

Optical imaging of breast cancer oxyhemoglobin flare correlates with neoadjuvant chemotherapy response one day after starting treatment

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Approximately 8–20% of breast cancer patients receiving neoadjuvant chemotherapy fail to achieve a measurable response and endure toxic side effects without benefit. Most clinical and imaging measures of response are obtained several weeks after the start of therapy. Here, we report that functional hemodynamic and metabolic information acquired using a noninvasive optical imaging method on the first day after neoadjuvant chemotherapy treatment can discriminate nonresponding from responding patients. Diffuse optical spectroscopic imaging was used to measure absolute concentrations of oxyhemoglobin, deoxyhemoglobin, water, and lipid in tumor and normal breast tissue of 24 tumors in 23 patients with untreated primary breast cancer. Measurements were made before chemotherapy, on day 1 after the first infusion, and frequently during the first week of therapy. Various multidrug, multicycle regimens were used to treat patients. Diffuse optical spectroscopic imaging measurements were compared with final postsurgical pathologic response. A statistically significant increase, or flare, in oxyhemoglobin was observed in partial responding ($n = 11$) and pathologic complete responding tumors ($n = 8$) on day 1, whereas nonresponders ($n = 5$) showed no flare and a subsequent decrease in oxyhemoglobin on day 1. Oxyhemoglobin flare on day 1 was adequate to discriminate nonresponding tumors from responding tumors. Very early measures of chemotherapy response are clinically convenient and offer the potential to alter treatment strategies, resulting in improved patient outcomes.

in vivo imaging | near-infrared | diffuse optics | tissue spectroscopy | therapeutic monitoring

An increasing number of patients diagnosed with locally advanced breast cancer undergo preoperative neoadjuvant chemotherapy (NAC) (1). Although disease-free and overall survival is approximately identical compared with postoperative therapy, primary NAC has been shown to downstage tumor grade and reduce tumor volume, leading to more breast-conserving surgeries (2–4). Patients who experience a pathological complete response (pCR) are associated with longer disease-free and overall survival (2, 5, 6). Unfortunately, between 8% and 20% of patients will have no clinical or pathologic response and will not benefit from months of treatment (2, 7). Noninvasive markers to predict response very early during therapy would help physicians make evidence-based changes to treatment strategies, potentially minimizing side effects and maximizing therapeutic outcome.

Current methods for measuring response, including palpation, mammography, ultrasound, and MRI, have shown limited success (8–11). These methods largely rely on anatomic information, which is generally insensitive to early, functional changes caused by chemotherapy. Alternatively, functional imaging technologies, including contrast-enhanced MRI, magnetic reso-

nance spectroscopy, and 18F-fluorodeoxy-glucose PET (FDG-PET), have shown some predictive capability by monitoring tumor metabolism or perfusion during treatment (12–18). For example, reductions in tumor FDG uptake measured after one cycle of chemotherapy have been shown to be predictive of a favorable outcome (19). Practical limitations, however, may prevent these techniques from being widely applied in clinical practice for this use (20). Current functional imaging modalities require exogenous contrast agents that may be poorly tolerated by some patients and are performed at significant expense (20, 21).

Diffuse optical spectroscopic imaging (DOSI) provides quantitative, functional information from breast tumors in a noninvasive manner using multiwavelength near IR light. Tissue concentrations (ct) of oxygenated hemoglobin (ctO₂Hb), deoxygenated hemoglobin (ctHHb), water (ctH₂O), and lipids as well as tissue oxygen saturation are measured using tomographic imaging instruments (22, 23) and hand-held probes (24–26). Because near IR light is nonionizing, it is possible to use DOSI to monitor physiological changes on a frequent basis without exposing tissue to potentially harmful radiation.

