

Inflammatory Breast Cancer as a Model Disease to Study Tumor Angiogenesis: Results of a Phase IB Trial of Combination SU5416 and Doxorubicin

Beth Overmoyer,^{1,2} Pingfu Fu,^{2,4} Charles Hoppel,^{2,5,6} Tomas Radivoyevitch,^{2,3} Robert Shenk,⁷ Marjie Persons,⁷ Paula Silverman,^{2,5} Kelly Robertson,² Nicholas P. Ziats,⁸ Jay K. Wasman,⁸ Fadi W. Abdul-Karim,⁸ John A. Jesberger,^{2,9} Jeffrey Duerk,^{2,9} Paul Hartman,³ Shelli Hanks,¹⁰ Jonathan Lewin,¹¹ Afshin Dowlati,^{2,5} Keith McCrae,^{2,5} Percy Ivy,¹² and Scot C. Remick^{2,5}

Abstract Purpose: We used inflammatory breast cancer (IBC) as a model disease to investigate biological changes associated with an antiangiogenesis agent, SU5416, combined with doxorubicin. **Experimental Design:** Patients with stage IIIB or IV IBC were treated neoadjuvantly with the combination of SU5416 and doxorubicin for induction therapy. The dose of SU5416 (administered on days 1 and 4, every 3 weeks) and doxorubicin (administered on day 1 every 3 weeks) were escalated in cohorts of three patients starting at 110 and 60 mg/m², respectively, for a total of five cycles leading up to mastectomy. Patients underwent serial assessment (pharmacokinetic sampling, biopsy of breast, tumor blood flow dynamic contrast-enhanced magnetic resonance imaging, plasma angiogenesis, and endothelial cell damage markers) prior to treatment, at the end of cycles no. 2 and no. 5, and after mastectomy. **Results:** Eighteen patients were enrolled; neutropenia was dose-limiting, and overall median survival was not reached (50 months of study follow-up). Four patients (22%) experienced congestive heart failure, which resolved and were likely attributable to a smaller volume of distribution and higher C_{max} of doxorubicin in combination with SU5416. We did observe a significant decline in tumor blood flow using Kep calculated by Brix (pretreatment versus post-cycle no. 5; *P* = 0.033), trend for a decline in tumor microvessel density after treatment, and low baseline levels of soluble intracellular adhesion molecule were associated with improved event-free survival. **Conclusions:** This study showed evidence of an unfavorable cardiac interaction between SU5416 and doxorubicin, which prohibits further investigation of this combination. However, this study supports the importance of using IBC as a model for investigating angiogenesis inhibitors.

Authors' Affiliations: ¹Connecticut Oncology & Hematology Associates/US Oncology, Torrington, Connecticut; ²Developmental Therapeutics Program and ³Translational Research Core Facility, Case Comprehensive Cancer Center, Departments of ⁴Biostatistics and Epidemiology, ⁵Medicine, ⁶Pharmacology, ⁷Surgery, ⁸Pathology, and ⁹Radiology, Case Imaging Research Center, University Hospitals Case Medical Center, Cleveland, Ohio; ¹⁰Department of Radiation Oncology, University of Arizona, Tucson, Arizona; ¹¹The Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins School of Medicine and The Johns Hopkins Hospital, Baltimore, Maryland; and ¹²Investigational Drug Branch, Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, Maryland

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Requests for reprints: Beth Overmoyer, Connecticut Oncology & Hematology Associates, 200 Kennedy Drive, Torrington, CT 06790. E-mail: beth.overmoyer@usoncology.com.

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The introduction of angiogenesis inhibitors into clinical oncology has resulted in considerable supposition concerning the effects of these targeted therapies on the tumor *in vivo*. Although the proliferation of all cancer requires an "angiogenic switch" wherein the ratio of proangiogenic to antiangiogenic factors is increased, some human tumors may present a more favorable model to examine the effects of therapeutic antiangiogenic agents (1–3).

Inflammatory cancer of the breast (IBC) is a subtype of breast cancer that occurs in ~1% to 5% of all breast cancers in the U.S. and is characterized by the rapid onset of characteristic pathognomonic features comprised of breast erythema, skin induration, and tumor emboli involving dermal lymphatics (4, 5). This diagnosis is associated with a poor prognosis, even with recent innovations in multimodality therapy including primary systemic chemotherapy, followed by surgery and radiation treatment. Surveillance, Epidemiology, and End Results data between 1988 and 2000 showed a median overall survival of 2.9 years, whereas recent long-term follow-up from

