A Phase I Dose-Escalation Trial of Trastuzumab and Alvespimycin Hydrochloride (KOS-1022; 17 DMAG) in the Treatment of Advanced Solid Tumors

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Abstract

Purpose: We conducted a phase I dose-escalation study to define the maximum tolerated dose (MTD), pharmacokinetics (PK), and pharmacodynamics of alvespimycin (17-DMAG), a heat shock protein 90 (Hsp90) inhibitor, given in combination with trastuzumab.

Experimental Design: Patients were treated with trastuzumab followed by intravenous alvespimycin on a weekly schedule. Hsp90 client proteins were measured at baseline and serially in peripheral blood lymphocytes (PBL) during cycle 1. Patients with advanced solid tumors progressing on standard therapy were eligible.

Results: Twenty-eight patients (25, breast; 3, ovarian) were enrolled onto three dose cohorts: 60 (n = 9), 80 (n = 13), and 100 mg/m² (n = 6). Dose-limiting toxicities (DLT) were: grade III left ventricular systolic dysfunction presenting as congestive heart failure in 1 patient (100 mg/m²), and reversible grade III keratitis in two patients (80 mg/m²). Drug-related grade III toxicity included one episode each of fatigue, diarrhea, myalgia, and back pain. Common mild to moderate toxicities included diarrhea, fatigue, myalgia, arthralgia, nausea, blurred vision, headache, back pain, and dry eyes. There was one partial response and seven cases of stable disease (range, 4–10 months), all in HER2+ MBC. In addition, an ovarian cancer patient had complete resolution of ascites and pleural effusion that lasted 24.8 months. There was no change in PK upon weekly dosing. Hsp70 effect continued to increase across four weeks and was most pronounced at 80 and 100 mg/m².

Conclusion: The combination of alvespimycin and trastuzumab is safe and tolerable at MTD. Antitumor activity was seen in patients with refractory HER2+ MBC and ovarian cancer. The recommended dose of alvespimycin for further study in this combination is 80 mg/m² weekly.

Introduction

Heat shock protein 90 (Hsp90) is a ubiquitously expressed molecular chaperone that regulates the folding, function, and viability of client proteins within cells under conditions of environmental stress. When Hsp90 is inhibited, its client proteins are rendered unstable and ultimately undergo ubiquitination followed by degradation via the proteasomal pathway (1–3). In addition, this inhibition results in an upregulation of Hsp70, hence both client protein degradation and induction of Hsp70 constitute the molecular signature of Hsp90 inhibition (3, 4).

A number of Hsp90 client proteins are important proteins in cell-specific oncogenic processes and include: mutant B-Raf (5), FLT3 (6), BCL-XL (7), EGF receptor (EGFR) harboring kinase mutations (8), BCR-ABL (9), mutant C-kit (10), activated Akt (11), and HER2 (12) among others. The prototype Hsp90 inhibitor, geldanamycin, is an ansamycin antibiotic which works by selectively binding to the ATP/ADP pocket of Hsp90 at the aminoterminal domain, thereby disrupting its client-chaperone function, and ultimately producing antitumor effects (13).

Tanespimycin [17-allylamino-17-demethoxygeldanamycin (17-AAG)], a clinically viable geldanamycin derivative, has shown antitumor activity in a variety of preclinical models (14–16). More specifically, in HER2+ SKBR3 breast cancer xenografts, treatment with 17-AAG inhibited tumor proliferation and was associated with depletion of HER2 and destabilization of Raf-1 and mutant p53 (17). Preclinical data also support the combination of trastuzumab plus 17-AAG in HER2+ breast cancer models where the combination has superior antitumor activity compared with either...
Translational Relevance

Heat shock protein 90 (Hsp90) inhibitors destabilize many oncoproteins in key signaling pathways, making them a unique anticancer strategy. Preclinically, HER2 is among the most sensitive client proteins of Hsp90 inhibition. Positive results from our phase II study of tanespimycin plus trastuzumab validated the role of Hsp90 inhibitors in HER2+ metastatic breast cancer (MBC). Alvespimycin, a water-soluble analog of tanespimycin, has an improved pharmacological and toxicity profile, and is associated with superior preclinical antitumor activity. This phase 1 trial of alvespimycin plus trastuzumab confirms the clinical effect of Hsp90 inhibitors in HER2+ MBC. Despite evidence of clinical activity, client protein degradation (as measured in peripheral blood lymphocytes) did not correlate with response. This illustrates the importance of pre- and posttreatment tumor biopsies rather than utilization of normal tissues as a surrogate marker of target modulation for the future clinical development of this class of agents.