Laboratory and commercial DOSI instruments have been used to characterize both normal and tumor breast tissue (25, 27–29), breast tissue metabolic changes after breast core biopsy (30), and response to neoadjuvant chemotherapy (22, 31–37). Previous neoadjuvant studies have focused on complete pathologic (pCR) response as a primary clinical endpoint and have shown that reductions in ctO₂Hb, ctHHb, and ctH₂O, apparent as early as the first week of primary therapy and continuing until surgery, are predictive of pathologic response (32, 35). These response metrics seem to be consistent between tumors despite chemotherapy regimen and mechanism of action (38).

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Conflict of interest statement: B.T. and A.C. report patents, which are owned by the University of California, that are related to the technology and analysis methods described in this study. The diffuse optical spectroscopic imaging instrumentation used in this study was constructed in a university laboratory using federal grant support (National Institutes of Health). The University of California has licensed diffuse optical spectroscopic imaging technology and analysis methods to two companies, FirstScan, Inc. and Volighten, Inc., for different fields of use, including breast cancer (FirstScan, Inc.). This research was completed without participation, knowledge, or financial support of either company, and data were acquired and processed from patients by coauthors unaffiliated with either entity. The Institutional Review Board and Conflict of Interest Office of the University of California, Irvine, have reviewed both patent and corporate disclosures and did not find any concerns.

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We present here clinical evidence that functional measurements made with DOSI observed the first day after administration of preoperative chemotherapy are predictive of therapy response in patients with primary breast cancer. In contrast to previous NAC studies, we focus here on identifying nonresponding (NR) patients within 1 d of their first infusion. Although the clinical endpoint NR has not been studied as extensively as pCR, the ability to identify these patients very early in treatment has unique implications for rapidly informing treatment strategy alterations.

Results

Table S1 indicates subject and tumor characteristics; 8 of 24 tumors achieved pCR, 11 tumors were classified as partial response (PR), and 5 tumors were classified as NR.

Responding Tumors Show a Flare in ctO₂Hb on Day 1 of Treatment. To determine time points at which diagnostically relevant functional changes occur during the first week of treatment, values of oxyhemoglobin, deoxyhemoglobin, water, and lipids were analyzed in subjects who were measured at least 3 d during the first week of treatment. **Table S1** indicates the 17 subjects who met this criterion, and it includes four NR, seven PR, and six pCR tumors. In these subjects, the percent change from baseline of ctO₂Hb was the only optical metric that discriminated responding subjects from nonresponding subjects, and the maximum difference in the mean oxyhemoglobin values between responders and nonresponders occurred on day 1 after the start of therapy. Fig. 1 shows the observed mean percent change from baseline of ctO₂Hb over the first week in responding and nonresponding groups.

Generalized estimating equations (GEE) models that incorporated therapy response, treatment regimen, measurement day, and interaction terms were used to fit the outcomes of oxyhemoglobin, deoxyhemoglobin, water, and lipid changes from baseline. Tumor and normal tissue measurements were modeled separately over the first 7 d of treatment for all 23 subjects. Sensitivity analyses were performed to assess the effect of outlying data points on the model outcomes (**SI Results**). Mean values for percent change from baseline and 95% confidence intervals for the means as predicted by the models for oxyhemoglobin in both tumor and normal tissue are shown in **Table 1**. All other outcomes are shown in **Table S2** and **Table S3**. At day 1, the differences between the predicted means for NR vs. PR (68.0%) and NR vs. pCR (68.9%) in oxyhemoglobin were larger than corresponding differences at other measurement days, and the 95% confidence intervals for the NR group did not overlap those intervals computed for the PR and pCR groups. The

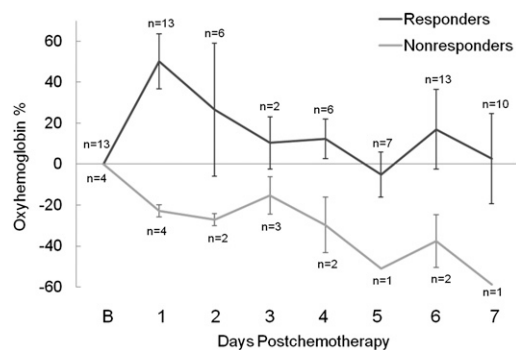


Fig. 1. Percent change in ctO₂Hb during the first 7 d of chemotherapy in responding and nonresponding tumors. The number of tumors measured at each day is indicated. The maximum separation between these groups occurred on day 1. Error bars represent SE.

