Supporting Information

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SI Materials and Methods

Diffuse Optical Spectroscopic Imaging Instrumentation. Design details and concepts of the diffuse optical spectroscopic imaging (DOSI) system are described elsewhere (1–3). Briefly, the instrument uses both frequency domain and continuous wave (CW) spectroscopy measurements in the near IR spectrum (650–1,000 nm) to determine the tissue optical scattering and absorption properties. Six diode laser sources (660, 680, 780, 810, 830, and 850 nm) are used for frequency domain illumination and are intensity-modulated between 50 and 500 MHz. Amplitude and phase of the detected signals are input into an analytical model of diffuse light transport to determine tissue scattering and absorption coefficients at these wavelengths. White light illumination is used for continuous wave spectroscopy; detected reflectance spectra are fit and scaled to frequency domain measurements so that absorption is determined continuously over the entire spectral range. Absolute tissue concentrations are calculated by using the Beer–Lambert law and known extinction coefficient spectra of deoxyhemoglobin (HHb), oxyhemoglobin (HbO_2), water, and lipids.

A handheld probe is used to acquire measurements in subjects. The probe housing contains frequency domain, CW illumination fibers, CW detection fiber, and avalanche photodiode for frequency domain detection. In breast tissue, the DOSI instrument measures tissue properties between 1 and 5 cm below the skin. Measurements represent average optical properties for the measurement tissue volume, typically several centimeters cubed.

Subject Measurements. This study is a retrospective analysis conducted in early 2010 of a subset of subjects with newly diagnosed, operative, primary breast cancer measured with DOSI during their neoadjuvant chemotherapy treatment between 2005 and 2009. Because there was almost no data on chemotherapy monitoring with optical techniques before the initiation of this study, attempts were made to make exploratory measurements of subjects as frequently as possible during their treatment. The 23 subjects included in this study are those subjects who were measured with DOSI at a minimum of baseline and day 1 after their first infusion, and 17 patients were measured at least three times during their first week of treatment. One subject has bilateral disease, and therefore, a total of 24 tumors was monitored. 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). Exclusion criteria included pregnant women and women who were less than 21 y old or more than 75 y old. All subjects were histologically diagnosed with invasive carcinoma before neoadjuvant treatment. Estrogen receptor (ER), progesterone receptor (PrR), and c-erbB2 (HER2) were immunohistochemically assessed from core biopsy. Positive HER2 status was confirmed using FISH analysis.

All subjects received neoadjuvant chemotherapy before surgical resection of tumors and were measured with the DOSI system before treatment (to establish a baseline measurement), 1 d after the start of treatment, and as many days as possible in the remaining first 7 d of treatment. Based on our previous findings, baseline measurements were obtained at least 10 d after diagnostic biopsies to minimize their impact on DOSI scans (4). Subjects were measured in a supine position. The DOSI probe was placed against the breast tissue, and sequential measurements were taken in a linear or rectangular grid pattern using 10-mm spacing. Measurements were taken to include the area of the underlying tumor determined by ultrasound and palpation as well as a margin of surrounding normal tissue. Contralateral normal breast measurements were collected from subjects with unilateral breast cancer.

Total measurement time varied between 20 min and 1 h per subject. Molar concentrations (ct) of oxyhemoglobin (ctO_2Hb), deoxyhemoglobin (ctHHb), water, and lipids were calculated at each measurement point. Maps (images) of oxyhemoglobin, deoxyhemoglobin, water, and lipids were constructed by a linear interpolation between measurement points. Repeat DOSI scans have been shown previously to be relatively insensitive to probe contact pressure fluctuations, displaying less than 5% average variation in test–retest studies of human subjects (5).

Fig. S1 shows a typical DOSI map created from discrete measurement points taken every 10 mm in a grid pattern over an 8 × 5-cm area of tissue containing a 34-mm invasive ductal carcinoma (IDC). This map shows a composite optical index of ctHHb, water, and lipids that has been previously shown to be useful for identifying tumors, and it is termed the tissue optical index (TOI) (6). The resulting map shows increased optical contrast over the tumor. Note that the DOS image shows tissue optical properties in the x–y plane (i.e., *en face*), whereas ultrasound shows x–z anatomic features.

Neoadjuvant Chemotherapy Regimen. The focus of this study concerned tumor functional changes that occurred after the first chemotherapy infusion; 20 of 23 subjects received doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2; AC therapy) at their first infusion. Of the remaining three subjects, two received paclitaxel, carboplatin, and trastuzumab (Pac+Carb+Her) at first infusion, and one received paclitaxel, carboplatin, and bevacizumab (Pac+Carb+Bev) at first infusion. Details of treatments are described below and in Table S1.