Patients and Methods

Eligibility

Patients were eligible if they were ≥18 years of age with histologically confirmed advanced solid tumor malignancy (irrespective of the HER2 expression) not curable with standard therapies, negative pregnancy test, Karnofsky performance status (KPS) of ≥70%, 2 weeks from prior radiation or chemotherapy (6 weeks for nitrosoureas), hemoglobin of at least 8.5 g/dL, absolute neutrophil count (ANC) of at least 1.5 × 10⁹ cells/L, platelet count of at least 75 × 10⁹/L, serum bilirubin no more than 2 × the upper limit of normal (ULN), AST and ALT no more than 2.5 × ULN, and serum creatinine no more than 2 × ULN; patients were required to have either measurable or evaluable disease, assessed with scans 4 weeks before initiation of treatment on the trial. Patients were excluded if they were pregnant or lactating, had a prior severe hypersensitivity reaction to trastuzumab, active central nervous system metastases, moderate dyspnea at rest or the need for supportive oxygen, New York Heart Association class III/IV congestive heart failure, left ventricular ejection fraction (LVEF) of <50%, a history of prior radiation including the heart in the field, myocardial infarction or active ischemia within 12 months, history of uncontrolled dysrhythmias, requirement for antiarrhythmics, left bundle branch block, baseline QTcF interval of >450 msec for men and 470 msec for women in the absence of a correctable electrolyte imbalance, congenital QTc prolongation, and prior malignancies except basal cell carcinoma of the skin and carcinoma in situ of either the uterine cervix or urinary bladder.

The study protocol was reviewed and approved by the institutional review boards of each participating center. Before entering the study, all patients gave written informed consent according to the institutional guidelines.

Drug administration

Patients received weekly therapy in 4-week cycles. On cycle 1/week 1, patients received intravenous trastuzumab as a 4 mg/kg loading dose over 90 minutes followed by alvespimycin as an intravenous infusion over 60 minutes. Patients whose last dose of trastuzumab was <21 days before enrollment received 2 mg/kg. Alvespimycin was administered intravenously over 1 hour in 3 dose levels: 60, 80, and 100 mg/m². There were no standard premedications for alvespimycin; however, supportive medications could be introduced if drug-related symptoms developed (e.g., antiemetics). Given the absence of data to support a specific sequence, we elected to administer trastuzumab first, followed immediately by alvespimycin as was done in other trials in this setting (27). Three patients were assigned to each cohort; however, up to 4 were allowed because of simultaneous screening at different sites. On subsequent weeks, trastuzumab 2 mg/kg was administered over 30 minutes, followed by alvespimycin.

Study requirements and assessments

A history, physical exam, and serum studies were required at screening and before each cycle. Serum studies included:

