

## Paclitaxel Decreases the Interstitial Fluid Pressure and Improves Oxygenation in Breast Cancers in Patients Treated With Neoadjuvant Chemotherapy: Clinical Implications

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Authors' disclosures of potential conflicts of interest are found at the end of this article.

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### A B S T R A C T

#### Purpose

It has been hypothesized that tumors with high interstitial fluid pressure (IFP) and/or hypoxia respond poorly to chemotherapy (CT) because of poor drug delivery. Preclinical studies have shown that paclitaxel reduces the IFP and improves the oxygenation ( $pO_2$ ) of tumors. Our aim is to evaluate the IFP and  $pO_2$  before and after neoadjuvant CT using sequential paclitaxel and doxorubicin in patients with breast cancer tumors of  $\geq 3$  cm.

#### Patients and Methods

Patients were randomly assigned, according to an institutional review board–approved phase II protocol, to receive neoadjuvant sequential CT consisting of either four cycles of dose-dense doxorubicin at 60 mg/m<sup>2</sup> every 2 weeks followed by nine cycles of weekly paclitaxel at 80 mg/m<sup>2</sup> (group 1) or vice versa, with paclitaxel administered before doxorubicin (group 2). Patients were re-evaluated clinically and radiologically. The IFP (wick-in-needle technique) and  $pO_2$  (Eppendorf) were measured in tumors at baseline and after completing the administration of the first and second drug.

#### Results

IFP and  $pO_2$  were measured in 54 patients at baseline and after the first CT. Twenty-nine and 25 patients were randomly assigned to groups 1 and 2, respectively. Paclitaxel, when administered first, decreased the mean IFP by 36% ( $P = .02$ ) and improved the tumor  $pO_2$  by almost 100% ( $P = .003$ ). In contrast, doxorubicin did not have a significant effect on either parameter. This difference was independent of the tumor size or response measured by ultrasound.

#### Conclusion

Paclitaxel significantly decreased the IFP and increased the  $pO_2$ , whereas doxorubicin did not cause any significant changes. Tumor physiology could potentially be used to optimize the sequence of neoadjuvant CT in breast cancer.

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### INTRODUCTION

Neoadjuvant chemotherapy (CT) followed by surgery and radiation is now a common approach for treating large primary breast cancers<sup>1</sup> and is increasingly being used for operable palpable breast cancers as well.<sup>2</sup> Because the use of neoadjuvant CT affords

an ideal opportunity to observe the response of a tumor in vivo, it is possible to distinguish responders from nonresponders. In fact, patients who have a complete pathologic response after neoadjuvant CT have a significantly better outcome.<sup>3</sup> Why some tumors fail to respond remains a challenge. It is important to find out whether the cancer

cells are intrinsically resistant to the drugs or whether penetration of the drugs into the tumors is suboptimal. Only by studying physiologic and molecular parameters together will we be able to distinguish these mechanisms. To date, efforts to determine the physiologic parameters restricting the delivery of therapeutic agents to tumor tissue in breast cancer patients have been modest.

The delivery of the drugs to the cancer cells within the tumor is one of the most important parameters in the success of CT.<sup>4</sup> Both a heterogeneous, poor blood supply and elevated interstitial fluid pressure (IFP) in tumors can be significant parameters affecting the access of therapeutic agents to neoplastic cells.<sup>5</sup> Interstitial hypertension reduces the driving force for the filtration of fluid and large molecules (eg, antibodies) in tumors and also leads to radially outward convection, which opposes inward diffusion.<sup>5</sup> Furthermore, when a high IFP is coupled with the high vascular permeability of the tumor vessels, the pressure gradient along vessels becomes less steep. This could reduce the tumor blood flow and induce perfusion heterogeneities, thus influencing the delivery of oxygen and the diffusion of small chemotherapeutic agents.<sup>6,7</sup>

There is an increased interest in studying the mechanisms that control the elevated IFP in solid tumors and investigating strategies that improve tumor perfusion; if these mechanisms were understood, it might lead to the ability to modify the IFP and thus improve the efficacy of therapy. The induction of apoptosis in experimental tumors with paclitaxel and docetaxel has been shown to reduce the microvascular pressure (MVP) and the IFP.<sup>8</sup> It also increased the diameter of tumor vessels without a change in tumor vascular density, thus suggesting that taxanes increase the blood flow, blood volume, and vascular surface area for exchange of small therapeutic agents in tumors.<sup>8</sup> If this hypothesis were valid in human tumors, one could speculate that tumors with high IFP might benefit from exposure to a taxane first to decrease IFP and improve blood flow and oxygenation ( $pO_2$ ) before treatment with other CT drugs to maximize tumor response.

It has also been recognized for many years that the  $pO_2$  status of tumors is an important factor affecting the cytotoxicity of radiation. The level of cellular  $pO_2$  is also an important factor in the action of many antineoplastic agents, several of which have been classified in vitro and in vivo by their selective cytotoxicity towards oxygenated and hypoxic tumor cells in animal models.<sup>9-11</sup> Whether intrinsic properties of tumor cells or metabolic factors micromilieu (including tissue  $pO_2$ ) are the dominant factors in the treatment outcome of radiation or CT is still a subject of controversy.<sup>12</sup> In an animal model, Milas et al<sup>13</sup> also found an increase in the tumor  $pO_2$  with taxanes, which was observed with direct measurements using the Eppendorf histograph.<sup>13</sup> If this were true in human tumors, one could speculate that tumors with low  $pO_2$  measurements might

benefit from exposure to a taxane first to improve  $pO_2$  before treatment with other CT drugs.

Given the findings of Milas et al<sup>13</sup> and Griffon-Etienne et al,<sup>8</sup> we hypothesized that paclitaxel, compared with doxorubicin, decreases the IFP and increases the tumor  $pO_2$ . This should potentially improve drug delivery and, hence, tumor response to neoadjuvant CT. In this prospective, phase II, randomized study, we evaluated the effect of paclitaxel and doxorubicin on the tumor IFP and  $pO_2$  in patients with palpable breast cancer treated by neoadjuvant CT.

## PATIENTS AND METHODS

### Patients

Patients with primary invasive breast cancer, as diagnosed by fine-needle aspirate or core biopsy were enrolled onto this study. To be eligible, patients had to have a palpable breast mass of  $\geq 3$  cm in size clinically, to be otherwise in good health, and to have no evidence of distant metastasis. This study was approved by the internal review board of the Massachusetts General Hospital and Dana-Farber/Partners Cancer Care. Written consent was obtained from each patient. After enrollment in the trial, breast tumor size was estimated by clinical examination and ultrasound (US). The  $pO_2$  and IFP were measured under US guidance in conjunction with a breast radiologist.

