Phase I/II Study of G3139 (Bcl-2 Antisense Oligonucleotide) in Combination with Doxorubicin and Docetaxel in Breast Cancer

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Abstract

Purpose: Preclinical data showed enhancement of breast cancer cell death when G3139 was combined with anthracyclines and taxanes. We evaluated the efficacy and safety of a Bcl-2 antisense oligonucleotide, G3139, in combination with doxorubicin (A) and docetaxel (T) in patients with locally advanced breast cancer (LABC).

Experimental Design: Following a brief phase I to determine the phase II dose, patients with locally advanced breast cancer received G3139 administered by continuous i.v. infusion for 5 to 7 days with bolus A (50 mg/m²) and T (75 mg/m²) administered on either day 3 or 6 of therapy with G3139. Cycles were repeated every 21 days × 6 in the neoadjuvant setting. Serial plasma samples were obtained for pharmacokinetic analysis. Tissue samples were obtained before and after therapy for pharmacodynamic analysis of Bcl-2 expression.

Results: Thirty patients (median age, 49 years; range, 24-71 years) received 160 cycles. During the phase I portion of the trial, the dose of G3139 was escalated from 3 to 7 mg/kg/d (i.v. for 5 days) in combination with AT. During the phase II portion of the trial, several doses and schedules of G3139 were evaluated. There were no pathologic complete responses. Pharmacodynamic studies showed limited Bcl-2 down-regulation in the primary tumors.

Conclusions: G3139 in combination with doxorubicin and docetaxel is well tolerated. No pathologic complete response was seen and pharmacodynamic studies showed very little down-regulation of Bcl-2 in primary tumors, perhaps related to issues with insufficient drug delivery to the intact tumor.

Neoadjuvant chemotherapy is the standard front-line treatment for patients with locally advanced breast cancer (LABC) followed by locoregional therapy in the form of surgery, with or without radiation. In patients with LABC, anthracycline-based neoadjuvant chemotherapy produces pathologic complete response (pCR) rates of ~12% (1). Docetaxel in combination with either doxorubicin or epirubicin as neoadjuvant chemotherapy results in pCR rate of 5.6% to 21% (2–6).

Therapy with both anthracyclines and taxanes induces apoptosis in breast cancer cells (7, 8). The BCL2 family of proteins includes important regulators of apoptosis that have been implicated in chemotherapy resistance (9). Approximately 40% to 80% of invasive breast carcinomas express Bcl-2 protein that can be detected using immunohistochemical methods (10–13). The prognostic role of Bcl-2 overexpression in patients with breast cancer is controversial. Several retrospective studies showed that Bcl-2 expression is not an independent prognostic indicator (12, 14). A study of breast cancer patients with 30-year follow-up data showed a more favorable 5-year disease-free survival for patients with Bcl-2 overexpression but similar long-term survival for patients with or without Bcl-2 overexpression (13). Although Bcl-2 may be a weak prognostic indicator for breast cancer, studies have shown that Bcl-2 expression can be associated with chemotherapy resistance (12, 15, 16). Preclinical models have established that down-regulation of Bcl-2 protein leads to an increase in apoptosis and improved response to chemotherapy in vivo (17).

G3139 (oblimersen, Genasense; Genta) is an 18-base phosphorothioate oligonucleotide complementary (antisense) to the first six codons of Bcl-2 mRNA. Treatment with G3139 leads to selective decrease in concentrations of Bcl-2 mRNA and protein levels in breast cancer cell lines (17). The BCL2 family of proteins includes important regulators of apoptosis that have been implicated in chemotherapy resistance (18, 19).

