Phase II Study of Bortezomib and Pegylated Liposomal Doxorubicin in the Treatment of Metastatic Breast Cancer

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Abstract

Background: Based on preclinical studies and a phase I trial of the combination of bortezomib and pegylated liposomal doxorubicin (PLD), which both showed activity in breast cancer, we conducted a phase II study of this regimen in patients with metastatic breast cancer. **Patients and Methods:** Patients received bortezomib 1.3 mg/m^2 on days 1, 4, 8, and 11 of an every-21-day cycle, along with PLD 30 mg/m^2 on day 4. The primary objective was to evaluate the response rate of this combination, while secondary objectives were to obtain further safety data about this combination, to evaluate the time to disease progression (TTP), and to evaluate response by the breast cancer subtype. **Results:** One of 12 evaluable patients had a partial response (8%), while 3 (25%) had stable disease. At 26 months follow-up, the median overall survival was 4.3 months (95% CI, 1.2-26.2) and the median TTP was 1.3 months (95% CI, 0.8-14.0 months). The combination was well tolerated, with the most common events including low-grade nausea and vomiting, neutropenia, and neuropathy, and no cardiac toxicity was seen. Of the 7 tumors subtyped, no association was seen between intrinsic subtype or receptor status and response. **Conclusion:** The combination of PLD and bortezomib was well tolerated but has minimal activity in heavily pretreated unselected metastatic breast cancer.

Introduction

Bortezomib (VELCADE®; Millennium Pharmaceuticals, Inc., San Diego, CA and Johnson & Johnson Pharmaceuticals Research & Development, L.L.C., Raritan, NJ) is a dipeptide boronic acid derivative that specifically inhibits the chymotrypsin-like activity of the proteasome. The proteasome is a large, multicatalytic protein-ase complex responsible for the degradation of many intracellular proteins, including targets such as IκB, p53, p21, and p27, and other proteins involved in the cell cycle and apoptosis-related signal transduction pathways.[1] Proteasome inhibition has antineoplastic effects through several distinct mechanisms, including inhibition of cell growth signaling pathways, inhibition of angiogenesis, and induction of apoptosis. Bortezomib has shown antitumor activity in preclinical models and clinical trials, most notably against multiple myeloma,[2,3] and is Food and Drug Association approved both for the treatment of multiple myeloma and mantle cell lymphoma. Activity in solid tumors has been seen as well, including in ovarian cancer,[4] prostate cancer,[5] renal cell carcinoma,[6] and non–small-cell lung cancer.[7] Preclinical studies in breast cancer models have demonstrated an antitumor effect of bortezomib as a single agent,[8-11] but in the clinical arena bortezomib has not shown activity, as judged by the lack of objective responses seen in 2 studies with a total of 24 patients with breast cancer.[12,13]

Proteasome inhibition is also a rational strategy to achieve chemosensitization and overcome chemoresistance,[14] and we therefore considered that a bortezomib-based combination regimen might show greater efficacy against breast cancer. This hypothesis was

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supported in part by preclinical studies, which suggested that bortezomib-containing combinations were more active preclinically.[8-11] In particular, bortezomib has been found to suppress expression of proteins involved in DNA damage repair pathways, resulting in enhanced sensitivity to anthracyclines.[15] Moreover, anthracyclines have been noted to suppress the induction of mitogen-activated protein kinase phosphatase (MKP)-1,[16] which is an antiapoptotic stress-response protein induced by proteasome inhibitors.[17] Indeed, bortezomib with pegylated liposomal doxorubicin (PLD) was shown to have synergistic activity in drug-naive models in vitro, and enhanced antitumor activity in vivo against breast cancer.[18,19] Pegylated liposomal doxorubicin has demonstrated clinical activity in breast cancer. In a phase II study of PLD at 45-60 mg/m^2 administered every 3-4 weeks to patients with anthracycline-naive breast cancer, the overall response rate (RR) was 31%.[20] Though RRs are lower in anthracycline pretreated breast cancer patients, additive dosing of PLD may be less cardiotoxic than the parent drug, doxorubicin.[21,22] The regimen of bortezomib and PLD has been extensively evaluated in patients with multiple myeloma, where it was shown to be both safe and effective in phase I,[23,24] and superior to bortezomib alone in a phase III trial.[25] Finally, in our previous phase I study of this combination targeting patients with solid tumors, we found that bortezomib 1.3 mg/m^2 on days 1, 4, 8, and 11, and PLD, 30 mg/m^2 on day 4, could be safely administered on an every-21-day schedule.[26] Evidence of a clinical benefit was seen in breast cancer patients in the phase I setting, and a phase II study was therefore designed to further evaluate the possible efficacy of this combination.