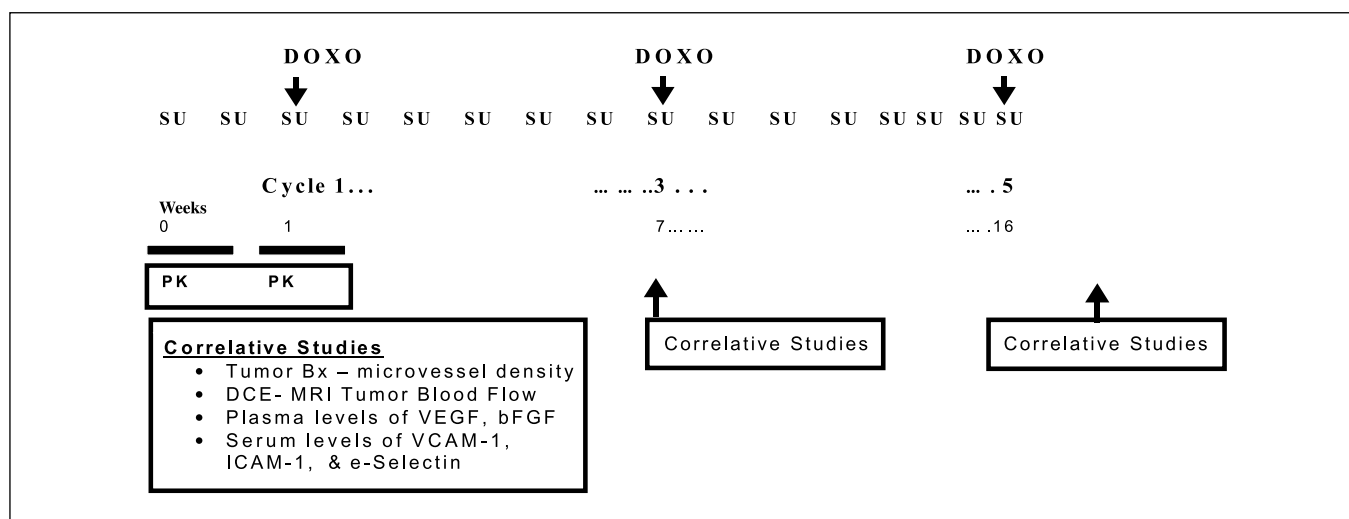


Fig. 1. Treatment scheme: SU5416 (SU) was given twice weekly beginning 1 wk prior to doxorubicin. Doxorubicin (DOXO) was given once every 21 d for a total of five cycles. Patients deemed surgically resectable underwent modified radical mastectomy after five cycles of treatment. Radiation therapy to chest and regional lymph nodes occurred after modified radical mastectomy. Hormone-responsive patients received tamoxifen after modified radical mastectomy.

the National Cancer Institute found patients with IBC to have a median overall survival of 3.8 years (6, 7).

Given the unique locally invasive properties and substantial potential for systemic metastasis associated with the natural history of this disease, it was hypothesized that the evaluation of the vascular component of IBC is likely to yield useful information that can subsequently be applied to the development of novel treatment strategies. For this reason, we used IBC as a model disease to investigate the biological changes associated with angiogenesis inhibitors. Unfortunately, treatment with single-agent angiogenesis inhibitors results in minimal disease response, therefore, these biological agents must be combined with conventional systemic treatment. This study explores the toxicity and efficacy of the combination of conventional chemotherapy for IBC, doxorubicin, with a novel antiangiogenesis agent, SU5416.

Vascular endothelial growth factor (VEGF) receptor signaling results in a cascade of cellular events stimulating vascular and lymphatic endothelial cell growth and survival (8). Specifically, the cytokine VEGF-A is overexpressed in invasive cancer, which is associated with microvasculature that overexpresses the two VEGF receptors, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR). In this way, the interaction of VEGF with its cellular receptors results in the proliferation of stromal and vascular endothelial cells resulting in a potent angiogenic environment (9, 10). Conversely, the inhibition of VEGF and/or VEGF receptor activity results in endothelial cell apoptosis and consequent reduction of vascularity (11). SU5416 (NSC 696819) is a small organic molecule that inhibits VEGF-mediated signaling through the Flk-1/KDR (VEGFR-2) tyrosine kinase receptor, which interferes with endothelial cell proliferation and neovascularization required for the propagation of cancer (12). This phase IB translational study was also designed to examine *in vivo* the biological effects on surrogate markers of angiogenesis when SU5416 is combined with doxorubicin in the primary treatment of IBC.

Patients and Methods

Patient eligibility. Patients with primary or secondary IBC, made by clinical or pathologic criteria, were eligible following histologic

confirmation. Patients could present with either stage IIIB or IV disease, had received less than three prior chemotherapies, or prior radiation; however, prior anthracyclines were not permitted. Measurable disease was not required. Adequate bone marrow, liver and renal function, and normal coagulation profile were required as previously reported (13, 14). Additional eligibility included Eastern Cooperative Oncology Group performance status <3 , ≥ 18 years of age, and use of acceptable and effective method of contraception if of reproductive potential. No previous or concurrent malignancy was allowed. The calculated left ventricular ejection function (LVEF) must have been $\geq 50\%$. Patients with brain metastasis, uncontrolled illnesses, and New York Heart Association classification III or IV heart disease were ineligible. All patients signed informed consent prior to undergoing any investigational procedure(s).

