Predicting Responses to Neoadjuvant Chemotherapy in Breast Cancer: ACRIN 6691 Trial of Diffuse Optical Spectroscopic Imaging

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Abstract

The prospective multicenter ACRIN 6691 trial was designed to evaluate whether changes from baseline to mid-therapy in a diffuse optical spectroscopic imaging (DOSI)–derived imaging endpoint, the tissue optical index (TOI), predict pathologic complete response (pCR) in women undergoing breast cancer neoadjuvant chemotherapy (NAC). DOSI instruments were constructed at the University of California, Irvine (Irvine, CA), and delivered to six institutions where 60 subjects with newly diagnosed breast tumors (at least 2 cm in the longest dimension) were enrolled over a 2-year period. Bedside DOSI images of the tissue concentrations of deoxy-hemoglobin (ctHHb), oxy-hemoglobin (ctHbO2), water (ctH2O), lipid, and TOI (ctHHb × ctH2O/lipid) were acquired on both breasts up to four times during NAC treatment: baseline, 1-week, mid-point, and completion. Of the 34 subjects (mean age 48.4 ± 10.7 years) with complete, evaluable data from both normal and tumor-containing breast, 10 (29%) achieved pCR as determined by central pathology review. The percent change in tumor-to-normal TOI ratio (%TOITN) from baseline to mid-therapy ranged from –82% to 321%, with a median of –36%. Using pCR as the reference standard and ROC curve methodology, %TOITN AUC was 0.60 (95% CI, 0.39–0.81). In the cohort of 17 patients with baseline tumor oxygen saturation (%SO2) greater than the 77% population median, %TOITN AUC improved to 0.83 (95% CI, 0.63–1.00). We conclude that the combination of baseline functional properties and dynamic optical response shows promise for clinical outcome prediction. Cancer Res; 76(20); 5933–44. ©2016 AACR.

Introduction

Neoadjuvant chemotherapy (NAC), or preoperative systemic therapy, offers unique opportunities for patient care and cancer drug development (1). In addition to improving breast tissue conservation and cosmetic outcome, NAC can cause downstaging of the presurgical tumor and reduce the required extent of axillary dissection (2). Studies have shown that pathologic complete response (pCR) predicts patient survival (3, 4), and the FDA now allows use of the pCR endpoint to support accelerated approval of drugs for high-risk early-stage breast cancer (5). Importantly, NAC enables tumor response and chemotherapy effectiveness to be assessed on an individual patient basis, allowing oncologists to optimize treatment strategy and improve patient outcome.

In current clinical practice, NAC response assessment is determined predominantly by serial physical examination, mammography, and/or ultrasound. Yeh and colleagues (6) showed that palpation, mammography, ultrasound, and MRI had 19%, 26%, 35%, and 71% agreement, respectively, with final pathologic response. These and other studies have shown that anatomic changes in tumor presentation are not reliable predictors of final pathologic state (7–9). Functional measurements of tumors from contrast-enhanced MRI (10), magnetic resonance spectroscopy (MRS; refs. 11, 12), and PET (13, 14) have shown substantial
improvement over conventional anatomic imaging. However, the
cost, radiation exposure, and/or potential toxicities of contrast
agents limit the frequency of these scans. Optical imaging offers a
low-cost, risk-free approach that could be used as a "bedside"
adjunct to these methods.

Currently, morphologic criteria are the only clinical care stan-
dards for evaluating therapeutic efficacy of NAC. Prior studies
have indicated that functional and molecular measures may
provide an early indicator of breast cancer therapy response in
the neo-adjuvant setting. Studies support the potential value of
proliferation biomarkers, including imaging markers (15), as
predictors of therapeutic response (16, 17). Other imaging studies
indicate that early changes in blood flow and tumor metabolism
predict subsequent response and provide an early marker for
cellular response to treatment (10–12, 18). Taken together, evi-
cence suggests that early (24 to 72 hours) tumor biochemical
changes precede volumetric changes in response to cancer ther-
apy (19, 20). These early biochemical changes may provide a
pathway for predicting final pathologic outcome (20). Early
classification of therapeutic effectiveness could increase patient
survival and minimize unnecessary damage to sensitive tissues
(e.g., heart, liver, brain) caused by ineffective therapeutic strate-
gies. This opportunity motivates the development of accurate
imaging predictors of pCR and pathologic nonresponse.