In the current report, we present the results of the phase II trial of bortezomib and PLD in breast cancer. The primary objective of this study was to evaluate the RR of this combination in metastatic breast cancer. Secondary objectives were to evaluate the time to progression (TTP) of disease in metastatic breast cancer patients undergoing treatment with this combination, to obtain further evidence of the safety of this combination in this population, and to compare any responses with the breast cancer intrinsic subtype.

Patients and Methods

Eligibility

This was an open label phase II trial of PLD and bortezomib in patients with histologically or cytologically confirmed metastatic breast cancer. Patients were required to have measurable disease by the Response Evaluation Criteria in Solid Tumors (RECIST).[27] All patients gave written, informed consent according to federal and institutional guidelines before treatment, and this study received approval from the University of North Carolina at Chapel Hill Institutional Review Board, and was conducted in accordance with the Declaration of Helsinki. Patients were recruited from the University of North Carolina Multidisciplinary Breast Cancer Clinic. Eligibility criteria included: age ≥ 18; Karnofsky performance status > 60%; a life expectancy of ≥ 3 months; no other concurrent antineoplastic treatment; adequate hematologic (platelets > 100,000/mm^3, hemoglobin ≥ 9.0 g/dL, and absolute neutrophil count ≥ 1500/mm^3), hepatic (aspartate aminotransferase and alanine aminotransferase ≤ 2 times the upper limit of normal; alkaline phosphatase ≤ 2 times the upper limit of normal, except if attributed to tumor; and total bilirubin ≤ the upper limit of normal), and renal (creatinine

≤ 2.5 mg/dL) function. A normal left ventricular ejection fraction (LVEF), defined as > 50% within 42 days before the first dose of study drug, was necessary. Also, patients could not be pregnant or nursing, had to be amenable to the use of appropriate contraception, could not have symptomatic brain metastases, any treated brain metastases had to have been stable for more that 3 months and did not require chronic steroids, and, according to the study investigators, patients could have no other coexisting medical problems of sufficient severity to limit full study compliance or cause undue risk. Patients were ineligible if they had a previous cumulative exposure to doxorubicin > 300 mg/m^2, or epirubicin > 540 mg/m^2; previous anthracycline treatment within the last 6 months; New York Heart Association Class II or greater heart failure, or clinical evidence of congestive heart failure, or a myocardial infarction within 6 months before enrollment; uncontrolled angina; electrocardiographic evidence of acute ischemia or active conduction system abnormalities; hypersensitivity to PLD, bortezomib, boron, or mannitol; more than 2 previous chemotherapy regimens for metastatic disease; or grade 2 peripheral neuropathy within 14 days before enrollment.

Treatment Plan

Pegylated liposomal doxorubicin from commercial stock was prepared as described in the package insert, and a dose of 30 mg/m^2 administered as a 60 to 90 minute infusion 1 hour after bortezomib administration on day 4 of each cycle. Bortezomib, a sterile, lyophilized powder in vials with mannitol, was reconstituted with normal saline to a drug concentration of 1 mg/mL. A dose of 1.3 mg/m^2 was administered by intravenous push over 3-5 seconds on days 1, 4, 8, and 11 of an every-21-day cycle. For grade 2 or higher neutropenia or thrombocytopenia, or hand-foot syndrome (HFS), or stomatitis, or for grade 3 or higher other nonhematologic toxicity, doses were held until recovery to grade 0-1. Held doses within a cycle were skipped and not delayed. In the case of toxicity observed on the first day of a new cycle, doses were delayed for up to 2 weeks. If grade 2 or higher bilirubin levels were seen, the PLD was dose reduced by 50% and the bortezomib continued at full dose. No inter- or intra-patient dose escalation was allowed.