1. A history, physical exam, and serum studies were required at screening and before each cycle.
2. A urine pregnancy test was performed at screening.
3. A pregnancy test was performed at each cycle.
4. A chest X-ray was performed at screening.
5. A chest X-ray was performed at each cycle.
6. A cardiac echocardiogram was performed at screening.
7. A cardiac echocardiogram was performed at each cycle.
8. An electrocardiogram was performed at screening.
9. An electrocardiogram was performed at each cycle.
10. A complete blood count was performed at screening.
11. A complete blood count was performed at each cycle.
12. A serum chemistry panel was performed at screening.
13. A serum chemistry panel was performed at each cycle.
14. A urinalysis was performed at screening.
15. A urinalysis was performed at each cycle.
16. A serum creatinine was performed at screening.
17. A serum creatinine was performed at each cycle.
18. A serum bilirubin was performed at screening.
19. A serum bilirubin was performed at each cycle.
20. A gamma-glutamyl transferase was performed at screening.
21. A gamma-glutamyl transferase was performed at each cycle.
22. An alanine aminotransferase was performed at screening.
23. An alanine aminotransferase was performed at each cycle.
24. A total cholesterol was performed at screening.
25. A total cholesterol was performed at each cycle.
26. A high-density lipoprotein cholesterol was performed at screening.
27. A high-density lipoprotein cholesterol was performed at each cycle.
28. A low-density lipoprotein cholesterol was performed at screening.
29. A low-density lipoprotein cholesterol was performed at each cycle.
30. A triglyceride was performed at screening.
31. A triglyceride was performed at each cycle.
32. A hemoglobin was performed at screening.
33. A hemoglobin was performed at each cycle.
34. A hematocrit was performed at screening.
35. A hematocrit was performed at each cycle.
36. An absolute neutrophil count was performed at screening.
37. An absolute neutrophil count was performed at each cycle.
38. A platelet count was performed at screening.
39. A platelet count was performed at each cycle.
40. A prothrombin time was performed at screening.
41. A prothrombin time was performed at each cycle.
42. A partial thromboplastin time was performed at screening.
43. A partial thromboplastin time was performed at each cycle.
44. A serum bilirubin was performed at screening.
45. A serum bilirubin was performed at each cycle.
46. A liver function test was performed at screening.
47. A liver function test was performed at each cycle.
48. A serum creatinine was performed at screening.
49. A serum creatinine was performed at each cycle.
50. A serum sodium was performed at screening.
51. A serum sodium was performed at each cycle.
52. A serum potassium was performed at screening.
53. A serum potassium was performed at each cycle.
54. A glucose was performed at screening.
55. A glucose was performed at each cycle.
56. A serum albumin was performed at screening.
57. A serum albumin was performed at each cycle.
58. A serum iron was performed at screening.
59. A serum iron was performed at each cycle.
60. A ferritin was performed at screening.
61. A ferritin was performed at each cycle.
62. A thyroxine was performed at screening.
63. A thyroxine was performed at each cycle.
64. A triiodothyronine was performed at screening.
65. A triiodothyronine was performed at each cycle.
66. A thyrotropin was performed at screening.
67. A thyrotropin was performed at each cycle.
68. A hemoglobin was performed at screening.
69. A hemoglobin was performed at each cycle.
70. A hematocrit was performed at screening.
71. A hematocrit was performed at each cycle.
72. An absolute neutrophil count was performed at screening.
73. An absolute neutrophil count was performed at each cycle.
74. A platelet count was performed at screening.
75. A platelet count was performed at each cycle.
76. A prothrombin time was performed at screening.
77. A prothrombin time was performed at each cycle.
78. A partial thromboplastin time was performed at screening.
79. A partial thromboplastin time was performed at each cycle.
80. A serum bilirubin was performed at screening.
81. A serum bilirubin was performed at each cycle.
CBC, full chemistry panel, liver function tests, serum electrolytes, uric acid, and lactose dehydrogenase. Three baseline electrocardiograms (EKG) were collected at least 5 minutes apart and forwarded to a central laboratory for determination of QTcF interval. EKGs were then carried out again on cycles 1 and 4, <120 minutes prior and 20 minutes after the alvespimycin infusions. Cardiac function was monitored with multiple gated acquisition scans (MUGA) or echocardiograms at baseline and every 8 weeks. Patients were evaluated for tumor response and disease progression using Response Evaluation Criteria in Solid Tumors (RECIST; ref. 28) every 2 cycles (8 weeks). All responses were confirmed with followup scans at 4 weeks.

Toxicity assessment
Toxicity assessment was carried out before and after each administration of alvespimycin and graded according to the National Cancer Institute Common Toxicity Criteria, version 3.0. Patients were evaluated for DLT during cycle 1 and this was defined as any drug-related (possible, probable, or definite) grade IV neutropenia, anemia, or thrombocytopenia lasting at least 7 days with adequate management, grade ≥III nausea, diarrhea, or vomiting that did not respond to maximal supportive care, grade ≥III nonhematologic toxicity (except fatigue) and treatment delay of >3 weeks because of prolonged recovery from a drug-related toxicity; or clinically significant change in cardiac function (sinus tachycardia >140 bpm; new occurrence atrial dysrhythmia; any ventricular dysrhythmia; QTcF >500 msec or an absolute increase of 60 msec; LVEF <40%; cardiac troponin >1ULN). If no DLT was observed in a cohort of 3 patients evaluable for dose-escalating decision (‘evaluable’ defined as having received 3 treatments in a 4-week period or having withdrawn as a result of drug-related toxicity), then the next dose level was evaluated. If 1 of 3 patients experienced a DLT, then the cohort was increased to 6 assessable patients. The maximum tolerated dose (MTD) was defined as the dose level producing DLT in no more than 1 of 6 patients.