strongest statistical significance for any of the outcomes was found for oxyhemoglobin at day 1 for NR vs. PR (nominal P value = 2.0×10^{-12} , multiple comparisons corrected P value = 2.7×10^{-11}) and NR vs. pCR (nominal P value = 2.5×10^{-6} , corrected P value = 3.6×10^{-5}).

Treatment type (cytotoxic, cytotoxic and bevacizumab, or cytotoxic and trastuzumab) did not contribute information to the models of percent change from baseline in tumor oxyhemoglobin (score statistic P value = 0.38), deoxyhemoglobin (P = 0.28), lipids (P = 0.069), or water (P = 0.46). Additionally, histology (invasive ductal carcinoma vs. invasive lobular carcinoma), Scarff–Bloom–Richardson grade, c-erbB2 status, estrogen receptor status, progesterone receptor status, age, and body mass index did not show any significant effects on the models (score statistic $0.071 < P < 0.99$ for all) except for age in the outcome of lipids (score statistic P value = 0.039).

In contrast to the tumor tissue, models of normal tissue revealed that the NR group was not simultaneously statistically different from both the PR and pCR groups for any of the measurement days for any outcome. To further explore the paired tumor and normal measurements, the difference between the percent change from baseline for the normal breast and the percent change from baseline for the tumor breast tissue was computed and used as the outcome variable for a GEE model. A near zero estimate for this outcome variable is expected if paired normal and tumor tissue experience the same trends (both in time and magnitude). For oxyhemoglobin, deoxyhemoglobin, water, and lipids, there were multiple measurement days in which the outcome variable was statistically different from zero, which was indicated by the confidence intervals of estimates (**Table S4**). Despite this result, it is still possible that normal measurements mimicked the temporal behavior of tumor measurements but at a reduced magnitude. For example, for the model outcome of oxyhemoglobin in normal tissue, there was an increase in the PR and pCR groups on day 1 (26.2% and 8.5%, respectively) (**Table 1**) and a decrease in the NR group (−4.3%), although a pairwise analysis between response groups did not indicate statistically significant differences after correcting for multiple comparisons.

Because the most significant differences between response groups occurred on day 1 after treatment, which was indicated by the GEE analysis, the following results focus on this time point. **Fig. 2** shows the observed mean percent change of ctO₂Hb, ctHHb, water, and lipids in all 23 study subjects (24 tumors) on day 1. An increase in ctO₂Hb was observed in both PR (44.5% ± 46.1% SD) and pCR (41.4% ± 39.1% SD) groups, and a decrease was observed in the NR (−22.5% ± 5.10% SD) group. This trend was mirrored in the contralateral normal measurements, with increases in ctO₂Hb in the PR (22.6% ± 43.1% SD) and pCR (12.6% ± 22.0% SD) groups and only small deviations from baseline in the NR (−0.4% ± 9.7% SD) group. **Fig. 3** shows absolute molar concentrations of ctO₂Hb in tumors at baseline and day 1 for NR, PR, and pCR groups.

Observed values of tumor ctHHb change were lowest in the NR group (−21.9% ± 17.1% SD) and trended higher in the PR (−5.6% ± 27.5% SD) and pCR groups (9.8% ± 39.3% SD). No trend was observed in ctHHb in contralateral normal tissue, and mean percent change at day 1 was close to zero for all three response groups. Values of water and lipids were not useful to discriminate response groups on day 1 of treatment.

