Predicting Breast Tumor Response to Neoadjuvant Chemotherapy with Diffuse Optical Spectroscopic Tomography prior to Treatment

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Abstract

Purpose: To determine whether pretreatment biomarkers obtained from diffuse optical spectroscopic tomographic (DOST) imaging predicts breast tumor response to neoadjuvant chemotherapy (NAC), which would have value to potentially eliminate delays in prescribing definitive local regional therapy that may occur from a standard complete 6- to 8-month course of NAC.

Experimental Design: Nineteen patients undergoing NAC were imaged with DOST before, during, and after treatment. The DOST images of total hemoglobin concentration (HbT), tissue oxygen saturation (StO2), and water (H2O) fraction at different time points have been used for testing the abilities of differentiating patients having pathologic complete response (pCR) versus pathologic incomplete response (pIR).

Results: Significant differences ($P < 0.001$, AUC = 1.0) were found between pCR patients versus pIR in outcome, based on the percentage change in tumor HbT within the first cycle of treatment. In addition, pretreatment tumor HbT (pretreatment HbT) relative to the contralateral breast was statistically significant ($P = 0.01$, AUC = 0.92) in differentiating pCR from pIR.

Conclusions: This is the first clinical evidence that DOST HbT may differentiate the two groups with predictive significance based on data acquired before NAC even begins. The study also demonstrates the potential of accelerating the validation of optimal NAC regimens through future randomized clinical trials by reducing the number of patients required and the length of time they need to be followed by using a validated imaging surrogate as an outcome measure. Clin Cancer Res; 20(23); 6006–15. ©2014 AACR.

Introduction

Breast cancer is the most common nonskin malignancy in women worldwide, and the second leading cause of female cancer mortality in the United States (1). A common treatment strategy is neoadjuvant chemotherapy (NAC) before surgery when tumor size is larger than 3 cm because of the opportunity to monitor the response of the primary disease which is expected to be representative of response of distant metastases well before they become clinically apparent (1). Clinical studies have shown that patients with a complete pathological response (pCR) to NAC experience longer disease-free survival. (2–4) However, as the pCR rate is only about 20% to 30%, (4) and the delay in definitive local therapy that may occur from a complete course of NAC can be long (up to 8 months; ref. 5), prediction of pCR before NAC and/or from early treatment response to stratify disease management is likely to improve the outcomes of patients and their long-term survival. In particular, if a prognostic marker was highly accurate of response to NAC and could successfully be utilized before therapy, significant benefits could occur to both the healthcare system and the patient through better disease management.

Conventional cancer imaging systems [mammography, ultrasound (5) and MRI (2, 6)] have been reported to assess response to treatment; however, the evaluations are typically based on changes in tumor volume which occur secondarily to physiologic variations, and usually require at least three cycles of treatment before an accurate determination can be reached (7). Functional imaging techniques such as dynamic contrast-enhanced MRI
Translational Relevance

The results of this study suggest that biomarkers obtained through diffuse optical spectroscopic tomographic imaging could be prognostic for response, potentially eliminating the delay in definitive local regional therapy that may occur from a complete 6- to 8-month course of neoadjuvant chemotherapy (NAC). The deeper implication of this information is that certain tumors are predisposed to responding to NAC, and that this predisposition should be known before choosing the therapy, similar to how immunohistochemistry is used today. The study also demonstrates the potential of accelerating the validation of optimal NAC regimens through future randomized clinical trials by reducing the number of patients required and the length of time they need to be followed by using a validated imaging surrogate as an outcome measure.