Based on these data, we hypothesized that inactivation of Bcl-2 using G3139 would improve the efficacy of AT chemotherapy and improve the rate of pCR over that historically seen in patients with LABC treated with neoadjuvant AT chemotherapy. We herein report the results of a
Translational Relevance

One of the main limitations of gene therapy for solid tumors is suboptimal drug delivery. In this study, we treated patients with inoperable locally advanced breast cancer with a Bcl-2 antisense oligonucleotide (G3139) in combination with neoadjuvant doxorubicin and docetaxel (AT). Our hypothesis was that down-regulation of Bcl-2 would improve the efficacy of chemotherapy in locally advanced breast cancer. We obtained serial tumor biopsies to determine whether Bcl-2 was down-regulated in the primary tumors after single-agent Bcl-2 antisense therapy. The AT/G3139 combination did not improve pathologic complete response compared with what has been shown with AT alone. Our translational studies showed minor changes in Bcl-2 inhibition in paired tissue samples, suggesting that the lack of response may be due to poor drug delivery or failure of the drug to affect the target. No prior studies have assessed the intratumoral effect of Bcl-2 antisense in human breast cancer.

Materials and Methods

Study design

This study was a prospective, open-label, phase I/II study conducted at The University of Texas M. D. Anderson Cancer Center between May 2003 and April 2005. For the phase I component of the trial, a conventional 3+3 study design was used with fixed doses of doxorubicin (A = 50 mg/m² i.v. every 21 d) and docetaxel (T = 75 mg/m² i.v. every 21 d) and three escalating dosing cohorts of G3139 (Table 1). No further dose escalation was initially planned after the 7 mg/kg/d dose of G3139 because this dose was known to be sufficient to down-regulate Bcl-2 protein in peripheral blood mononuclear cells (PBMC) based on previous single-agent studies in humans (20). No intrapatient dose escalation was allowed. All patients enrolled onto a given dose level were observed for dose-limiting toxicity for a minimum of 21 d before initiation of accrual to higher dosing cohorts. Dose-limiting toxicity was defined as any grade 3 or 4 nonhematologic toxicity (excluding nausea/vomiting or bone pain that was responsive to symptomatic management), any grade 2 toxicity (excluding alopecia) that did not resolve by day 22 of therapy, grade 4 neutropenia lasting >8 d, grade 3 or 4 neutropenia on day 21, grade 4 neutropenia associated with infection, grade 4 thrombocytopenia, and grade 3 thrombocytopenia associated with significant blood loss.

The phase II component of the trial was designed to determine if the addition of G3139 to neoadjuvant AT would improve pCR in previously untreated patients with LABC (stage IIIA-C). Patients with LABC treated at the recommended phase II dose cohort in the phase I portion of the trial were considered evaluable for efficacy in the phase II portion of the trial.

After obtaining baseline tumor measurements, patients were evaluated for clinical response after completion of three and six cycles of neoadjuvant chemotherapy. Response was evaluated using physical exam, mammogram, and ultrasound of the breast. After three cycles of therapy, patients with stable or responding tumors received three additional cycles of therapy and then underwent surgical resection. Surgery was done 4 to 6 wk after completion of the last cycle of chemotherapy. Surgical resection was followed by radiation and/or adjuvant systemic therapy as clinically indicated. Patients with progressive disease after three cycles discontinued protocol therapy and underwent additional treatment as clinically indicated. When initial efficacy analyses showed a lower incidence of pCR than would be expected with AT alone, the phase II portion of the study was modified to explore additional dosing schedules of G3139 as shown in Table 1.

Thirty-one patients signed informed consent for protocol participation and 1 patient withdrew consent before therapy administration. As such, the analysis reported represents data collected through July 2007 for 30 patients (phase I = 9; phase II = 21). The protocol was reviewed by the institutional review board and all patients provided written informed consent.