Diffuse optical imaging (DOI) technology, a potential can-
diate to address this clinical need, uses red and near-infrared (NIR)
light (~600–1,000 nm) to probe tissue absorption and scattering
properties at depths up to several centimeters (21). Absorption
and scattering properties in this regime characterize tissue struc-
ture and function. Analogous to MRS and MRI, diffuse optical
spectroscopic imaging (DOSI) is a specialized DOI technology
that performs high-resolution spectroscopy from 650 to 1000 nm,
where oxy- and deoxy-hemoglobin, water, and lipid have promi-
nent absorption features. These endogenous components vary
significantly in abundance and molecular state between breast
and normal tissue (22–26) and unique cancer-specific absorption
signatures not found in normal breast have been observed (27).
DOSI-measured tumor water concentration and water binding state were shown to scale with the Nottingham
Bloom–Richardson histopathology score and appear to be propor-
tional to tissue cellularity and extracellular matrix composi-
tion (22, 28). Hemoglobin, water, and lipid components have been shown to be sensitive to microvasculature, cellular
metabolism, angiogenesis, edema, hypoxia, and necrosis in sev-
eral single-center studies and provide tumor contrast that can change significantly with the growth and regression of tumors
(22, 29–35). Quantitative, optical functional endpoints are easy
to interpret and offer objective measures to predict therapeutic
outcome and minimize patient toxicity. For instance, tissue
optical index (TOI; compound function of deoxy-hemoglobin,
water, and lipid tissue concentrations), which describes tissue
metabolism, structure, and cellularity, has been shown to be a
promising imaging endpoint that significantly decreases by mid-
therapy and can be used for predicting NAC response (36).
Response to chemotherapy significantly correlated with a decrease
in TOI by mid-therapy. The biological origins of this TOI signal
change are NAC-induced reductions in tumor cellular density and
edema (reduced water content), re-normalization of breast adi-
pose displaced by the tumor (increased lipid fraction), and
decreased levels of deoxycyhemoglobin due to a loss of metabo-
lically active tumor cells. Any DOI instrument that uses the
appropriate wavelength bands to retrieve hemoglobin, water, and
lipid information can calculate TOI. Because DOSI is a relatively
inexpensive, portable, risk-free technology, it can be used fre-
quently at the bedside, potentially creating new opportunities for
patients to receive personalized treatment and for physicians to
gain insight into response mechanisms. These features have been
highlighted in several single-center studies and they motivated
American College of Radiology Imaging Network (ACRIN)
6691, which was designed to evaluate whether quantitative
DOSI measurements can be conducted in a prospective, multi-
center trial setting to predict therapeutic response in breast
cancer patients undergoing NAC. The primary aim was to
evaluate whether the baseline to mid-therapy change of the
DOSI-derived TOI could predict pCR. The secondary aims
investigate the utility of additional DOSI measurements of
tumor biochemical composition, obtained both at baseline and
other time-points, for predicting pCR.

Patients and Methods

Subject eligibility and enrollment

Seven institutions were approved to enroll a total of 60 female
breast cancer patients: Dartmouth Hitchcock Medical Center
(Dartmouth), Massachusetts General Hospital (MGH), Univer-
sity of California Irvine (UCI), University of California San
Francisco (UCSF), University of Pennsylvania (UPenn), Boston
University (BU), and MD Anderson Cancer Center (MDACC).
Enrollment began in June 2011 and completed in June 2013. All
institutions activated concurrently, except MDACC and BU, which
joined the study in January and May 2013, respectively.

Eligible subjects were females age 18 years or older with biopsy-
proven invasive breast cancer of at least 2 cm in the greatest
dimension (as measured by palpation or standard breast imaging
techniques), who planned to receive NAC followed by surgery. No
restrictions were placed on menopausal status, tumor stage, or
tumor subtype/pathology. For this study, the chemotherapy reg-
imen was determined by the treating oncologist, and regimens
containing at least one cytotoxic chemotherapeutic agent were
required. Neoadjuvant hormonal therapy or other targeted agents
alone in the absence of cytotoxic chemotherapy was not allowed.
Potential subjects who received previous treatments, such as
chemotherapy, endocrine therapy, radiation, or surgery (exclud-
ing breast augmentation) to the involved breast were excluded
from the study. In addition, women who were pregnant or nursing
were excluded. All subjects provided written informed consent.
The HIPAA-compliant protocol and informed consent were
approved by the American College of Radiology Institutional
Review Board, the NCI Cancer Therapy Evaluation Program
(CTEP), and each site’s Institutional Review Board.

