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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**Statement of translational relevance:**

Heat shock protein 90 (Hsp90) inhibitors destabilize many oncoproteins in key signaling pathways making them a unique anti-cancer 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) (Modi et al CCR, 2011). Alvespimycin, a water-soluble analog of tanespimycin, has an improved pharmacological and toxicity profile, and is associated with superior pre-clinical anti-tumor activity. This phase 1 trial of alvespimycin plus trastuzumab confirms the class 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 post treatment 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.
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), an 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 (PBLs) 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 3 dose cohorts: 60 (n = 9), 80 (n = 13), and 100 mg/m² (n = 6). Dose-limiting toxicities (DLT) were: grade 3 left ventricular systolic dysfunction presenting as congestive heart failure in 1 patient (100mg/m²), and reversible grade 3 keratitis in 2 patients (80mg/m²). Drug-related grade 3 toxicity included 1 episode each of fatigue, diarrhea, myalgia and back pain. Common mild to moderate toxicities included diarrhea, fatigue, myalgia, arthralgia, nausea, blurry vision, headache, back pain and dry eyes. There was 1 partial response and 7 cases of stable disease (range, 4-10 months), all in HER2+ MBC. Additionally, 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 4 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 80mg/m² weekly.
INTRODUCTION

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). Additionally, 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-6 (7), epidermal growth factor 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 to either agent given alone (18). This translated clinically to a positive phase II trial of tanespimycin plus trastuzumab for patients with HER2+ MBC. This combination produced a RECIST-defined response rate of 22% and clinical benefit of 59% in patients with trastuzumab-refractory disease and validated Hsp90 inhibition as an anti-cancer strategy. (19)

To overcome the formulation issues with tanespimycin, alvespimycin was developed as its water-soluble analog. Alvespimycin is associated with a longer plasma half-life, greater oral bioavailability, and less extensive metabolism. In preclinical studies, its favorable PK also produced superior antitumor activity and less toxicity compared to 17-AAG (20-22).

Phase I trials of alvespimycin explored various dosing schedules including: twice weekly (23), daily × 3 or 5 days every 3 weeks (1.5–46 mg/m²) (24), weekly (2.5–80 mg/m²) (25), and twice weekly × 2 weeks or every 3 weeks (8–32 mg/m2) (26). Pharmacokinetic data from these trials revealed a drug half-life of 24 ± 15 hours with a dose-dependent linear increase in exposure (area under curve ,AUC), and lack drug accumulation with repeated dosing (23, 24). However, due to the adverse safety profile observed with the more frequent dosing schedules, combined with our prior successful experience with weekly tanespimycin, we elected to use a weekly...
schedule for the current trial (19). The primary objective of our phase I study was to determine the MTD and a recommended phase II dose of alvespimycin when administered as a weekly infusion in combination with standard doses of trastuzumab to patients with advanced solid malignancies. PK and pharmacodynamics (PD) analyses were undertaken to evaluate the effect of Hsp90 inhibition on its client proteins in PBLs.

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 X 10⁹ cells/L, platelet count of at least 75 X 10⁹/L, serum bilirubin no more than 2 X the upper limit of normal (ULN), AST and ALT no more than 2.5 X ULN, and serum creatinine no more than 2 X ULN; patients were required to have either measurable or evaluable disease, assessed with scans 4 weeks prior to 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, 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 4mg/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 one 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 (eg. 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 (19). Three patients were assigned to each cohort; however, up to four 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 prior to each cycle. Serum studies included: CBC, full chemistry panel, liver function tests, serum electrolytes, uric acid, and lactose dehydrogenase. Three baseline electrocardiograms (EKG’s) were collected at least 5 minutes apart and forwarded to a central laboratory for determination of QTcF interval. EKG’s were then performed again on Cycle 1 and 4, < 120 minutes prior and 20 minutes following 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) (27) every two cycles (8 weeks). All responses were confirmed with follow-up scans at 4 weeks.

**Toxicity Assessment**

Toxicity assessment was performed prior to 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 4 neutropenia, anemia or thrombocytopenia lasting at least 7 days with adequate management, grade ≥ 3 nausea, diarrhea or vomiting that did not respond to maximal supportive care, grade ≥ 3 non-hematologic toxicity (except fatigue) and treatment delay of > 3 weeks due to 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 > UNL). If no DLT was observed in a cohort of three patients evaluable for dose-escalating decision (“evaluable” defined as having received three treatments in a 4-week period or having withdrawn as a result of drug-related toxicity), then the next dose level was evaluated. If one of three patients experienced a DLT, then the cohort was increased to six assessable patients. The maximum-tolerated dose (MTD) was defined as the dose level producing DLT in no more than one of six patients.

