Published in final edited form as:

Breast Cancer Res Treat. 2012 February ; 131(3): . doi:10.1007/s10549-011-1866-7.

A phase II study of 17-allylamino-17-demethoxygeldanamycin in metastatic or locally advanced, unresectable breast cancer

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Abstract

Heat shock protein 90 (Hsp90) is an attractive target for breast cancer treatment, as it is required for the proper folding and stabilization of several proteins known to be involved in breast cancer growth and development. These proteins include the epidermal growth factor receptor, human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER), progesterone receptor (PR), and src. 17-Allylamino-17-demethoxygeldanamycin (17-AAG) is an intravenous Hsp90 inhibitor in development for breast cancer treatment. We conducted a phase II study of 17-AAG 220 mg/m² on days 1, 4, 8, and 11 every 21 days in patients with metastatic and locally advanced breast cancer. Since we expected the molecular effects of Hsp90 inhibition to extend beyond just ER, PR, and HER2 down regulation and to impact a variety of other cellular proteins, patients were not selected based on ER, PR, or HER2 status. Eleven patients, including 6 patients with triple

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Ethical standards This study was performed within and conducted in compliance with the current laws of the United States of America.

Conflict of interest Lawrence Flaherty discloses that he has participated as an advisor to Bristol-Myers Squibb in relation to other agents. The remaining authors declare that they have no conflict of interest.

negative breast cancer, were enrolled and treated. There were no responses and 3 patients had stable disease as their best response. Five patients developed grade 3/4 toxicities, which were primarily hepatic and pulmonary. Based on these results, we do not recommend further study of 17-AAG at this dosing schedule or in unselected breast cancer patients.

Keywords

17-AAG; 17-allylamino-17-demethoxygeldanamycin; Breast cancer; Hsp90; Heat shock protein 90

Introduction

17-allylamino-17-demethoxygeldanamycin (17-AAG) is an inhibitor of heat shock protein (Hsp) 90, interacting with its ATP-binding pocket and preventing its normal functions [1]. Hsp90 primarily acts as a chaperone protein, promoting the proper folding of a variety of proteins involved in cell growth signaling processes. Hsp90 also functions to stabilize some of these proteins, preventing their degradation [2]. Several of the proteins requiring Hsp90 function are known to be important in breast cancer, including the estrogen receptor (ER), the progesterone receptor (PR), HER2, Akt, the epidermal growth factor receptor (EGFR), and src [2–4]. Preclinical studies have shown significantly higher levels of staining for cytoplasmic/nuclear Hsp90, as well as higher levels of Hsp90 mRNA, in malignant breast tissue compared with normal breast tissue [5]. In addition, higher levels of Hsp90 have been associated with poor prognosis in breast cancer patients [6].

Several phase I studies evaluating different dosing schedules of single agent 17-AAG in adults and children have been performed [7–15]. The results reported here are of a phase II study using the twice weekly dosing schedule of 17-AAG administration on days 1, 4, 8, and 11 every 21 days, which was considered to be the most promising of these regimens for breast cancer. The maximum tolerated dose on the twice weekly schedule used for this study was 220 mg/m² per dose (880 mg/m² per cycle) [15]. Toxicities observed at this dose level included grade 2 diarrhea, skin irritation, and blurry vision.

We evaluated 17-AAG for patients with metastatic breast cancer in a multicenter phase II clinical trial. Given the number of other potential breast cancer targets expected to be affected by 17-AAG, patients were not selected for participation based on ER, PR, or HER2 status. The endpoints of this trial were to evaluate the overall response rate (OR) and progression-free survival (PFS).

Patients and methods

Eligibility criteria

Patients were required to have pathologically confirmed primary breast adenocarcinoma, locally advanced or metastatic disease not amenable to treatment with curative intent, measurable disease by response evaluation criteria in solid tumors (RECIST), progressive disease following hormonal therapy in appropriate tumors, and progressive disease following treatment with both an anthracycline and taxane, if not contraindicated. No other antineoplastic agents were allowed while on this study, including trastuzumab; however, zoledronic acid for bone metastasis or hypercalcemia was permitted. Patients with active brain metastases were ineligible. Patients were required to be at least 18 years of age and have a life expectancy of at least 3 months with ECOG performance status (PS) of 0–2. Normal hematologic, renal, and liver function was required. Patients with a venous thrombotic event within 6 months, New York Heart Association (NYHA) class III or IV