Patient selection

Phase I component. Patients were required to have histologically or cytologically confirmed invasive carcinoma of the breast and to have either stage III or IV disease; a Karnofsky performance score of at least 60; normal organ function; no prior therapy with G3139, taxane, or anthracycline; and normal cardiac function as measured by echocardiogram or multigated acquisition before administration of protocol

Table 1. Dose escalation cohorts studied

<table>
<thead>
<tr>
<th>Dose level</th>
<th>G3139</th>
<th>Doxorubicin/docetaxel (AT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3 mg/kg/d continuous i.v. infusion for 5 d</td>
<td>Doxorubicin 50 mg/m² i.v. over 15 min*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Docetaxel 75 mg/m² i.v. over 60 min</td>
</tr>
<tr>
<td>2</td>
<td>5 mg/kg/d continuous i.v. infusion for 5 d</td>
<td>Docetaxel 75 mg/m² i.v. over 60 min</td>
</tr>
<tr>
<td>3</td>
<td>7 mg/kg/d continuous i.v. infusion for 5 d</td>
<td>Docetaxel 75 mg/m² i.v. over 15 min*</td>
</tr>
<tr>
<td>Phase II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7 mg/kg/d continuous i.v. infusion for 5 d</td>
<td>Docetaxel 75 mg/m² i.v. over 60 min</td>
</tr>
<tr>
<td>4</td>
<td>7 mg/kg/d continuous i.v. infusion for 7 d</td>
<td>Docetaxel 75 mg/m² i.v. over 60 min</td>
</tr>
<tr>
<td>5</td>
<td>9 mg/kg/d continuous i.v. infusion for 7 d</td>
<td>Docetaxel 75 mg/m² i.v. over 60 min</td>
</tr>
</tbody>
</table>

*Chemotherapy given on day 3.
†Chemotherapy given on day 6.
therapy. Patients could have received up to three prior chemotherapy regimens for breast cancer (excluding anthracyclines and taxanes), either as adjuvant/neoadjuvant therapy or as therapy for metastatic disease.

**Phase II component.** Patients were required to have newly diagnosed, noninflammatory LABC (stage IIA-C) with cytologic or histologic confirmation of invasive disease. Patients were required to have a palpable breast mass, a Karnofsky performance score of at least 60, adequate organ and cardiac function, and no evidence of distant metastasis.

**Treatment schedule**

G3139. G3139 was provided free of charge to all patients by the National Cancer Institute, Cancer Therapy Evaluation Program. G3139 was administered through a central line as a continuous i.v. infusion using an ambulatory infusion pump for 5 to 7 d beginning on day 1 of each cycle. Actual weight was used to determine the dose of G3139, regardless of obesity or cachexia. Dosing cohorts for the phase I portion of the trial are listed in Table 1. For the phase II portion of the trial, the initial dose of G3139 was 7 mg/kg/d given by continuous infusion over 5 d. On day 3, the infusion was interrupted and chemotherapy was administered. When initial efficacy analyses showed a lower incidence of pCR than would be expected with AT alone, the phase II portion of the study was modified to explore additional dosing schedules of G3139 as shown in Table 1.

**Chemotherapy.** Patients received premedication (dexamethasone, 8 mg orally twice daily for a total of six doses, starting 24 h before chemotherapy administration) to prevent acute anaphylactic reaction and reduce fluid retention associated with the administration of docetaxel. Antiemetics were recommended as prophylaxis to reduce the risk of nausea and vomiting. AT chemotherapy was administered according to the following schedule: 50 mg/m² doxorubicin as a 15-min i.v. infusion followed immediately by a 1-h i.v. infusion of 75 mg/m² docetaxel. Treatment cycles were repeated every 21 d.

After a patient in the first dosing cohort developed febrile neutropenia, 6 mg prophylactic pegfilgrastim (Neulasta, Amgen) was given s.c. 24 h after administration of AT.

**Measurement of response and toxicity**

Response was evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST; ref. 21). In the phase I portion of the trial, measurable metastatic lesions were defined as those that could be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm with conventional techniques (computed tomography, magnetic resonance imaging, and X-ray) or as >10 mm with spiral computed tomography scan. Mammography and ultrasound were used (in addition to physical examination) to measure the target lesions involving the affected breast and lymph node basin. pCR was defined as no evidence of residual invasive tumor in the breast and axillary lymph nodes at the time of definitive surgery. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria (version 3.0).3 Patients enrolled in the phase I portion of the trial received cardiac assessment by echocardiogram or multigated acquisition after every two cycles of therapy. Because no cardiac toxicity was identified in this group of patients, those receiving therapy in the phase II portion of the trial underwent cardiac assessment after completion of six cycles of therapy just before surgical resection.