**Dose Delays and Modifications**

A 3-week delay in treatment was permitted to allow recovery from toxicities. In case of non-hematologic toxicity, treatment was held until recovery to ≤ grade 2. Dose reductions were made if a patient experienced a DLT or required a 2-3 week dose delay for toxicity-related failure to meet the re-treatment 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 (60mg/m²) then the patient was allowed to be re-treated at 40mg/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, 72 hours following the end of infusion. Pre-dose samples were also drawn prior to the 2nd and 3rd infusions on days 8 and 15. Plasma samples were kept on ice during collection and centrifugation and then split into 2 cryovials prior to freezing at -70-80°F. Plasma concentrations of alvespimycin and its metabolites were determined by a validated liquid chromatography-mass spectrometry (LC/MS/MS) bioanalytical method developed and performed by a central analytical laboratory. A seven-point standard curve, ranging from 0.2 ng/mL to 500 ng/mL, was used as a calibration curve for alvespimycin quantifications. The lower limit of quantification (LLOQ) was at 0.2ng/mL and the highest quality control sample at 400 ng/mL. Non-compartmental 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**
Pharmacodynamic 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. Based on pre-clinical studies which suggested that client proteins could be degraded within 3-6 hours of drug exposure, serum samples for PBLs were drawn pre-treatment on weeks 1 – 4 and 4, 24 and 72 hours post alvespimycin infusion during weeks 1 and 4 to assess the timing and duration of client protein degradation (28). Blood was collected and lymphocytes isolated by Ficoll-Paque density gradient centrifugation. Cell isolation was performed 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 prior to and following initiation of therapy was performed.

**RESULTS**
Twenty-eight patients (25 MBC and 3 advanced ovarian cancer) were enrolled into 3 dose cohorts (60mg/m² [n=9], 80mg/m² [n=13], 100mg/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 pre-treated (including prior chemotherapy and
endocrine therapy) with a median of 7.5 therapies (range 2 to 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 three 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. Co-incidentally, 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. While 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 three episodes of DLT (summarized in table 2): two cases of reversible grade 3
keratitis at 80 mg/m² and one case of grade 3 left ventricular systolic dysfunction presenting as
congestive heart failure at 100 mg/m². The latter patient was heavily pre-treated 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 a left ventricular ejection
fraction (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 beta blockers and furosemide and
also with antibiotics for possible super-imposed pneumonia. Three days later, her symptoms
had resolved, she was saturating 98% on room air and was discharged from the hospital on
beta blockers and a 7 day course of Levofoxacin. 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 two 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 grade 1-3 toxicities. Based on this, a decision was taken to expand the
80 mg/m² dose cohort to enroll a total of 13 patients. The two cases of reversible grade 3
keratitis (DLT) observed at the 80 mg/m² dose cohort were noted in the expansion phase.
Interestingly, re-treatment with dose reduction was successful in only one patient, as; keratitis
recurred upon re-initiation of therapy at a dose of 60 mg/m² in the second patient. Both
patients had nearly double the plasma exposure to alvespimycin compared to the mean of the remaining patients in the cohort (AUC\text{inf} of 13028 and 14878 ng/mL*hr compared to 8106 ng/mL*hr for the remaining patients in the cohort); the reason for which is not clear. Grade 3 toxicity other than DLT included one 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 1-2 in severity. Neurotoxicity, hepatotoxicity, hypersensitivity reactions, alopecia and myelosuppression were not observed.

**Pharmacokinetics**
PK evaluations were performed using plasma samples obtained from 24 patients. Table 4 lists the PK parameters for alvespimycin at week 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 prior to study) was administered prior to the alvespimycin infusion.

PK analysis revealed a greater than dose proportional exposure over the dose range tested. There was considerable intra-cohort variation in exposure, with CV\% ranging from 15% to 59\% for AUC\text{inf}. In addition, C\text{max} versus dose plot shows that C\text{max} increases in a linear but greater than dose proportional manner to an increase in dose in week 1 (figure 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 1155 L. Average half-lives ranged from approximately 16 to 20 hours, and did not vary with dose or dosing day. Following three weeks of intravenous dosing, PK for alvespimycin in week 4 was in general consistent with the PK in week 1.