### Overview of Treatment Plan

After undergoing baseline studies, the patients were given CT with sequential single-agent doxorubicin (four cycles at 60 mg/m<sup>2</sup> every 2 weeks) and paclitaxel (nine cycles at 80 mg/m<sup>2</sup> weekly). The patients were randomly assigned to the following two groups: group 1 received doxorubicin first followed by paclitaxel, and group 2 received paclitaxel first followed by doxorubicin. After the first phase of CT (doxorubicin or paclitaxel), each patient underwent breast US and  $pO_2$  and IFP measurements. If there was a complete clinical response (based on clinical and radiologic evaluation), a complete excision was carried out at this point, and the patient was administered the second CT agent (paclitaxel for group 1 and doxorubicin for group 2) as an adjuvant treatment. In the original protocol, patients also proceeded to surgery if the tumor had shrunk to  $\leq 2$  cm in size after the first CT drug. Later, the protocol was revised so that, if residual tumor was clinically palpable or radiologically present, the second CT agent was administered before surgery. After the second drug, the patients who had not had definitive surgery had repeat US,  $pO_2$ , and IFP measurements and then proceeded to definitive surgery. Breast-conserving therapy was usually feasible and recommended. Further CT was administered at the discretion of the treating physician. This article addresses the data on the changes in IFP and  $pO_2$  caused by CT. Because the protocol is still open and accruing patients, clinical outcome at the time of the writing of the manuscript could not be addressed.

### IFP Measurements

All IFP measurements were performed under local anesthesia using sterile techniques, before the protocol-required core biopsy and with the patient in a supine position. With the patient in this position, the tumor is at approximately heart level, and thus,

**Table 1.** Baseline Clinical Characteristics of Both Groups of Patients Who Participated in the Study

Variable	% of Patients		P
	Group 1* (n = 30)	Group 2† (n = 27)	
Age, years			.19
Mean	50.4	47.9	
SD	8.2	9.2	
Menopausal status			.68
Perimenopausal	6.9	3.7	
Postmenopausal	44.8	37.0	
Premenopausal	48.3	59.3	
Clinical T stage			.58
T2	60.0	70.4	
T3	40.0	29.6	
Clinical N stage			.60
N0	46.7	55.6	
N1	53.3	44.4	
ER positive	73.3	74.1	.99
PR positive	60.0	77.8	.17
HER2/ <i>neu</i> positive	30.0	11.1	.10
Histology, invasive ductal carcinoma	83.3	88.9	.71

Abbreviations: SD, standard deviation; ER, estrogen receptor; PR, progesterone receptor.  
 \*Group 1: doxorubicin followed by paclitaxel.  
 †Group 2: paclitaxel followed by doxorubicin.

the contribution of the hydrostatic load to the tumor IFP is minimized. IFP was measured with the wick-in-needle (WIN) technique.<sup>14</sup> The patient's oxygen saturation and arterial blood pressure were also measured. The WIN technique involves using a sterile 23-gauge hypodermic needle with a 3-mm side hole at 5 mm from the tip. Nylon filaments from surgical sutures (6-0 Ethilon; Ethilon Inc, Somerville, NJ) are placed within the needle. To take the pressure measurements, the needle is connected to a pressure transducer (Model No. 1290C; Agilent Technologies, Palo Alto, CA) by polyethylene tubing filled with sterile heparinized (70 U/mL) saline and an electronic data acquisition and recording system (Viridia 24/26 C, Model No. 1205A/AR; Hewlett Packard, Palo Alto, CA). Before the pressure measurements were taken in each patient, the pressure transducer setup was calibrated. The IFP was measured in at least three to four different tracks in different directions in the tumor and one track in normal breast tissue (away from the tumor) using the same WIN set-up. Each IFP track had a US documentation of its location within or outside the tumor. Every attempt was made to avoid overtly necrotic or cystic regions. The IFP was measured before the pO<sub>2</sub> to avoid disturbing the tumor microenvironment.<sup>15</sup> The IFP was measured on three different occasions, at baseline, after the first chemotherapeutic drug, and after the second drug.

**Interstitial pO<sub>2</sub> Measurements**

The steps used by Vaupel et al<sup>16</sup> were followed. Briefly, sterile polarographic needle electrodes were used (pO<sub>2</sub> histography, model KIMOC-6650; Eppendorf, Hamburg, Germany). Calibrations were performed in sterile phosphate-buffered solution (pH 7.4) immediately before and after pO<sub>2</sub> measurements in the tissues. Calibration in air and nitrogen is done to define the parameters of the linear relationship between pO<sub>2</sub> and electrode current

**Table 2.** Average Tumor IFP and pO<sub>2</sub> at Baseline and After the First CT Drug

Measures	Group 1*	Group 2†	All Patients
<b>IFP</b>			
No. of patients	29	25	54
IFP at baseline, mmHg			
Mean	6.2	6.9	6.5
95% CI	4.3 to 8.1	5.0 to 8.8	5.2 to 7.8
IFP after first drug, mmHg			
Mean	7.0	4.4	5.8
95% CI	5.2 to 8.9	2.6 to 6.3	4.5 to 7.2
Change in IFP, mmHg‡			
Mean	0.8	-2.5	-0.7
95% CI	-2.0 to 3.6	-5.0 to 0.1	-2.6 to 1.2
P§	.52	.02	.32
<b>pO<sub>2</sub></b>			
No. of patients	22	24	46
pO <sub>2</sub> at baseline, mmHg			
Mean	13.8	14.1	14.0
95% CI	7.3 to 20.4	7.7 to 20.4	9.6 to 18.3
pO <sub>2</sub> after first drug, mmHg			
Mean	14.3	27.6	21.2
95% CI	8.5 to 20.1	21.3 to 33.8	16.7 to 25.8
Change in pO <sub>2</sub> , mmHg			
Mean	0.5	13.5	7.3
95% CI	-8.2 to 9.2	4.9 to 22.0	1.1 to 13.5
P	.99	.001	.01
HF2.5 at baseline, %			
Mean	32.0	31.6	31.8
95% CI	19.4 to 44.5	20.2 to 43.1	23.7 to 39.9
HF2.5 after first drug, %			
Mean	24.9	7.3	15.5
95% CI	13.6 to 36.1	2.2 to 12.4	9.2 to 21.7
Change in HF2.5, %			
Mean	-7.1	-24.3	-16.3
95% CI	-21.6 to 7.4	-36.7 to -11.9	-25.7 to -6.9
P	.32	.003	.002