Fig. 4 shows representative ctO₂Hb maps at baseline and day 1 for three different study subjects, one NR, one PR, and one pCR. All of the maps show a 6 × 6-cm measurement area that includes tumor and a surrounding normal margin. The approximate tumor location, determined by ultrasound and palpation, is indicated by a dotted circle. In each example, baseline ctO₂Hb values in the region corresponding to tumor were elevated over

Table 1. Mean values and 95% confidence limits for percent change from baseline for 7 d after the start of chemotherapy in oxyhemoglobin in tumor and normal tissue as predicted by a GEE model

Day	pCR (n = 8)	PR (n = 11)	NR (n = 5)
Tumor			
1	44.25 (17.89, 70.6)	43.42 (24.12, 62.71)	-24.61 (-34.71, -14.51)
2	-1.4 (-20.46, 17.67)	22.76 (-8.43, 53.96)	-30.22 (-42.86, -17.59)
3	23.01 (6.63, 39.4)	14.81 (-14.59, 44.21)	-20.63 (-41.6, 0.34)
4	-7.52 (-46.74, 31.7)	24.75 (-13.23, 62.72)	-29.22 (-46.29, -12.15)
5	-15.68 (-40.26, 8.9)	16.6 (-17.3, 50.5)	-41.62 (-55.48, -27.77)
6	-4.05 (-21.81, 13.71)	26.2 (-19.9, 72.3)	-37.78 (-55.63, -19.94)
7	-21.55 (-44.96, 1.85)	1.21 (-40.13, 42.54)	-49.38 (-63.24, -35.52)
Normal			
1	8.5 (-7.85, 24.85)	26.19 (-2.57, 54.96)	-4.33 (-10.5, 1.83)
2	-2.43 (-15.09, 10.23)	3.17 (-11.28, 17.61)	-14.42 (-25.79, -3.05)
3	4.57 (-2.03, 11.17)	-9.74 (-24.19, 4.7)	-13.2 (-22.7, -3.7)
4	-15.05 (-30.15, 0.05)	19.39 (-12.92, 51.7)	-19.78 (-32.3, -7.26)
5	-13.62 (-32.85, 5.6)	7.71 (0.62, 14.8)	-25.33 (-29.2, -21.46)
6	-3.09 (-17.57, 11.39)	6.12 (-8.05, 20.29)	-25.01 (-31.05, -18.97)
7	-16.23 (-36.71, 4.26)	-11.19 (-24.51, 2.14)	-29.78 (-33.65, -25.91)

the surrounding normal tissue, an observation that was previously shown (25).

ctO₂Hb Magnitude and Spatial Extent Discriminate Nonresponders on Day 1 of Therapy. Fig. 5 *Left* shows a scatter plot of tumor ctO₂Hb change from baseline. Perfect separation of NR tumors from both PR and pCR tumors is achieved using the single feature of ctO₂Hb change at day 1.

The spatial extent of elevated oxyhemoglobin was calculated for 16 subjects with sufficiently large measurement areas. The area of elevated ctO₂Hb expanded in PR (57.4% ± 27.7% SD) and pCR (47.7% ± 33.6% SD) subjects and decreased in NR subjects (-33.4% ± 30.3% SD). The change in ctO₂Hb spatial extent is shown in Fig. 5 *Middle*. When magnitude and spatial extent were combined into a single metric, PR subjects experienced a 139.0% ± 114.3% SD increase, pCR subjects experienced an 88.3% ± 62.5% SD increase, and NR subjects experienced a -47.3% ± 23.1% SD decrease in this metric. This combined metric was able to perfectly discriminate NR from PR and pCR, and this finding is shown in Fig. 5 *Right*.

