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# A Phase I/II Study of Suberoylanilide Hydroxamic Acid (SAHA) in Combination with Trastuzumab (Herceptin) in Patients with Advanced Metastatic and/or Local Chest Wall Recurrent HER2-Amplified Breast Cancer: A trial of the ECOG-ACRIN Cancer

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## Abstract

**Purpose—**Suberoylanilide hydroxamic acid (SAHA; vorinostat), a small molecule inhibitor of histone deacetylase (HDAC), attenuates signaling pathways known to confer trastuzumab resistance. A combination of SAHA and trastuzumab may be a promising strategy to improve the efficacy of trastuzumab against breast cancer. In this Phase I/II study, we evaluated the toxicity and response rate after treatment with SAHA and trastuzumab in patients with HER2 overexpressing metastatic breast cancer with trastuzumab-resistant progressive disease.

**Methods—**In Phase I, the SAHA dose was modified in cohorts of 3–6 patients to find the dose level at which 0 or 1 patients experienced a dose-limiting toxicity (DLT) during the first cycle of therapy. In the Phase II study, response to the recommended dose identified in Phase I was based

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The authors declare that they have no conflict of interest.

on the Response Evaluation Criteria in Solid Tumors (RECIST). Overall survival and time to progression were also evaluated.

**Results—**The recommended dose was determined to be 200 mg twice a day on days 1–14 and IV trastuzumab 6 mg/kg on day 1 of a 21-day cycle (n= 6). The Phase II study (n=10) was terminated when the pre-planned efficacy evaluation found that none of the patients in the primary analysis set responded to combination SAHA and trastuzumab treatment.

**Conclusions—**In patients with HER2–positive metastatic breast cancer who had relapsed or progressed during trastuzumab therapy, we observed no DLTs with SAHA 200 mg twice daily combined with trastuzumab; however, there was insufficient statistical evidence that adding SAHA reversed trastuzumab resistance in these patients.

### Keywords

histone diacetylase; toxicity; anticancer therapy; tumor; resistance

## Introduction

Suberoylanilide hydroxamic acid (SAHA; NSC 701852; vorinostat) is a small molecule inhibitor of histone deacetylase (HDAC) that directly binds the enzyme's active site in the presence of a zinc ion [1]. The action of HDACs on nucleosomal histones leads to tight coiling of chromatin and silencing of expression of various genes, including those implicated in the regulation of cell survival, proliferation, differentiation, and apoptosis [2]. HDACs also act as members of a protein complex to recruit transcription factors to gene promoter regions, including those of tumor suppressors, and HDACs also affect the acetylation status of specific cell cycle regulatory proteins [3]. Because aberrant HDAC activity has been implicated in a variety of cancers, several HDAC inhibitors have been developed as targeted anticancer therapeutics and are currently being evaluated in clinical trials. Trichostatin and butyric acid were among the first HDAC inhibitors to be administered to patients, but were found to be clinically unsuitable due to potency and formulation issues [4, 5]. Depsipeptide was originally selected for clinical study based on its antiproliferative effects; subsequently, it was discovered to be an antagonist of HDACs [6] and was the first HDAC inhibitor to demonstrate clinical efficacy [7]. Among the HDAC inhibitors currently in clinical trials, SAHA is the most potent HDAC inhibitor, targeting most human Class 1 and Class 2 enzymes [8, 9], and can be administered orally with excellent bioavailability. SAHA was the first HDAC inhibitor approved by the FDA for an oncologic indication in cutaneous T-cell lymphoma, with activity also seen in Hodgkin's lymphoma and other hematologic malignancies [10]. Objective responses to SAHA monotherapy were not observed in a Phase II study in metastatic breast cancer resistant to conventional therapy, although 4 patients experienced stable disease [11].