Abbreviations: IFP, interstitial fluid pressure; pO<sub>2</sub>, oxygen pressure; CT, chemotherapy; HF, hypoxic fraction.  
 \*Group 1: doxorubicin followed by paclitaxel.  
 †Group 2: paclitaxel followed by doxorubicin.  
 ‡Change in IFP: difference between the IFP measured at baseline and after the first CT drug.  
 §Associated P values (using two-sided Wilcoxon exact test).  
 ||Change in pO<sub>2</sub>: difference between the pO<sub>2</sub> measured at baseline and after the first CT drug.

at room temperature and barometric pressure. This relationship is then used to determine the pO<sub>2</sub> from the probe current recorded during the patient measurements, after correcting for the difference in temperature.

All pO<sub>2</sub> measurements were performed under local anesthesia using sterile techniques, before the protocol-required core biopsy and with the patient in a supine position. Local anesthesia (3 to 4 mL of 1% lidocaine without epinephrine) was injected in the subcutaneous tissue at the site of the entrance of the trocar and in the peritumor area. To avoid any disturbance of the tumor microenvironment, there was no lidocaine injected inside the tumor before the pO<sub>2</sub> measurements, making it unlikely that local anesthesia would have altered the pO<sub>2</sub> measurements. After local anesthesia, a trocar (outer diameter, 0.8 mm) equipped

**Table 3.** Average Tumor IFP and pO<sub>2</sub> at Baseline and After the First and Second CT Drugs

Measures	Group 1*	Group 2†	All Patients
<b>IFP</b>			
No. of patients	21	20	41
IFP at baseline, mmHg			
Mean	5.2	7.4	6.2
95% CI	2.8 to 7.5	5.1 to 9.6	4.6 to 7.8
IFP after first drug, mmHg			
Mean	7.2	3.7	5.5
95% CI	5.0 to 9.4	2.3 to 5.1	4.1 to 6.9
Change in IFP #1, mmHg‡			
Mean	2.1	-3.7	-0.7
95% CI	-1.4 to 5.6	-6 to -1.4	-3.0 to 1.5
P§	.19	.002	.39
IFP after second drug, mmHg			
Mean	4.7	4.5	4.6
95% CI	2.4 to 7.1	2.7 to 6.3	3.2 to 6.0
Change in IFP #2, mmHg			
Mean	-2.5	0.8	-0.9
95% CI	-5.4 to 0.4	-1.6 to 3.2	-2.8 to 1.0
P§	.04	.63	.21
Change in IFP #3, mmHg¶			
Mean	0.4	-2.9	-1.6
95% CI	-4.4 to 3.5	-5.4 to -0.4	-3.9 to 0.7
P§	.41	.01	.02
<b>pO<sub>2</sub></b>			
No. of patients	17	15	32
pO <sub>2</sub> at baseline, mmHg			
Mean	11.9	10.9	11.4
95% CI	4.7 to 19.0	4.8 to 17.0	6.9 to 15.9
pO <sub>2</sub> after first drug, mmHg			
Mean	11.2	27.3	18.7
95% CI	5.3 to 17.2	19.2 to 35.3	13.3 to 24.2
Change in pO <sub>2</sub> #1, mmHg#			
Mean	-0.6	16.4	7.4
95% CI	-10.8 to 9.6	6.5 to 26.2	0 to 14.7
P§	.88	.009	.05
pO <sub>2</sub> after second drug, mmHg**			
Mean	22.7	13.9	18.5
95% CI	16.0 to 29.3	7.1 to 20.7	7.1 to 20.7
Change in pO <sub>2</sub> #2, mmHg			
Mean	11.4	-13.4	-0.2
95% CI	2.2 to 20.6	-24.6 to -2.1	-8.3 to 7.9
P§	.01	.03	.82
Change in pO <sub>2</sub> #3, mmHg††			
Mean	10.8	3.0	7.1
95% CI	-0.2 to 21.9	-7.6 to 13.6	-0.2 to 14.5
P§	.05	.55	.05
<b>pO<sub>2</sub>, HF2.5‡‡</b>			
HF2.5 at baseline, %			
Mean	33.4	39.8	36.6
95% CI	15.7 to 51.0	23.4 to 56.2	25.3 to 47.9
HF2.5 after first drug, %			
Mean	31.1	8.6	19.9
95% CI	16.9 to 45.3	1.5 to 15.7	11.2 to 28.5
Change in HF2.5, %§§			
Mean	-2.3	-31.2	-16.7
95% CI	-22.4 to 17.9	-48.4 to -13.9	-30.3 to -3.1
P§	.94	.01	.03

(continued on following page)

**Table 3.** Average Tumor IFP and pO<sub>2</sub> at Baseline and After the First and Second CT Drugs (continued)

Measures	Group 1*	Group 2†	All Patients
HF2.5 after second drug, %			
Mean	14.4	26.2	20.3
95% CI	4.4 to 24.5	6.9 to 45.4	9.9 to 30.7
Change in HF2.5 #2, mmHg			
Mean	-16.7	17.5	0.4
95% CI	-35.4 to 2.0	-1 to 36.1	-13.6 to 14.4
P§	.10	.05	.97
Change in HF2.5 #3, mmHg¶¶			
Mean	-19.0	-13.6	-16.3
95% CI	-37.4 to -0.5	-31.7 to -0.9	-31.7 to -0.9
P§	.05	.20	.02

Abbreviations: IFP, interstitial fluid pressure; pO<sub>2</sub>, oxygen pressure; CT, chemotherapy; HF, hypoxic fraction.

\*Group 1: doxorubicin followed by paclitaxel.

†Group 2: paclitaxel followed by doxorubicin.

‡Change in IFP #1: difference between the IFP measured at baseline and after the first CT drug.

§Associated P values (using two-sided Wilcoxon exact test).

||Change in IFP #2: difference between the IFP measured after the first and after the second CT drug.

¶¶Change in IFP #3: difference between the IFP measured at baseline and after the second CT drug.