Treatment plan. Prior to initiating combined therapy, SU5416 alone was administered i.v. via central venous access, over 1 h on days 1 and 4 for 1 week. The combined regimen then began with doxorubicin administered i.v. over 5 to 10 min on day 1 of each 21-day cycle for a total of five cycles (cumulative dose of doxorubicin equaling 300-375 mg/m²). SU5416 was given immediately following the administration of doxorubicin, and continued to be administered twice weekly as described, throughout each treatment cycle for a total of 15 weeks (Fig. 1). Cytokine support was not permitted. End of study is defined as the completion of administration of SU5416 and doxorubicin prior to mastectomy.

Actual body weight was used when calculating dosages. The dose of doxorubicin and SU5416 were escalated as follows: level 1 (nine patients), doxorubicin 60 mg/m², SU5416 110 mg/m²; level 2 (three patients), doxorubicin 75 mg/m², SU5416 110 mg/m²; level 1a (six patients), doxorubicin 60 mg/m², SU5416 145 mg/m². Dose levels accrued in patient cohorts of three per standard dose-escalation procedure. Dose-limiting toxicity was defined as any grade 3 or 4 nonhematologic or grade 4 hematologic event(s). SU5416 is administered in cremaphor, therefore, dexamethasone premedication was prescribed 12 and 6 h prior to SU5416. The starting dose of dexamethasone was 10 mg orally, however, this was tapered to a maximum of 2 mg if tolerated. Both H1- and H2-blockers were also given prior to SU5416 infusion. All patients had indwelling central line catheters that required concurrent low-dose (2 mg) warfarin administration.

Pharmacokinetics of SU5416 and doxorubicin. SU5416 (NSC 696819) was supplied by the Cancer Therapy Evaluation Program/National Cancer Institute. Baseline pharmacokinetics of SU5416 was

done during week 0 of cycle 1; pharmacokinetic analysis of SU5416 and doxorubicin were done during week 1 of cycle 1. Plasma samples for the pharmacokinetics of SU5416 were collected prior to the infusion and at 30, 60, 65, 70, 75, 90, 105, 120, 180, 300, and 420 min after the start of the infusion. Plasma samples for the pharmacokinetics of doxorubicin were collected at time points 0, 5, 10, 15, 30, 60, 120, 240, 480, 720, and 1,440 min after doxorubicin administration. SU5416 was analyzed with a high-performance liquid chromatographic method developed and validated by SUGEN (15), and doxorubicin was determined by a modified high-performance liquid chromatography/tandem mass spectrometry method (16). The pharmacokinetic analysis has been described elsewhere (14).

Safety and clinical efficacy assessments. Patients were fully evaluated on day 1 of every cycle, and monitored every week for toxicity using the National Cancer Institute Common Toxicity Criteria, version 2.0.¹³ Due to the nature of IBC, measurable disease was not required, therefore, the clinical end point was defined as achieving the ability to undergo surgical mastectomy following completion of treatment.

Dynamic contrast-enhanced magnetic resonance imaging. Patients underwent a total of three dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) examinations to estimate tumor perfusion: prior to beginning treatment, after two cycles, and prior to surgery (after five cycles). DCE-MRI was done at 1.5 T using an interleaved, multislice FLASH gradient echo sequence (TR = 37 ms; TE = 4 ms; flip angle, 30 degrees; matrix, 256 × 256; FOV = 320 mm). Repeated acquisitions were used to obtain 90 to 120 T₁-weighted image sets through five adjacent 5-mm axial slices of tissue. Three baseline sets were acquired, after which, a 30-s infusion of 0.1 mmol/kg dose of Gd-DTPA was started, immediately followed by a 30-s saline flush. Infusion timing and dosage is rigidly controlled with a programmable power injector. Native precontrast T₁ was estimated at each voxel using two T₁-weighted spin-echo image sets acquired using different TR (400 and 800 ms), while holding all other imaging variables constant. Using a specialized breast receiver coil to permit relocation of the region of interest in each patient, the same slices of breast were scanned thrice at the set time points previously defined. The spatial sensitivity of the coil excludes the descending aorta, thus were unable to generate arterial concentration versus time curves, and instead used the pharmacokinetic model of Brix et al. (17) to obtain Kep (min⁻¹), the efflux rate constant.