All patients received pyridoxine at 200 mg orally daily beginning up to 1 week before the initiation of therapy. As premedications, patients received ondansetron 24 mg orally, ranitidine 50 mg intravenous (I.V.), dexamethasone 20 mg orally, and diphenhydramine 50 mg orally before dosing of PLD. Alternative and/or additional antiemetics could be used at the discretion of one of the study investigators. No premedications were administered before or after dosing with bortezomib.

Toxicity Assessment

Complete blood cell counts, serum chemistries, and adverse events were evaluated on day 1 of each cycle, and were graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 3.0. If during treatment the LVEF fell to ≤ 45%, or the LVEF fell by 20% from the baseline level, PLD was discontinued. For patients with cumulative doxorubicin dosing of < 350 mg/m^2, or of epirubicin < 540 mg/m^2, cardiac monitoring was performed at the physician's discretion. For patients with cumulative doxorubicin dosing of ≥ 350 mg/m^2, or epirubicin of ≥ 540 mg/m^2, cardiac monitoring was performed every 2 cycles.

Table 1	Baseline Patient Characteristics
Characteristic, n (%)	**Value**
Age, Years	
Median	51
Range	40-66
Race	
Caucasian	10 (77)
Black or African-American	2 (15)
American Indian	1 (8)
ECOG Performance Status	
0	5 (38)
1	8 (62)
Number of Previous Chemotherapy Lines (Metastatic)	
0	3 (23)
1	6 (46)
2	4 (31)
Previous anthracycline	10 (77)
Previous taxane	12 (92)
Metastatic Sites	
Liver	11 (85)
Lung	10 (77)
Bone	9 (69)
Lymph nodes	3 (23)
Other (skin, peritoneum)	2 (15)
ER/PgR positive	9 (69)
ER/PgR negative	4 (31)
HER2/*neu* overexpressed	5 (39)
Triple negative	3 (23)

Abbreviations: ECOG = Eastern Cooperative Oncology Group; ER = estrogen receptor; PgR = progesterone receptor

Response Criteria

Tumor assessments were performed every 6 weeks (2 cycles), or at the follow-up visit if the patient was removed or withdrew from therapy in mid-cycle. Response was evaluated using the RECIST criteria.

For breast cancer patients treated on this or the previous phase I study who had archival tumor biopsy specimens available, the Prediction Analysis of Microarray 50-gene classifier (PAM50) intrinsic subtyping assay was performed on RNA from formalin-fixed paraffin-embedded (FFPE) tissue blocks, as previously described.[28]

Statistical Analysis

Response rate was the primary endpoint. For this patient population, we used as the benchmark for PLD response the 10% RR seen in the previous phase II study of the single agent in previously anthracycline treated patients.[21] The null hypothesis was that the RR would be 10% or less, with a RR of interest being 30%. A two-stage approach with early stopping rules was used with 12 patients enrolled in stage I.[29] The accrual goal was 40 patients with at least 35 eligible and evaluable patients.

Figure 1a	The Kaplan-Meier Curves for Overall Survival With the Number of Patients at Risk

| Number of Patients | 13 | 7 | 6 | 6 | 4 | 4 | 2 | 2 | 2 | 0 |
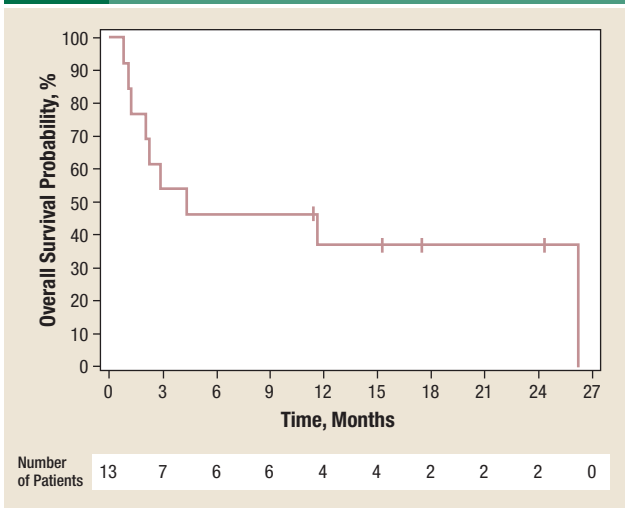
At a follow-up of 26 months, the median overall survival was 4.3 months (95% CI, 1.2-26.2).