Twenty of twenty-three subjects received AC therapy i.v. every 14 d for two to four cycles. This treatment was followed three to four cycles of weekly paclitaxel [80 mg/m^2; either cremophore-bound or albumin-bound (nab-paclitaxel)] and carboplatin (Pac+Carb). Subjects with positive HER2/neu status received concurrent trastuzumab therapy at a 4-mg/kg loading dose followed by a maintenance dose of 2 mg/kg weekly for 10–12 cycles. Seven subjects with negative HER2/neu status received a regimen of concurrent Pac+Carb combined with bevacizumab (10 mg/kg every 2 wk for five to six cycles). Seven subjects received pegfilgrastim support s.c. 24 h or later after the first chemotherapy dose and after day 1 DOSI measurements.

Briefly, these chemotherapy regimens are indicated as follows: AC alone (n = 1), AC followed by Pac+Carb (AC→Pac+Carb; n = 7), AC followed by Pac+Carb and trastuzumab (AC→Pac+Carb+Tras; n = 6), AC followed by Pac+Carb and bevacizumab (AC→Pac+Carb+Bev; n = 6), concurrent Pac+Carb and bevacizumab (Pac+Carb+Bev; n = 1), and concurrent Pac+Carb and trastuzumab (Pac+Carb+Tras; n = 2).

Criteria of Treatment Response. Treatment response criteria were similar to those criteria defined in the National Surgical Adjuvant Breast and Bowel Project protocol (7). Baseline tumor size was determined by clinical ultrasound or MRI dependent on availability. Final assessment of pathologic therapeutic response in breast tumor was determined from standard pathology. The histological response in the resected lymph nodes was not evaluated for treatment response. Criteria of treatment response

were previously described (6). Briefly, treatment response was stratified into a tertiary classification scheme of pathologic complete response (pCR), partial response (PR), and no response (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 dimension were considered PR, and subjects with a less than 50% reduction were considered NR.

Analysis. To determine the change in oxyhemoglobin, deoxyhemoglobin, water, and lipids over the first week of treatment, the mean values of these quantities inside a region corresponding to the tumor were computed. This region was determined based on ultrasound, local increases in ctHHb and water, and decreases in lipids. This combination of metrics, designated as the TOI, has been previously shown to be a consistent indicator of tumor location (6). Mean values were also computed from contralateral normal breast measurements. Absolute and percent changes in ctO_2Hb, ctHHb, water, and lipids over the first week of treatment were statistically compared with their baseline values.

It is important to note that, because DOSI is not a tomographic instrument such as MRI or PET, surface measurements represent average tissue properties over a large volume, typically 10 cm^3, for the probe geometry used (8). This property of DOSI means that even measurements taken over a known tumor location include properties that may be averaged between tumor and surrounding tissue. Although previous studies have shown that DOSI measurements do produce sufficient contrast to localize tumors (9, 10), it is not possible at this time to fully separate the contributions of tumor tissue and immediately adjacent normal tissue on the dynamic changes observed in this study.

It was noted that, over the first week of treatment, changes occurred in both the magnitude and the spatial extent of elevated oxyhemoglobin values in the tumor region. To quantify the spatial expansion or contraction of these values, the number of discrete measurement points with values above a set threshold was computed at each measurement date for each subject. The threshold was calculated from the baseline measurement as the mean value in the normal tissue surrounding the tumor. If the number of measurement points above this threshold increased at subsequent measurement dates, then the spatial extent was determined to increase. If the number of measurement points decreased, then the spatial extent also decreased. Measurements were taken using 1-cm spacing in the x and y directions, and therefore, expansion and contraction of areas were described in units of centimeters squared and percent change from baseline.

A combined magnitude/spatial extent metric was calculated as the product of the mean tumor value and the number of measurement points above the threshold. This combined metric was compared with baseline values for each subject.

Generalized Estimating Equations. To take into account the correlation between values for oxyhemoglobin, deoxyhemoglobin, water, and lipids measured on different days for individual subjects, the generalized estimating equations (GEE) method was applied with subjects as clusters, an exchangeable correlation structure, and a normal model with an identity link function. Separate models were fit to longitudinal data with the outcome variables of oxyhemoglobin, deoxyhemoglobin, water, and lipids. Each outcome variable was represented as a percent change from baseline. Predictors included chemotherapy response (NR, PR, and pCR), treatment (cytotoxic, cytotoxic and bevacizumab, and cytotoxic and trastuzumab), and measurement day. Models were examined that included variables representing interactions between these predictors. Sensitivity analyses were performed to assess the effect of outliers on the main findings of the paper. Potential outliers were identified using cluster deletion diagnostics previously described for the GEE method in the work by Preisser and Qaqish (11). For each outcome, the studentized distance measure of the influence of the i^{th} cluster on overall model fit (MCLS statistic) was examined. For each outcome, clusters having an MCLS value above the 95th percentile for the statistic were further investigated with sensitivity analysis. Clusters were removed from the models to investigate the effect on SEs of parameter estimates. Clusters were removed if their exclusion led to a mean reduction in the SE of estimates for regression parameters, including the interaction between response group and measurement day. The statistical significance of comparisons between chemotherapy response groups for the main findings (e.g., the outcome of oxyhemoglobin on day 1 after infusion) was then compared between models including and excluding the identified outliers.