DOSI

Instrumentation. DOSI measurements were performed with sys-
tems that combine frequency domain photon migration (FDPDM)
and broadband NIR (650–1000 nm) spectroscopy for quantita-
tive, model-based measurement of tissue absorption and scatter-
ing properties and recovery of tissue oxy-hemoglobin (ctHbO2),
deoxy-hemoglobin (ctHHb), water, and lipid. The full technical
details of the system are described elsewhere (37). Standardized
procedures were developed for data collection and to monitor
instrument performance. Three sets of three calibration measure-
ments on two custom-fabricated solid breast-tissue simulating
phantoms (Institut National d’Optique, Quebec, Canada; and UCI Irvine Beckman Laser Institute, CA) and on one reflectance standard (SRS-99-020, Labsphere Inc.) were performed before, midway and after each subject measurement. The breast tissue simulating phantoms and reflectance standards used at all sites were identical. These measurements were used for determining instrument response-function, cross-calibration between instruments, and monitoring instrument performance over the duration of the study (38). Dartmouth, MGH, UCI, UCSF, and UPenn used identical DOSI instruments. MDACC and BU, which joined the trial about 1.5 years after the first patient enrollment, used a smaller, portable version with equivalent features and performance to the original five DOSI instruments. The handheld DOSI probe that is scanned on the breast for data collection was identical for all instruments. A group of trainers from UCI, who developed the DOSI instruments, traveled to each site for initial instrument setup and training.

Spectroscopy. The approach to DOSI data acquisition and analysis has been previously described (22), and is briefly summarized here. Concentrations of oxy-hemoglobin (ctHbO2), deoxy-hemoglobin (ctHHb), water (ctH2O), and bulk lipid were calculated by fitting a linear combination of their known molar extinction coefficient spectra to the tissue μa values derived from model-based fits of DOSI data (22). From these quantities, the total blood volume (THb = ctHHb + ctHbO2), percent oxygen saturation (StO2), and TOI were calculated. The StO2 is the ratio of ctHbO2 to THb. TOI is defined as TOI = ctHHb × ctH2O/lipid. Prior single-center studies have shown that TOI can provide high tumor to normal (T/N) tissue contrast and that changes in T/N TOI are sensitive to chemotherapy response (22, 36). Quantitative images of these local tissue concentrations and contrast function were formed on a generic three-dimensional (3D) breast surface for visualization.

Imaging procedures

DOSI scans were performed prior to the start of the chemotherapy treatment (baseline), 5 to 10 days after the first cycle (early-), at a change in chemotherapy regimens or mid-way through a single regimen protocol (mid-), and within 3 weeks after completion of the chemotherapy treatment but prior to surgery (post-therapy). The mid-therapy DOSI measurement was performed at least 5 days after the completion of the last cycle prior to the mid-point, and prior to the first cycle after the mid-point. Early- and post-therapy scans were optional as they were not required to analyze the primary aim.

DOSI measurements were performed using a standard protocol. Subjects were measured in a supine or reclining position. The DOSI probe was placed against the breast tissue, and sequential measurements were recorded in a rectangular grid pattern using 10-mm spacing. Both breasts were specified to be measured at each visit. The dimension of the grids ranged from 7 × 7 to 15 × 16 cm² (i.e., 49 to 240 measurement locations). The grid size was chosen to fully encompass the tumor anatomical extent (by ultrasound or palpation) and to capture surrounding normal tissue. The grid on the noncancerous contralateral breast (normal side) was mirrored from the grid on the cancerous breast (tumor side). An example of DOSI imaging grid locations and the resulting images is provided in Fig. 1. For consecutive DOSI visits, individual static landmarks such as moles or freckles were recorded on a transparency sheet and provided a fixed reference to coregister the imaging grid at follow-up DOSI sessions. After initial processing performed centrally by the UCI/ACRIN core lab (see section “Image Analysis”), DOSI data from each session were...
plotted using grid coordinates and heatmap functions in MATLAB. Linear interpolation was used to account for 10-mm spacing of grid points. The colored 2D maps were then overlaid on a 3D textured mesh of a realistic breast anatomic model with grid points drawn on it. The initial 3D image was obtained by using an X-box Kinect scanner for windows and accompanying Software Development Kit (Microsoft). Colored heatmaps obtained from MATLAB were warped and overlaid on the textured mesh using Photoshop CS6 Extended (Adobe) to create the final images.

**Histopathologic assessment**

Histopathologic analysis of surgical specimens was performed locally by each institution’s pathologist. One central pathologist (UCI core) then reviewed all pathologic reports to assess individual tumor response. The binary determination of pCR or non-pCR was made based on evaluation of the pathology reports, and if needed, standard-of-care imaging reports. All determinations were made blinded to DOSI imaging data. pCR was standardized and defined as no residual of invasive disease present in the surgically resected tissue (breast and lymph nodes) as per FDA guidelines (http://www.fda.gov/downloads/drugs/guidancocompliance/ucm305501.pdf). Non-pCR included both partial and nonresponders. For the purposes of this report, only pCR status (0 or 1) was used to evaluate clinical outcome. Partial response characteristics will be further characterized in future analyses of secondary aims.