Materials and Methods

The NIRST imaging system has been developed at Dartmouth during more than a decade of research, and was first approved for experimental breast imaging studies at Dartmouth in 2001 (20, 21). In this study, subjects provided informed consent as part of the protocol approved by the Dartmouth Institutional Review Board (IRB) to use the latest NIRST system (12), which the IRB designated as a nonsignificant risk (NSR) device according to FDA guidelines. The study was Health Insurance Portability and Accountability Act (HIPAA) compliant. Subjects enrolled in this study received NAC recommended by their medical oncologist, typically any of several taxane/anthracycline chemotherapy regimens. More specifically, the regimens frequently used at the time these patients were enrolled in this study. For women with Her2+ LABC, the regimen used was TAC [docetaxel (Taxotere), doxorubicin (Adriamycin), and cyclophosphamide], or one of several other anthracycline/taxane combination regimens. For women with locally advanced Her2− disease, patients generally were treated with AC [doxorubicin (Adriamycin), and cyclophosphamide]/paclitaxel (Taxol)/trastuzumab (Herceptin)], given exactly as in CALGB 49909/NCCTG 9831, which was the first large adjuvant trial demonstrating the impact of adjuvant trastuzumab (22). Table 1 lists clinical information on the 19 subjects enrolled, including age, radiologic breast density, menopause status, initial pathologic diagnosis/receptor status, initial tumor region of interest (ROI), and surgical pathology outcome (complete or incomplete response, pCR or pIR) after treatment. The mean age of participants was 49 years old (range 27–70). The majority of subjects, 58% (11/19), were premenopausal and most, 74% (14/19), had mammographically dense breasts, including heterogeneously dense (H) and extremely dense breasts (E) compositions. All of these women completed their NAC and surgery as part of standard-of-care breast cancer management.

For each subject, baseline clinical images (primarily contrast MRI) of the diseased breast were acquired as a part of standard clinical care. The details of the MRI parameters have been described previously (12). DOST was performed before the start of NAC (pretreatment, baseline), approximately days 7, 14, and 21 in cycles 1 and 2, and within 7 days before the second half of treatment was initiated (midpoint). These time points were selected because they were representative of the biologic time periods during which treatment variations were most likely to be observed based upon earlier studies (13). After all treatment cycles were completed, DOST images of both breasts were obtained several days before surgery. Histopathologic characteristics of the resected tissue were evaluated after surgery (17), and a complete (pCR) or incomplete (pIR) pathologic response to NAC was determined and compared with the DOST results.
As described previously (12, 23), subjects were prone on a padded examination table during each DOST imaging session with the breast to be imaged pendant within the fiber-optic array. The circular fiber array was composed of 48 fiber bundles positioned in three planes that were separated vertically by a distance of 1.5 cm, and their diameters each decreased by 4 mm from chest wall towards the nipple. During the pretreatment imaging session, the tumor location was marked on the breast surface. The height of the fiber bundle planes was adjusted with a vertical motor so that the middle plane of the array was placed on the breast surface mark designating the tumor location. The diameter of the fiber array was controlled by 16 motors that translated each fiber bundle radially in synchrony to ensure the bundles contacted the breast uniformly. The distance from the chest wall to the top fiber plane and the diameter of the circular planes were recorded. In all subsequent DOST imaging sessions, the chest wall distance was kept the same for both the abnormal and contralateral breasts, whereas the diameter of the abnormal breast varied depending on the changes in tumor and breast size that occurred during treatment for each subject.

Frequency modulated near-infrared light from 6 laser diodes was delivered sequentially to illuminate the breast with an average optical power of less than 30 mW. A total of 240 measurements of transmitted light amplitude and phase were acquired for each wavelength of light and all detector positions within each plane of the fiber bundle.

<table>
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<th>Breast density</th>
<th>Initial Pathology (ER/PR/Her2)</th>
<th>Size/ROI (mm)</th>
<th>Treatment regimen</th>
<th>Surgical pathology</th>
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<td>TAC</td>
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array. The data acquisition time for the three measurement planes for all six wavelengths was approximately 7 minutes. A spectrally constrained chromophore and scattering reconstruction method was used to separate the tissue absorption and scattering properties, and recover $H_bT$, $StO_2$, and $H_2O$ images (24). In this method, chromophore constraints were incorporated into the reconstruction algorithm to estimate oxyhemoglobin, deoxyhemoglobin, and $H_2O$, whereas an empirical approximation to Mie scattering theory was used to constrain the elastic scattering properties. A series of spectral images was recovered for each plane (25), and the plane maximally overlapping the tumor was used for analysis.