#Change in pO<sub>2</sub> #1: difference between the mean pO<sub>2</sub> measured at baseline and after the first CT drug.

\*\*Change in pO<sub>2</sub> #2: Difference between the mean pO<sub>2</sub> measured after the first and after the second CT drug.

††Change in pO<sub>2</sub> #3: Difference between the mean pO<sub>2</sub> measured at baseline and after the second CT drug.

‡‡HF2.5: percentage of measurements less than 2.5 mmHg.

§§Change in HF2.5 #1: difference between the mean HF2.5 measured at baseline and after the first CT drug.

||||Change in HF2.5 #2: difference between the mean HF2.5 measured after the first and after the second CT drug.

¶¶¶Change in HF2.5 #3: difference between the mean HF2.5 measured at baseline and after the second CT drug.

with a hypodermic needle was advanced to an initial depth of approximately 2 mm into the tissue of interest where the oxygen-sensitive probe was then placed. All electrode tracks were performed leaving the trocar at one puncture site and guiding the oxygen probe from this site in different directions (usually three tracks: starting at 0 and conducted at approximately -45 or +45 degrees direction). The electrode automatically moved through the tissue in preset steps of 1 mm, immediately followed by a backward step of 0.3 mm to minimize compression effects caused by the forward motion of the electrode. The length of the track depended on the tumor size (varied between 1 and 5 cm). Each pO<sub>2</sub> track had a US documentation of its location within or outside the tumor. Depending on the tumor size, a different number of tracks was used, and 50 to 200 pO<sub>2</sub> readings were obtained. Po<sub>2</sub> histograms (ie, pO<sub>2</sub> frequency distributions) were obtained with a class width of 2.5 mmHg. We also assessed other parameters for tumor hypoxia, including the percentage of pO<sub>2</sub> values ≤ 2.5, ≤ 5.0, and ≤ 10.0 mmHg (hypoxic fraction [HF] 2.5, HF5 and HF10, respectively). The distribution of the measured pO<sub>2</sub> values was characterized in our analysis by the median pO<sub>2</sub> and by HF2.5. pO<sub>2</sub> was measured before starting CT, after the first drug, and after completing all CT just before surgery. Every patient had a track of normal tissue measurements located in the same breast at distance from the tumor site.

### Statistical Analysis

The randomization was stratified based on clinical tumor size (≤ 5 cm and > 5 cm) and the presence or absence of suspicious palpable axillary lymph nodes. This was done so that both treatment arms were well balanced regarding the clinical presentation. We recorded and tabulated simple statistics (means, medians, and standard deviations for IFP and pO<sub>2</sub>). Negative pO<sub>2</sub> values less than 0 mmHg and values more than 100 mmHg were not included in the analysis. However, we also performed a sensitivity analysis

that included all the discarded pO<sub>2</sub> measurements to make sure that the qualitative results of the present study are not affected by the inclusion of negative pO<sub>2</sub> measurements. We used the Spearman test to evaluate the correlation between the median pO<sub>2</sub>, HF2.5, HF5, and HF10. We used the Wilcoxon test to compare pO<sub>2</sub> or IFP measurements and their changes between treatment groups. Similarly, pO<sub>2</sub> or IFP changes were tested for statistical significance with a paired Wilcoxon test.<sup>17</sup> P ≤ .05 was considered statistically significant. The tumor response was evaluated using the US tumor volume; this was calculated from the three dimensions (L1, L2, and L3) using the following equation: L1 × L2 × L3 × 3.14/6.<sup>18</sup>

## RESULTS

### Patient Population

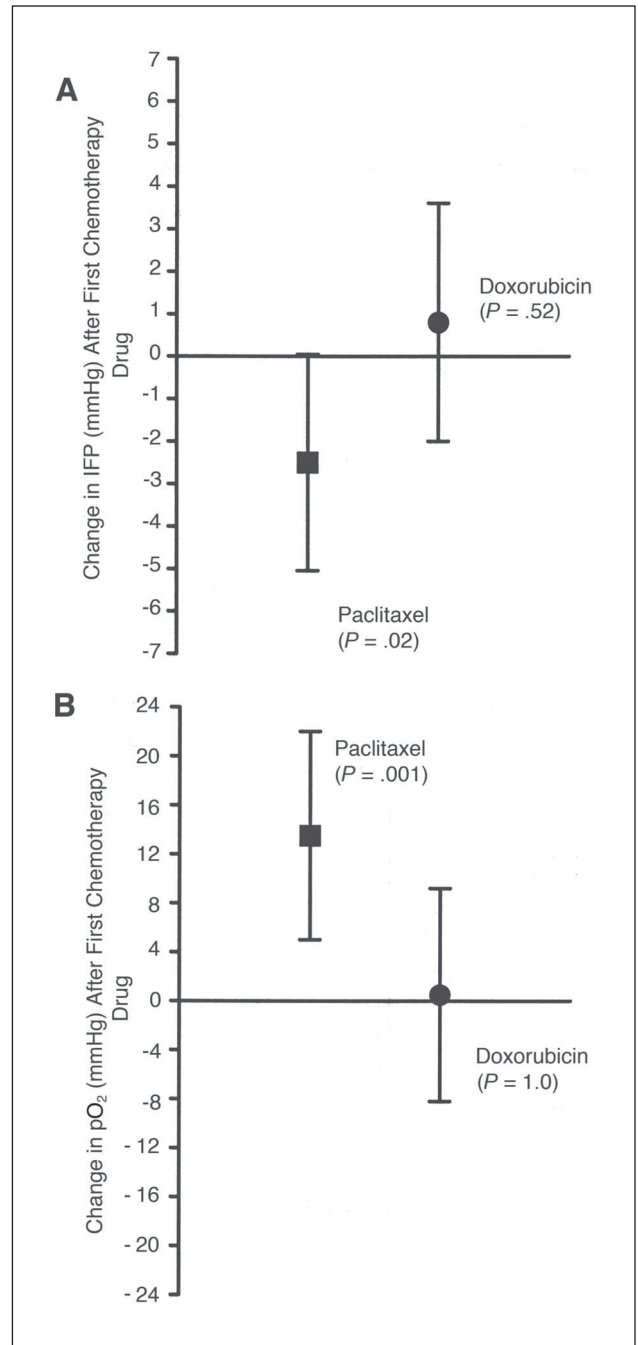
At the time of the analysis, 59 patients were enrolled onto the study (30 patients in group 1 and 29 patients in group 2) who completed at least the first course of CT. Two patients refused to continue after the first CT. The baseline clinical characteristics of the remaining 57 patients are listed in Table 1; no significant differences were found between study groups. Some measurements at baseline or after first CT failed for technical reasons that were extraneous to the disease or patient status. We report the results for patients for whom the measurements were performed at baseline and after the first CT; 54 patients had both IFP measurements, and 46 patients had both pO<sub>2</sub> measurements. There were no significant differences between both groups with respect to baseline IFP or pO<sub>2</sub> (Table 2). Of those 54 and 46 patients, three measurements of IFP and