Despite recent progress in our understanding of its genetic and molecular basis, advanced and metastatic breast cancer remains incurable [12]. Approximately 25% of breast cancers have amplification and over-expression of HER2/neu oncogene [13], which encodes a member of the epidermal growth factor (EGFR) family of tyrosine kinases [14]. HER2 positive breast cancer has a poor prognosis, making Her-2 a promising target for new

therapies, including the monoclonal antibody trastuzumab. However, previous reports have indicated that responses to trastuzumab occur in only a minority (25–30%) of Her-2 overexpressing breast cancers [15, 16]; one potential mechanism of resistance to trastuzumab involves Her-2–independent increased activity of AKT [17–19].

HDAC inhibitors that are hydroxamic acid analogues, including SAHA, have been shown to induce p21WAF1 and p27KIP1, inhibit cell growth, and induce apoptosis of breast cancer cells [20–23]. Studies have also demonstrated that SAHA induces acetylation of hsp90, which inhibits the binding of hsp90 to ATP and destabilizes the chaperone complex with its target proteins, including Her-2, AKT, and c-Raf [22, 24], leading to ubiquitination and proteasomal degradation [22, 24]. Since SAHA attenuates the levels of pAKT and c-RAF-1, it may abrogate the signaling pathways known to confer trastuzumab resistance. Both SAHA and trastuzumab have also been shown to inhibit vascular endothelial growth factor levels and exert anti-angiogenic effects [25, 26]. Based on these findings, we and others have hypothesized that a combination of SAHA and trastuzumab may be a promising strategy to reverse trastuzumab resistance in trastuzumab pre-treated HER2 overexpressing breast cancers.

Therefore, the Phase I objective of the present study was to determine the recommended dose of SAHA used in combination with trastuzumab based on toxicity endpoints in patients with HER2 overexpressing metastatic breast cancer who had progressive disease after prior trastuzumab. The objective of the Phase II portion of the study was to evaluate response rate, overall survival, and time to progression after treatment with the Phase I-recommended dose of SAHA combined with trastuzumab.

## Methods

### Study design

This was a phase I/II study to determine a recommended dose of SAHA used in combination with trastuzumab and to evaluate the response rate and toxicity of the combination therapy in patients with HER2 overexpressing metastatic breast cancer who had progressive disease after prior treatment with trastuzumab (ClinicalTrials.gov identifier: NCT00258349).

### Study participants

Patients with histologically confirmed HER2 overexpressing breast cancer, defined locally by immunohistochemical analysis (with 3+ indicating positive status), fluorescence in situ hybridization (with an amplification ratio ≥2.0 indicating positive status), or both. Efficacy analysis was performed only for those subjects with centrally confirmed HER2 overexpressing breast cancer. Evidence of measurable metastatic disease and/or chest wall recurrence were required for inclusion in the study. Patients may have received prior radiation therapy; however, the only site of measurable disease must not have been irradiated, except in the case of chest wall recurrence previously treated with adjuvant radiation therapy. Eligible patients must have had documented disease recurrence or progression while receiving trastuzumab or must have had relapsed within 3 months of completing the last dose of adjuvant trastuzumab or trastuzumab for metastatic disease.

Patients must have had adequate organ and marrow function, defined as (1) CBC values (obtained within 14 days prior to registration) of ANC ≥1500/μL, platelet count ≥100,000/$mm^3$, and hemoglobin ≥9 g/dL; and (2) prestudy chemistry values (obtained within 4 weeks prior to registration) of serum creatinine ≤1.5 g/dL, ALT ≤2×ULN, AST ≤2×ULN, and total bilirubin ≤1.5 mg/dL. Eligible patients must have had adequate cardiac function as defined by left ventricular ejection fraction less than or equal to the lower limit of institutional normal obtained within 6 weeks prior to registration, as assessed by an echocardiogram and nuclear scan and no evidence of PR prolongation or AV block on ECG performed within 4 weeks prior to registration.