For image analysis, the tumor ROI was defined by the interpretation of the radiologist of the contrast MR coronal plane (acquired before initiation of therapy) corresponding to the same midplane tumor distance from the chest wall used to position the DOST instrumentation. The entire tumor area, the entire area outside of the tumor ROI, and the entire area of the contralateral breast, in the designated plane were defined as tumor, nontumor, and contralateral ROIs, respectively. The mean values of $H_bT$, $S_tO_2$, and $H_2O$ of each ROI were found from the reconstructed images. To minimize baseline variations from intersubject variability of breast density affecting the absolute $H_bT$, $StO_2$, and $H_2O$ parameters, ratios relative to the pretreatment average of the contralateral breast (ROI/contralateral whole breast) were formed. In addition, ratios of the mean values in the ROI relative to outside the ROI were defined as tumor contrast. At each time point, a two-sample $t$ test was used to determine whether significant changes occurred between each time point and the baseline in $H_bT$, $StO_2$, and $H_2O$ in the pCR and pIR patients. Receiver operating characteristic (ROC) curves were formed and area under the ROC curve (AUC) was obtained to illustrate graphically the performance of DOST at baseline, after the first cycle of NAC and after later sessions. We use 2,000 stratified bootstrap replicates to estimate the 95% confidence interval for the AUC. The Bonferroni correction was used to adjust for multiple comparisons.

Results

Figure 1A and B show a set of DOST images of a pCR case prior (same day, but before the first infusion, \[ Pre-TX \]

\[ H_mT/Pre-TX,Contrat \]

\[ ROI \]

\[ Bilgrd \]

\[ Cntrlat \]

\[ Post-TX \]

\[ MRI \]

\[ NIRT \]

\[ C1,D7 \]

\[ Post-TX \]

\[ NIRT \]

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\[ ROI \]

\[ Bilgrd \]

\[ Cntrlat \]

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pretreatment), during [on day 7 of cycle 1 (C1, D7)], and after NAC (posttreatment, 7 days after the last infusion and 22 days before surgery), as well as an axial contrast MR image (30 days) before NAC was started. This 66-year-old woman had a large palpable lump in the upper central portion of the left breast. MR imaging showed the lump size was 59 x 43 x 53 mm, and the biopsy results indicated it was ER^-, PR^-, Her2neu^+ (ACR category 6) invasive ductal carcinoma (IDC) mixed with ductal carcinoma in situ (DCIS). The chemotherapy regimen this subject received was four cycles of paclitaxel (Taxol) and trastuzumab (Herceptin), followed by four cycles of FEC [5-flourouracil, epirubicin, and cyclophosphamide]/trastuzumab (Herceptin)]. The DOST images are shown in Fig. 1A with Hb T and H2O concentrations in the ROI decreasing after the first infusion, and eventually no contrast in Hb T and H2O in the ROI was detectable after NAC was completed.

Figure 1C–E show Hb T, StO2, and H2O at different time points of NAC. The mid-point session occurred on (C4, D14), which is 7 days before the first infusion of the second half of the NAC regime. During the first half of NAC, Hb T (Fig. 1C), StO2 (Fig. 1D), and H2O (Fig. 1E) in the ROI decreased from 2.47 to 1.41, 1.13 to 0.91, and from 1.98 to 1.02, respectively, for the three parameters. Furthermore, the early percentage changes of Hb T, StO2, and H2O between (C1, D7) and the pretreatment session were −110%, −15%, and −25%, respectively. The pretreatment contrasts in Hb T, StO2, and H2O for this patient were 1.3, 1.0, and 1.2, respectively. The surgical pathology showed no residual IDC or DCIS existed in the excised specimen, and confirmed the case was a pCR.