Tumor biopsy and microvessel density. Serial biopsies of affected breast tissue were required to perform tumor microvessel density determinations. Core biopsies of the affected breast were obtained pretreatment, after two cycles (week 6), and at the time of mastectomy (after cycle five). The biopsy samples were submitted in saline, cut into carefully labeled sections and then formalin-fixed or flash-frozen. Samples were processed for histologic analysis (H&E staining) to assess for the presence of tumor. Sections of tumor were stained by immunohistochemistry for factor VIII/vWF for microvessel density (MVD) determination by previously published methods (13, 18). Slides were read independently by three pathologists (N. Ziats, F. Abdul-Karim, and J. Wasman). Concordance measures were used in place of variance components for continuous variables in assessing observer agreement.

Plasma markers of angiogenesis: basic fibroblast growth factor and VEGF. Plasma levels basic fibroblast growth factor (bFGF) and VEGF were measured prior to receiving treatment of SU5416 and doxorubicin, following two cycles of treatment, and at the end of therapy; i.e., following five cycles of therapy. Plasma collection was strictly followed to avoid artificial results from platelet activation. After discarding the first 2 mL, blood was collected directly into siliconized tubes that contained a cocktail of citrate, theophylline, adenosine, and dipyridamole (Becton Dickinson) which is specifically designed to inhibit platelet activation. The filled vacutainer tube was immediately centrifuged at 3,000 rpm for 10 min at 4°C. The plasma was removed and immediately re-centrifuged to remove residual platelets,

then aliquotted and stored at -80°C. As a control, we measured β-thromboglobulin, a platelet α-granule-specific protein used as a marker of platelet activation, both in plasma and in serum obtained simultaneously from the same patient. Plasma samples in which the β-thromboglobulin levels were >5% of the serum levels were discarded. Measurement of plasma levels of bFGF and VEGF were done using commercially available ELISA kits (R&D Systems). All samples were assayed in triplicate. The sensitivity of these assays for bFGF and VEGF was at <1 and <9 pg/mL, respectively. A PC-driven Perkin-Elmer HTS 7000 fluorescent plate reader was used, which provided high sensitivity and an expanded linear range (6.4-20,000 pg/mL) for antigen detection.

Endothelial cell markers: E-selectin, vascular cell adhesion molecule 1 (VCAM-1), and intracellular adhesion molecule 1. In addition, plasma levels of E-selectin, VCAM-1, and ICAM-1 were obtained prior to the initiation of therapy, on day 1 of cycle 1 combined therapy, at the end of two cycles, and at the end of treatment (after cycle five). Methods have been published elsewhere (13, 19).

Statistical analysis. Sample size calculations were based on a minimum of nine patients treated with the doxorubicin-SU5416 combination, and a minimum of six patients treated at the maximal tolerated dose, ensuring that common toxicities seen in 30% of patients were rarely missed ($P = 0.12, n = 6$; $P = 0.04, n = 9$) and very common toxicities (those occurring in 50% of patients) were almost never missed ($P = 0.016$). Spearman rank correlation coefficient and scatter plot were used to examine the association between two continuous measurements (20). Event-free survival was defined as the time from registration to date of cancer progression, development of a new cancer, or death, and censored at the date of last follow-up for those without events. Overall survival was defined as time from registration to death, and censored at the time of last assessment for survivors. The event-free survival and overall survival were first estimated by Kaplan-Meier method (21). Univariate Cox proportional hazards models (22) were used to examine the effect of continuous measurements [such as age, tumor size, number of lymph nodes involved, ICAM, VCAM, VEGF, E-selectin, β-thromboglobulin and bFGF, area under the curve (AUC) C_{max}] on overall survival and event-free survival. The profiles over time of ICAM, VCAM, E-selectin, β-thromboglobulin, VEGF, bFGF, AUC, and C_{max} for SU5416 and AUC for doxorubicin were examined by box plot or bar plot. Their difference over time was examined by

Table 1. Patient demographics: patients presented *de novo* with primary or secondary IBC

Characteristic	Number
Age (y)	
Median	54
Range	30 – 79
Sex	
Female	17
Male	1
Menopausal status	
Premenopausal	7
Postmenopausal	10
Clinical stage at presentation	
IIIB	10 (56%)
IV	8 (44%)
Estrogen receptor	
Positive	12 (67%)
Negative	6 (33%)
HER2-neu (3+ immunohistochemistry)	
Positive	8 (44%)

NOTE: No patients had received prior therapy, including trastuzumab; performance status for all patients was ≤1.