**Image analysis**

A single analyst from UCI processed the deidentified raw DOSI data centralized from all participating institutions, and recovered the mean and SD in tumor and normal tissue of all DOSI parameters. All DOSI data processing, including model-based fits to raw data, calculation of breast tissue composition, and image generation, was performed blinded with respect to clinical outcome on deidentified data by the UCI data core team. These processed data were transferred to the central ACRIN facility for further statistical analysis by the ACRIN Biostatistics Center. From the DOSI images, two regions of interest (ROI) representing tumor and normal tissue were defined using an automated algorithm. DOSI parameters were determined by calculating the mean of the measurement locations in each ROI. In this work, we focus on the $T/N$ ratio, which accounts for chemotherapy-induced changes in normal tissue.

As described in previous studies, the tumor ROI consisted of the full-width-at-half-maximum (FWHM) region around the baseline TOI tumor peak (36). The DOSI measurement locations that had greater TOI values than a threshold equal to half the peak were included in the tumor ROI. In addition, a continuity rule was implemented to define the tumor ROI: any location calculated as part of the ROI but spatially disconnected (>2 cm away) was excluded from the ROI, and any location not meeting the threshold but surrounded by at least three other included points was incorporated. The tumor ROI size was fixed for all time-points, but the tumor ROI could be translated up to 2 cm at later time-points to account for a shift in tumor TOI enhancement (due to asymmetric tumor shrinkage or grid misplacement on the breast). This method was designed to capture the entire tumor region, but because the tumor size was longitudinally fixed, it overestimates the tumor extent at later time-points in responders. The normal tissue ROI was selected in the contralateral breast to mirror the ipsilateral tumor ROI.

**Statistical analysis**

Summary statistics including the mean, SD, median, and range were computed for all DOSI markers. Logistic regression was used to model the association between percent change in $T/N$ TOI ratio ($\%T/OI_N$) from baseline to mid-therapy and pathologic response status (pCR vs. non-pCR). The resulting OR and 95% CI were reported. In addition, a receiver operating characteristic (ROC) curve was derived for $\%T/OI_N$ with pathologic response status as the reference standard. Area under the ROC curve (AUC) and the associated 95% confidence interval were computed empirically. If the lower limit of the 95% CI for AUC was at least 0.50, then the marker was considered capable of differentiating pCR vs. non-pCR.

To account for the heterogeneous cohort, the predictive power of $\%T/OI_N$ was evaluated further on two subsets of patients stratified by baseline tumor tissue oxygen saturation (StO2), which is an optical parameter reflective of the tumor biophysical state. Tumor baseline StO2 has been shown to be a promising predictor for pCR at early time-points (33, 39). The median value of baseline StO2 was used to stratify the subsets, with ROC curve and corresponding AUC reported for each subset. Evaluation of baseline StO2 alone and in combination with midpoint $\%T/OI_N$ was a planned secondary aim of the study.

ACRIN 6691 was powered to detect an odds ratio of 3.0 using a two-sided hypothesis test, a type I error rate of 0.05, and 90% power (PASS 2008). The resulting sample size was determined to be 47 evaluable subjects. All tests were two-sided with $P$ value < 0.05 considered statistically significant. Statistical computations were done using SAS 9.3 (SAS Institute) and R v3.1.0 (R project, http://www.r-project.com).

**Results**

**Enrollment information**

The target accrual was met with 60 subjects enrolled in ACRIN 6691. As depicted in Fig. 2, three subjects withdrew from the study. An additional 12 subjects were not included in the imaging analysis because of the following DOSI scan issues: mandatory baseline DOSI non-evaluable ($n = 8$), mandatory mid-therapy DOSI non-evaluable ($n = 3$). A DOSI scan was considered non-evaluable in case of unrealistic physiological values, or incorrect instrument configuration. This decision was made on blinded, de-identified data using instrument calibration and raw data QC reports. Of the 45 eligible subjects with evaluable DOSI scans, one surgical pathology report after chemotherapy was not available. For another 10 subjects, the DOSI scan of the contralateral breast was not performed due to insufficient time during the measurement session to complete the normal side breast scan, which was required in this analysis for $T/N$ values. The analysis, therefore, included 34 subjects with evaluable DOSI scans and available pathologic response data from five different institutions (Supplementary Table S1). Measurements at the two mandatory time-points to assess the primary aim, baseline, and mid-therapy were performed in 97% and 88% of all enrolled subjects, respectively (Supplementary Table S2). Despite being optional, measurements at early-therapy and post-therapy were performed in 73% and 82% of all enrolled subjects, respectively.