Figure 2A and B contain a set of DOST images from a pIR case where pretreatment (on the same day, before the first infusion), during [on day 14 of cycle 1 (C1, D14)], and posttreatment (18 days after the last infusion and a day before the surgery), as well as contrast MR images (20 days) before the start of NAC are presented. The axial contrast MR images in Fig. 2B revealed that this 56-year-old woman had an index invasive breast cancer with dimensions of 5.4 x 3.4 x 5.2 cm in the lateral right breast with rim-enhancing skin metastases overlying the index tumor at 9:00 en face (top image). In addition, she had a subpectoral lymph node metastasis (lower image) at 12 measuring 2.5 x 1.9 x 3.0 cm. The biopsy results proved the lesion was an ER^+, PR^+, HER2neu^+ (ACR
Category 6) mucinous IDC. The chemotherapy regimen this subject received consisted of four cycles of doxorubicin (Adriamycin)/cyclophosphamide followed by four cycles of paclitaxel (Taxol). The subepidermal lymph node metastasis could not be imaged by DOST due to its proximity to the chest wall (See Fig. 2B, lower MR image). As the rim-enhancing skin metastases exist overlying the index tumor, (see Fig. 2B, top MR image), the tumor ROI in DOST was evaluated as the sum in this case. In the DOST images shown in Fig. 2A, HbT and StO2 in the ROI increased, whereas H2O decreased in the first cycle of NAC, but residual contrast in HbT and H2O were observed in the images after NAC was completed.

To quantify the HbT, StO2, and H2O changes, Fig. 2C–E present graphs of these values at different time points relative to the NAC treatment cycles. The midpoint imaging session occurred on the same day, but before the first infusion of the second half of the NAC regime. During the first course of NAC, HbT (Fig. 2C), StO2 (Fig. 2D), and H2O (Fig. 2E) in the ROI fluctuated with an overall decrease from 1.47 to 0.72, 1.19 to 1.00, and from 2.26 to 2.23, respectively. However, the early percentage changes in HbT, StO2, and H2O were normalized to the mean of the pretreatment baseline for analysis. The early percentage changes in HbT, StO2, and H2O for this patient were 1.4, 1.0, and 1.3, respectively. The pathology results on the surgical specimen revealed that residual IDC and DCIS were present in the lesions, although with some treatment effects, and confirmed the case was a pIR. In addition, IDC was identified in the tissue between the skin metastases and the index tumor; hence, the two represented one contiguous cancer with a maximum diameter of 65 mm. The pathologic results confirmed the validity of the ROI definition we used for the DOST image analysis.

Of the 19 patients who finished all imaging exams and were included in the analysis, 9 subjects were confirmed as pCR, whereas 10 were confirmed as pIR. Figure 3 shows boxplots of early percentage changes in tumor HbT in Fig. 3A, pretreatment HbT in Fig. 3B, and pretreatment contrasts in StO2 (cStO2) in Fig. 3C, and H2O (cH2O) in Fig. 3D, respectively. The early ΔHbT in Fig. 3A represents the change in the pretreatment image and the image which was acquired in the 2-week time frame after the first cycle (C1, D7), and the first day of cycle 2 (C2, D1). To allow intersubject comparisons of HbT, StO2, and H2O, the HbT, StO2, and H2O values were normalized to the mean of the contralateral breast from the pretreatment baseline image on a subject-by-subject basis. The red lines in the boxes represent the medians of the 9 pCR and 10 pIR subjects, respectively. The two ends of the line through each box indicate the full range of each property. Means/medians of early ΔHbT% in the pCR and pIR cases were -74%/−43% and 21%/20%, respectively. The P value for difference in these means between the pCR and pIR groups was 0.0003. As shown in Fig. 3B, means/medians of pretreatment HbT in the pCR and pIR cases were 2.2%/2.0% and 1.5%/1.5%, respectively. The difference in mean pretreatment HbT between the pCR and pIR groups was significant with a P of 0.01. The box plots of pretreatment cStO2 and cH2O in Fig. 3C and D showed the means/medians in pCR and pIR groups were 1.1/1.0 and 1.4/1.3, and 1.0/1.0 and 1.2/1.1, respectively. Statistical tests for differences in mean pretreatment cStO2 and cH2O between the pCR and pIR groups indicated marginal significance (P values of 0.07 and 0.06, respectively).