**Statistical considerations for phase II portion of the trial**

The outcome of primary interest on phase II was the achievement of pCR in both the primary tumor and lymph nodes. If a patient failed to undergo surgery for any reason, that patient was counted as failure with regard to the primary end point. Patients with LABC treated at the recommended dose on the phase I trial were evaluated for efficacy and included in the phase II portion of the trial. Under an assumption of 30% pCR rate with the G3139/AT combination, a sample size of 60 patients would provide a 90% credible interval of width 0.19 for estimating pCR rate. Study results were monitored beginning after pathologic findings were known for the first 12 patients, with a recommendation of trial termination if there was <10% probability of pCR results at least equal to those obtained on the previous doxorubicin plus docetaxel trial at M. D. Anderson (14% pCR in 88 patients).4 Following these rules for termination, simulation results (based on 10,000 replications) indicated the expected sample size to be 12, 60, 60, 60, and 60 patients if the true pCR rate was 5%, 10%, 15%, 20%, and 30%, respectively. Probabilities of early termination were 0.84, 0.44, 0.19, 0.08, and 0.02, respectively.

Safety analysis was conducted primarily on the intent-to-treat population (all patients who received at least one dose of therapy). Safety variables included incidence of overall treatment-emergent adverse events, laboratory tests (clinical chemistry, hematology, and urinalysis), vital signs, physical exams, and patient reported symptoms.

**Correlative studies**

**Pharmacokinetic analysis.** Plasma samples (10 mL) were collected before the start of the doxorubicin infusion and 0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 h after the start of the doxorubicin infusion on day 3. The samples were split, and 5 mL of blood were used for doxorubicin analysis and 5 mL for docetaxel analysis. The results were subjected to multiple linear regression analysis and described by an open two-compartment model as previously published (22, 23).

**Tissue processing.** During cycle 1 of therapy, patients with LABC were asked to undergo serial fine-needle aspirations (FNA) before administration of G3139 and 2 to 5 d after initiation of G3139. The second FNA was obtained before initiation of AT chemotherapy. The cells obtained were divided onto seven glass slides using a spreader slide. The spreader slide was stained with Diff-Quik (Allegiance) for an immediate microscopic interpretation of the specimen adequacy. The first glass slide was immediately fixed in 95% ethanol and then stained.

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3 http://ctep.info.nih.gov

4 Unpublished data.
with H&E. Fresh tissue samples were divided and either stored in RNA later (Ambion) or snap frozen for protein analysis.

**Quantification of Bcl-2 protein in primary tumor.** Snap-frozen breast cancer tissue specimens were homogenized in PBS and centrifuged at 12,000 × g for 20 min at 4°C. The protein concentration of the supernatant was determined by the bicinchoninic acid method (Pierce Chemical). Bcl-2 expression was measured using an ELISA kit (BioSource International) according to the manufacturer’s instructions. A standard curve was generated to calculate the concentration of Bcl-2 on each sample.

**Quantification of Bcl-2 mRNA in primary tumor.** Total RNA was extracted from thawed samples using a modified protocol of the RNeasy Mini kit (Qiagen, Inc.) as previously described. RNA yield and quality was assessed by loading 1.0 μL from each sample into a NanoDrop ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Inc.).

Expression levels of β-actin and Bcl-2 were measured by reverse transcription-PCR (RT-PCR) as previously published (24). The following primer sequences were used for these reactions: β-actin, 5’-CCGGGAATTCGCTGCAGATC-3’ (forward) and 5’-GATGGAGGTGAAGTAGTGCTG-3’ (reverse); Bcl-2, 5’-TCCAGTTATGTTGACAACACC-3’ (forward) and 5’-CTCCACGTCATCTCCATCT-3’ (reverse; Sigma Genosys). To allow more accurate comparison, RNA was diluted so that each matched pair of samples was analyzed using equivalent concentrations. The RT-PCR data were analyzed using Statistical Package for the Social Sciences statistical software (SPSS, Inc.) and expressed as mean ± SE. Differences between means were evaluated by independent t tests, with statistical significance assigned at P < 0.05.