Dose delays and modifications
A 3-week delay in treatment was permitted to allow recovery from toxicities. In case of nonhematologic toxicity, treatment was held until recovery to ≤grade II. Dose reductions were made if a patient experienced a DLT or required a 2- to 3-week dose delay for toxicity-related failure to meet the retreatment criteria (similar to eligibility criteria with respect to performance status, bone marrow, hepatic and renal function, serum electrolytes, and chemistries). A maximum of 2 dose reductions were allowed. If a patient had a study defined DLT at the starting dose level (60 mg/m²) then the patient was allowed to be retreated at 40 mg/m² upon discretion of the Investigator and the sponsor. Patient requiring dose delays ≥3 weeks were removed from the study. A cardiac DLT also necessitated removal of the patient from study.

PK assessment
PK blood samples (2 mL each into EDTA-containing tubes) for determination of plasma concentrations of the parent compound and its plasma metabolite were collected during cycle 1 at the following times relative to the 1st and 4th infusion: before treatment, 30, and 55 minutes after start of infusion, and 5, 15, 30, 60 minutes, and 2, 4, 6, 24, and 72 hours after the end of infusion. Predose samples were also drawn before the second and third infusions on days 8 and 15. Plasma samples were kept on ice during collection and centrifugation and then split into 2 cryovials before freezing at −70°C to 80°F. Plasma concentrations of alvespimycin and its metabolites were determined by a validated liquid chromatography-mass spectrometry (LC/MS/MS) bioanalytical method developed and carried out by a central analytical laboratory. A 7-point standard curve, ranging from 0.2 to 500 ng/mL, was used as a calibration curve for alvespimycin quantifications. The lower limit of quantification (LLOQ) was at 0.2 ng/mL and the highest quality control sample at 400 ng/mL. Noncompartmental analysis was applied to the individual plasma alvespimycin concentration data using WinNonLin (Pharsight Corporation, Version 5.0.1).

PD assessment of Hsp90 client proteins in PBLs
PD assessment were undertaken to evaluate the effects of alvespimycin on Hsp90 client proteins in peripheral blood lymphocytes. PBLs were isolated from serum samples (8 mL per time point collected into CPT tubes), which were obtained during cycle 1 only. On the basis of preclinical studies, which suggested that client proteins could be degraded within 3 to 6 hours of drug exposure, serum samples for PBLs were drawn pretreatment on weeks 1 to 4 and 24, and 72 hours post-alvespimycin infusion during weeks 1 and 4 to assess the timing and duration of client protein degradation (29). Blood was collected and lymphocytes isolated by Ficoll–Paque density gradient centrifugation. Cell isolation was carried out locally by each participating center. Samples were analyzed via either Western blot or Luminex analysis (see further details for PD methods and analysis in Appendix 1). A descriptive analyses to compare the levels of signaling proteins before and after initiation of therapy was carried out.

Results
Twenty-eight patients (25 MBC and 3 advanced ovarian cancer) were enrolled into 3 dose cohorts [60 mg/m² (n = 9), 80 mg/m² (n = 13), 100 mg/m² (n = 6)]. Patient demographics are presented in Table 1. The trial was designed to enroll patients with advanced solid malignancies irrespective of their HER2 status, however, the vast majority of the patients enrolled with MBC had HER2+ disease. Patients were heavily pretreated (including prior chemotherapy and endocrine therapy) with a median of 7.5 therapies (range, 2–21). All 24 patients with HER2+ MBC were treated with a median of 4 prior trastuzumab-based therapies. A total of 111 cycles were delivered, with the median number of 2 cycles (range, 0–24). Median cycles...
administered per dose cohort equaled 3, 2, and 1.5 for the 3 dose cohorts, respectively.

**Overall safety**

Nine patients were treated on the first dose cohort instead of the planned 3 to 6 patients. Initially, 5 patients were simultaneously screened and enrolled between the 3 institutions at the first cohort. Coincidentally, at the same time, the Cancer Therapy Evaluation Program (CTEP) issued a safety alert regarding pulmonary DLT in other phase I CTEP-sponsored alvespimycin trials. This led to an amendment of the current protocol to include this information and safety parameters regarding pulmonary toxicity. Although waiting for this amendment to be approved at all sites, 4 additional patients were enrolled to obtain further safety data at this dose level before escalating to the next dose.