congestive heart failure, or a myocardial infarction within 1 year were excluded. Any medications known to prolong the QTc were not permitted during the study. After the first 8 patients were enrolled, the protocol was amended to exclude patients with the following conditions: any history of dysrhythmia (uncontrolled or requiring antiarrhythmic drugs), any history of serious ventricular arrhythmia, prolonged QTc, left ventricular ejection fraction (LVEF) < 40%, prior history of cardiac or pulmonary toxicity after other antineoplastic agents (anthracyclines, bleomycin, BCNU), ongoing grade 2 pulmonary or cardiac symptoms, prior history of chest irradiation, history of chronic obstructive pulmonary disease and/or restrictive pulmonary disease, meeting Medicare criteria for home oxygen, dyspnea, dyspnea on exertion, and paroxysmal nocturnal dyspnea. Patients with active infection, including known HIV positivity, were excluded. Patients of childbearing potential were required to have a negative serum pregnancy test and agree to adequate birth control measures. The use of medications interacting with cytochrome p450 3A was avoided. All patients signed a written, informed consent form.

Study procedures

Before treatment, patients had a complete history and physical examination (PE), including assessment of PS, LVEF assessment (MUGA scan), and baseline laboratory evaluation with a complete blood count (CBC: white blood count with differential, hemoglobin, and platelet count), serum chemistry panel (CMP: sodium, potassium, bicarbonate, chloride, calcium, BUN, creatinine, glucose, albumin, bilirubin, transaminases), and appropriate serum tumor markers. Baseline QTc was determined by calculating the mean of 3 EKG measurements performed manually the day of treatment. After initial reports of cardiopulmonary toxicity, the study was amended to require pulmonary function testing with DLCO before treatment. Baseline tumor measurements with appropriate radiographic studies were obtained within 4 weeks of treatment.

Patients received 17-AAG 220 mg/m² IV over 2 h on days 1, 4, 8, and 11 of a 21-day cycle. The infusion was stopped and an EKG obtained for any cardiac symptoms experienced during infusion. The infusion was restarted and administered over a total time of 6 h upon resolution of the symptoms at the discretion of the treating physician; however, all infusions were required to be completed within 8 h of mixing. Doses were reduced to 165 mg/m² and, if necessary, to 110 mg/m² for grade 3 hematologic toxicities or grade 2 non-hematologic toxicities. Doses were held, then restarted at the reduced dose levels for grade 4 hematologic toxicities and grade 3 or 4 non-hematologic toxicities. 17-AAG was discontinued for grade 4 diarrhea, grade 4 transaminitis, unresolved EKG changes, occurrence of ventricular arrhythmia, confirmed troponin I elevation, significant decrease in LVEF, or when more than 2 dose reductions would have been required for any toxicity. Oral 5HT receptor antagonists were administered before each infusion for nausea prophylaxis and patients were given appropriate oral anti-emetics for home use. Patients were instructed to use loperamide as needed for diarrhea.

During treatment, patients were assessed with history, PE, PS, CBC, and CMP on day 1 of each cycle. CBC and CMP were also performed on days 8 and 15 of cycle 1. An EKG was repeated after each infusion during cycle 1. Disease assessment with appropriate radiographic studies and serum tumor markers were repeated every 6 weeks, following each 2 cycles of 17-AAG. Response assessments were performed using RECIST criteria.

Patients continued study treatment indefinitely with stable or responsive disease. Patients were removed from the study for disease progression and for unacceptable toxicity.

Statistical methods

This study was designed using Simon's optimal two-stage design with 10% type I error and 90% power to distinguish an OR (complete response [CR] and partial response [PR]) of 5% from one of 20%. Under these assumptions, a total of 37 response-evaluable participants were required. The trial was designed to stop at the end of the first stage if there were no responses among the first 12 response-evaluable participants.

Precision of the estimated response rate is computed as a 95% confidence interval using Wilson's method. Progression is defined as either disease progression or death. Progression-free and overall survivals are estimated using Kaplan–Meier methods.