Figure 1b	The Kaplan-Meier Curves for Time to Progression With the Number of Patients at Risk

| Number of Patients | 13 | 3 | 3 | 3 | 3 | 2 | 1 | 1 | 1 | 0 |
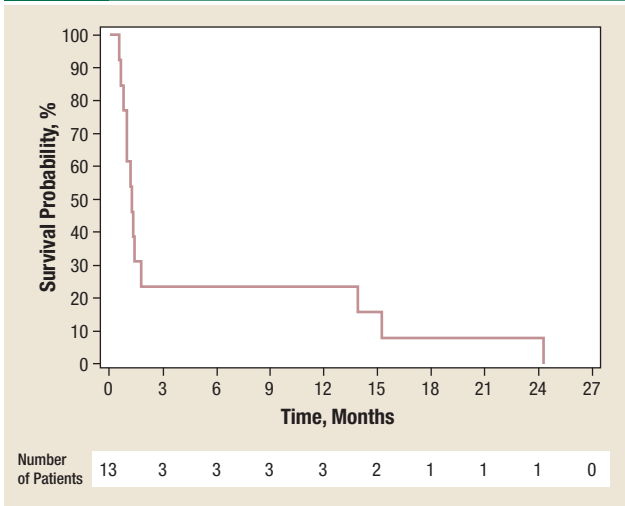
At a follow-up of 26 months, the median time to progression was 1.3 months (95% CI, 0.8-14).

Using the Simon optimal 2-stage design, if only 1 of the first 12 evaluable patients responded (complete response [CR] or partial response [PR]), the trial would be terminated based on the failure to reject the null hypothesis. If 2 or more of the first 12 patients responded, then an additional 23 patients would have been enrolled at stage II. The null hypothesis would be rejected if 6 or more of the 35 patients responded. This 2-stage design yielded an overall α level of 0.10; the power is 90% when the true RR is 30%. For RR assessment, only patients who completed an entire first cycle of therapy were considered evaluable for response.

Overall survival (OS) was defined from the date of enrollment to the date of death from any cause, or censored at the date of last follow-up. Time to progression (TTP) was defined as the time from the date of enrollment to the date of clinically or radiographically evident progression. The survival analysis was based on intention-to-treat principle

Table 2 Drug-Related Toxicities

Adverse Event	All Grades Number (%)	Grade 3 Number (%)
Nausea	6 (46)	1 (8)
Neuropathy	6 (46)	0
Diarrhea	5 (39)	1 (8)
Vomiting	5 (39)	0
Neutropenia	4 (31)	2 (15)
Thrombocytopenia	4 (31)	2 (15)
Fatigue	4 (31)	0
Rash	4 (31)	1 (8)
Lymphopenia	3 (23)	1 (8)
Constipation	3 (23)	0
Arthralgias/Myalgias	3 (23)	0
Headache	2 (15)	0
Oral Sores	2 (15)	0
Abdominal Pain	2 (15)	0
Thrush	1 (8)	0
Taste Alteration	1 (8)	0
Anorexia	1 (8)	0
Anemia	1 (8)	1 (8)
Leukopenia	1 (8)	0
Pruritus	1 (8)	0
Hot Flashes	1 (8)	0
Shingles	1 (8)	0
Redness at Injection Site	1 (8)	0
Hand-Foot Skin Reaction	1 (8)	0
Skin Hyperpigmentation	1 (8)	0
Allergic Rhinitis	1 (8)	0
Cardiac Palpitations	1 (8)	0
Hypertension	1 (8)	0
Fever	1 (8)	0
Dyspnea	1 (8)	0
Cough	1 (8)	0
Blurred Vision	1 (8)	0
Red Eyes	1 (8)	0
Elevated Creatinine	1 (8)	0
Hypoalbuminemia	1 (8)	1 (8)
Proteinuria	1 (8)	0