There were 3 episodes of DLT (summarized in Table 2): 2 cases of reversible grade III keratitis at 80 mg/m² and 1 case of grade III left ventricular systolic dysfunction presenting as congestive heart failure at 100 mg/m². The latter patient was heavily pretreated with 12 lines of prior chemotherapy in the metastatic setting including anthracyclines in both the neoadjuvant and again in the metastatic setting, as well as receiving numerous lines of prior trastuzumab and lapatinib. Her baseline echocardiogram showed an LVEF of 52%. After her second weekly dose of alvespimycin plus trastuzumab, the patient was evaluated in the emergency department for shortness of breath, and was noted to be tachycardic with an oxygen saturation of 87% on room air. A repeat MUGA scan showed LVEF of 37% with no evidence of any wall motion abnormalities. Her CT scan showed no evidence of pulmonary embolism but did reveal new and increased moderate-sized bilateral pleural effusions associated with pulmonary edema. There was also evidence of tumor progression in the soft tissues. The patient was treated with β-blockers and furosemide and also with antibiotics for possible superimposed pneumonia. Three days later, her symptoms had resolved, she was saturating 98% on room air and was discharged from the hospital on β-blockers and a 7-day course of Levaquin. In spite of her initial clinical improvement in the hospital, she unfortunately had rapid progression of disease, with a precipitous decline in her performance status, and eventually passed away less than 2 months after her hospitalization with no repeat ECHO/MUGA scans on file.

It is important to highlight that in addition to this DLT at 100 mg/m², this dose level was in general difficult to tolerate and 5 of the 6 patients on this dose elected to discontinue therapy in <6 weeks for grades I to III toxicities. On the basis of this, a decision was taken to expand the 80 mg/m² dose cohort to enroll a total of 13 patients. The 2 cases of reversible grade III keratitis (DLT) observed at the 80 mg/m² dose cohort were noted in the expansion phase. Interestingly, retreatment with dose reduction was successful in only 1 patient, as keratitis recurred upon reinitiation of therapy at a dose of 60 mg/m² in the second patient. Both patients had nearly double the plasma exposure to alvespimycin compared with the mean of the remaining patients in the cohort (AUCinf of 13,028 and 14,878 ng/mL.hours

### Table 1. Patient demographics data (N = 28)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>53</td>
</tr>
<tr>
<td>Range 31–75</td>
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</tr>
<tr>
<td>Age, 18–65 y</td>
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<td>Age, &gt;65 y</td>
<td>3</td>
</tr>
<tr>
<td>Gender</td>
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<tr>
<td>Male</td>
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</tr>
<tr>
<td>Female</td>
<td>28</td>
</tr>
<tr>
<td>KPS, %</td>
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</tr>
<tr>
<td>Median 80–100</td>
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<tr>
<td>Tumor types</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>25</td>
</tr>
<tr>
<td>HER2 + MBC (IHC 3+/FISH &gt; 2)</td>
<td>23</td>
</tr>
<tr>
<td>HER2 1+</td>
<td>1</td>
</tr>
<tr>
<td>HER2 2+</td>
<td>1</td>
</tr>
<tr>
<td>HER2 status unknown</td>
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</tr>
<tr>
<td>Ovarian</td>
<td>3</td>
</tr>
<tr>
<td>HER2 status known</td>
<td>0</td>
</tr>
<tr>
<td>HER2 status unknown</td>
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</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>26</td>
</tr>
<tr>
<td>American Indian or Alaska Native</td>
<td>1</td>
</tr>
<tr>
<td>Black or African heritage</td>
<td>1</td>
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<tr>
<td>Asian (other Pacific Islander)</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
</tr>
<tr>
<td>No. of prior chemotherapy and hormonal agents</td>
<td></td>
</tr>
<tr>
<td>Median 7</td>
<td></td>
</tr>
<tr>
<td>Range 2–21</td>
<td></td>
</tr>
<tr>
<td>Trastuzumab-based therapy for HER2+ MBC</td>
<td></td>
</tr>
<tr>
<td>No. of patients who received prior trastuzumab</td>
<td>24</td>
</tr>
<tr>
<td>Median no. of trastuzumab-containing regimens</td>
<td>4</td>
</tr>
<tr>
<td>Range 1–9</td>
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</table>

Abbreviations: HER, human epidermal growth factor receptor; IHC, immunohistochemistry.