**Results**

**Patient characteristics.** Thirty-one women were enrolled. One patient withdrew informed consent before therapy administration and was excluded from analysis. The patient characteristics are summarized in Table 2. The median age was 49 years. Most patients (93%) enrolled carried a diagnosis of LABC. Two patients with stage IV disease were treated in the phase I portion of the trial. The majority of the patients were premenopausal (n = 18, 60%) and/or had clinical stage IIIC disease (n = 16, 53%). No patients had received prior therapy for breast cancer.

**Dose administration.** Seventeen patients (57%) received six cycles of therapy without dose reduction. Eight patients (27%) received six cycles of therapy but required a 20% dose reduction in either doxorubicin, docetaxel, or both for toxicity, which included nonneutropenic fever (n = 1), neutropenic fever (n = 2), elevated transaminases (n = 1), dehydration (n = 1), neuropathy (n = 2), and diarrhea (n = 1). Three patients (10%) received three cycles of therapy before discontinuation for repeated neutropenic fever (n = 1), death (n = 1), and lack of response (n = 1). Two patients (7%) received only one cycle of therapy before withdrawing consent for study participation.

**Toxicity.** Ten patients were registered on the phase I component of the study. One patient withdrew consent before therapy administration and was excluded from analysis. The patient characteristics are summarized in Table 2. The median age was 49 years. Most patients (93%) enrolled carried a diagnosis of LABC. Two patients with stage IV disease were treated in the phase I portion of the trial. The majority of the patients were premenopausal (n = 18, 60%) and/or had clinical stage IIIC disease (n = 16, 53%). No patients had received prior therapy for breast cancer.

**Table 3. Overall drug-related nonhematologic adverse events occurring in ≥5% patients treated**

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade 1</th>
<th></th>
<th>Grade 2</th>
<th></th>
<th>Grade 3</th>
<th></th>
<th>Grade 4</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No. patients</td>
<td>%</td>
<td>No. patients</td>
<td>%</td>
<td>No. patients</td>
<td>%</td>
<td>No. patients</td>
<td>%</td>
</tr>
<tr>
<td>Constipation</td>
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<tr>
<td>Diarrhea</td>
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<td>30</td>
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<td>0</td>
<td>1</td>
<td>3</td>
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<td>0</td>
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<tr>
<td>Drug fever</td>
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<td>20</td>
<td>5</td>
<td>17</td>
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<tr>
<td>Fatigue</td>
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<td>7</td>
<td>7</td>
<td>23</td>
<td>21</td>
<td>70</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Heartburn/dyspepsia</td>
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<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Hot flashes</td>
<td>6</td>
<td>20</td>
<td>2</td>
<td>7</td>
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<td>Infection, nonneutropenia</td>
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<td>0</td>
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<td>7</td>
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<td>Myalgia</td>
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<td>53</td>
<td>3</td>
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<td>Nausea</td>
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<td>13</td>
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<td>3</td>
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<td>13</td>
<td>2</td>
<td>7</td>
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<td>3</td>
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<tr>
<td>Vomiting</td>
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<td>4</td>
<td>13</td>
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<tr>
<td>Alanine aminotransferase</td>
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<td>27</td>
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<td>0</td>
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</table>