pO<sub>2</sub> (baseline, after first CT drug, and after second CT drug) were performed in 41 and 32 patients, respectively (Table 3). Thirteen patients did not have the IFP measured for the third time after the second CT drug (four patients had surgery performed after completing the first CT drug per original protocol, three had complete response by US and tumor could not be found, two refused the third measurement, one was taken off the protocol, and three are still receiving the second CT drug). Fourteen patients did not have the pO<sub>2</sub> measurement after the second CT drug (three patients had surgery performed after completing the first CT drug per original protocol, three had complete response by US and tumor could not be found, two refused the third measurement, one was taken off the protocol, the pO<sub>2</sub> measurements failed in two patients because of technical problems, and three are still receiving the second CT drug). The clinical tumor size varied between 3 and 10 cm, with a mean of 5 cm (standard deviation, 1.7 cm) and a median of 4 cm.

**Effect of Doxorubicin and Paclitaxel on the IFP**

In all patients, except the first patient, the tumor IFP was measured in three to four tracks. For each patient, the IFP was also measured in the normal tissue of the same breast, at a distance from the tumor site. The mean and median normal tissue IFP values were 1.05 and 0.5 mmHg, respectively. For the whole population, there was a highly significant difference between the baseline IFP in normal tissue and tumor (1.05 and 6.5 mmHg, respectively;  $P < .0001$ ). Paclitaxel used as first drug in group 2 patients significantly decreased the overall mean IFP from 7.0 mmHg (95% CI, 5.2 to 8.9 mmHg) to 4.4 mmHg (95% CI, 2.6 to 6.3 mmHg;  $P = .02$ ; Table 2 and Fig 1A). There was a decrease in IFP in 17 of 25 patients (Fig 2, upper panels). In contrast, in patients treated with doxorubicin first, the mean IFP before and after doxorubicin was not significantly different (Table 2 and Fig 1A).

For the 41 patients with IFP measurements at the three different time points (Table 3), doxorubicin plus paclitaxel significantly decreased the tumor IFP by 25% (absolute decrease of 1.6 mmHg, 95% confidence limits [CL], -3.9, 0.7 mmHg;  $P = .02$ ). When this overall decrease in IFP was broken down by group, the IFP of group 1, who were treated with doxorubicin first, changed by 8% (absolute change, +0.4 mmHg; 95% CL, -4.4, 3.5 mmHg;  $P = .41$ ); however, patients of group 2, who received paclitaxel first, had a significant decrease in IFP by 39% (absolute decrease of 2.9 mmHg; 95% CL, -5.4, -0.4;  $P = .01$ ). Therefore, patients treated with paclitaxel first had a significant decrease in IFP measured after completion of all CT, whereas patients who started with doxorubicin did not (Fig 3A and 3B). The effect of paclitaxel in reducing the IFP was significant, whether used as a first drug (in group 2) or as a second

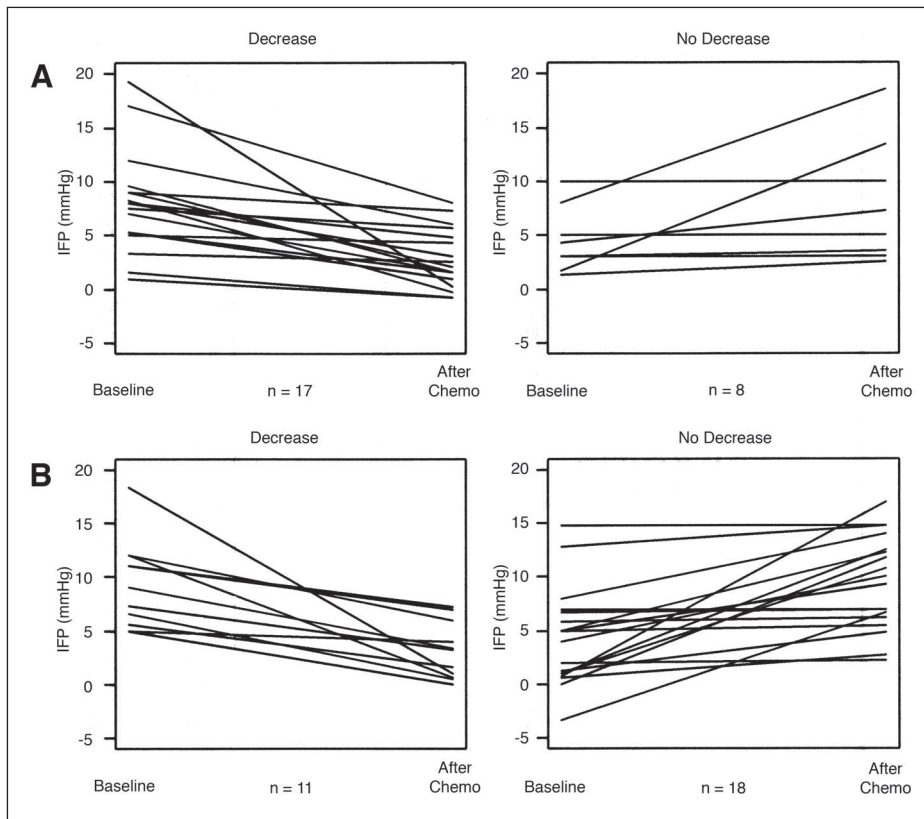


**Fig 1.** Overall change and 95% CI in tumor (A) interstitial fluid pressure (IFP) and (B) oxygen pressure (pO<sub>2</sub>) in breast cancer patients who had both pre- and postfirst chemotherapy measurements available. Paclitaxel decreased the IFP and increased the pO<sub>2</sub> significantly, whereas doxorubicin had no significant effect.

drug (in group 1). This was in contrast to the effect of doxorubicin (Table 3 and Fig 3A and 3B).