### Table 2. DLT by cohort

<table>
<thead>
<tr>
<th>DLT</th>
<th>Cohort 1: dose 60 mg/m²</th>
<th>Cohort 2: dose 80 mg/m²</th>
<th>Cohort 3: dose 100 mg/m²</th>
</tr>
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<tbody>
<tr>
<td>Keratitis</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviation: DLT, dose-limiting toxicity.
compared with 8,106 ng/mL-hours for the remaining patients in the cohort); the reason for which is not clear. Grade III toxicity other than DLT included 1 episode of fatigue, diarrhea, myalgia, and back pain each. All grade drug-related toxicities for each dose cohort are summarized in Table 3. Overall, diarrhea (64%), fatigue (61%), headache (50%), and arthralgia (46%) were the most frequent toxicities and were predominantly grades I to II in severity. Neurotoxicity, hepatotoxicity, hypersensitivity reactions, alopecia, and myelosuppression were not observed.

### Pharmacokinetics

PK evaluations were carried out using plasma samples obtained from 24 patients. Table 4 lists the PK parameters for alvespimycin at weeks 1 and 4. Trastuzumab (2 mg/kg dosage or 4 mg/kg loading dose for patients who had not received their last dose of trastuzumab within 21 days before study) was administered before the alvespimycin infusion. PK analysis revealed a greater than dose proportional exposure over the dose range tested. There was considerable intracohort variation in exposure, with CV% ranging from 15% to 59% for AUC_{inf}. In addition, $C_{max}$ versus dose plot shows that $C_{max}$ increases in a linear but greater than dose proportional manner to an increase in dose in week 1 (Fig. 1). Given the variability between subjects, dose-proportionality could not be ruled out statistically. Volume of distribution did not vary with dose or dosing day, and ranged from approximately 142 to 1,155 L. Average half-lives ranged from approximately 16 to 20 hours, and did not vary with dose or dosing day. After 3 weeks of intravenous dosing, PK for alvespimycin in week 4 was in general consistent with the PK in week 1.

### Pharmacodynamics

PBls data were available for only 19/28 patients. Three patients received only 1 dose of the study drug and then

#### Table 3. Adverse events by cohort

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Grade I/II</th>
<th>Grade III/IV</th>
<th>Grade I/II</th>
<th>Grade III/IV</th>
<th>Grade I/II</th>
<th>Grade III/IV</th>
<th>All grades</th>
<th>Grade I/II</th>
<th>Grade III/IV</th>
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<tr>
<td>Diarrhea</td>
<td>5</td>
<td>10</td>
<td>3</td>
<td>19</td>
<td>64</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fatigue/asthenia</td>
<td>6</td>
<td>8</td>
<td>2</td>
<td>14</td>
<td>50</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>4</td>
<td>8</td>
<td>2</td>
<td>10</td>
<td>36</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>32</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>29</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myalgia</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>29</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Blurred vision</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>29</td>
<td>0</td>
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<tr>
<td>Dry eye</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>8</td>
<td>29</td>
<td>0</td>
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<tr>
<td>Back pain</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>21</td>
<td>4</td>
<td></td>
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#### Table 4. Pharmacokinetics

<table>
<thead>
<tr>
<th>Parameter Geo-Mean (CV% - GM)</th>
<th>Week 1 ($n = 24$)</th>
<th>Week 4 ($n = 18$)</th>
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<tr>
<td>Clearance, L/h</td>
<td>16.9 ± 7.7</td>
<td>18.3 ± 7.2</td>
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<tr>
<td>$V_z$, L</td>
<td>440.0 ± 189.2</td>
<td>441 ± 233.1</td>
</tr>
<tr>
<td>$t_{1/2}$, h</td>
<td>19.3 ± 7.8</td>
<td>17.0 ± 7.1</td>
</tr>
<tr>
<td>At 80 mg/m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{max}$</td>
<td>1,633 ± 981</td>
<td>1,237 ± 477</td>
</tr>
<tr>
<td>AUC_{sys}, ng/mL h</td>
<td>8,905 ± 3,469</td>
<td>9,087 ± 3,966</td>
</tr>
</tbody>
</table>

Abbreviations: AUC_{sys}, area under the plasma concentration–time curve to infinity; $C_{max}$, mean plasma concentration; $Cl$, systemic clearance calculated as dose/AUC_{sys}; $t_{1/2}$, apparent terminal phase half-life; $V_z$, apparent volume of distribution during the terminal elimination phase.