CYP450 inducers or inhibitors must have been discontinued 1 week prior to initiating protocol treatment. Patients must not have had chemotherapy or radiotherapy within 3 weeks prior to entering the study. Patients must have recovered from all adverse events related to prior chemotherapy or radiation. Patients must not have been receiving any other investigational agents, nor might they have received any other investigational agents within 4 weeks prior to entering the study. Patients must not have had a history of untreated brain metastasis or brain metastasis currently undergoing radiation. Patients with brain metastasis representing the sole site of disease were not eligible. Patients with previously treated brain metastasis who had responded to brain radiotherapy and/or surgery and had continued in response were eligible, provided the brain was not the only site of measurable disease. Patients must not have had a history of allergic reactions attributed to compounds of similar chemical or biologic composition to SAHA or other agents used in study. Patients must not have had taken valproic acid, another histone deacetylase inhibitor, for at least 2 weeks prior to registration.

Patients who met inclusion criteria provided informed consent prior to enrollment.

## Study treatment

In the Phase I portion of the trial, the SAHA dose was to be modified in cohorts of 3–6 patients based on the number of patients who experienced a dose-limiting toxicity (DLT) event at each dose level during the first cycle of therapy. If 0 or 1 patient out of 3 developed a DLT at a given SAHA dose, then 3 additional patients were to be treated at the same dose level. If 0 or 1 patient out of these 6 patients developed a DLT, this SAHA dose was to be determined to be the recommended dose for the Phase II study. If 2 or more patients developed a DLT at a given SAHA dose, the dose level was to be reduced. Specifically, the first 3–6 patients enrolled in the study were to be treated with 200 mg of SAHA orally twice a day (total 400 mg/day), on days 1–14 per 21-day cycle, in addition to IV trastuzumab (6 mg/kg) on day 1 per cycle (Arm A). If excessive toxicity was observed in Arm A, the dose was to be reduced in subsequent cohorts of 3–6 patients to 300 mg/day (100 mg AM, 200 mg PM) on days 1–14 (Arm B). If excessive toxicity was observed in Arm B, the dose was to be reduced again to 200 mg/day (100 mg twice a day) on days 1–14 (Arm C). If excessive toxicity was observed at this dose level, the study was to be closed to accrual.

Based on the Phase I study findings, the recommended dose of SAHA was administered orally twice a day, days 1–14 per 21-day cycle, in addition to IV trastuzumab (6 mg/kg) on

day 1 in the Phase II study (Arm D). Patients continued on the Phase II protocol treatment until unacceptable toxicity or progression of disease.

## Study outcomes

All adverse events were documented in both the Phase I and Phase II studies. Toxicity was assessed using the NCI Common Toxicity Criteria (CTC) Version 3.0. Patients were assessed weekly for DLTs, defined as: (1) excessive bone marrow function toxicity: Grade 3–4 thrombocytopenia and/or Grade 3–4 neutropenia lasting more than 7 days, Grade 3–4 febrile neutropenia, or Grade 4 anemia; (2) excessive GI toxicity: Grade 4 anorexia, Grade 4 diarrhea and/or vomiting not responding to maximal therapy or prophylaxis, Grade 3 diarrhea and/or vomiting persisting more than 24 hours despite maximum treatment, or any symptoms requiring sustained IV fluid replacement or hospitalization for IV fluid replacement (Grade 3 dehydration); (3) excessive general toxicity: Grade 3–4 fatigue, or fatigue, asthenia or malaise leading to a decrease in ECOG performance status to 3 or 4 (or 2 if the baseline performance status was 0).