**Subject and tumor characteristics**

Table 1 presents the characteristics of all enrolled subjects ($n = 60$), and those subjects included in the analysis of the primary aim.
Among analyzed subjects, the average age was 48.4 ± 10.7 years (range 30–67 years), with 50% premenopausal, 3% perimenopausal, and 47% postmenopausal. The racial distribution was 56% White, 24% Black, 12% Asian, and 9% of unknown race; 15% of analyzed subjects were of Hispanic or Latino ethnicity. Prechemotherapy tumor characteristics are also reported in Table 1. IDC accounted for approximately 85% of the tumors (with or without a DCIS component, and including 6% of cases mixed with an ILC component) in the analysis group. Notably, approximately 71% of the tumors in the analysis group were ER⁺.

The chemotherapy protocols undergone by the subjects were not controlled in this study and, as a result, were highly heterogeneous. Treatments for all subjects were based on cytotoxic therapies. Targeted agents, such as Avastin (bevacizumab) or Herceptin (trastuzamab), could be used in combination of the cytotoxic agents. Hormonal therapies were not allowed. A total of 17 different chemotherapy protocols, including 12 different drugs, were administered in the 34 evaluable cases. HER2 status was known in 25 of 34 (74%) analyzable subjects. Trastuzamab was administered to 10 of 11 (91%) HER2⁺ tumors, and bevacizumab was administered to 1 of 2 (50%) triple-negative tumors.

Pathologic outcome statistics
At surgery, 10 (29%) pCR and 24 (71%) non-pCRs were included in the analysis group. A similar distribution of 15 (25%) pCRs was observed in all 60 enrolled subjects, with 5 (8%) not undergoing surgery/lost to follow-up.

DOSI during NAC
Figure 3 presents two typical examples of a pCR and a non-pCR measured using DOSI during NAC. The corresponding %TOITN is shown across NAC for both responses. Both subjects exhibited relatively high baseline TOI levels of approximately three to four at tumor and areolar regions. The pCR subject demonstrated a greater %TOITN decrease at midpoint than the non-pCR subject (−24.6% and −14.1%, respectively). Peak areolar TOI levels for subjects remained approximately the same throughout therapy.

Response prediction—TOIT/N change from baseline to mid-therapy
Among analyzable subjects, the distribution of %TOITN from baseline to mid-therapy ranged from −82.4% to 320.8%, with a median of −36.2%. The corresponding mean (SD) was −25.9% (67.2%). Figure 4A presents the distribution of %TOITN from baseline to mid-therapy by pathologic response category. On average, the pCR group exhibited a greater decrease in %TOITN than the non-pCR group; however, large variability occurred in %TOITN in the non-pCR group.

The protocol-planned logistic regression analysis demonstrated an OR for pCR prediction of 1.4 (95% CI, 0.7–2.9; P = 0.40) per
Table 1. Subject and tumor characteristics from initial pathology

<table>
<thead>
<tr>
<th>Analysis group</th>
<th>All enrolled subjects</th>
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<tr>
<td><strong>Age, years</strong></td>
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<tr>
<td>Mean ± SD (range)</td>
<td>48.4 ± 10.7 (30–67)</td>
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<tr>
<td>Menopausal status, n (%)</td>
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<tr>
<td>Pre-</td>
<td>17 (50)</td>
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<tr>
<td>Peri-</td>
<td>1 (3)</td>
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<tr>
<td>Post-</td>
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<tr>
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<tr>
<td>Race, n (%)</td>
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<tr>
<td>White</td>
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<tr>
<td>Black/African-American</td>
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<tr>
<td>Asian</td>
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<tr>
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<td>Ethnicity, n (%)</td>
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<tr>
<td>Non-Hispanic/Latino</td>
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<td>Histologic findings, n (%)</td>
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<tr>
<td>IDC</td>
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<td>KR status, n (%)</td>
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<td>Positive</td>
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<td>HER2 status, n (%)</td>
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<td>1</td>
<td>8 (24)</td>
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<tr>
<td>2</td>
<td>8 (24)</td>
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<tr>
<td>3</td>
<td>4 (12)</td>
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<td>Fish status, n (%)</td>
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<tr>
<td>Nonareolar tumor</td>
<td>17 (50)</td>
</tr>
<tr>
<td>N/A (DOSI not performed)</td>
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</table>

NOTE: Data are numbers of subjects, with percentages in parentheses.