From the final GEE model for a given outcome and tissue type, the estimated percent change from baseline for the PR and pCR response groups, adjusted for covariates, was compared with the estimated change from baseline of the NR response group at each of the 7 measurement d. The Bonferroni method was applied to maintain an experimentwise significance level of 0.05, with a comparisonwise significance level of 0.00357. Additionally, for each outcome, the estimated percent change from baseline was compared between the two treatment groups at a significance level of 0.05.

Longitudinal GEE models also were fit to assess the relationship between change from baseline for each of the four outcomes and demographic and clinical variables including age, Scarff–Bloom–Richardson (SBR) grading status, histology type [IDC vs. invasive lobular carcinoma (ILC)], HER2 status, ER status, PR status, and body mass index, which were adjusted for variation in tissue type, treatment, response, and measurement day.

Finally, for patients that had measurements made on both tumor breast tissue and normal breast tissue, we computed the difference between the percent change from baseline for the normal breast tissue and the percent change from baseline for the tumor breast tissue. This difference was then treated as the outcome variable for a GEE model with predictors of response group, treatment group, measurement day, and interaction between treatment group and measurement day. Outliers were identified by examining cluster deletion diagnostics with the MCLS statistic, and sensitivity analyses were performed to determine the effect of outlying data points on the models. Comparison of tumor and normal tissue was made using the score statistic at a significance level of 0.05. These methods were applied for analysis of paired data for oxyhemoblobin, deoxyhemoglobin, lipids, and water.

One of twenty-three subjects had bilateral breast cancer; one tumor achieved a pCR, and the other achieved a PR. Because these tumors achieved different responses, they had different SBR grades, and it is known that bilateral tumors frequently have disparate biology (even in the same subject) (12), for purposes of the GEE analysis, these tumors were treated as having come from different subjects but with the same demographic and treatment information.

SI Results

Two subjects were identified as outlying clusters for the outcome of oxyhemoglobin, two subjects were identified as outlying clusters for the outcome of tumor deoxyhemoglobin, two subjects were identified as outlying clusters for the outcome for tumor lipids, and one subject each was identified as an outlying cluster for the outcomes of normal tissue oxyhemoglobin, normal tissue deoxyhemoglobin, normal tissue lipids, and normal tissue water. Outcomes of the model excluding outliers are shown in Table S3, and specific outliers are identified. The exclusion of outliers did improve the statistical significance of the main findings of the paper but did not change the overall conclusions. For example, the P values obtained comparing the difference in means for the

outcome of oxyhemoglobin change on day 1 between response groups are nominal P values with outliers for PR vs. NR = 2.0×10^{-12}, P value without outliers = 3.6×10^{-16}, nominal P values with outliers for pCR vs. NR = 2.5×10^{-6}, and P value without outliers = 1.6×10^{-13}. The results presented in the text include outliers.

1. Jakubowski D, Bevilacqua F, Merritt S, Cerussi A, Tromberg B (2009) Quantitative absorption and scattering specta in thick tissues using broadband diffuse optical spectroscopy. *Biomedical Optical Imaging*, eds Fujimoto JG, Farkas DL, (Oxford University Press), pp 330–355.
2. Bevilacqua F, Berger AJ, Cerussi AE, Jakubowski D, Tromberg BJ (2000) Broadband absorption spectroscopy in turbid media by combined frequency-domain and steady-state methods. *Appl Opt* 39:6498–6507.
3. Pham TH, Coquoz O, Fishkin JB, Anderson E, Tromberg BJ (2000) Broad bandwidth frequency domain instrument for quantitative tissue optical spectroscopy. *Rev Sci Instrum* 71:2500–2513.
4. Tanamai W, et al. (2009) Diffuse optical spectroscopy measurements of healing in breast tissue after core biopsy: Case study. *J Biomed Opt* 14:014024.
5. Cerussi A, et al. (2009) Effect of contact force on breast tissue optical property measurements using a broadband diffuse optical spectroscopy handheld probe. *Appl Opt* 48:4270–4277.
6. Cerussi A, et al. (2007) Predicting response to breast cancer neoadjuvant chemotherapy using diffuse optical spectroscopy. *Proc Natl Acad Sci USA* 104:4014–4019.
7. Rastogi P, et al. (2008) Preoperative chemotherapy: Updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. *J Clin Oncol* 26: 778–785.
8. Tromberg BJ, et al. (2005) Imaging in breast cancer: Diffuse optics in breast cancer: Detecting tumors in pre-menopausal women and monitoring neoadjuvant chemotherapy. *Breast Cancer Res* 7:279–285.
9. Cerussi A, et al. (2006) In vivo absorption, scattering, and physiologic properties of 58 malignant breast tumors determined by broadband diffuse optical spectroscopy. *J Biomed Opt* 11:044005.
10. Cerussi AE, et al. (2001) Sources of absorption and scattering contrast for near-infrared optical mammography. *Acad Radiol* 8:211–218.
11. Preisser JS, Qaqish BF (1996) Deletion diagnostics for generalised estimating equations. *Biometrika* 83:551–562.
12. Cavaliere A, Bellezza G, Scheibel M, Vitali R, Sidoni A (2004) Biopathological profile of multiple synchronous homolateral and bilateral breast cancers. *Pathol Res Pract* 200:9–12.