**Pharmacodynamics**
PBMC data were available for only 19/28 patients. Three patients received only one dose of the study drug and then discontinued for reasons unrelated to the study. Other samples were not assessable/available due to 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 100mg/m\text{2}. This upregulation was time dependent and reached levels of 2.0-2.5 folds above baseline (figure 2A) 72 hours following the drug administration. The Hsp70 effect continued to increase across the 4 week evaluation period, albeit a qualitative trend. With regards to client proteins, Akt and pAkt levels in dose cohort 1 and 2 were not substantially altered (figure 2B and 2C), however patients in cohort 3 did show a non-significant 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); three patients withdrew consent for personal reasons and one was taken off study due to 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 one patient with HER2+ MBC had a confirmed PR. Clinical benefit rate (CR+ PR + SD>6 months) was noted in 7/24 (29\%) evaluable
patients (N = 6 HER2 + MBC [1PR and 5 SD] and N = 1 [1SD] ovarian cancer) and are described below. 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 non-measurable disease achieved SD for 6, 8, 9 and 10 months respectively. Details of three cases with the greatest evidence of activity are presented below:

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 80mg/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 80mg/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 non-measurable 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 60mg/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 non-measurable 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 due to symptomatic deterioration and a rising 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 phase I studies in an attempt to optimize the therapeutic index of this agent (23-26). Based on our previously reported positive phase II results with tanespimycin in combination with trastuzumab, we investigated the weekly dosing of alvespimycin in this study.

At our defined MTD, the combination of alvespimycin and trastuzumab was well-tolerated and the overall toxicity profile was similar to what has been reported with the 17-AAG compound, with the exception of ocular toxicity. Two patients treated at 80mg/m² had dose limiting grade
3 reversible keratitis. Pacey et al also reported ocular toxicity in four patients who experienced 10 ocular adverse events, presenting as grade 2 or less blurred vision, dry eyes and keratitis, conjunctivitis or ocular surface disease in their phase I trial of alvespimycin monotherapy; the majority of these occurring at a weekly dose of 80 mg/m² or higher (25). To date, of the 200 patients with advanced malignancies who received alvespimycin on various trials, 25 patients experienced a drug-related ocular toxicity (typically presenting as blurry vision). While the etiology for this unusual toxicity is not known, it appears to be dose and schedule dependent. Possible mechanisms include an impairment of the tear production (either the composition or quantity of tears) and secondary keratitis, impairment of corneal endothelial cell health, or direct retinal epithelial cell damage as described in animal models (29). It is notable that second generation, non-geldanamycin, small molecule Hsp90 inhibitors have also produced ocular toxicities (30) and in fact the oral Pfizer compound SNX-5422 was discontinued from further development based on excessive eye toxicity and the potential for irreversible retinal damage (31). Whether the ocular toxicity is a class effect for all Hsp90 targeting compounds remains to be elucidated.

One patient developed symptomatic congestive heart failure after the second dose of alvespimycin and trastuzumab at 100 mg/m². Similar to the experience with other anti-HER2 therapies such as trastuzumab, cardiotoxicity is a potential concern with HSP90 inhibitors as HER2 is a very sensitive client protein. In the present case, while no definitive associations can be drawn given the other confounding factors such as prior anthracyclines and concurrent trastuzumab exposure, this case reinforces the need to continue cardiac monitoring of patients on HSP90 inhibitors. To date, there have been no other reports of clinically symptomatic congestive heart failure with alvespimycin or any other HSP90 inhibitor.

Depletion of client proteins and induction of Hsp70 are the hallmarks of Hsp90 inhibition and these have been studied, largely in surrogate tissues, as PD endpoint 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 and 100 mg/m². Akt and pAkt client protein degradation was time dependent and was demonstrated 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 to normal cells (32, 33). Hence monitoring PBLs as a PD endpoint, although reproducible and easily accessible, is not predictive of tumor-specific activity. Alternatively, pre and post-treatment tumor biopsies
have also been evaluated to ascertain Hsp90 inhibition. Pacey et al reported that Hsp90 was inhibited (defined as Hsp72 induction and CDK4 depletion) in tumor samples taken from patients 24 hours after 80mg/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 X 5 versus daily X 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 (both by 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, amongst the patients with HER2+ MBC, there was one 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. Additionally, one 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 (34) and it is possible that this patient could have had HER2 positive disease which may explain her response to the study treatment. Alternatively, preclinical data also suggests that the anti-multiple receptor tyrosine kinase activity achieved by Hsp90 inhibition alone could also be effective in ovarian cancer. Unfortunately there was insufficient tumor tissue available to retrospectively determine the HER2 status of the patient’s cancer in this case, hence (35) 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 (36).
Many second generation Hsp90 inhibitors like 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. While pre-clinical studies suggest a superior therapeutic index, the tolerability and safety profile of these next generation Hsp90 inhibitors are yet to be fully evaluated.