Table 3 Intrinsic Breast Cancer Subtype and Response

Study Phase	Subtype	Cycles Received	Response
II	Basal	1	PD
II	Luminal A	7	PR
II	Luminal B	6	SD
II	Basal	2	PD
II	Basal	2	PD
I	Basal	4	PD
I	Basal	2	PD

Abbreviations: PD = progressive disease; PR = partial response; SD = stable disease

Results

Patients

Thirteen patients were enrolled and received an average of 2.8 cycles per patient (range, 1-7 cycles). Of the 10 patients who received 2 or more cycles, 8 missed 1 or more doses of bortezomib for an average dose intensity of 83% in the first 2 cycles for these patients. Table 1 lists the patient characteristics. The median age was 51 years, with the range between 40-66 years of age. This population was heavily pretreated, with 31% having received 2 lines of chemotherapy in the metastatic setting. A significant percentage of tumors (39%) were human epidermal growth factor receptor 2 (HER2) overexpressing, and 23% of patients were "triple negative," or estrogen receptor (ER), progesterone receptor (PgR), and HER2 negative. All of the patients had invasive ductal carcinoma, and none had invasive lobular carcinoma.

Efficacy

Of the first 13 patients, 1 did not receive a complete cycle of therapy because of progression of an unrelated disease, and she was therefore considered unevaluable for response and was replaced. One of 12 evaluable patients had a PR (8%), and 3 of 12 had stable disease (SD; 25%). Of the patients with SD, 1 remained stable for 20 weeks, and 2 for 8 weeks. All others had progressive disease on treatment (66%). The 1 responding patient had hormone receptor positive, HER2 not overexpressed breast cancer, and had been treated previously with an anthracycline. She remained on study for 6 months, and was discontinued because of an inability to comply with frequent visits, and a desire to discontinue intravenous therapy.

At a follow-up of 26 months, 9 of the 13 patients on study had died, for a median OS of 4.3 months (95% CI, 1.2-26.2). The median TTP was 1.3 months (95% CI, 0.8-14). Figure 1 shows the Kaplan-Meier curves for OS and TTP.

Toxicity

No grade 4 adverse events (AEs) were seen. Of the grade 3 events thought to be possibly, probably, or likely related to therapy, thrombocytopenia and neutropenia were seen in 2 patients, and hypoalbuminemia, nausea, diarrhea, anemia, lymphopenia, and hand-foot syndrome were seen in 1 patient each. As shown in Table 2, the most frequent toxicities of any grade were nausea, neuropathy, vomiting, neutropenia, diarrhea, fatigue, rash, and thrombocytopenia, which were all seen in > 30% of patients. All 13 patients

and included all patients (intent to treat [ITT] cohort, n = 13). Analysis of toxicities was based on the ITT cohort using adverse events thought to be possibly, probably, or likely related to the therapy. The OS and TTP curves were computed by the Kaplan-Meier method. The hazard ratios for the corresponding 95% CIs were calculated by Cox proportional hazards methods. The statistical software package used was SAS version 9.2 (SAS Institute, Inc., Cary, NC).

had at least 1 AE; however, 5 of 13 (38%) had a grade 3 AE. No cardiac toxicity was observed.

Intrinsic Subtype Prediction

Archival tumor tissue from 5 patients in this study and 2 patients treated in the phase I trial were available to be subtyped (Table 3). Of the 7 typed, none of the basal breast cancers (typically, ER/PgR/HER2 negative) responded.