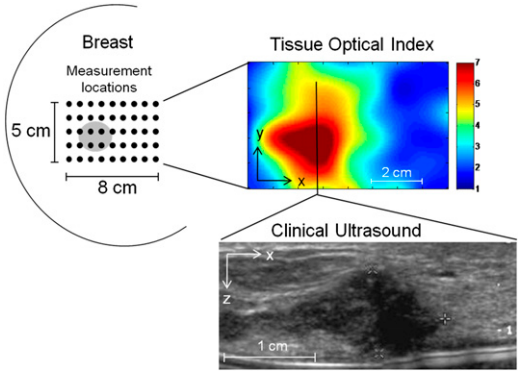

Fig. S1. Diagrammatic representation of the measurement procedure. DOSI measurements are taken in a grid or line pattern with a handheld probe in the *x–y* plane (*en face*). Measurements are taken every 10 mm over a tissue region previously determined by ultrasound and/or palpation to contain a tumor and include a surrounding normal margin. Measurements are also taken of the corresponding contralateral normal breast. In this example, an 8 × 5-cm region of tissue was measured containing a stage 2 IDC measured to be 34 mm in the greatest dimension. Maps of ctO_2Hb, ctHHb, water, and lipids are constructed from the measurement points. In this example, the map shows a composite metric termed the TOI, which is combination of ctHHb, water, and lipids; values above three are typical of tumors. A local increase in optical contrast is observed where the tumor is located. A clinical ultrasound measurement, which displays the tumor in the *x–z* plane, is shown for comparison.

Table S1. Subject characteristics and treatment regimens

Side	Age (y)	Size at max (mm)	TNM stage	Histology	SBR grade	ER	PrR	HER2	Treatment response	Treatment regimen	First day use of targeting therapy	Measured at least three times in week 1
Rt	43	40	T2N3M0	IDC	7	+	+	–	NR	AC→Pac+Carb	No	No
Rt	48	20	T1N1M0	ILC	6	+	+	–	NR	Pac+Carb+Bev	Yes	Yes
Rt	56	17	T1N0M0	IDC	4	+	+	–	NR	AC	No	Yes
Rt	57	20	T2N1M1	IDC	6	+	+	–	NR	AC→Pac+Carb	No	Yes
Rt	60	31	T2N1M0	IDC	7	–	–	+	NR	AC→Pac+Carb+Tras	No	Yes
Lt	71	34	T2N1M0	IDC	6	+	+	+	PR	Pac+Carb+Tras	Yes	Yes
Lt	55	20	T2N2M1	IDC	7	+	+	+	PR	AC→Pac+Carb+Tras	No	No
Lt	61	90	T4N2M0	IDC	8	+	+	–	PR	AC→Pac+Carb+Bev	No	No
Lt	63	60	T4N2M1	IDC	9	+	+	–	PR	AC→Pac+Carb+Bev	No	No
Rt	63	27	T2N1M0	IDC	5	ND	ND	ND	PR	AC→Pac+Carb+Bev	No	Yes
Rt	43	46	T2N0M0	ILC	6	+	–	–	PR	AC→Pac+Carb+Bev	No	Yes
Rt	33	43	T4N1M0	IDC	6	+	+	+	PR	AC→Pac+Carb+Tras	No	Yes
Lt	61	40	T2N0M0	ILC	6	+	+	–	PR	AC→Pac+Carb+Bev	No	Yes
Rt	41	38	T2N0M0	IDC	7	–	–	–	PR	AC→Pac+Carb+Bev	No	Yes
Lt	62	30	T4N2M0	IDC	7	+	+	–	PR	AC→Pac+Carb	No	Yes
Lt	41	35	T2N1M0	IDC	7	+	+	–	PR	AC→Pac+Carb	No	No
Lt	37	30	T3N1M0	IDC	ND	–	–	+	pCR	Pac+Carb+Tras	Yes	Yes
Lt	56	30	T3N1M0	IDC	7	–	–	+	pCR	AC→Pac+Carb+Tras	No	No
Lt	63	29	T2N2M0	IDC	8	+	+	–	pCR	AC→Pac+Carb+Bev	No	Yes
Rt	36	15	T1N1M0	IDC	7	+	+	–	pCR	AC→Pac+Carb	No	Yes
Lt	57	27	T2N0M0	IDC	7	+	+	+	pCR	AC→Pac+Carb+Tras	No	Yes
Lt	53	55	T3N1M0	IDC	6	–	–	+	pCR	AC→Pac+Carb+Tras	No	No
Rt	32	29	T2N1M0	IDC	8	–	–	–	pCR	AC→Pac+Carb	No	Yes
Lt	50	66	T4N2M0	ILC	ND	+	+	–	pCR	AC→Pac+Carb	No	Yes

AC, doxorubicin + cyclophosphamide; Bev, bevacizumab; Pac, paclitaxel; Carb, carboplatin; Tras, trastuzumab; ER, estrogen receptor; PrR, progesterone receptor; HER2, c-erbB2; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; Lt, left; Rt, right; ND, not described; NR, no response; PR, partial response; pCR, pathological complete response; SBR, Scarff–Bloom–Richardson score.