**Table 4. Overall drug-related hematologic adverse events occurring in ≥5% patients treated**

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade 1</th>
<th></th>
<th>Grade 2</th>
<th></th>
<th>Grade 3</th>
<th></th>
<th>Grade 4</th>
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<tbody>
<tr>
<td></td>
<td>No. patients</td>
<td>%</td>
<td>No. patients</td>
<td>%</td>
<td>No. patients</td>
<td>%</td>
<td>No. patients</td>
<td>%</td>
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<tr>
<td>Neutropenia</td>
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<td>3</td>
<td>2</td>
<td>7</td>
<td>7</td>
<td>23</td>
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<td>23</td>
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<td>Thrombocytopenia</td>
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<td>0</td>
<td>4</td>
<td>13</td>
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<tr>
<td>Infection or fever with neutropenia</td>
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<td>0</td>
<td>3</td>
<td>10</td>
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</table>
and severe pulmonary edema. In the opinion of the examining serositis of the small intestine, cardiomegaly, hepatomegaly, the 9 mg for 7 days dosing cohort. Autopsy revealed severe home 14 days after receiving her third cycle of chemotherapy in receiving protocol therapy. The patient was found deceased at alone. One death occurred in a 50-year-old patient actively Tables 3 and 4 Toxicity was similar to that expected with AT the phase I and phase II portions of the trial are as listed in 50/75, day 3) with growth factor support. Toxicity data for both escalation of G3139 was continued. No dose-limiting toxicities were observed on dose levels 2 and 3. No cardiac toxicity was observed during the phase I portion of the trial.

The recommended phase II dose was G3139 at 7 mg/kg/d × 5 days in combination with doxorubicin and docetaxel (AT 50/75, day 3) with growth factor support. Toxicity data for both the phase I and phase II portions of the trial are as listed in Tables 3 and 4 Toxicity was similar to that expected with AT alone. One death occurred in a 50-year-old patient actively receiving protocol therapy. The patient was found deceased at home 14 days after receiving her third cycle of chemotherapy in the 9 mg for 7 days dosing cohort. Autopsy revealed severe acute diverticulitis with multifocal rupture and patchy acute serositis of the small intestine, cardiomegaly, hepatomegaly, and severe pulmonary edema. In the opinion of the examining pathologist, the cause of death was most likely acute heart failure secondary to cardiomegaly and hypertension. This condition was complicated by severe acute diverticulitis, which may have been exacerbated by the immunosuppressive effects of chemotherapy; however, the role of chemotherapy in the patient’s death was not defined at the time of autopsy.

**Efficacy.** In the phase I portion of the study, all patients with LABC developed a partial response to therapy. One of the two patients with metastatic breast cancer also had a partial response to therapy as measured by RECIST. The second patient with stage IV breast cancer discontinued therapy after one cycle due to complications of fatigue, vomiting, and catheter-related infection.

The response rates for the phase II portion of the trial are shown in Table 5. Although no patients developed a pCR in the breast and axillary lymph nodes, one patient with a 5.7-cm primary tumor had no residual tumor in the breast, at the time of surgical resection. Most patients treated had a confirmed partial response.

**Pharmacokinetics.** Doxorubicin and docetaxel pharmacokinetic variables (mean ± SD) are available for three patients treated with G3139 at 3 mg/kg/d for 5 days and three patients treated with G3139 at 5 mg/kg/d for 5 days. All six patients received doxorubicin and docetaxel on day 3 (doxorubicin plasma clearance, 52.2 ± 26.4 L/h/m²; Vss,1 4.91 ± 740 L/m²; T1/2a, 37.4 ± 18.8 hours; docetaxel plasma clearance, 35.8 ± 11.4 L/h/ m²; Vss 378 ± 240 L/m²; T1/2a, 11.7 ± 8.2 hours). These pharmacologic values are within the range previously reported for each agent administered alone. These data suggest that the combination of G3139, doxorubicin, and docetaxel does not alter the pharmacologic profile of either doxorubicin or docetaxel.

**Pharmacodynamics.** The first 12 patients on the phase II component of the trial were treated with G3139 given as 7 mg/kg/d infusion for 5 days, and AT chemotherapy (50/75) on day 3. Breast cancer tissue was obtained by FNA from five of these patients before initiation of the G3139 infusion and on day 6. The response to therapy as measured by RECIST. The second patient with stage IV breast cancer developed a partial response to therapy. One of the two patients with metastatic breast cancer also had a partial response to therapy as measured by RECIST. The second patient with stage IV breast cancer discontinued therapy after one cycle due to complications of fatigue, vomiting, and catheter-related infection.
the three patients, the Bcl-2 down-regulation was also noted at the protein level using ELISA.