In the Phase II study, treatment response was based on the Response Evaluation Criteria in Solid Tumors (RECIST). Tumor lesions at baseline were classified as measurable (accurately measured in at least one dimension as >20 mm with conventional techniques or as >10 mm with spiral CT scan) or non-measurable. Complete response (CR) was defined as the disappearance of all target lesions (maximum of 5 lesions per organ and 10 lesions total, selected based on size at baseline). Partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameters of target lesions. CR or PR was confirmed by repeat assessments performed no less than 4 weeks after the criteria for response were first met. Progressive disease (PD) was defined as at least a 20% increase in the sum of the longest diameters of target lesions and stable disease (SD) was defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD. The best response rate was based on response of target and non-target tumors and duration of response. Overall survival, defined as the time from registration to death from any cause, and time to progression (appearance of new tumors) were also evaluated.

## Statistical methods

Descriptive statistics were used to characterize patients at baseline. Best response rate was reported and its confidence interval was computed by the method of exact binomial confidence interval. The Kaplan-Meier method was used to estimate the overall survival and disease progression-free rates over time. Standard errors of the estimates were calculated using Greenwood’s formula. The confidence interval of the median overall survival and median time to progression were constructed using the method by Brookmeyer and Crowley.

For the Phase II study, a two-stage plan was employed to distinguish between a response rate (CR+PR) of 10%, which would be considered unworthy of further study, and a response rate of 30%, which would be considered worthy of further study. Adopting the optimal design of Simon et al [27], if 0 or 1 of the first 12 eligible patients (including the patients who were treated at the recommended Phase II SAHA dose level during the initial Phase I safety evaluation) showed a response, the study was stopped, with the conclusion that the regimen

was not effective. If 2 or more patients responded, 23 additional eligible patients were accrued, for a total of 35. If 6 or more of these 35 patients responded, the regimen was considered worthy of further study. With this design, the study would be stopped early with probability of 66% if the true response rate was 10% and with a probability of 9% if the true response rate was 30%. Allowing up to 10% of patients to be false HER2 overexpressors based on the retrospective central review and up to 5% ineligibility rate for other reasons, a total of 35 patients were planned to be enrolled in the Phase II study, including the 6 patients from the Phase I study treated with the Phase II recommended SAHA dose.

## Results

### Phase I safety evaluation

The Phase I study was activated on August 23, 2006 at five institutions (Fox Chase Cancer Center, Albert Einstein College of Medicine/Montefiore Hospital, Vanderbilt University, University of Alabama Birmingham, and Johns Hopkins University). Six patients with confirmed Her-2–positive breast cancer were treated at the starting dose of SAHA 200 mg twice daily on days 1–14 combined with 6 mg/kg trastuzumab on day 1 every 21 days (Arm A). There were no DLTs at this dose level, eliminating the need to reduce the SAHA dose. The Arm A dose was determined to be the recommended dose for the Phase II study.

### Phase II treatment response

The Phase II study was activated on June 28, 2007 and suspended on October 4, 2007 for the pre-planned efficacy evaluation. Due to a low response rate in this efficacy evaluation, the study was formally terminated on August 27, 2009. A total of 16 patients were enrolled in the Phase II study; all patients were from ECOG. One patient was ineligible because the measurement of lesions was completed after registration. Five patients were Her-2–negative from the ECOG central review; of these, 1 patient's HER2 status from the central review was not available due to insufficient tissue. Therefore, 10 eligible patients were confirmed as HER2–positive by the central review and were included in the primary efficacy analysis of the Phase II study; this set of patients was named the primary analysis set. Efficacy analysis (response, overall survival, time to progression) included the primary analysis set (n=10). Toxicity was assessed in both the primary analysis set plus the 6 patients from the Phase I safety evaluation (N=16).

### Patient characteristics

Table 1 presents the baseline characteristics of the primary analysis set (n=10). The median age was 54 years, ranging from 42 to 69 years. Three of the patients were pre-menopausal or younger than 50 years of age. Four patients had an ECOG performance status of 1 and 6 patients had a status of 0. At initial diagnosis, 2 patients were ER-positive and/or PR-positive. Four patients had 4 or 5 metastatic sites, and the other 6 patients had 1 to 3 metastatic sites.