Table S2. Mean values and 95% confidence limits for percent change from baseline for 7 d after the start of chemotherapy in tumor and normal tissue for all outcomes as predicted by GEE models

		Tumor			Normal		
Outcome	Days	pCR	PR	NR	pCR	PR	NR
	1	44.25 (17.89, 70.6)	43.42 (24.12, 62.71)	−24.61 (−34.71, −14.51)	8.5 (−7.85, 24.85)	26.19 (−2.57, 54.96)	−4.33 (−10.5, 1.83)
	2	−1.4 (−20.46, 17.67)	22.76 (−8.43, 53.96)	−30.22 (−42.86, −17.59)	−2.43 (−15.09, 10.23)	3.17 (−11.28, 17.61)	−14.42 (−25.79,3.05)
	3	23.01 (6.63, 39.4)	14.81 (−14.59, 44.21)	−20.63 (−41.6, 0.34)	4.57 (−2.03, 11.17)	−9.74 (−24.19, 4.7)	−13.2 (−22.7, −3.7)
ctO_2Hb	4	−7.52 (−46.74, 31.7)	24.75 (−13.23, 62.72)	−29.22 (−46.29, −12.15)	−15.05 (−30.15, 0.05)	19.39 (−12.92, 51.7)	−19.78 (−32.3, −7.26)
	5	−15.68 (−40.26, 8.9)	16.6 (−17.3, 50.5)	−41.62 (−55.48, −27.77)	−13.62 (−32.85, 5.6)	7.71 (0.62, 14.8)	−25.33 (−29.2, −21.46)
	6	−4.05 (−21.81, 13.71)	26.2 (−19.9, 72.3)	−37.78 (−55.63, −19.94)	−3.09 (−17.57, 11.39)	6.12 (−8.05, 20.29)	−25.01 (−31.05, −18.97)
	7	−21.55 (−44.96, 1.85)	1.21 (−40.13, 42.54)	−49.38 (−63.24, −35.52)	−16.23 (−36.71, 4.26)	−11.19 (−24.51, 2.14)	−29.78 (−33.65, −25.91)
	1	15.1 (−13.92, 44.12)	−10.2 (−22.08, 1.67)	−20.22 (−31.46, −8.97)	−16.37 (−29.94, −2.79)	8.2 (−30.53, 46.92)	−11.82 (−17.86, −5.77)
	2	−13.83 (−23.56, −4.11)	−9.61 (−30.01, 10.79)	−20.31 (−35.84, −4.78)	−17.23 (−30.26, −4.19)	−12 (−29.04, 5.04)	−13.38 (−18.3, −8.46)
	3	−5.8 (−19.05, 7.45)	−54.69 (−73.99, -35.38)	−4.3 (−31.87, 23.28)	−13.91 (−22.18, −5.64)	−35.77 (−52.81, −18.73)	−11.43 (−19.6, −3.25)
ctHHb	4	17.73 (−8.4, 43.86)	13.28 (−7.99, 34.55)	−24.44 (−36.46, −12.42)	6.36 (−5.18, 17.91)	21.94 (−5.82, 49.71)	−5.39 (−11.1, 0.31)
	5	−5.81 (−33.71, 22.08)	8.46 (−11.59,28.52)	−38.16 (−51.32, −25)	4.64 (−18.93, 28.21)	7.6 (−38.68, 53.87)	−8.47 (−13.36, −3.59)
	6	−2.66 (−19.16, 13.84)	8.26 (−21.41, 37.92)	−26.99 (−48.36, −5.61)	3.26 (−12.07, 18.59)	−7.89 (−16.31, 0.53)	−6.34 (−10.8, −1.88)
	7	−4.52 (−20.17, 11.13)	17.95 (−16.29, 52.18)	−43.58 (−56.74, −30.42)	0.69 (−14.86, 16.24)	−3.14 (−32.33, 26.06)	−11.58 (−16.46, −6.69)
	1	6.55 (−0.98, 14.07)	3.6 (−3.31,10.51)	−1.54 (−8.65, 5.58)	−6.7 (−13.07, −0.33)	−1.26 (−4.67, 2.16)	−3.72 (−6.93, −0.52)
	2	−11.46 (−29.5, 6.57)	−16.04 (−28.01, −4.06)	−3.39 (−8.5, 1.73)	−2.49 (−4.46, −0.51)	−2.48 (−4.99, 0.03)	−3.78 (−5.18, −2.37)
	3	−23.48 (−29.75, −17.22)	−30.45 (−34.7, −26.2)	1.5 (−9.06, 12.06)	−8.56 (−11.26, −5.86)	−27.29 (−29.8, −24.78)	−1.77 (−6.76, 3.22)
Lipid	4	−21.77 (−45.66, 2.13)	−4.25 (−21.53, 13.02)	8.5 (−1.01, 18)	0.01 (−10.05, 10.07)	19.36 (−6.12, 44.83)	−0.34 (−2.2, 1.52)
	5	−19.57 (−35.55, −3.58)	2.91 (−10.14, 15.96)	14.12 (9.49, 18.76)	8.05 (1.94, 14.16)	6.25 (1.11, 11.39)	3.73 (1.05, 6.41)
	6	−6.67 (−24.27, 10.92)	−7.83 (−25.12, 9.46)	12.02 (8.81, 15.23)	−1.37 (−10.36, 7.63)	0.15 (−2.79, 3.09)	3.81 (2.55, 5.07)
	7	−7.06 (−19.55, 5.43)	6.26 (−0.1, 12.61)	14.2 (9.56, 18.83)	−2.07 (−9.21, 5.08)	0.92 (−3.44, 5.29)	−7.45 (−10.13, −4.77)
	1	6.99 (−6.55, 20.52)	−1.54 (−12.23, 9.15)	−5.88 (−18.34, 6.57)	−9.85 (−21.23, 1.52)	11.99 (−14.15, 38.12)	0.35 (−7.11, 7.8)
	2	−8.32 (−27.87, 11.22)	−12.91 (−46.84, 21.03)	3.02 (−12.43, 18.48)	4.26 (−6.9, 15.42)	0.79 (−9.97, 11.56)	4.09 (−2.16, 10.35)
	3	4.88 (−8.42, 18.18)	−23.69 (−34.17, −13.2)	17.34 (−9.18, 43.87)	−0.09 (−8.85, 8.68)	−23.04 (−33.8, −12.28)	6.53 (1.58, 11.48)
Water	4	3.01 (−13.09, 19.12)	15.54 (−2.52, 33.59)	−8.17 (−23.73, 7.4)	1.99 (−8.31, 12.28)	21.88 (−9.9, 53.67)	2.63 (−4.29, 9.56)
	5	−14.96 (−38.25, 8.33)	9.31 (−5.43, 24.05)	−20.65 (−38.39, −2.92)	1.17 (−14.4, 16.73)	−2.09 (−11.7, 7.51)	−1.09 (−6.7, 4.53)
	6	−5.49 (−19.64, 8.66)	4.42 (−22.24, 31.07)	1.75 (−34.55, 38.06)	1.19 (−12.78, 15.15)	−1.18 (−13.04, 10.69)	0.72 (−7.42, 8.87)
	7	−18.19 (−34.36, −2.02)	−9.79 (−27.96, 8.37)	−23.75 (−41.49, −6.01)	−5.61 (−18.17, 6.94)	0 (−17.87, 17.88)	−7.53 (−13.14, −1.91)