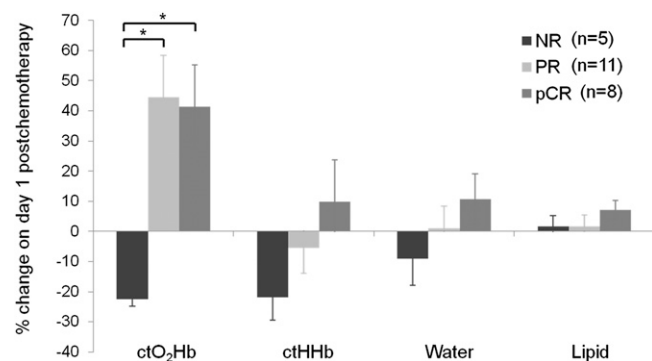


Fig. 2. Percent change in ctO₂Hb, ctHb, water, and lipids on day 1 compared with baseline. Based on longitudinal GEE models, statistically significant differences, noted with asterisks, were found for NR vs. PR (nominal P value = 3.6×10^{-16} , multiple comparisons corrected P value = 5.0×10^{-15}) and NR vs. pCR (nominal P value = 1.6×10^{-13} , corrected P value = 2.2×10^{-12}), which were adjusted for differences in tissue type and treatment. Error bars represent SE.

Discussion

We have shown that significant functional changes occur after the first day of preoperative chemotherapy and that these changes are correlated with therapy response. The presence of a flare (increase in magnitude and spatial extent above baseline) in oxyhemoglobin values was indicative of either a partial clinical response or a pathologic complete response. These two patient groups represent individuals who are likely to benefit from improved rates of breast-conserving surgery and/or improved overall and disease-free survival (2–4, 6).

Zhou et al. (33), in collaboration with our group, previously published a case study in which early vascular response was observed in a patient receiving neoadjuvant cytotoxic therapy who had achieved a partial overall response. A transient increase followed by a decrease in blood flow and metabolic rate of oxygen consumption measured using diffuse correlation spectroscopy and DOSI was observed on day 3 of therapy (this subject was not measured on day 1 or 2 of therapy). Others have shown similar findings of early functional changes in animal models using FDG-PET. In three separate studies, an early transient

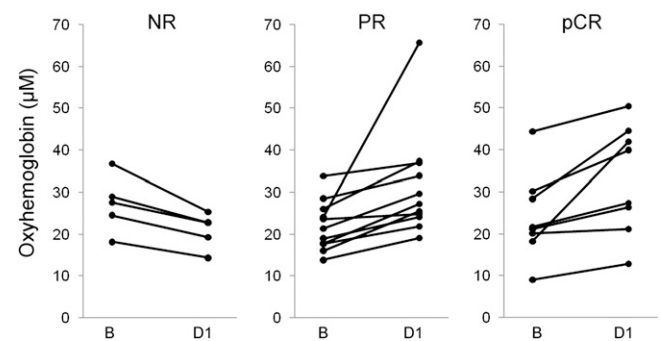


Fig. 3. Absolute values of tumor ctO₂Hb at baseline and day 1 for the different response groups. Baseline and day 1 values in the NR group were 27.2 μM (±6.8 SD) and 20.9 μM (±4.3 SD), respectively, and this represents an average change of -22.5%. Baseline and day 1 values in the PR group were 22.0 μM (±5.9 SD) and 31.5 μM (±12.8 SD), respectively, with an average change of 44.5%. Baseline and day 1 values in the pCR group were 24.1 μM (±10.4 SD) and 33.1 μM (±13.0 SD), respectively, with an average change of 41.4%.