### Treatment compliance

Among the primary analysis set, 6 patients completed 2 cycles of treatment, 2 patients completed 3 cycles, 1 patient completed 7 cycles, and 1 patient completed only 1 cycle.

Eight patients were off treatment due to disease progression; 1 was due to toxicity and 1 was due to patient withdrawal.

### Toxicity

Table 2 summarizes counts of the observed toxicity for all 16 patients in the Phase I and II studies. One patient had Grade 3 decreased platelets and another patent had Grade 4 decreased platelets. Two patients experienced Grade 4 dyspnea. Ten patients experienced Grade 1 or 2 diarrhea without prior colostomy.

### Best response rate

Of the 10 patients with HER2–positive disease in the primary analysis set, no patient (0%, 90% CI [0%, 26%]) had CR or PR, 1 (10%, 90% CI [0.5%, 39%]) patient had SD, 8 (80%) patients had PD, and 1 patient's response was not evaluable due to non-protocol therapy prior to disease progression and no disease assessment after baseline. The patient with unknown HER2 status from the central review had PD. Among the 5 patients with centrally confirmed HER2–negative disease, 1 had a PR and the others had PD.

### Overall survival

Among the primary analysis set of 10 patients, 9 (90%) died and 1 was alive after a follow-up of 30 months. Among all 16 patients treated, 2 were alive and 14 (87.5%) died. Figure 1 shows the Kaplan-Meier curve and 95% confidence interval for overall survival among the patients in the primary analysis set (n=10). The median survival time was 9.3 months (95% CI: 5.1, 24.7).

### Time to progression

All 16 patients had documented disease progression. Figure 2 shows the Kaplan-Meier curve and 95% confidence interval for time to progression among the patients in the primary analysis set (n=10). The median time to progression was 1.5 months (95% CI: 1.3, 3.7).

## Discussion

E1104 was a phase I/II study to determine the recommended dose of SAHA that could be used with minimal toxicity in combination with trastuzumab and to evaluate the response rate of combination therapy in patients with HER2 overexpressing metastatic breast cancer who had progressive disease after prior treatment with trastuzumab. In the Phase I study, the recommended dose of SAHA was determined to be 200 mg twice a day for days 1–14 of a 21-day cycle. The Phase II study of 16 patients treated with this combination therapy was terminated when the pre-planned efficacy evaluation suggested a low response rate. Among the 10 patients with centrally confirmed Her-2–positive breast cancer included in the primary analysis set, none responded to the treatment. Thus, there is insufficient statistical evidence that adding the HDAC inhibitor, SAHA, reverses trastuzumab resistance in these patients.

Similar to our study, another Phase I/II study of SAHA in combination with paclitaxel and bevacizumab found no dose-limiting toxicities at the recommended dose of SAHA 300 mg

twice daily. For the primary efficacy analysis in 44 patients treated at with this SAHA dose, the objective response rate was 55% and SAHA was found to induce tubulin acetylation and Hsp 90 inhibition [28].

SAHA was also shown to reduce tamoxifen resistance in ER-positive breast cancer. In a Phase II study of 43 women with ER-positive breast cancer and progression while on tamoxifen, treatment with 400 mg of vorinostat daily for 3 weeks of a 4-week cycle plus 20 mg tamoxifen daily led to a 19% objective response rate, and 40% of patients experienced a response or stable disease after 24 weeks of treatment [29]. The authors also reported that HDAC2 expression in PBMC may be predictive of tamoxifen response and that histone deacetylation may be a useful pharmacodynamic marker for efficacy [29]. In our study, there were insufficient patient samples to perform similar correlative analyses.

More recently, preclinical work has shown that another HDAC inhibitor, SNDX-275 (entinostat), enhances the efficacy of trastuzumab in HER2 overexpressing breast cancer cell lines and exhibits the potential to overcome trastuzumab resistance [30]. SNDX-275 has also shown activity in metastatic hormone receptor-positive breast cancer in combination with an aromatase inhibitor and is currently being evaluated in a Phase III trial in this population [31]. There are no clinical data available currently with entinostat in HER2–positive breast cancer.