SD increase in %TOI$_{IN}$. As strong evidence of model lack of fit existed using continuous %TOI$_{IN}$, we explored using −40% to dichotomize the population for the purpose of logistic regression, which was approximately equal to the median %TOI$_{IN}$ across all subjects. Patients who had a 40% or larger decrease in TOI$_{IN}$ from baseline to mid-therapy were more likely to be complete responders than patients who experienced smaller decreases or increases, with an estimated odds ratio for pCR prediction of 4.7 (95% CI, 0.9–23.0; P = 0.059; see Table 2). The ROC AUC for pCR using %TOI$_{IN}$ was 0.60 (95% CI, 0.39–0.81), suggesting this marker does not exhibit good discriminatory ability for pCR in the entire cohort (Fig. 5A).

In the subset analysis, subjects were further stratified using the median tumor StO$_2$ at baseline (76.9%) as another binary marker. Note that this median baseline tumor StO$_2$ is similar to the threshold value reported by Ueda and colleagues (39), 76.7%, which has been shown to correlate with NAC response. Fig. 4B and C shows the distributions of %TOI$_{IN}$ from baseline to mid-therapy by pathologic response category in each subset. Although %TOI$_{IN}$ in patients with low baseline tumor StO$_2$ did not correlate with pathologic response, a large decrease in %TOI$_{IN}$ from baseline to mid-therapy in patients with high baseline tumor StO$_2$ was associated with pCR. For 17 subjects with baseline StO$_2$ greater than 76.9%, AUC was 0.83 (95% CI, 0.63–1.00; Fig. 5C). These subjects were more likely to be complete responders than patients who experienced smaller decreases or increases, with an estimated OR for pCR prediction of 16.5 (95% CI, 1.1–250.2; P = 0.043; see Table 2).

**Discussion**

ACRIN 6691 is the first multicenter breast imaging trial utilizing DOSI technology. As its primary aim, the trial was designed to evaluate whether %TOI$_{IN}$ from baseline to mid-therapy was predictive of the final pathologic response of breast cancer during NAC. DOSI measurements were successfully performed longitudinally throughout NAC at six different institutions across the United States.

Standard-of-care NAC response is currently assessed using changes in anatomic tumor size. However, anatomic assessment has been shown to be an unreliable metric of pathologic response and arises after a measurable change in functional characteristics (7, 19, 20). Functional imaging techniques, such as dynamic contrast enhanced (DCE) MRI and PET have shown better predictive value than anatomic imaging methods (10, 40), but they are costly, involve lengthy scan times, and require injection of a contrast agent, which limit availability and measurement frequency. DOSI is a portable, noninvasive intrinsic-signal technique that monitors functional tumor response during NAC and may predict early clinical outcome. Frequent longitudinal DOSI measurements were first reported to reveal dynamic tumor physiology (41), and validated the TOI endpoint in a single-site 34-subject study by showing %TOI$_{IN}$ is significantly different at mid-therapy for pCR versus non-pCR NAC patients (36). Additional studies by several groups have confirmed the sensitivity of diffuse optical methods to breast cancer NAC response using both baseline and dynamic response measures (30–32, 42–48). Although these studies were completed with different instruments and NAC protocols, their common findings indicate that tumor cell metabolism and vascularity are significantly altered during chemotherapy and optical methods are sensitive to several markers of these changes. For example, NAC-induced cellular damage alters tumor oxygen consumption and lowers tumor ctHbO$_2$, vascular damage causes a decrease in tumor ctHHb, and progressive loss of cellularity and edema results in a decrease in tumor water content.

The current multicenter clinical trial was devised to evaluate NAC response using a single, standardized DOSI platform and TOI endpoint. Extensive validation and performance assessment features were built into the study to evaluate the potential use of DOSI technologies in patient management. As expected, we observed that the 10 subjects achieving pCR exhibited dramatic
decreases in TOI\textsubscript{TN} from baseline to mid-therapy. However, although many non-pCR tumors exhibited lesser TOI\textsubscript{TN} changes, significant TOI\textsubscript{TN} decreases were, nonetheless, observed in some of the non-pCR tumors. Although a previous single-site study using identical DOSI technology showed %TOI\textsubscript{TN} was significantly different at mid-therapy for pCR versus non-pCR patients (36), the variability among non-pCR subjects limited the ability of %TOI\textsubscript{TN} to discriminate between pCR and non-pCR in the multicenter setting. This outcome may be due to factors that were not controlled for in this study including diverse tumor biology, widely varying chemotherapy regimens, and the fact that pathologic response is performed on a microscopic level. Nevertheless, pCR has been recognized by the FDA as a validated endpoint for evaluating drug efficacy and it is a surrogate for 5-year survival. The pCR endpoint has been used in a similar manner to test and validate several imaging technologies including PET, MRI, and ultrasound for monitoring and predicting NAC response. Several previous ACRIN NAC trials of experimental imaging endpoints have been based on pCR including ACRIN 6657 for MRI and ACRIN 6688 for PET.