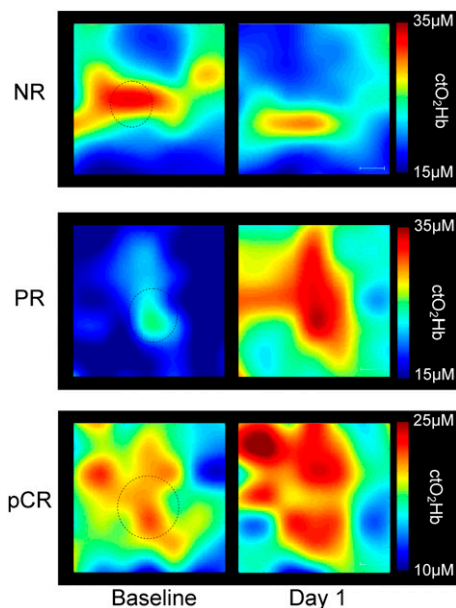


Fig. 4. ctO_2Hb maps from three different subjects at baseline and day 1 after the start of neoadjuvant chemotherapy. Each map shows a 6×6 -cm measurement area that includes the tumor and a surrounding normal margin. (Scale bar: 1 cm.) The circles represent the location and approximate anatomic size of the tumors determined by ultrasound. (Top) An example of a 17-mm tumor that did not respond to chemotherapy. Mean tumor ctO_2Hb dropped 21.6% at day 1, and spatial extent decreased by 54.3%. (Middle) An example of a partial response. Tumor was 20 mm before chemotherapy. Mean tumor ctO_2Hb increased 53.1% at day 1, and spatial extent increased by 142.1%. (Bottom) An example of a pathologic complete response. Tumor was 30 mm before chemotherapy. Mean tumor ctO_2Hb increased 5.6% at day 1, and spatial extent increased by 4.5%.

increase in tumor FDG uptake was observed during the first week after chemotherapy, independent of tumor growth. This observation was followed by a rapid decrease in FDG uptake and subsequent tumor regression (39–41). The cause of the observed flare was uncertain; it was hypothesized that exposure to chemotherapy transiently induced glucose hypermetabolism in tumors, and cancer cells, inefficiently adapted to the induced metabolic stress, underwent apoptotic cell death through metabolic catastrophe (42, 43).

The biologic origin of the oxyhemoglobin flare in responding subjects observed in this study may stem from one or both of the following factors: (i) a rapid decrease in cellular metabolism caused by cytotoxic chemotherapy-induced cell damage and a subsequent decrease in oxyhemoglobin conversion to deoxy-

hemoglobin, and/or (ii) increased perfusion to tissue. Previous investigations have shown significant apoptotic activity and decreased proliferation within 24 h after anthracycline therapy (44–46). A drop in deoxyhemoglobin would also be expected to accompany decreased metabolism; however, only a small drop was observed in the PR group, and an increase was observed in the pCR group.

The response may be better explained by perfusion changes caused by an acute inflammatory response induced by cell damage and death. Previous studies have shown that clinical outcome in breast cancer patients is associated with elevations of proinflammatory serum biomarkers after taxane treatment (47–50). Tumor cell exposure to anthracyclines and some platin drugs has been shown to induce immunostimulatory apoptosis and presentation of damage-associated molecular patterns, including calreticulin, heat-shock proteins, and high-mobility group box 1 proteins, before, during, or after apoptosis (51–54). These damage-associated molecular patterns signal the release of proinflammatory cytokines and inflammatory molecules, including IL-1, IL-6, TNF α , nitric oxide, and histamine, which stimulate innate and adaptive immunity and induce vascular changes. The vascular hallmarks of acute inflammation include increased vascular permeability and vessel dilation, which transiently increases perfusion to the injured area over the course of hours to days (55–57). Although DOSI does not directly measure blood flow, the concomitant increase in total hemoglobin observed during oxyhemoglobin flare does suggest an increase in perfusion.

NR patients may not elicit the same immune response because of a higher proportion of chemoresistant or nonimmunostimulatory tumor cells. Additionally, NR patients may have severely limited perfusion and be especially devoid of vascular reactivity because of loss of smooth muscle cell coverage and innervations (58). This loss could also inhibit drug-induced cell/vascular damage and subsequent inflammatory flare.