Table S3. Results of sensitivity analyses after exclusion of outliers

		Tumor			Normal		
Outcome	Days	pCR	PR	NR	pCR	PR	NR
ctO_2Hb*	1	27.66 (16.98, 38.34)	33.74 (24.26, 43.22)	−25.29 (−34.5, −16.07)	12.02 (−2.83, 26.88)	10.49 (2.22, 18.75)	−4.2 (−13.58, 5.18)
	2	−9.19 (-24.52, 6.15)	5.26 (−4.14, 14.66)	−29.64 (−39.64, −19.64)	−0.41 (−10.28, 9.45)	−4.04 (−9.87, 1.8)	−16.24 (−30.85, −1.63)
	3	16.49 (5.77, 27.2)	−0.9 (−10.3, 8.5)	−20.47 (−38.63, −2.31)	5.24 (−1.25, 11.74)	−16.95 (−22.78, −11.11)	−13.36 (−28.1, 1.38)
	4	10.64 (2.24, 19.05)	8.13 (−18.66, 34.93)	−29.33 (−49.74, −8.93)	−11.16 (−28.36, 6.04)	17.74 (−11.71, 47.18)	−21.17 (−30.29, −12.05)
	5	−28.4 (-42.07, −14.72)	−0.21 (−19.62, 19.2)	−44.05 (−56.56, −31.54)	−10.67 (−27.64, 6.29)	6.05 (1.64, 10.46)	−24.82 (−29.31, −20.33)
	6	−9.67 (-23.23, 3.9)	0.35 (−11.35, 12.04)	−39.34 (−55.4, −23.28)	−1.05 (−15.27, 13.17)	2.45 (−6.56, 11.46)	−23.18 (−26.98, −19.37)
	7	−19.31 (-38.82, 0.2)	−20.9 (−38.14, −3.66)	−51.81 (−64.31, −39.3)	−14.93 (−35.18, 5.33)	−16.26 (−26.17, −6.34)	−29.27 (−33.76, −24.78)
ctHHb†	1	−1.34 (-13.86, 11.18)	−14.2 (−25.3, −3.1)	−19.47 (−29.67, −9.28)	−11.69 (−22.55, −0.82)	−13.97 (−21.99, −5.95)	−11.81 (−21.55, −2.07)
	2	−15.89 (-26.43, −5.35)	−23.9 (−38.1, −9.7)	−18.95 (−34.34, −3.56)	−13.26 (−22.5, −4.02)	−18.77 (−28.11, −9.42)	−14.95 (−18.93, −10.98)
	3	−9.67 (-20.47, 1.13)	−67.26 (−81.46, −53.06)	−3.79 (−32.01, 24.42)	−9.58 (−16.64, −2.51)	−42.53 (−51.88, −33.19)	−11.65 (−14.86, −8.45)
	4	10.66 (-10.39, 31.71)	2.3 (−14.93, 19.53)	−23.62 (−34.76, −12.47)	10.15 (−2.47, 22.77)	19.07 (−14.69, 52.83)	−6.55 (−16.26, 3.15)
	5	−13.1 (-42.11, 15.9)	−2.29 (−18.23, 13.64)	−37.29 (−49.17, −25.42)	7.68 (−12.26, 27.62)	25.01 (−19.77, 69.79)	−8.12 (−14.72, −1.52)
	6	−3.32 (-18.43, 11.79)	−7.93 (−24.3, 8.45)	−27.16 (−45.89, −8.43)	6.67 (−8.07, 21.41)	−11.71 (−19.01, −4.42)	−4.92 (−10.53, 0.69)
	7	−4.97 (-20.15, 10.21)	−0.64 (−25.89, 24.61)	−42.72 (−54.59, −30.84)	3.23 (−11.03, 17.5)	−9.57 (−45.21, 26.08)	−11.23 (−17.82, −4.63)