¹³ <http://ctep.info.nih.gov>

Table 2. Toxicity presented below was captured throughout all five cycles of treatment ($n = 21$)

Toxicity	Highest grade	No. of patients	Comments
Headache	1-2	16	Only occurred with cycle 1
Thrombus	3	4	Two, catheter-related; two, deep vein thrombosis
Neutropenia	4	2	
Gastrointestinal bleeding	3	1	
Hyperglycemia	4	1	
Decrease in LVEF	3	4	Occurred after therapy
Congestive heart failure	4	3	Among the four patients with LVEF change

Kruskal-Wallis test or paired t test between any two time points. All of the statistical analyses were carried out with SAS (SAS Institute) and $P < 0.05$ was considered significant.

Results

Patients. A total of 18 patients presenting with *de novo* IBC (including one male) were enrolled from April 2000 to October 2002 (Table 1). Seventeen patients completed study treatment; one patient was removed after two cycles because of patient preference.

Toxicity. The dose-limiting toxicity of the combination SU5416 and doxorubicin was neutropenia, and the maximum tolerated dose was determined to be 60 mg/m² for doxorubicin and 145 mg/m² for SU5416 (Table 2). One patient experienced grade 4 hyperglycemia due to the dexamethasone premedication. Two patients experienced grade 3 thrombosis of the central venous catheter, and two patients developed deep vein thrombosis, one in the setting of progressive metastasis. One patient experienced grade 3 upper gastrointestinal tract bleeding believed to be due to chronic steroid administration. Sixteen patients (89%) experienced grade 1 to 2 headache with the first administration of SU5416. Headache was treated with narcotic analgesics and/or sumatriptan succinate.

Four (22%) patients experienced a significant reduction in calculated LVEF, all following completion of treatment. Three patients experienced symptoms associated with congestive heart failure, a median of 37 days (range, 17-420 days) after completing combined treatment. One patient was found to have an asymptomatic reduction in LVEF 42 days after completing treatment. The median decrease in LVEF from baseline was 28%; all were less than the lower limit of normal (i.e., 50%). All patients received medical management with resolution of cardiac toxicity within a median time-frame of 32 days (range, 20-180 days). One patient was lost to follow-up.

Pharmacokinetic analysis. Seven patients completed pharmacokinetic analysis of SU5416 administered at a dose equaling 110 mg/m². When SU5416 was administered alone, the $t_{1/2}$ was 27.8 min, the C_{max} was 3,749 ng/mL, the AUC was 292 min ng/mL, the Vd was 16 L/m², and the Cl was 406 mL/min m². With the coadministration of doxorubicin at a dose of 60 mg/m², the $t_{1/2}$ was 19.9 min, the C_{max} was 3,927 ng/mL, the AUC was 268 min ng/mL, the Vd was 12.8 L/m², and the Cl was 441 mL/min m². There was a statistically significant reduction in $t_{1/2}$ ($P = 0.008$) and Vd ($P = 0.04$) of SU5416 when doxorubicin was coadministered.

Doxorubicin pharmacokinetics were evaluated in all 18 patients. There was no statistical difference in variables due

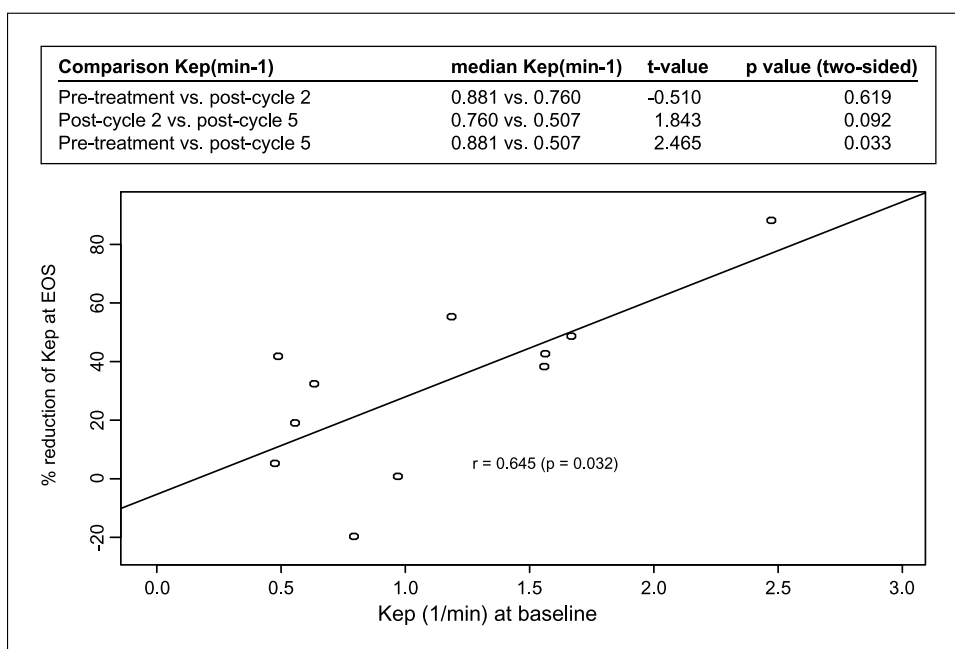


Fig. 2. Baseline Kep versus percentage of reduction of Kep at the end of the study.