Discussion

The primary goal of this study was to evaluate the RR of the combination of bortezomib and PLD in metastatic breast cancer, with the secondary objectives being to estimate the TTP of disease with this treatment, and its tolerability. In our phase I study, RECIST measurable disease was not required, but 2 of 19 breast cancer patients (11%) had either a CR or PR, and 2 others showed clinical benefit.[26] With only 1 of the 12 patients responding in this phase II study, the trial was terminated according to the designed early-stopping rules, given the failure to reject the null hypothesis. This study suggests that the combination of PLD and bortezomib is no more efficacious than single-agent PLD or single-agent bortezomib in metastatic breast cancer.[12,13,30,31]

Other combination trials conducted concurrently have yielded mixed efficacy results. A phase I combination of bortezomib with weekly paclitaxel that enrolled 3 patients with metastatic breast cancer did not show evidence to suggest enhanced efficacy above what would be expected of single-agent paclitaxel.[30] Similarly, a phase I/II study of bortezomib with capecitabine in metastatic breast cancer showed a 15% overall RR.[31] However, a phase I/II study combining docetaxel with bortezomib in metastatic breast cancer was more promising, and revealed a RR of 38% at the maximum tolerated dose.[32]

In this phase II study, our population was heavily pretreated, and the majority had received a previous anthracycline. Furthermore, this was an unselected population that had a higher percentage of HER2 overexpression than the anticipated 20%.[33] These factors may have contributed to the lower RR, especially because of the activity of the p44/42 mitogen-activated protein kinase pathway, which is induced by HER2 signaling, has been shown to oppose proteasome inhibitor-mediated cell death.[17] The low-dose intensity may also have contributed to the poor RR, and also comments on the tolerability of this combination. Interestingly, of the subtyped tumors, none of the patients with basal breast cancers showed evidence of a response to this combination. Basal breast cancer is typically "triple negative," and the hormone receptor–negative subtypes traditionally have a worse prognosis.[34-36] Basal breast cancer may be associated with breast and ovarian cancer susceptibility (BRCA) 1 pathway dysfunction, and also dysregulation of intracellular proteolysis given the role of BRCA1 as an E3 ubiquitin ligase.[37,38] It is thus tempting to speculate that, because such tumors appear to already be tolerating some dysfunction of the ubiquitin-proteasome pathway, they may be less sensitive to other approaches targeting this protein turnover mechanism.

Unfortunately, our encouraging preclinical studies were not predictive of clinical activity for this drug regimen in this case. This may have been in part a result of the use of drug-naive breast cancer models in vitro, which were likely more intrinsically drug-sensitive. In contrast, our heavily pretreated population likely had breast cancer in which multiple drug resistance pathway were activated, reducing the likelihood that a 2-drug regimen would be active, especially in those patients who had previously received anthracyclines. Finally, proteasome inhibitors like bortezomib activate apoptosis through the c-Jun-N-terminal kinase (JNK), but they also induce MKP-1, which inactivates JNK and protects tumors from programmed cell death.[17,18,39-44] MKP-1 may also play a role in tumorigenesis, and its overexpression has shown to be associated with poor patient outcomes in a number of malignancies, including breast cancer.[45-47] Unfortunately, a specific, clinically-relevant inhibitor of MKP-1 is not available, and though anthracyclines suppress MKP-1 preclinically,[16] it is likely that the doses used in our study were not sufficient to suppress the promoter of this phosphatase. Combined with the known baseline overexpression of MKP-1 in many breast cancers,[48,49] this inability to suppress MKP-1 may also have contributed to the lack of activity of this regimen clinically. If this is the case, it may indicate that, once targeted MKP-1 inhibitors are available, future studies of these agents in combination with chemotherapeutics may be warranted.

Conclusion

The combination of PLD and bortezomib had minimal activity in heavily pretreated, unselected metastatic breast cancer, with a RR of 8%. The regimen is tolerable and the toxicities in this study are commensurate with those seen in our previous phase I study.[26] Further studies of PLD/bortezomib in this population are not warranted, but the ubiquitin-proteasome pathway remains an interesting target, and studies of patients earlier in their disease course, or with novel, irreversible proteasome inhibitors, may uncover a role for this class of drugs in our armamentarium against breast cancer.[50-52]

Disclosures

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