### Discussion

This study has shown that G3139 can be safely administered in doses up to 9 mg/kg/d for 7 days in combination with standard doses of doxorubicin and docetaxel (AT). Toxicity was similar to that previously reported with neoadjuvant AT with the exception of a higher rate of grade 3 fatigue (70% versus 28%; ref. 2). Although fatigue was commonly reported as a treatment-related adverse event in single-agent phase I trials of G3139, it was usually low grade (25, 26). Additionally, phase I or II trials of G3139 in combination with chemotherapy also did not show such high rates of severe fatigue, including one trial combining G3139 with docetaxel (27–31). This unexpected rate of toxicity cannot be explained by changes in chemotherapy clearance, as pharmacokinetic studies showed no significant change in the expected metabolism of doxorubicin or docetaxel (22, 23). Despite the high level of fatigue, most patients (57%) completed six cycles of protocol therapy with no dose reductions. No cardiac toxicity was seen in the patients who underwent serial evaluation by echocardiogram or multigated acquisition; however, one patient died unexpectedly while receiving protocol therapy and autopsy revealed cardiomegaly and pulmonary edema. Although the examiner could not directly relate the patient’s death to complications of chemotherapy, it is certainly feasible that the patient’s cardiopulmonary findings were the result of therapy-related cardiac toxicity.

Of the nine patients treated in the phase I portion of the trial, eight patients had confirmed partial responses as measured by RECIST, including one of two patients with metastatic breast cancer. In the phase II portion of the trial, confirmed radiographic responses were observed in 13 of the 21 patients treated (62%). One patient treated in the 7 mg/kg/d for 7 days dosing cohort had a pathologically confirmed complete response within the breast but had residual disease within the lymph nodes at the time of surgery. No patients achieved a complete pathologic response within the breast and draining axillary lymph nodes (pCR) as defined by the primary end point within the efficacy analysis of the protocol. Although these data support that there was no additional efficacy with the combination, the lack of pCR in the patients treated suggests that the combination therapy may, in fact, be worse than chemotherapy alone. This worsening of clinical outcome has been shown in other solid tumor types, such as small cell lung cancer, where a randomized phase II trial revealed a statistically significant worse overall survival for patients receiving G3139 in combination with carboplatin and etoposide (32).

Pharmacodynamic analysis of Bcl-2 expression in paired breast cancer samples obtained by FNA before treatment and after 3 days of G3139 (before administration of chemotherapy) showed no alteration in target gene expression with the 7 mg/kg/d dosing schedule. Based on these results and lack of clinical efficacy seen during administration of combination therapy to the first 12 patients enrolled on the phase II portion of the trial, additional samples were obtained from patients treated at a higher dose of G3139 (9 mg/kg/d) for 7-day duration. All of the patients sampled in the higher dosing cohort (n = 3) had inhibition of Bcl-2 expression by RT-PCR (range, 4.28% inhibition; Table 6). Despite this shown inhibition of Bcl-2, no patients treated in this dosing cohort experienced pCR.

The lack of efficacy shown by this study is likely multifactorial. In paired pretreatment and posttreatment tumor biopsies, treatment with G3139 did not significantly alter Bcl-2 expression in the majority of breast tumors sampled. Significant inhibition (>15%) was shown in two of three patients treated in the 9 mg/kg over 7 days dosing cohort, but this was not associated with improved response. To our knowledge, this is the first clinical trial to show the effects of G3139 on Bcl-2 (using both mRNA and protein expression) in breast cancer. One additional study in patients with non–Hodgkin’s lymphoma also showed similar rates of decreased Bcl-2 expression in serial lymph node biopsies. In that study, 2 of 11 paired samples of lymph node biopsies showed significant (>15%) decrease in Bcl-2 expression (25).

In contrast, a second study of G3139 given in combination with dacarbazine in patients with metastatic melanoma showed a much higher rate of Bcl-2 inhibition with 10 of 12 patients showing inhibition of Bcl-2 >15% (range, 40-70% inhibition; ref. 33). This study also showed response rates of 21% (one complete response and two partial responses in 14 patients treated).