Lipid‡	1	5.22 (-1.5, 11.95)	2.72 (−5.03, 10.47)	−0.21 (−5.89, 5.47)	−6.46 (−12.76, −0.16)	−0.11 (−3.16, 2.93)	−3.48 (−6.32, −0.64)
	2	−8.96 (-27.17, 9.26)	−14.63 (−26.78, −2.48)	−1.77 (−5.73, 2.19)	0.37 (−2.72, 3.45)	−2.98 (−5.41, −0.55)	−4.13 (−5.36, −2.9)
	3	−19.64 (-24.99, −14.28)	−29.39 (−34.57, −24.22)	2.03 (−6.28, 10.33)	−2.49 (−5.38, 0.4)	−27.79 (−30.23, −25.36)	−2.02 (−6.4, 2.36)
	4	−2.78 (-7.95, 2.38)	−3.23 (−19.52, 13.06)	9.72 (−1.09, 20.53)	−1.33 (−10.87, 8.2)	4.08 (−2.17, 10.32)	−1.29 (−4.58, 2)
	5	−9.66 (-20.84, 1.51)	3.25 (−9.6, 16.09)	15.91 (11.79, 20.03)	6.97 (−0.12, 14.05)	9.76 (7.92, 11.6)	3.97 (2.16, 5.79)
	6	2.27 (-9.51, 14.06)	−0.03 (−12.69, 12.62)	12.08 (8.42, 15.74)	−0.14 (−9.02, 8.73)	0.17 (−3.7, 4.04)	3.91 (2.45, 5.37)
	7	−2.88 (-14.67, 8.92)	8.4 (1.13, 15.67)	15.98 (11.86, 20.11)	−1.46 (−8.05, 5.14)	0.61 (−5.31, 6.54)	−7.21 (−9.02, −5.39)
Water§	1	6.99 (-6.55, 20.52)	−1.54 (−12.23, 9.15)	−5.88 (−18.34, 6.57)	−7.21 (−17.22, 2.8)	−2.47 (−10.62, 5.68)	0.25 (−7.44, 7.95)
	2	−8.32 (-27.87, 11.22)	−12.91 (−46.84, 21.03)	3.02 (−12.43, 18.48)	6.18 (−4.42, 16.79)	−2.9 (−10.79, 4.99)	3.21 (−4.23, 10.65)
	3	4.88 (-8.42, 18.18)	−23.69 (−34.17, −13.2)	17.34 (−9.18, 43.87)	3.45 (−5.77, 12.67)	−26.73 (−34.63, −18.84)	6.4 (−0.37, 13.17)
	4	3.01 (-13.09, 19.12)	15.54 (−2.52, 33.59)	−8.17 (−23.73, 7.4)	3.52 (−4.9, 11.93)	4.67 (−14.75, 24.09)	3.25 (−2.23, 8.74)
	5	−14.96 (-38.25, 8.33)	9.31 (−5.43, 24.05)	−20.65 (−38.39, −2.92)	2.26 (−11.74, 16.25)	1.37 (−8.32, 11.05)	0.71 (−3.7, 5.12)
	6	−5.49 (-19.64, 8.66)	4.42 (−22.24, 31.07)	1.75 (−34.55, 38.06)	2.53 (−11.33, 16.38)	−1.22 (−11.67, 9.22)	2.32 (−5.12, 9.75)
	7	−18.19 (-34.36, −2.02)	−9.79 (−27.96, 8.37)	−23.75 (−41.49, −6.01)	−4.51 (−16.03, 7.02)	5.37 (−7.06, 17.8)	−5.73 (−10.14, −1.32)

Mean values and 95% confidence limits for percent change from baseline for 7 d after the start of chemotherapy in tumor and normal tissue for all outcomes as predicted by GEE models.