Results

Eleven patients were enrolled and treated between January 2005 and January 2008. As a result of the number of grade 3/4 toxicities experienced by nearly half the patients and a lack of response, the decision was made to close the study to further accrual before all 12 planned patients were enrolled. Patient characteristics are shown in Table 1. Nine patients had invasive ductal carcinoma, one had inflammatory breast cancer, and one had adenocarcinoma with medullary features. Six patients had triple negative breast cancer and none were known to be HER2-positive. The majority of patients had either lung or liver metastasis. This was a heavily pretreated group; while the median number was 3, patients had received up to 15 prior treatment regimens for metastatic disease. All patients enrolled had PS of 0 or 1.

Seven of the 11 patients completed at least 2 cycles of 17-AAG and were evaluable for response. Three of these patients had stable disease as their best responses and four progressed. The PFS and overall survival for these response-evaluable patients were 1 month (95% CI: 1, –) and 10 months (95% CI: 6,19), respectively. Of the 3 patients with stable disease after 2 cycles, two subsequently progressed after 4 cycles of 17-AAG. The third was removed from study treatment during cycle 3 for grade 3 hyperbilirubinemia and transaminitis (AST). This may have been due to progressive malignant liver infiltration from known hepatic metastases, or it may have been due to toxicity of the 17-AAG.

Four patients were unable to complete a full cycle of 17-AAG due to toxicity. Two of these patients were removed from study treatment after 2 doses. This was due to grade 4 transaminitis (AST) in one and an empyema requiring hospitalization in the other. The patient who developed transaminitis had known liver metastasis, but as the transaminitis improved after withdrawing 17-AAG, it was felt to be treatment related. It is possible that the empyema was related to the 17-AAG; however, this patient had a large pleural effusion, requiring thoracentesis twice in the 2 weeks before enrollment, and did not develop leukopenia with 17-AAG administration. One patient developed a hypersensitivity reaction with hypotension, chest pain, and shortness of breath at the very beginning of the second 17-AAG infusion and was removed from study treatment at that time. The fourth patient withdrew consent after 1 dose of treatment for quality of life reasons. This patient had experienced grade 2 anorexia, fatigue, and nausea following the first dose of 17-AAG. Overall, five of the 11 patients treated on this study experienced grades 3/4 toxicity. All reported grade 3/4 toxicities are listed in Table 2. Four patients, including one who did not complete a full cycle of 17-AAG, required dose reductions to 165 mg/m² for grade 3/4 toxicities. One additional patient was dose reduced to 165 mg/m² for grade 2 transaminitis (AST and ALT) after cycle 1. This patient did not develop further liver toxicity.

Due to a high reported rate of pulmonary complications in study patients receiving 17-AAG on different studies nationally, the protocol was amended to restrict patients with pre-

existing pulmonary conditions from participation. Seven patients, all enrolled in this study before that amendment, had documented pleural effusions at the time of enrollment. Grade 3/4 pulmonary toxicities occurred in four of them (2 worsened pleural effusions, empyema, and pneumonia) and a relationship to 17-AAG treatment cannot be excluded. None of the patients enrolled after the amendment had such pulmonary compromise before enrollment and none developed any pulmonary toxicity, except one patient with grade 1 cough.

Discussion

Breast cancer continues to be a leading cause of cancer death among women and, although survival rates have been improving, there is still no known cure for metastatic breast cancer. In the past 10–15 years, there has been a movement toward targeted therapies with the hope that they would be more effective, possibly curative, and less toxic than traditional chemotherapeutic agents. 17-AAG was developed for clinical use with this intent.

17-AAG is an ansamycin antibiotic that has been shown to inhibit Hsp90 by blocking its ATP-binding pocket, maintaining it in the ADP-bound conformation, thus preventing its normal functions as a chaperone protein [1, 2]. Hsp90 is a chaperone for a wide variety of signaling proteins, many of which are known to be important in breast cancer, such as ER, PR, HER2, EGFR, Akt, and src [2–4]. Loss of Hsp90 function leads to ubiquitination and degradation of some of these proteins, resulting in lower levels within the cancer cells [16, 17]. Hsp90 inhibition with 17-AAG, in particular, was shown to have antineoplastic effects in HER2 expressing mouse xenografts [18]. Furthermore, in breast cancer cell line studies, 17-AAG was able to cause growth arrest and changes within the cells suggestive of differentiation to a more normal epithelial phenotype [19]. These cells subsequently underwent apoptosis.