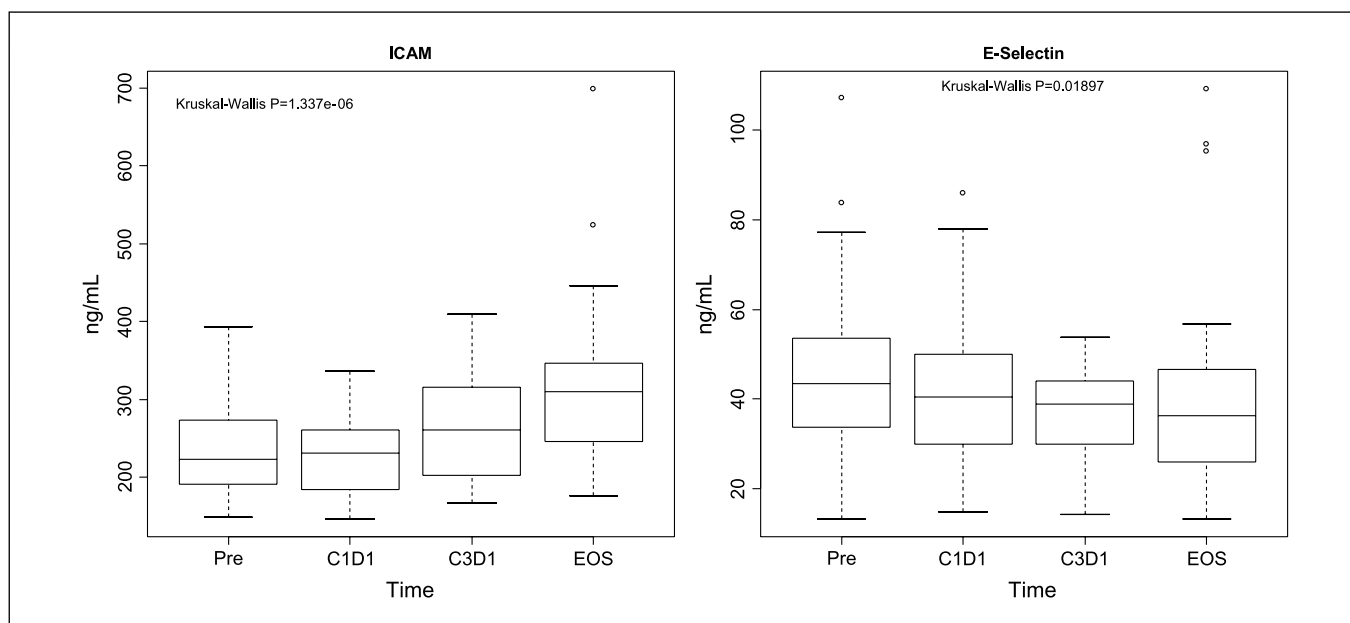


Fig. 3. Markers of angiogenesis: changes in serum ICAM levels and soluble E-selectin levels with treatment by SU5416 and doxorubicin.

to dose of doxorubicin (60 or 75 mg/m²) or SU5416 (110 or 145 mg/m²). The $t_{1/2}$ was 15.9 min, the C_{max} was 4,183 ng/mL, the AUC was 901 min ng/mL, the Vd was 322.1 L/m², and the Cl was 768.8 mL/min m². The Vd seems to be smaller than that shown in previously reported studies, which may account for the higher C_{max} of doxorubicin found in combination with SU5416 (23, 24).

DCE-MRI imaging of tumor blood flow. Eighteen patients underwent DCE-MRI imaging. Fifteen patients (83%) were evaluable for analysis. Among the three unevaluable patients, two had suboptimal T1 data, and one patient had uncorrectable motion artifact. Changes in DCE-MRI image analysis are summarized in Fig. 2. The level of DCE-MRI (Kep) stayed approximately the same from onset of treatment (baseline) to the end of cycle 2. However, by the end of the study (cycle 5), the Kep level was significantly lower than its baseline value ($P = 0.033$). The baseline Kep was also significantly associated with the percentage of reduction of Kep level at the end of study ($P = 0.032$).

Tumor microvessel density analysis. Sixteen patients were evaluable for changes in tumor MVD. There was a trend that suggested a decrease in MVD after treatment, regardless of the magnification (i.e., $\times 200$ or $\times 400$). There was also a significant positive association between baseline MVD and the percentage reduction of MVD at the end of the study ($r = 0.769$, $P = 0.0003$). The median reduction of MVD was 39.3% under $\times 200$ magnification and 26.1% under $\times 400$ magnification.