*One PR and one pCR were excluded in the tumor group (rows 10 and 24 in Table S1, respectively). One PR was excluded in the normal group (row 7 in Table S1).

†One PR and one pCR were excluded in the tumor group (rows 10 and 24 in Table S1, respectively). One PR was excluded in the normal group (row 12 in Table S1).

‡One PR and one pCR were excluded in the tumor group (rows 14 and 17 in Table S1, respectively). One PR was excluded in the normal group (row 12 in Table S1).

§One PR was excluded in the normal group (row 12 in Table S1).

Table S4. Mean difference between percent change from baseline in DOSI measures for tumor and normal tissue and 95% confidence limits for the mean difference as predicted by GEE models

		Tumor − normal		
Outcome	Days	pCR	PR	NR
ctO_2Hb	1	38.91 (16.35, 61.48)	2.38 (−20.98, 25.73)	−20.5 (−27.42, −13.58)
	2	5.05 (−18.75, 28.85)	−5.59 (−23.46, 12.29)	−15.48 (−29.09, −1.86)
	3	25.49 (14.29, 36.7)	1.17 (−16.71, 19.04)	−5.14 (−22.67, 12.39)
	4	9.92 (−10.77, 30.61)	−14 (−39.01, 11)	−11.68 (−19.64, −3.71)
	5	4.21 (−35.16, 43.57)	−15.15 (−33.26, 2.96)	−20.77 (−30.79, −10.74)
	6	7.42 (−8.11, 22.94)	−15.09 (−32.24, 2.06)	−11.9 (−35.99, 12.19)
	7	3.29 (−7.86, 14.44)	−21.68 (−41.91, −1.44)	−24.08 (−34.1, −14.06)
ctHHb	1	39.2 (13.66, 64.74)	−25.87 (−62.29, 10.54)	−6.76 (−15.51, 1.99)
	2	4.97 (−15.46, 25.39)	−11.26 (−32.04, 9.51)	−5.74 (−16.9, 5.41)
	3	10.29 (−2.94, 23.51)	−30.85 (−51.63, −10.08)	8.93 (−13.02, 30.88)
	4	22.55 (5.29, 39.82)	−15.18 (−40.64, 10.27)	−17.43 (−27.95, −6.9)
	5	7.88 (−12.94, 28.69)	−5.26 (−55.32, 44.8)	−28.42 (−39.05, −17.79)
	6	0.44 (−16.69, 17.56)	−1.56 (−24.13, 21.01)	−18.5 (−37.93, 0.92)
	7	3.88 (−12.25, 20)	21.51 (−11.09, 54.1)	−30.73 (−41.36, −20.1)
Lipid	1	10.54 (0.22, 20.86)	2.15 (−2.41, 6.71)	2.24 (−4.63, 9.11)
	2	−1.67 (−14.04, 10.71)	−5.45 (−11.96, 1.07)	1.26 (−3.66, 6.18)
	3	−17.66 (−25.04, −10.28)	−2 (−8.51, 4.52)	3.57 (−6.28, 13.42)
	4	−23.12 (−43.75, −2.5)	−17.97 (−59.85, 23.92)	10.01 (3.26, 16.77)
	5	−27.29 (−46.89, −7.68)	−3.92 (−14.92, 7.08)	10.38 (5.3, 15.45)
	6	2.92 (−11.94, 17.79)	−7.03 (−30.42, 16.36)	7.78 (3.59, 11.97)
	7	−6.91 (−17.72, 3.91)	3.07 (−4.86, 11.01)	21.63 (16.55, 26.7)
Water	1	17.3 (4.63, 29.97)	−21.48 (−41.63, −1.33)	−6.05 (−12.62, 0.53)
	2	−4 (−15.88, 7.87)	1.11 (−4.58, 6.79)	−4.32 (−9.11, 0.47)
	3	6.6 (−0.53, 13.72)	−8.98 (−14.67, −3.3)	13.35 (−11.89, 38.59)
	4	3.52 (−3.87, 10.9)	−22.46 (−56.41, 11.5)	−19.45 (−27.79, −11.12)
	5	−3.02 (−10.1, 4.07)	0.43 (−8.26, 9.12)	−35.59 (−43.64, −27.55)
	6	−2.9 (−17.71, 11.92)	−14.03 (−26.33, −1.73)	0.07 (−49.72, 49.85)
	7	−6.01 (−16.35, 4.32)	−25.18 (−54.03, 3.67)	−32.25 (−40.3, −24.21)