**Effect of Doxorubicin and Paclitaxel on pO<sub>2</sub>**

pO<sub>2</sub> was measured in 46 patients at baseline and after the first CT drug. The average number of tracks was 4.9



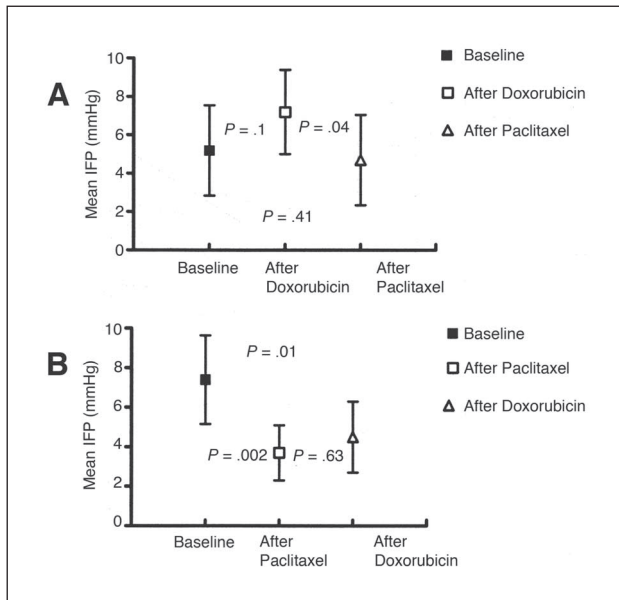
**Fig 2.** Change in tumor interstitial fluid pressure (IFP) from baseline to after paclitaxel (upper panels) or after doxorubicin (lower panels) in individual patients. N, number of patients in each group; chemo, chemotherapy.

(range, two to 11 tracks). The mean and median numbers of  $pO_2$  readings per tumor were 123 and 128, respectively. The correlation between the median  $pO_2$  and the HF2.5, HF5, and HF10 was highly significant ( $P \leq .000001$ ), with Spearman coefficients of  $-0.85$ ,  $-0.92$ , and  $-0.90$ , respectively. Therefore, we used the median  $pO_2$  to represent the  $pO_2$  status for each tumor, although data on the HF2.5 is also presented (Tables 2 and 3). The median  $pO_2$  in tumors varied from 0.8 to 62.6 mmHg. In normal tissue, the mean of the median  $pO_2$  measurements was 49 mmHg compared with 14.3 mmHg in tumors ( $P < .00001$ ). Figure 4 shows the effect of paclitaxel or doxorubicin administered as a first drug on the median  $pO_2$  of individual patients. In 19 of 24 patients, paclitaxel increased the median  $pO_2$ , whereas in five of 24 patients, the median  $pO_2$  did not change or decreased (Fig 4, upper panel). In contrast, the effect of doxorubicin was more variable; the median  $pO_2$  increased in 10 of 22 patients and did not change or decreased in 12 of 22 patients (Fig 4, lower panel). After paclitaxel, the overall median  $pO_2$  significantly increased ( $P = .001$ ) from a mean of 14.1 mmHg (95% CI, 7.7 to 14.1) to 27.6 mmHg (95% CI, 21.3 to 27.6), and the HF2.5 decreased from 33.9% to 7.6% ( $P = .001$ ; Table 2). After doxorubicin, the overall change in the mean  $pO_2$  and the HF2.5 was not significant (Fig 1B and Table 2).

In 32 patients,  $pO_2$  was measured at baseline and at two time points after doxorubicin or paclitaxel in group 1 (doxorubicin followed by paclitaxel) and group 2 (paclitaxel followed by doxorubicin). In group 1, doxorubicin did not significantly change the tumor  $pO_2$ ; however, paclitaxel used as a second drug significantly increased the  $pO_2$  compared with doxorubicin ( $P = .01$ ) or baseline ( $P = .05$ ; Fig 5 and Table 3). In group 2, paclitaxel increased the  $pO_2$  by 250% (from a mean of 10.9 mmHg to 27.3 mmHg;  $P = .009$ ), and after doxorubicin (used as a second drug), the  $pO_2$  dropped by almost 50% (from 27.3 mmHg to 13.9 mmHg;  $P = .03$ ; Fig 5 and Table 3).

### US Tumor Volume and Response to CT

To evaluate whether the changes in IFP and  $pO_2$  observed after doxorubicin or paclitaxel were a result of differential changes in tumor volume, the US tumor volume was calculated from the three dimensions based on the previously mentioned formula.<sup>18</sup> The mean percent decrease in US tumor volume after the first CT drug was 29.2% after doxorubicin and 35.8% after paclitaxel. This difference was not significant ( $P = .29$ ). The mean percent decrease in US tumor volume from postfirst to postsecond CT drug was 42.7% after paclitaxel and 52.8% after doxorubicin ( $P = .79$ ). The mean percent decrease in US tumor volume from baseline to postsecond CT drug was 79.5% for



**Fig 3.** Tumor interstitial fluid pressure (IFP) in patients who had three measurements available (baseline, after first drug, and after second drug) in patients who received (A) doxorubicin followed by paclitaxel (group 1) or (B) paclitaxel followed by doxorubicin (group 2). For group 1, the difference between baseline and after second drug was not significant ( $P = .41$ ); for group 2, it was significant ( $P = .01$ ).

group 1 and 70.6% for group 2. This difference was also not significant ( $P = .99$ ).

### Correlation Between Changes in IFP and Changes in $pO_2$

With the Spearman test, there was no significant correlation between the change in IFP and the change in  $pO_2$  ( $r = 0.07$ ,  $P = .65$ ). When only patients who had a decrease in IFP were selected, the correlation between the decrease in IFP and the change in  $pO_2$  was still not significant ( $r = 0.01$ ,  $P = .98$ ).

## DISCUSSION

Neoadjuvant CT has been shown in randomized trials to be equivalent to adjuvant CT in terms of clinical benefit.<sup>2,19</sup> A number of trials have shown excellent response rates to regimens that combine an anthracycline with a taxane.<sup>20</sup> We used single-agent therapy in the neoadjuvant setting in a dose-dense regimen<sup>21</sup> to correlate the response to each individual drug with biologic parameters. This report details the initial data showing the effect of paclitaxel and doxorubicin on IFP and  $pO_2$  in breast cancer patients treated with neoadjuvant CT.