Clinical trials measuring change in expression of Bcl-2 in PBMCs have shown a much higher frequency (50-100%) of significant Bcl-2 inhibition than that seen with serial tumor biopsies (25, 28, 29). The reasons for the disparity between PBMCs and tumor tissue are not completely known and could be related to insufficient drug penetration into tumor tissue or differences in drug effect between PBMCs and cells within an intact tumor. Previously published reports of drug efficacy in breast cancer cell lines would suggest drug exposure rather than differing cellular response as the cause of discrepancy (34). If this is the case, caution should be used as PBMCs may not be appropriate surrogate markers for drug activity in all tumor types. Differences in tumor vascularity and subsequent drug penetration are also plausible explanations for the differences.

### Table 6. Bcl-2 change after G3139 treatment 9 mg/kg/d × 7 d

<table>
<thead>
<tr>
<th>Acc. no.</th>
<th>FNA date</th>
<th>Bcl-2/actin mRNA (RT-PCR)</th>
<th>Change after G3139</th>
<th>Bcl-2 protein (ELISA)</th>
<th>Change after G3139</th>
<th>Response by imaging</th>
<th>Path response</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>1/19/05 (Pre)</td>
<td>44.68</td>
<td>-4.19%</td>
<td>0.327</td>
<td>-12%</td>
<td>N/A</td>
<td>N/A (early death)</td>
</tr>
<tr>
<td>27</td>
<td>1/31/05 (Post)</td>
<td>42.81</td>
<td>-19.15%</td>
<td>0.29</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>28</td>
<td>2/2/05 (Pre)</td>
<td>33.98</td>
<td>-27.5%</td>
<td>0.22</td>
<td>-28%</td>
<td>PR</td>
<td>Residual disease</td>
</tr>
<tr>
<td>29</td>
<td>3/3/05 (Pre)</td>
<td>36.76</td>
<td>-</td>
<td>0.38</td>
<td>N/A</td>
<td>MR</td>
<td>Residual disease</td>
</tr>
</tbody>
</table>
in drug effect seen in our study versus that reported by Jansen et al. (33) in patients with malignant melanoma. Additionally, although others have suggested that >15% inhibition of Bcl-2 in tumor tissue is significant, the amount of Bcl-2 inhibition necessary to induce apoptosis may vary based on cancer cell type. Thus, it is certainly possible that much higher levels of inhibition (>40%), such as that reported by Jansen et al., are necessary to induce apoptosis in solid tumors.

It is difficult to explain the possibility of worsening outcome from the combination therapy. It is unlikely, based on the pharmacokinetic analysis, that chemotherapy clearance was affected by G3139; however, the possibility of nontarget effects of the investigational agent as a cause for worsening outcome cannot be excluded.

Based on the data presented, we conclude that the combination of G3139 with doxorubicin and docetaxel is feasible but has limited efficacy in patients with LABC, most likely due to the inability of G3139 to induce a biologically meaningful down-regulation of Bcl-2 levels in primary breast tumors using the doses, route, and schedules included in this study. The exact cause of inactivity remains undefined and future studies with this compound in solid tumors should be designed to determine actual drug concentrations in tumor tissue in addition to the biological effects of drug administration. Bcl-2 remains an interesting target for breast cancer therapy and further exploration into agents that inhibit Bcl-2 through differing mechanisms of action and/or improved tumor penetration is warranted. Recently, single-agent administration of G3139 over 2 hours at a total dose up to 900 mg has been safely achieved with peak plasma concentrations 10-fold higher than that observed with 7 to 9 mg/kg/d continuous infusion (35). This is an important clinical observation, as higher peak plasma concentrations were associated with greater intratumoral concentrations of G3139 in vitro studies (36). Thus, future studies exploring alternative dosing schedules of G3139 in combination with chemotherapy are feasible for the treatment of patients with advanced breast cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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