Changes in serum markers of angiogenesis. All 18 patients were assessed for changes in soluble ICAM-1, soluble VCAM-1, soluble E-selectin, plasma VEGF, and bFGF during treatment. There was a statistically significant increase in soluble ICAM-1 levels progressing from a pretreatment median concentration of 223.4 ng/mL to a posttreatment median concentration of 310.0 ng/mL ($P < 0.00001$). Conversely, there was a statistically significant decrease in soluble E-selectin levels, with a median pretreatment value of 43.3 ng/mL decreasing to a median

posttreatment value of 36.2 ng/mL ($P = 0.019$; Fig. 3). There was no significant change in soluble VCAM-1 concentration with treatment ($P = 0.415$). There was an overall increase in plasma VEGF levels with treatment that was statistically significant ($P < 0.0001$; Fig. 4). There was no significant change in bFGF levels.

Clinical response. Of 21 patients enrolled in the trial, 19 patients (90%) exhibited a clinical disease response: 6 patients had a clinical complete response (32%), 13 patients had a clinical partial disease response (68%). One patient experienced disease progression both locally and systemically following cycle 5 of treatment (5%), one patient withdrew from the study after two cycles for socioeconomic reasons. Of the 19 patients who subjectively responded to treatment, 17 patients (89%) were rendered surgically resectable, i.e., able to undergo mastectomy, this was the definition of objective disease response. Of the two patients who did not meet criteria for surgical resection, one patient was male; the other patient presented with extensive local disease extending onto the abdominal wall. At the time of surgery, the median number of pathologically involved lymph nodes was eight (range, 0-29) and the median tumor size was 1.4 cm (range, 0.2-10 cm). There were no complete pathologic responses.

The median follow-up of this study is 50 months (range, 43-57 months). Overall, the median event-free survival was 31 months (range, 7-54 months), and the median overall survival was not reached. Separating the clinical outcome between those presenting with stage IIIB and stage IV disease, median event-free survival was 32 months (range, 11-54 months) and 16 months (range, 7-48 months), respectively; and the median overall survival was 47 months (range, 13-57 months) and 31 months (range, 9-55 months), respectively.

A univariate Cox proportional hazards model was used to examine the effects of other continuous factors on event-free survival. Only soluble ICAM levels at baseline were marginally significantly related to event-free survival ($P = 0.058$). With a

10 ng/mL increase in ICAM level, the hazard of having an event increased by 13%. The percentage reduction of MVD was marginally associated with event-free survival, but was not statistically significant. The percentage reduction of MVD at the end of the study compared with baseline MVD was significantly related to overall survival. Under higher magnification ($\times 400$), every 10% reduction of MVD was associated with a reduction in the hazard of dying by 12% ($P = 0.012$). Under lower magnification ($\times 200$), every 10% reduction of MVD was associated with a reduction in the hazard of dying by 9% ($P = 0.013$).

Missing Kep values were correlated with patients who had events, and this precluded our ability to use the Cox model to test for significant correlations between event-free survival and the covariates Kep (cycle 5) minus Kep (cycle 2) or Kep (baseline); log-rank testing after dichotomization of Kep differences also failed for this reason.

There is a moderate correlation between the increase in VEGF and the reduction in Kep ($r = 0.455$), which is marginally significant ($P = 0.16$). Other correlations between increasing VEGF and decreasing MVD ($r = -0.08$), and between increasing ICAM levels and decreasing Kep ($r = 0.29$) and decreasing MVD ($r = 0.33$) are weak.

Discussion

IBC is associated with a poor prognosis because of the substantial rate of both local and systemic recurrences. The diffuse nature of its local involvement within the breast, dermal lymphatics, and regional lymph nodes is highly suggestive of a disease mechanism closely associated with angiogenesis activity. Colpaert and colleagues examined 35 patients with IBC and showed a high amount of endothelial cell proliferation associated with significant angiogenesis (25). In addition, preclinical data shows a significantly more rapid growth of breast cancer tumors when they possess an angiogenic phenotype compared with a nonangiogenic phenotype (26). These data support the use of IBC as a clinical model in the investigation of angiogenesis inhibitors.