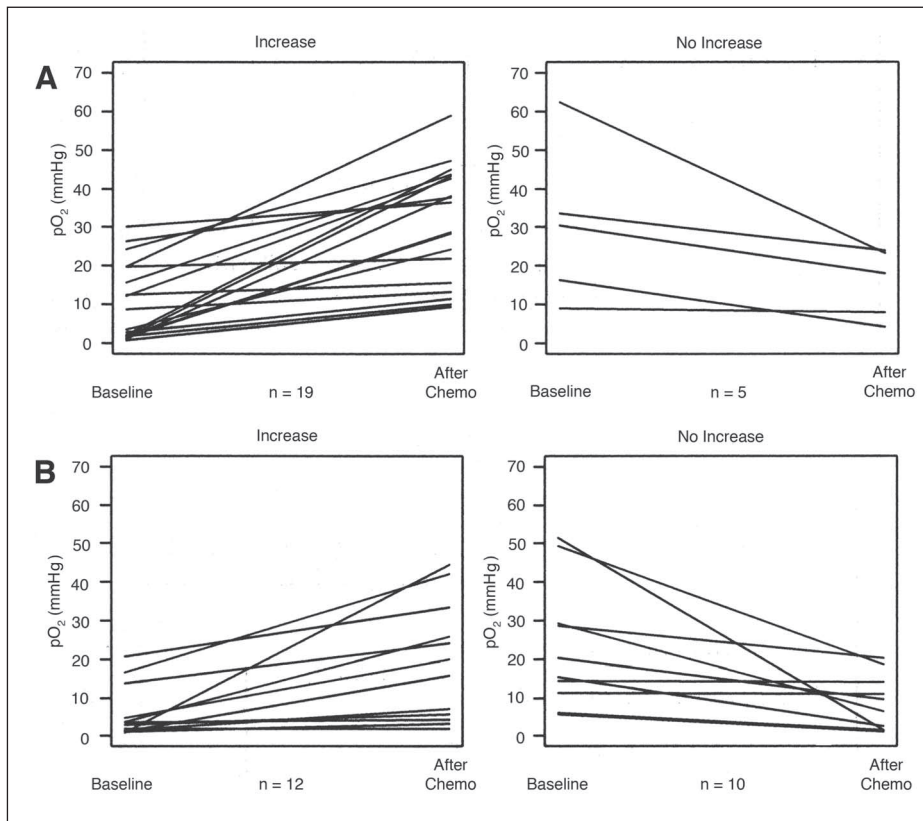
Paclitaxel significantly reduced the IFP whether it was used as first (group 2) or second drug (group 1; Figs 1A, 3A, and 3B). In contrast, after doxorubicin treatment, the overall change in IFP was not significant. This significant reduction in IFP induced by paclitaxel, in contrast to doxorubicin, was

not caused by differential changes in tumor volume; both drugs had similar effects on the tumor volume.

Dexamethasone is commonly given as a prophylactic before paclitaxel administration, to minimize any hypersensitivity reaction to cremophor. It could be argued that dexamethasone treatment is the cause of the decrease in IFP after paclitaxel. However, in the study of Kristjansen et al, in experimental tumors,<sup>21A</sup> the administration of dexamethasone between day 1 and 4 significantly reduced the IFP on day 4 and the pressure was not different from baseline on day 7. In our patients, dexamethasone was generally administered before infusion of the paclitaxel during the first 3 to 5 weekly cycles with a dose decrease each cycle if there was no evidence of allergic reaction. Our data showed that the IFP was measured more than 1 week (mean  $\pm$  standard deviation,  $15 \pm 6.0$  days) after the last paclitaxel cycle. Furthermore, dexamethasone is also used prior to each doxorubicin cycle to reduce nausea. Therefore, it is very unlikely that the decrease in IFP would be due to dexamethasone.

IFP in normal tissues and tumors is regulated by vascular permeability, lymphatic drainage, and the MVP, which is dependent on the arteriovenous pressure difference of the microcirculation and the architecture of the vascular network.<sup>22</sup> In tumors, the high vascular permeability and absence of a functional lymphatic drainage increases the accumulation of plasma proteins in the interstitium, thus increasing the oncotic pressure and IFP.<sup>23</sup> Other mechanisms, like the compression of blood vessels by tumor cell proliferation in a confined space, can reduce the diameter of blood vessels and, thus, increase the vascular resistance plus both the MVP and IFP.<sup>8,24</sup> Paclitaxel-induced apoptosis increases the diameter of tumor vessels and reduces both the MVP and IFP.<sup>8</sup> The downregulation of tumor vascular endothelial growth factor (VEGF) expression by paclitaxel<sup>25,26</sup> could also be partly responsible for the reduction in IFP, although it is not clear whether this regulation is intrinsic to the tumor cells or secondary to features of the tumor physiology. Inhibition of VEGF signaling with a blocking antibody reduces the permeability of the tumor vasculature<sup>27</sup> and the IFP in experimental tumors<sup>28</sup> and in rectal tumors in patients.<sup>29</sup> In contrast, doxorubicin can increase VEGF mRNA and vascular permeability in tumors,<sup>30</sup> which may explain the increase in IFP in tumors with pretreatment IFPs less than 5 mmHg (Fig 2, lower panel). Thus, the paclitaxel-induced reduction in tumor IFP may be a result of the decompression of blood vessels by increase in apoptosis<sup>8</sup> and a decrease in VEGF levels.<sup>29</sup>

Paclitaxel increased the  $pO_2$  significantly when used as either the first or second drug (Fig 3A and 3B). In contrast, when administered first, doxorubicin did not change the overall  $pO_2$  (Fig 1B). When doxorubicin was administered after paclitaxel, it significantly reduced the median  $pO_2$  and, thus, could not maintain the higher oxygen levels induced