Measurements from the contralateral normal breasts support the idea of a systemic or tissue-specific vascular/inflammatory response. Although not statistically significant, responding subjects seemed to exhibit an oxyhemoglobin flare in the contralateral normal breast, whereas nonresponding subjects showed almost no change at day 1. The magnitude of the flare in the normal breast was reduced compared with tumor measurements. The magnitude of oxyhemoglobin flare in both tumor and contralateral normal tissue may reflect individual subject vascular and immune responsiveness to cellular stress, which might be closely related to chemosensitivity. It is also of note that, because DOSI measurements represent the average optical properties of the measurement volume, it is possible that surrounding normal tissue may contribute to the observed flare in tumor locations.

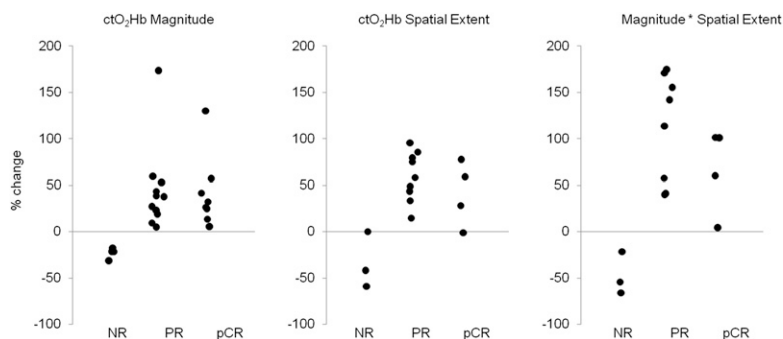


Fig. 5. Percent change in ctO_2Hb magnitude (concentration), spatial extent, and magnitude \times spatial extent for NR, PR, and pCR tumors. In all three cases, perfect separation was achieved between nonresponding and responding tumors.

This study is limited because of the small number of subjects and lack of additional confirmatory methods. The additional measurements of glucose metabolism, blood flow, and inflammatory markers would help to elucidate the underlying biological basis of flare response. Another limitation of this study is that several different therapy regimens were explored. It is of note, however, that the flare reaction seemed to be indicative of response regardless of age, body mass index, and tumor subtypes, and no differences were seen between treatment regimens. It should be emphasized that this study was retrospective and correlative in nature, and future prospective validations are necessary to confirm the predictive nature of oxyhemoglobin flare.

In conclusion, we observed a significant tumor ctO₂Hb flare reaction on the first day after neoadjuvant chemotherapy in responding subjects. This flare was adequate to perfectly discriminate nonresponding subjects from both partial and pathologic complete responders. Furthermore, the majority of responding subjects showed ctO₂Hb flare in the contralateral normal breast, whereas nonresponding subjects did not. The implementation of these measurements in the clinic may be used to monitor and strategically alter treatment strategies for breast cancer patients by rapidly identifying likely nonresponders. This implementation could lead to the development of new approaches for managing patients and expediently assessing chemosensitivity for individual subjects.

Materials and Methods

A detailed description of materials and methods is available in *SI Materials and Methods*.

Briefly, DOSI, which is a near IR optical imaging technique, was used to measure tissue concentrations of oxyhemoglobin, deoxyhemoglobin, water, and lipids in breast cancer patients undergoing neoadjuvant chemotherapy (Fig. S1). Changes in the magnitude and spatial dynamics of these quantities in tumor and normal breast tissue were statistically compared with overall response to chemotherapy in 23 subjects with 24 tumors. The GEE method was used to analyze the correlation between values for oxyhemoglobin, deoxyhemoglobin, water, and lipids measured on different days for individual subjects. Treatment response was stratified into a tertiary classification scheme of pCR, PR, and NR. Subjects with no residual carcinoma after therapy were considered pCR. Subjects with a 50% or greater reduction in tumor size determined from the maximum tumor dimensions were considered PR, and subjects with a less than 50% reduction were considered NR. All subjects provided informed consent and participated in this study under a clinical protocol approved by the Institutional Review Board at the University of California, Irvine (02-2306).

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