The importance of studying individual NAC treatment response is supported by recent work, which suggests that pCR may not be correlated with disease-free survival in certain NAC agents (49). The %TOI\textsubscript{TN} variability we observe is primarily a result of the diverse responses observed in the non-pCR group, which includes subjects who have a significant tumor burden, as well as subjects with only small clusters of remaining tumor cells. A more refined clinical assessment of the non-pCR group using a continuously variable endpoint, such as the residual cancer burden (50) or the Miller-Payer system (51), could lead to a better understanding of the non-pCR TOI\textsubscript{TN} changes, and to a better separation of %TOI\textsubscript{TN} between response groups. The total number of non-pCR subjects is too small, however, to conduct such an examination in this study. In addition, 17 out

Figure 3.
A, DOSI images throughout NAC for a pCR (6691-08) and a non-pCR (6691-13) subject. Scale bar illustrates TOI values. B, percent change in TOI\textsubscript{TN}, HHb\textsubscript{TN}, H\textsubscript{2}O\textsubscript{TN}, Lipid\textsubscript{TN} from baseline for both subjects. Error bars represent the SD of multiple measurement locations defined by the ROI for each subject (25 and 28 locations for 6691-08 and 6691-13, respectively).
of the 34 evaluable tumors were involved to some degree in the areolar complex. In premenopausal females, the areolar region is metabolically active, and, in some cases, it has been shown to exhibit NAC induced changes in metabolism measured using DOSI (52). Our results suggest that there is a trend toward differential performance of %TOITN in areolar versus nonareolar tumors, with better discriminatory ability in non-areolar tumors (ROC AUC = 0.52; 95% CI, 0.18–0.85 vs. ROC AUC = 0.70; 95% CI, 0.43–0.97); however, given the small sample size in each subset (N = 17), there was large variability in the point estimates and this difference was not statistically significant (P = 0.41). A larger sample size would be needed to confirm this trend. Nevertheless, the large magnitude of tumor changes observed in non-pCR subjects with retroareolar tumors may, in part, be due to the response of metabolically active areolar breast tissue combined with adjacent involved tumor. These factors impact the magnitude of the tumor response measured using DOSI and they underscore the importance of controlling the environment (tumor type, chemotherapy protocol, etc.) to optimally predict therapeutic response.

Hypoxia, characterized in part by low StO2 levels, is often present in locally advanced breast tumors due to a combination of abnormal tumor microvessels, elevated tumor metabolism, and high tumor osmotic pressures (53). Previous studies have shown that prechemotherapy tumor oxygen saturation (39) and total hemoglobin (48) may be good prognostic tools for
predicting therapeutic outcome. Here, the data also suggest that tumor oxygen saturation plays an important role in predicting pCR. Improved pCR prediction was observed when performing the primary analysis on tumors with baseline tumor oxygen saturation greater than the median value (77%). This prediction was not possible in subjects with baseline tumor oxygen saturation <77%. These results suggest that baseline tumor oxygen saturation may be important in stratifying the population for evaluating DOSI response. Overall, baseline tumor StO2 appears to be an important imaging biomarker that can be used in combination with dynamic changes to predict clinical outcome. However, the underlying origin of this response is not yet known. The predictive power of the %TOITN dynamic response may be restricted to well-oxygenated tumors because of technical reasons related to DOSI, or because of the biological advantage of oxygenation. Establishing threshold criteria for baseline DOSI measurements could play an important role in interpreting the tumor dynamic response and, ultimately, employing DOSI to improve patient outcome. Establishing predictive thresholds is expected to be sensitive to both the technology employed and the patient population. Their practical use in clinical management and outcome prediction will depend on whether baseline parameters can be clearly defined both within and across technology platforms and this should be the focus of future studies.

To assess the uniqueness of DOSI endpoints, our results were evaluated for the possible confounders of pretreatment molecular subtype and tumor size. Both have been used previously to stratify patient therapeutic response predictions (54, 55). No association was found between baseline tumor oxygen saturation and molecular subtype in the 31 of 34 evaluable patients where these data were available ($P = 0.41$ for ER status and $P = 1.0$ for PR status from Fisher exact test). Low correlation also occurred between baseline tumor oxygen saturation and tumor size (measured by ultrasound) in the 19 of the 34 patients where baseline tumor size was available (Spearman correlation $= -0.39$, $P = 0.10$).

Studies have shown that diffuse optical techniques are sensitive to breast tissue composition changes during the menstrual cycle (56–58). To account for these biological changes and for chemotherapy-induced changes in normal tissue, the tumor-to-normal ratio was reported. This resulted in 10 subjects excluded from the analysis due to the absence of contralateral tissue measurement. As part of the secondary aims, changes of tumor values rather than T/N ratio will be reported in future analysis. However, the preliminary results were not as promising as the ratiometric analysis.