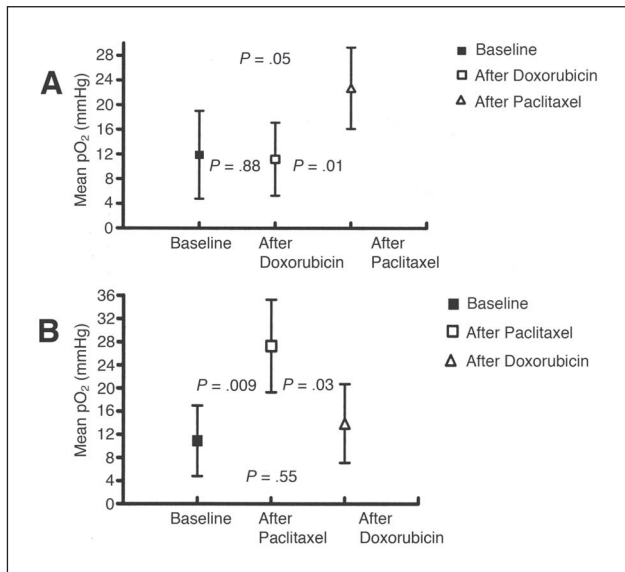
**Fig 4.** Change in mean tumor oxygen pressure ( $pO_2$ ) from baseline to after paclitaxel (upper panels) or after doxorubicin (lower panels) in individual patients. N, number of patients in each group; chemo, chemotherapy.

by paclitaxel (Fig 5A). Thus, paclitaxel was clearly more effective than doxorubicin at reducing tumor hypoxia and, therefore, maintaining normal  $pO_2$  levels in breast tumors. It should be noted that several patients did not have a third measurement because of various reasons, as mentioned in Patients and Methods. Some patients responded clinically completely to CT, and there was no tumor detected under US. Although unlikely, it is possible that biases could be present when analyzing the changes in IFP and  $pO_2$  from the second to the third measurements because of the lack of the third measurement for those patients.

Tissue oxygen levels are regulated by oxygen consumption, blood flow rate, and hemoglobin saturation in blood vessels. Based on mathematical simulations, changes in the HF of tumors are 10-fold more sensitive to changes in oxygen consumption than alterations in perfusion rate.<sup>31</sup> After paclitaxel-induced apoptosis, the improvement in tumor  $pO_2$  is most likely a result of the significant cell loss, which leads to a reduction in oxygen consumption.<sup>13</sup> In addition, paclitaxel-induced cell loss leads to blood vessel decompression and larger vascular diameters, which may also improve blood flow and oxygen delivery.<sup>8</sup> Similar to paclitaxel, doxorubicin induces tumor cell apoptosis<sup>32,33</sup>; however, it also reduces tumor blood flow,<sup>34,35</sup> which may counter-balance or negate the advantage associated with a reduction in oxygen consumption.

The lack of correlation between the changes in IFP and the changes in  $pO_2$  were striking, and although tumor cell killing and vessel decompression might seem to be important for  $pO_2$  as well IFP, there was no correlation in these observed changes. This was also found by Milosevic et al<sup>36</sup> in patients with cervical cancer. This observation would imply that exploiting the physiologic changes in a tumor for therapeutic gain should take into account which parameter is altered. Drugs with oxygen concentration–dependent cell killing would be used after the initial drug has normalized the  $pO_2$ , whereas large molecules, such as antibodies like trastuzumab, could potentially be more susceptible to the level of IFP.

A recent study by Milosevic et al<sup>36</sup> has shown that the tumor IFP can predict survival in patients with cervix cancer treated by radiation therapy, independently of other clinical prognostic factors and tumor oxygen measurements, suggesting that it is a potential useful parameter of response. A recent update<sup>37</sup> showed that the group of patients with oxic and low IFP tumors had a significantly higher disease-free survival than the other groups. Preclinical studies, with a variety of CT agents, have shown that a reduction in tumor IFP can be associated with improved blood flow and  $pO_2$  and enhanced accumulation of molecules in tumors.<sup>38,39</sup> Indeed, prostaglandin E1 and STI-571 inhibition of platelet-derived growth factor receptors decreased the



**Fig 5.** Tumor oxygen pressure ( $pO_2$ ) in patients who had three measurements available (baseline, after first drug, and after second drug) in patients who received (A) doxorubicin followed by paclitaxel (group 1) or (B) paclitaxel followed by doxorubicin (group 2). For group 1, the difference between baseline and after second drug was significant ( $P = .05$ ); for group 2, it was not significant ( $P = .55$ ).

IFP and enhanced the uptake of the low molecular mass compound 51 chromium-EDTA in tumors in rats.<sup>38,40</sup> In these studies, the exact mechanism responsible for the decrease in IFP or the enhanced accumulation of small molecules was not identified. The reduction in tumor IFP with paclitaxel could also be associated with an enhanced penetration of small molecules or drugs into tumors.

The hypothesis raised by our study is that tumors with a decrease in IFP and an increase in  $pO_2$ , obtained after the initial drug, would ultimately have a better overall response because of the improved penetration of the second drug and the improved cytotoxicity of oxygen-dependent drugs (ie, doxorubicin and cyclophosphamide). It is possible that hypoxic tumors and/or tumors with high IFP could be selected to start with paclitaxel CT to improve the physiologic status, and then other drugs could be administered to maximize the overall response. The individualization of preoperative CT sequencing in the treatment of patients with breast cancer based on tumor physiology could potentially improve the tumor response to CT and ultimately improve survival.

Our data shows that paclitaxel decreases the IFP and increases the  $pO_2$  significantly in patients with palpable breast tumors. In contrast, the overall effect of doxorubicin on the IFP and the  $pO_2$  was not significant. Doxorubicin significantly decreased the  $pO_2$  in tumors exposed to paclitaxel. These changes were independent of the tumor response to both drugs as evaluated by US. This study was not

powered to demonstrate a significant difference in clinical outcome based on the sequencing of drugs, but it could be a hypothesis-generating study to evaluate the various sequencing of CT drugs in the neoadjuvant CT setting to maximize the tumor response and ultimately improve survival. In the future, tailored treatment protocols could be designed for individuals, based on pretreatment characteristics or changes in the physiologic profile of their tumors during therapy, to improve the tumor response to treatment. These data suggest that tumors with high initial IFP and/or low initial  $pO_2$  would be better treated with paclitaxel first to reduce the IFP and increase the  $pO_2$  and, thus, improve the drug delivery of subsequent CT in particular large molecules such as trastuzumab. Knowledge of the tumor pressure and  $pO_2$  throughout the course of therapy might allow the selection and scheduling of anticancer treatments selectively cytotoxic to oxygenated cells as well as hypoxic cell cytotoxins. If the hypothesis that tumors with high IFP have a better clinical outcome when agents such as paclitaxel are used first can be substantiated by this study, then the concept of physiologic monitoring of a tumor during therapy could become a useful adjunct in a world where greater individualization of therapy is becoming more widely accepted as a means to improve cancer treatment.

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