## Conclusion

In this population of patients with HER2–positive breast cancer who had either relapsed or progressed during trastuzumab therapy (either alone or in combination with chemotherapy), we observed no dose-limiting toxicities with 200 mg twice daily for 14 days of a 21-day cycle combined with trastuzumab; however, there was insufficient statistical evidence that adding SAHA reversed trastuzumab resistance in these patients.

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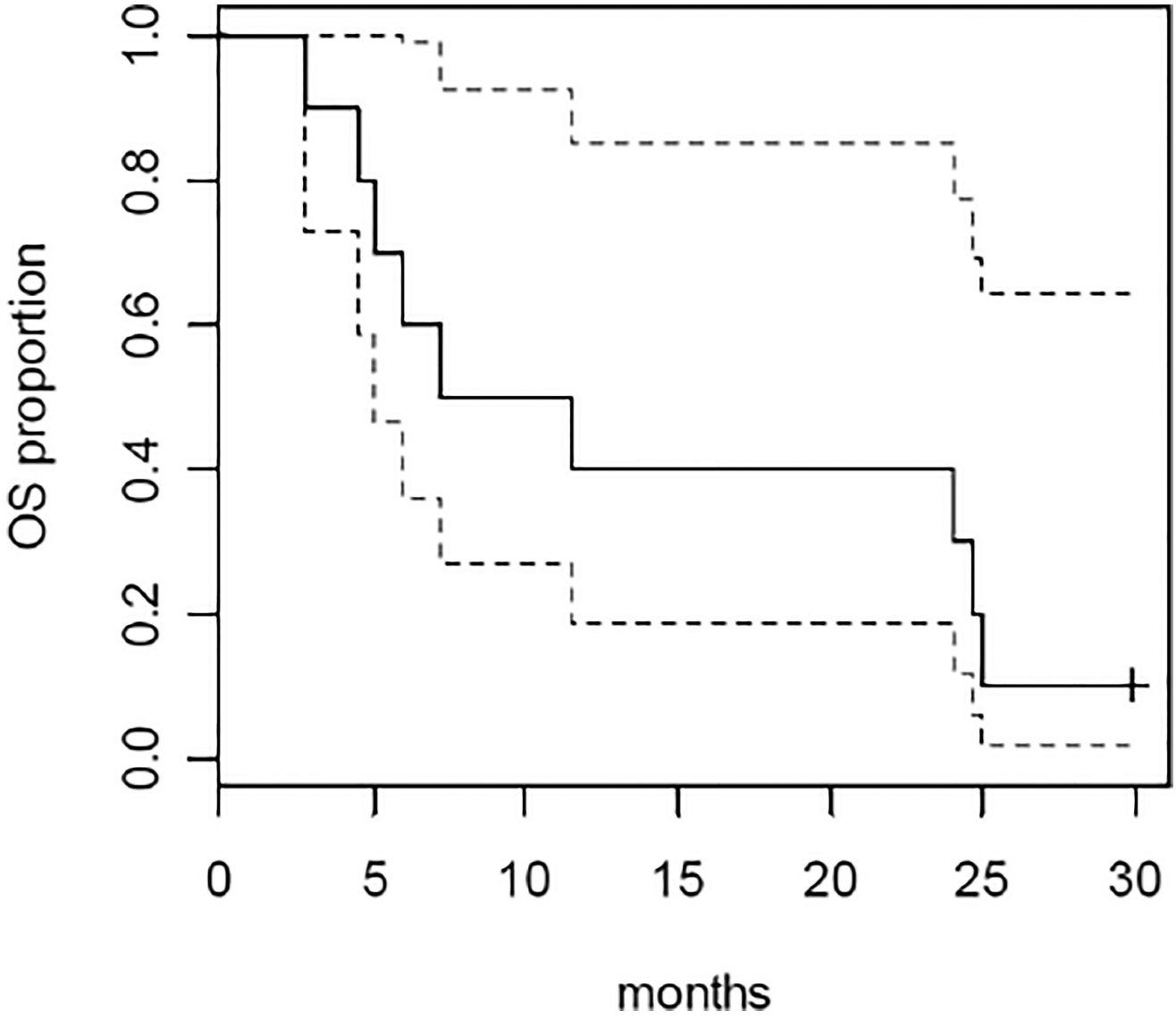