**Study limitations**

Important study limitations were the diversity of the patient population, heterogeneity of tumor molecular subtypes, and...
lack of standardized chemotherapy regimens. In addition, the adequate sample size was determined to be 47 subjects; however, only 34 of the 60 enrolled cases were assessed as analyzable. Because this study was the first multicenter trial that tested longitudinal DOSI measurements during NAC, much effort was focused on instrument and procedure standardization. Each instrument was fabricated and tested at University of California Irvine, using a common set of tissue-simulating phantoms before delivery to the study site. Preliminary analysis demonstrated <1% variability in broadband DOSI over 1 hour (typical patient measurement time-frame), <5% variability over 4 months (typical chemotherapy measurement time-frame), and <6% variability over the 2-year study duration. Although operators at all institutions were trained to perform standard measurement procedures, clinical DOSI data were in general of poor quality for the first few subjects. Data quality improved with more practice and feedback, nevertheless, the number of nonevaluable cases was primarily due to operator error that led to incorrect instrument settings and poor data quality, as well as clinical work-flow time pressures that resulted in lack of normal side data on several subjects. Also, a large disparity in subject enrollment occurred across institutions: UCI and MDACC enrolled about two thirds of the subjects, whereas MGH and UCSF enrolled a total of three and two subjects, respectively. As the technique and instrument become more standardized, and through the experience gained with this study, ease-of-use is expected to improve, and the number of nonevaluable cases is expected to decline in future multicenter studies. Finally, tumor dimensions by ultrasound matching the DOSI scan date at ±2 weeks (at baseline and mid-therapy) were available for only 12 subjects. Of those, only two were pCR, limiting our ability to make a DOSI-ultrasound comparison.

Future analysis

Analysis of ACRIN 6691 secondary aims is ongoing, including examining other DOSI dynamic response endpoints, baseline parameters, and molecular subtype. Further analyses will also explore the development of new indices to report tumor changes. Finally, the tumor ROI used in this study was obtained with an algorithm based on the FWHM around the baseline (pre-chemotherapy) TOI peak. Although some groups have explored the impact of different ROIs on NAC prediction of DOI (31, 32), the number of nonevaluable cases was primarily due to operator error that led to incorrect instrument settings and poor data quality, as well as clinical work-flow time pressures that resulted in lack of normal side data on several subjects. Also, a large disparity in subject enrollment occurred across institutions: UCI and MDACC enrolled about two thirds of the subjects, whereas MGH and UCSF enrolled a total of three and two subjects, respectively. As the technique and instrument become more standardized, and through the experience gained with this study, ease-of-use is expected to improve, and the number of nonevaluable cases is expected to decline in future multicenter studies. Finally, tumor dimensions by ultrasound matching the DOSI scan date at ±2 weeks (at baseline and mid-therapy) were available for only 12 subjects. Of those, only two were pCR, limiting our ability to make a DOSI-ultrasound comparison.

Conclusion

As an experimental cancer imaging technology, DOSI has been tested for the first time in ACRIN 6691, an independently-executed, prospective multicenter trial of breast cancer NAC. The results demonstrated that although a range of responses to NAC were observed in this heterogeneous patient population, subjects exhibiting a greater drop in %TOI_TN from baseline to mid-therapy were more likely to have a pCR to NAC. Although this change alone was not a strong predictor of clinical outcome for the full patient population, its performance was substantially improved when patients were stratified according to baseline tumor oxygen saturation levels. Importantly, the same dynamic %TOI_TN change was unable to predict pCR in subjects with baseline tumor oxygen saturation lower than the median value. This finding suggests that stratification using baseline tumor properties, especially oxygenation, can be used to improve DOSI therapy response predictions for individual subjects, particularly among diverse patient populations and NAC treatment strategies.

Disclosure of Potential Conflicts of Interest

B.J. Tromberg reports receiving a commercial research grant from LG Electronics, has ownership interest (including patents) in intellectual property related to breast imaging assigned to UCI Regents, and is a consultant/advisory board member for Hamamatsu Photonics. A.E. Cerussi has ownership interest (including patents) in intellectual property related to breast imaging assigned to UIC Regents. W. Yang is a is a consultant/advisory board member for General Electric Healthcare and Devisor. K.D. Paulsen has ownership interest (including patents) in Intellectual Property related to breast imaging, CairnSurgical, and MIST, Inc. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

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Other (review and analysis of pathology reports, contribution of study definitions): P.M. Carpenter

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