Lastly, trastuzumab has been shown to have an additive or synergistic effect in combination therapies and continued benefits in spite of progression (37, 38); 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 (39, 40).
REFERENCES


Table 1: Patient Demographics Data (N=28)

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<td>American Indian or Alaska Native</td>
<td>1</td>
</tr>
<tr>
<td>Black, or African heritage</td>
<td>1</td>
</tr>
<tr>
<td>Asian (other Pacific islander)</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
</tr>
<tr>
<td><strong>No. of prior chemotherapy and hormonal agents</strong></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>7</td>
</tr>
<tr>
<td>Range</td>
<td>2 to 21</td>
</tr>
<tr>
<td><strong>Trastuzumab-based therapy for HER2+ MBC</strong></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</td>
<td>1 to 9</td>
</tr>
</tbody>
</table>

HER: human epidermal growth factor receptor; IHC: immunohistochemistry; MBC: metastatic breast cancer; FISH: fluorescence in situ hybridization.

Table 2: DLT by Cohort

<table>
<thead>
<tr>
<th>DLT</th>
<th>Cohort 1: Dose 60mg/m2</th>
<th>Cohort 2: Dose 80mg/m2</th>
<th>Cohort 3: Dose 100mg/m²</th>
</tr>
</thead>
<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>

DLT: dose-limiting toxicity
Table 3: Adverse Events by Cohort

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Cohort 1: Dose 60mg/m² N=9</th>
<th>Cohort 2: Dose 80mg/m² N=13</th>
<th>Cohort 3: Dose 100mg/m² N=6</th>
<th>Total N=28</th>
<th>% of patients with specified adverse event combined for all 3 cohorts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1/2 Grade 3/4</td>
<td>Grade 1/2 Grade 3/4</td>
<td>Grade 1/2 Grade 3/4</td>
<td>Grade 1/2 Grade 3/4</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>5  1</td>
<td>10  0</td>
<td>3  0</td>
<td>19</td>
<td>64 4</td>
</tr>
<tr>
<td>Fatigue/Asthenia</td>
<td>6  1</td>
<td>8  0</td>
<td>3  0</td>
<td>18</td>
<td>61 4</td>
</tr>
<tr>
<td>Headache</td>
<td>4  0</td>
<td>8  0</td>
<td>2  0</td>
<td>14</td>
<td>50 0</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>8  0</td>
<td>3  0</td>
<td>2  0</td>
<td>13</td>
<td>46 0</td>
</tr>
<tr>
<td>Nausea</td>
<td>2  0</td>
<td>6  0</td>
<td>2  0</td>
<td>10</td>
<td>36 0</td>
</tr>
<tr>
<td>Myalgia</td>
<td>3  0</td>
<td>6  0</td>
<td>0  1</td>
<td>10</td>
<td>32 4</td>
</tr>
<tr>
<td>Blurred Vision</td>
<td>3  0</td>
<td>4  0</td>
<td>1  0</td>
<td>8</td>
<td>29 0</td>
</tr>
<tr>
<td>Dry Eye</td>
<td>4  0</td>
<td>3  0</td>
<td>1  0</td>
<td>8</td>
<td>29 0</td>
</tr>
<tr>
<td>Back pain</td>
<td>3  0</td>
<td>2  0</td>
<td>1  1</td>
<td>7</td>
<td>21 4</td>
</tr>
</tbody>
</table>

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>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance (L/hr)</td>
<td>16.9 ± 7.7</td>
<td>18.3 ± 7.2</td>
</tr>
<tr>
<td>Vz (L)</td>
<td>440.9 ± 189.2</td>
<td>441 ± 233.1</td>
</tr>
<tr>
<td>t1/2 (hr)</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>Cmax</td>
<td>1633 ± 981</td>
<td>1237 ± 477</td>
</tr>
<tr>
<td>AUC_{inf} (ng/ml*hr)</td>
<td>8905 ± 3469</td>
<td>9087 ± 3966</td>
</tr>
</tbody>
</table>

Clearance- Systemic clearance calculated as Dose/ AUC_{inf}; Vz- Apparent volume of distribution during the terminal elimination phase; t1/2- Apparent terminal phase half life; Cmax- Mean Plasma concentration; AUC_{inf} Area under the plasma concentration-time curve to infinity
Figure 1. AUC_{inf} by dose cohorts (60, 80 and 100mg/m^2)

Figure 2A. Summary of the Hsp70 protein Level in all Patients

Figure 2B. Summary of the total-Akt Level in all Patients

Figure 2C. Summary of the pAkt Level in all Patients.
Figure 1. $AUC_{inf}$ by dose cohorts (60, 80 and 100mg/m²)
Figure 2A. Summary of the Hsp70 protein Level in all Patients
Figure 28. Summary of the total-Akt Level in all Patients

![Graph showing total Akt levels across different cohorts](image-url)
Figure 2C. Summary of the Phospho-Akt Level in all Patients
Clinical Cancer Research

A Phase I Dose-escalation trial of Trastuzumab and Alvespimycin Hydrochloride (KOS-1022; 17 DMAG) In the Treatment of Advanced Solid tumors


Clin Cancer Res Published OnlineFirst July 10, 2012.

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