**Figure 1.**
Kaplan-Meier plot of overall survival with 95% confidence interval for the primary analysis set (n=10).

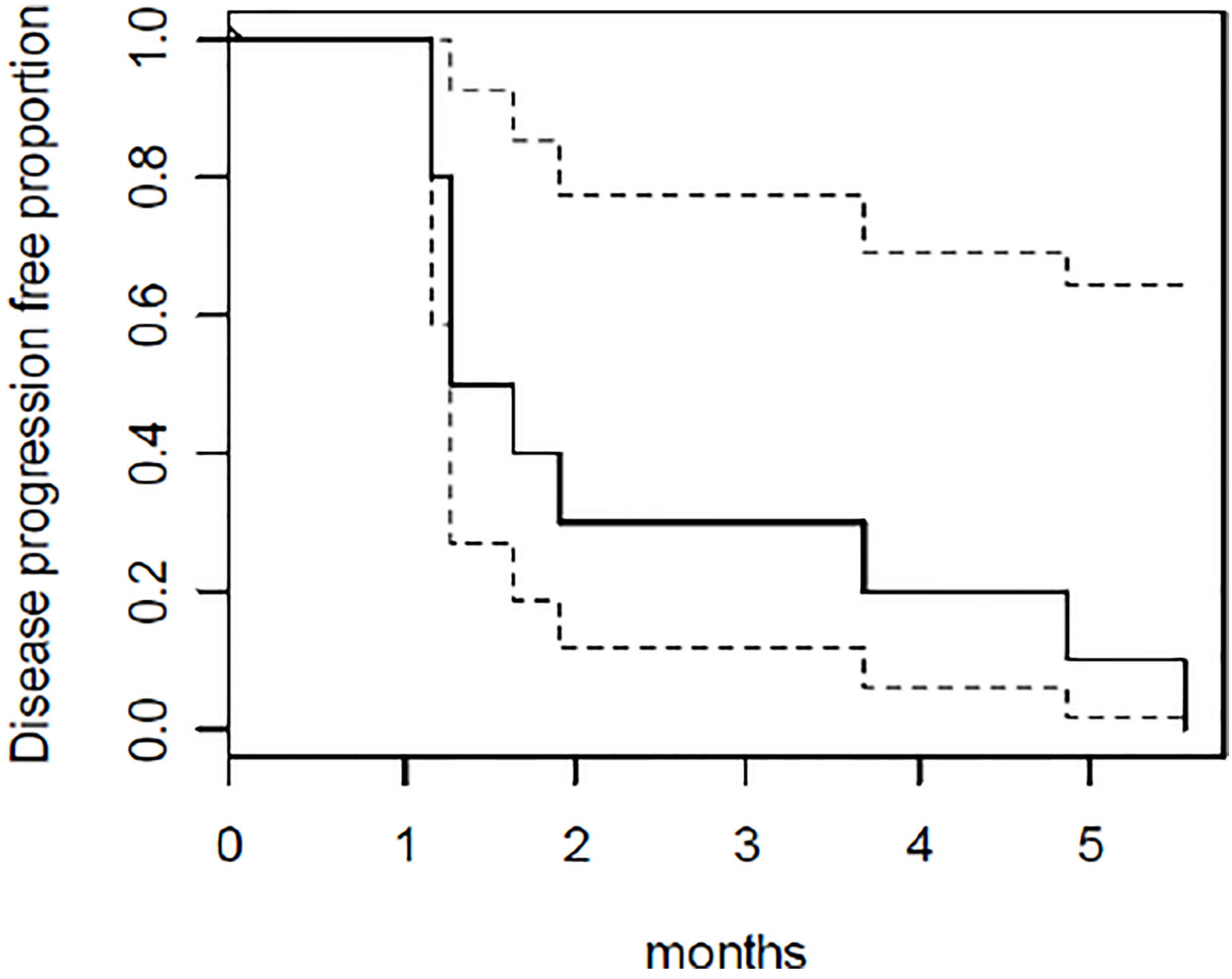


**Figure 2.**
Kaplan-Meier plot of time to disease progression with 95% confidence interval for the primary analysis set (n=10).

## Table 1

Patient Characteristics (primary analysis set, n=10)

| **Total (No.)** | **10** |
|---|---|
| Median age (range, years) | 54 (42,69) |
| ECOG PS | |
| 0 | 4 (40%) |
| 1 | 6 (60%) |
| Pre-menopausal or <50 years of age | 3 (30%) |
| ER+ and/or PR+ | 2 (20%) |
| Involved sites (No.) | |
| 1 | 2 (20%) |
| 2 | 1 (10%) |
| 3 | 3 (30%) |
| 4 | 2 (20%) |
| 5 | 2 (20%) |
| Prior chemotherapy | |
| Yes | 9 (90%) |
| Missing | 1 (10%) |
| Prior hormonal therapy | |
| Yes | 2 (20%) |
| No | 7 (70%) |
| Missing | 1 (10%) |
| Prior immunotherapy | |
| Yes | 9 (90%) |
| Missing | 1 (10%) |
| Prior radiation therapy | |
| Yes | 6 (60%) |
| No | 3 (30%) |
| Missing | 1 (10%) |
| Prior surgery | |
| Yes | 9 (90%) |
| Missing | 1 (10%) |

**Table 2**

Adverse Events in All Study Patients (n=16)

| | Grade | | | |
|---|---|---|---|---|