#### Figure 1. AUC_{inf} by dose cohorts (60, 80, and 100 mg/m²).

AUC_{inf}, area under the plasma concentration–time curve to infinity.
discontinued for reasons unrelated to the study. Other samples were not assessable/available because of collection problems at the sites. Hsp70 protein induction (a marker of HSP90 inhibition) was seen at all dose levels but was most pronounced at 80 and 100 mg/m². This upregulation was time dependent and reached levels of 2.0- to 2.5-folds above baseline (Fig. 2A) 72 hours after the drug administration. The Hsp70 effect continued to increase across the 4-week evaluation period, albeit a qualitative trend. With regard to client proteins, Akt and pAkt levels in dose cohorts 1 and 2 were not substantially altered (Fig. 2B and C), however, patients in cohort 3 did show a nonsignificant decline in these protein levels in a time-dependent manner.

**Antitumor effects**

Of the 28 patients enrolled (25 MBC and 3 ovarian cancer), 24 were evaluable for efficacy (21 MBC and 3 ovarian cancer); 3 patients withdrew consent for personal reasons and 1 was taken off study because of DLT (congestive heart failure) after receiving one cycle. Of the 24 patients with evaluable disease, 18 had measurable disease (16 MBC and 2 ovarian cancer). Per RECIST, there were no complete responses (CR) and 1 patient with HER2⁺ MBC had a confirmed PR. Clinical benefit rate (CR + PR + SD > 6 months) was noted in 7 of 24 (29%) evaluable patients [N = 6 HER2⁺ MBC (1 PR and 5 SD) and N = 1 (1SD) ovarian cancer] and are described later. Median time to progression was 2.1 months (range, 0.03–24.8).

**Evaluable MBC patients (N = 21)**

Of the 21 patients with MBC evaluable for efficacy, 16 had measurable disease. Of these 16 patients, 1 achieved a PR and 3 others had SD for 4, 5, and 7 months, respectively. Four additional patients with nonmeasurable disease...
achieved SD for 6, 8, 9, and 10 months respectively. Details of 3 cases with the greatest evidence of activity are presented later:

The patient who achieved PR by RECIST after 2 cycles was treated with 5 prior regimens, including 3 trastuzumab-containing regimens. Her PR was confirmed after another 2 cycles of therapy (31% and 52% decrease in the liver metastases, respectively) and she continued on study for a total of 5 months at 80 mg/m². The second patient also had measurable disease (treated with 11 prior regimens including 5 with trastuzumab and 2 with lapatinib) and achieved SD for 5 months at 80 mg/m², showing a 10% reduction in the tumor mass with radiographic changes consistent with tumor necrosis, a 64% reduction in serum CEA, and a 63% reduction in serum Ca 27.29. The final patient had nonmeasurable lymphangitic disease in the lung (treated with 13 prior regimens including progression on single-agent lapatinib and 3 prior trastuzumab containing therapies) and had complete resolution of this by positron emission tomography/computed tomography (PET/CT) and with significant improvement in dyspnea. This patient was treated at 60 mg/m² for 6 months.

**Evaluable ovarian cancer (N = 3)**

Of the 3 patients with ovarian cancer (HER2 status unknown), 2 had measurable disease. The patient with nonmeasurable disease (treated with 13 prior regimens) showed near complete resolution of ascites and pleural effusion at the end of cycle 2 accompanied by decrease in the serum Ca-125 levels by 83%. This patient received a total of 24 cycles and was ultimately taken off study because of symptomatic deterioration and an increasing Ca-125 level.