The large number of potential breast cancer targets for which Hsp90 inhibition using 17-AAG might be effective and the observation that there is a higher affinity for 17-AAG in tumor tissues than in normal tissues made it a very promising agent for treatment of breast cancer. Indeed, 17-AAG has recently shown significant benefit in combination with trastuzumab for HER2-positive breast cancer patients who have previously progressed on trastuzumab [20]. We evaluated single agent 17-AAG in 11 patients with metastatic breast cancer. The patients were not selected by ER, PR, or HER2 status and 6 of the 11 patients had triple negative breast cancer. Since many triple negative breast cancers may depend upon EGFR, Akt, and src, these patients were expected to be as likely to respond as those expressing ER or PR, or overexpressing HER2.

No responses were seen among our patients and 5 of the 11 patients treated had grade 3/4 toxicity. Four patients had grade 3/4 pulmonary toxicity, all of which occurred in the 8 patients treated before amending the eligibility criteria. All of these patients with grade 3/4 pulmonary toxicity had known pleural effusions at the time of enrollment and one patient required home oxygen with exertion. Although these patients all had good performance status, the pre-existing pleural disease likely contributed to the unexpected level of pulmonary toxicity in this study. Only 3 patients received more than 2 cycles of 17-AAG and one of these did not complete the third cycle due to disease progression versus liver toxicity. Given the lack of response and apparent toxicity, this study was terminated early. We do not believe that 17-AAG warrants further study in HER2-negative breast cancer patients.

Since this study was begun, there has been some pre-clinical evidence that Hsp90 inhibition may paradoxically increase activated Akt and src levels, which would be expected to drive breast cancer growth, rather than arrest it [21]. It is possible that this could account for the

early progression observed in the majority of patients on this study who completed 2 cycles of therapy; however, the low number of patients treated overall precludes any definite conclusions about the effect of 17-AAG. Another study in a HER2-overexpressing cell line showed that 17-AAG is able to down regulate Akt, making those cells more sensitive to paclitaxel. These data, along with the beneficial results seen with 17-AAG in combination with trastuzumab, support its continued study in HER2-positive patients. In addition, Hsp90 remains a promising target for all breast cancer patients and other less toxic Hsp90 inhibitors remain in development.

Acknowledgments

This study was funded through a grant from the National Institutes of Health, U01 CA062487.

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Table 1

Patient characteristics

	Median (range)
Age	54 years (38–73 years)
# Prior regimens for metastasis	3 (1–15)
Performance status (ECOG)	1 (0–1)
No. of patients (%), <i>N</i> = 11	
Gender: female	11 (100)
Race	
White	9 (82)
Black	2 (18)
Receptor status	
ER/PR-positive	1 (9)
ER-positive/PR-negative	2 (18)
ER-negative/PR-positive	1 (9)
HER2-positive	0 (0), 1 unknown
ER/PR/HER2-negative	6 (55)
Sites of disease	
Liver	4 (36)
Pleura/lung	8 (73)
Lymph nodes	9 (82)
Bone	4 (36)
Skin	2 (18)
Prior treatment (adjuvant/metastatic)	
Anthracycline based	11 (100)
Taxane based	11 (100)
Capecitabine based	10 (91)
Gemcitabine	5 (45)
Vinorelbine	4 (36)
CMF	4 (36)
Bevacizumab	1 (9)
Lapatinib	1 (9)
Aromatase Inhibitor	7 (64)
Tamoxifen	2 (18)
Fulvestrant	2 (18)
Oophorectomy	2 (18)
Other hormonal (megace, estradiol)	1 (9)

Table 2

Grade 3/4 toxicities

	No. of patients, <i>N</i> = 11
Anemia	1
Dehydration	1
Cough	1
Elevated alkaline phosphatase	2
Elevated AST	2
Fatigue	1
Hyperbilirubinemia	1
Hyperglycemia	1
Hypersensitivity reaction	1
Hypokalemia	1
Hypotension	1
Nausea	1
Pleural effusion	2
Pulmonary infection with normal ANC	2