| | **1/2** | **3** | **4** | **5** |
| Hemoglobin | 0 | - | - | - |
| Leukocytes | 3 | - | - | - |
| Neutrophils | 1 | - | - | - |
| Platelets | 2 | 1 | 1 | - |
| Hypertension | 1 | - | - | - |
| Hypotension | 1 | - | - | - |
| Fatigue | 6 | - | - | - |
| Insomnia | 1 | - | - | - |
| Weight loss | 3 | - | - | - |
| Alopecia | 1 | - | - | - |
| Nail changes | 1 | - | - | - |
| Rash: acne/acneiform | 1 | - | - | - |
| Anorexia | 5 | - | - | - |
| Constipation | 1 | - | - | - |
| Dehydration | 2 | - | - | - |
| Diarrhea w/o prior colostomy | 10 | - | - | - |
| Muco/stomatitis by exam, oral cavity | 1 | - | - | - |
| Nausea | 6 | - | - | - |
| Taste disturbance | 1 | - | - | - |
| Vomiting | 5 | - | - | - |
| Alkaline phosphatase | 1 | - | - | - |
| ALT, SGPT | 2 | - | - | - |
| AST, SGOT | 2 | - | - | - |
| Hypocalcemia | 1 | - | - | - |
| Creatinine | 5 | - | - | - |
| Hyperglycemia | 5 | - | - | - |
| Hypokalemia | 3 | - | - | - |
| Hyponatremia | 1 | - | - | - |
| Dizziness | 2 | - | - | - |
| Dyspnea | 1 | 2 | - | - |
| Pneumonitis/pulmonary infiltrates | 1 | - | - | - |
| WORST DEGREE | 10 | 1 | 1 | - |