**Discussion**

The Hsp90 chaperone protein integrates multiple critical oncogenic pathways justifying the focus on developing Hsp90 inhibitors for advanced malignancies. Building on the positive results achieved with tanespimycin, alvespimycin was developed as a more potent and water soluble analog of this compound. Various schedules of daily and weekly dosing of alvespimycin have been evaluated in a number of different trials (3, 4, 15, 16). In our study, we evaluated the treatment-induced changes in client proteins and Hsp70 in PBLs. Hsp70 levels were induced in a time-dependent manner with the most pronounced effect at 80 mg/m². Akt and pAkt client protein degradation was time dependent and was shown only at 100 mg/m². These qualitative effects suggest that the study drug achieved biologically effective plasma concentrations and affected the target in PBLs, however, there was no correlation with clinical response which was noted at 80 mg/m². Other trials, that evaluated the effect of alvespimycin on Hsp90 mRNA expression (23), Hsp90, Hsp70, and ILK levels (24) in PBLs, also showed large variability in effect. This is not surprising as there is growing evidence that cancer cells are highly sensitive to Hsp90 inhibition with preferential accumulation of these inhibitors in tumor cells when compared with normal cells (33, 34). Hence, monitoring PBLs as a PD endpoint, although reproducible and easily accessible, is not predictive of tumor-specific activity. Alternatively, preand post treatment tumor biopsies have also been evaluated to ascertain Hsp90 inhibition. Pacey and colleagues reported that Hsp90 was inhibited (defined as Hsp72 induction and CDK4 depletion) in tumor samples taken from patients 24 hours after 80 mg/m² of weekly alvespimycin, and thus considered this to be their recommended
phase II dose and schedule (25). In contrast, a second phase I trial that evaluated daily × 5 versus daily × 3 intravenous infusions of alvespimycin, also incorporating pre- and post-treatment tumor biopsies at 24 hours, showed no consistent client protein degradation suggesting that this dose and schedule may not be optimal for further development (24). Taken together, these studies illustrate the importance of incorporating tumor biopsies in all future trials to assess target modulation and thereby define and deliver the optimal biologically active dose of Hsp90 inhibitor therapy.

Our study was designed to enroll all patients with advanced solid malignancies who were otherwise candidates for phase I trials irrespective of their HER2 status. Given the overall low-toxicity rate for trastuzumab, it was deemed (by both the individual Institutional Review Boards and the Food and Drug Administration) that the administration of this agent in the setting of a Phase 1 trial using a safe dose and schedule outweighed the potential risks to HER2-negative patients. Although most of the patients eventually enrolled did have HER2+ breast cancer, the trial also enrolled 3 ovarian cancer patients (HER2 status unknown), none of whom had any adverse effects related to trastuzumab. With regards to efficacy, among the patients with HER2+ MBC, there was 1 confirmed PR with tumor regression in hepatic metastases and 7 other patients achieved SD that lasted 4, 5, 6, 7, 8, 9, and 10 months, respectively. In addition, 1 of the ovarian cancer patients also achieved a prolonged period of stable disease measuring 24.8 months. It is known that HER2 is a potential therapeutic target in ovarian cancer (35) and it is possible that this patient could have had HER2- disease which may explain her response to the study treatment. Alternatively, preclinical data also suggests that the antimultiple receptor tyrosine kinase activity achieved by Hsp90 inhibition alone could also be effective in ovarian cancer (36). Unfortunately there was insufficient tumor tissue available to retrospectively determine the HER2 status of the patient’s cancer in this case, hence the basis of her response to therapy cannot be further clarified at this point. Certainly the potential for this combination therapy and for Hsp90 inhibitors to be active in other tumor types warrants investigation in future trials.

Based on our study, the recommended phase II dose for alvespimycin is 80 mg/m² when given in combination with trastuzumab. In addition to our study, complete responses with alvespimycin therapy have been reported in other trials in castrate refractory prostate cancer (25) and in patients with refractory acute myeloid leukemia (26). Despite these encouraging results, ocular toxicity was a concern for further development of this agent and ultimately the development of alvespimycin has been suspended by the sponsor (37).

Many second generation Hsp90 inhibitors such as NVP-AUY922, STA-9090/ganetespib, ATI-13387, and PU-H71 among others, with more potent antitumor effects and lacking the hepatotoxicity of geldanamycin derivatives, are currently under clinical investigation. Although preclinical studies suggest a superior therapeutic index, the tolerability, and safety profile of these next generation Hsp90 inhibitors are yet to be fully evaluated.

Finally, trastuzumab has been shown to have an additive or synergistic effect in combination therapies and continued benefits in spite of progression (38, 39), hence the relative individual contributions of alvespimycin and trastuzumab in this combination regimen cannot be ascertained from this trial. To clarify this issue, single-agent phase II trials of Hsp90 inhibitors in patients with metastatic breast cancer are currently underway (40, 41).

Disclosure of Potential Conflicts of Interest
L.S. Rosen has commercial research support from Kosan. B.P. Schneider is a consultant/advisory board member for Genentech. No potential conflicts of interest were disclosed by the other authors.

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A Phase I Dose-Escalation Trial of Trastuzumab and Alvespimycin Hydrochloride (KOS-1022; 17 DMAG) in the Treatment of Advanced Solid Tumors

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