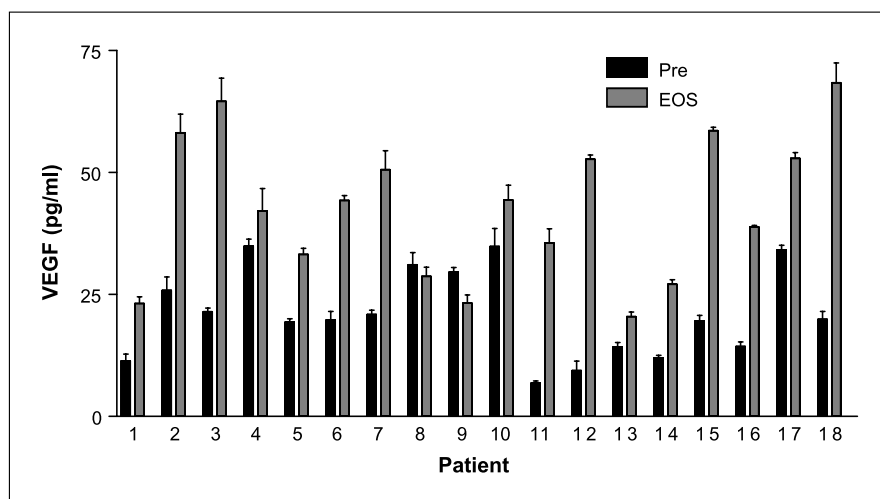
Interestingly, preclinical data shows a higher level of bFGF secreted by breast cancer cells with an angiogenic phenotype, and no difference in VEGF levels secreted by either phenotype

(26). Patients receiving combination VEGFR-2 inhibitor SU5416 and doxorubicin had an overall increase in plasma VEGF levels with ongoing therapy, whereas there was no change in bFGF levels. These changes were not correlated with clinical response to treatment. Only soluble ICAM levels correlated with event-free survival, with higher soluble ICAM levels associated with shorter event-free survival. A similar observation was recently reported from the large randomized phase III trial reported by the Eastern Cooperative Oncology Group of combination carboplatin/paclitaxel with or without bevacizumab in non-small cell lung cancer. These investigators observed that low soluble ICAM levels were both prognostic and predictive of clinical outcomes; in essence, low soluble ICAM levels may guide the selection of an antiangiogenic agent to a doublet cytotoxic chemotherapy platform (27). The phase I trial reported herein comprises a much smaller sample, but nonetheless, replicates findings from a larger phase III study. A scientific explanation for these changes is challenging, and therefore, these results should be viewed as hypothesis-generating; prompting further investigation of similar correlative studies using different angiogenesis inhibitors, with the goal of identifying a demonstrable "angiogenic" profile that may guide the selection of angiogenic therapy and/or correspond to clinical outcomes.

We investigated the effect of combination SU5416 (NSC 696819), a small-molecule tyrosine kinase inhibitor of Flk-1/KDR (VEGFR-2), and conventional chemotherapy (doxorubicin) on biological correlates examined during the treatment of IBC. Although SU5416 and doxorubicin treatment was associated with a significant effect on tumor blood flow, as shown by changes seen in DCE-MRI imaging (Kep), we were unable to correlate these changes with clinical outcome due to the inability of several patients to undergo the complete sequence of imaging studies. However, our results are supported by similar findings with bevacizumab, a humanized monoclonal antibody against VEGF that has been evaluated in the treatment of IBC (28).

Weidner and coworkers showed an association between microvessel density in breast cancer specimens and clinical outcome (29). This was not confirmed by a review of two Cancer and Leukemia Group B clinical trials (8541/8869) involving cyclophosphamide/doxorubicin/5-fluorouracil chemotherapy

Fig. 4. Markers of angiogenesis: changes in plasma VEGF levels with treatment by SU5416 and doxorubicin.



in the treatment of lymph node–positive breast cancer. This review did not find a correlation between increased microvessel density and poorer overall survival (30). Another study has reported a decrease in tumor MVD following chemotherapy, which however, did not correlate with tumor response (31). These studies show the inherent difficulty in interpreting tumor microvessel density, and we experienced similar problems. However, our results do suggest a trend between a reduction in tumor microvessel density and an improvement in event-free survival and overall survival. One complicating feature of this analysis was the absence of tumor within several biopsy specimens. Our hypothesis was that IBC diffusely involves the entire breast, and therefore, evidence of angiogenesis should be present regardless of the direct proximity of tumor cells. The use of microvessel density was not a robust indicator supporting this hypothesis. It is possible that documentation of other

biomarkers within breast specimens may be a more sensitive indicator of the widespread involvement of the breast in inflammatory cancer.

The combination of SU5416 and doxorubicin was well-tolerated among our patients with IBC. However, this study showed evidence of an unfavorable cardiac interaction between SU5416 and doxorubicin, which precludes further safe investigation of this combination. These results lend support to developing a scientifically cautious approach to combining cardiotoxic chemotherapy with angiogenesis inhibitors given the physiologic overlap of mechanisms of angiogenesis between the cardiovascular system and neoplasia. This study, however, supports the importance of using IBC as a model for the clinical investigation of angiogenesis inhibitors because this disease lends itself to easy access of tumor tissue and the ability to study the pharmacodynamic effects of novel therapies.

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