number of citations, *JCO* ranks second among 141 oncology journals and ranks 25th among all 6,598 scientific journals surveyed. *JCO* articles were cited more than 97,000 times in 2008—a 20% increase over the previous year.

JCO has published so much research-changing and practice-changing science over the years that, in 2008, a *JCO* article was cited every 6 minutes, on average, in another peer-reviewed journal.

If you want to have your research read by the largest, most discerning international audience, you need to publish in *JCO*. And if you want to read the most important research in clinical oncology, you need to subscribe to *JCO*.

To **submit a manuscript**, visit submit.jco.org.
jco.org/subscriptions.

To **subscribe or activate**, visit

American Society of Clinical Oncology