Figure 4 contains ROC analysis of sensitivity versus specificity as quantified by the normalized AUC for early ΔHbT% in (Fig. 4A), pretreatment HbT in (Fig. 4B), pretreatment cStO2 in (Fig. 4C), and cH2O in (Fig. 4D). The AUC values were 1.0 and 0.92 for early ΔHbT, indicating excellent potential for high sensitivity and specificity with these diagnostic measures. The P values of pretreatment cStO2 and cH2O were not significant in differentiating the pCR from pIR groups; however, the AUC of 0.8 for StO2 and AUC of 0.74 for H2O indicated fair potential for diagnostic accuracy when using these tests. To investigate the cut-off for early ΔHbT% and pretreatment HbT, the positive predictive value (PPV) was calculated. In this small patient group, less than a 40% reduction in HbT during the first NAC cycle or 1.5 in pretreatment HbT would be needed to achieve a perfect PPV = 1.0.

Table 2 summarizes the means and SDs of age, early ΔHbT, and pretreatment HbT, StO2, and H2O in the ROI.
and their contrast levels. The patient counts for positive and negative, \( P \) values, and AUC of menopause status, ER, PR, Her2, early \( \Delta HbT \), and pretreatment \( HbT \), \( StO2 \), and \( H2O \) in the ROI and their contrast levels for differentiating the pCR and pIR groups are presented in this table as well.

**Table 2.** Summary of means and SDs for age, early \( \Delta HbT \), and pretreatment \( HbT \), \( StO2 \), and \( H2O \) in the ROI and their contrasts

<table>
<thead>
<tr>
<th></th>
<th>Pretreatment ( HbT )</th>
<th>Pretreatment ( StO2 )</th>
<th>Pretreatment ( H2O )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( HbT )</td>
<td>ROI Contrast</td>
<td>ROI Contrast</td>
<td>ROI Contrast</td>
</tr>
<tr>
<td>( StO2 )</td>
<td>1.00 (^{a})</td>
<td>-0.60</td>
<td>-0.60</td>
</tr>
<tr>
<td>( H2O )</td>
<td>0.02 (^{a})</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>( pCR )</td>
<td>50.1 ± 3.1</td>
<td>5/4</td>
<td>3/6</td>
</tr>
<tr>
<td>( pIR )</td>
<td>48.3 ± 3.3</td>
<td>3/7</td>
<td>8/2</td>
</tr>
<tr>
<td>( ER )</td>
<td>2.7</td>
<td>2/7</td>
<td>6/3</td>
</tr>
<tr>
<td>( PR )</td>
<td>1.1 ± 0.02</td>
<td>1.1 ± 0.02</td>
<td>1.1 ± 0.02</td>
</tr>
<tr>
<td>( Her2 )</td>
<td>1.4 ± 0.08</td>
<td>1.4 ± 0.08</td>
<td>1.4 ± 0.08</td>
</tr>
</tbody>
</table>

**Figure 4.** AUC curves are shown for early percentage changes of tumor \( HbT \) between the pretreatment (pre-Tx) and the last imaging session in the first NAC cycle (A), and pretreatment \( HbT \) (B), pretreatment contrast in \( StO2 \) (C), and pretreatment contrast in \( H2O \) (D).

**Discussion**

The data in Fig. 3A offers improved power for statistically valid separation of \( \Delta HbT \) between subjects who had pCR versus pIR relative to our previous pilot study (12), and is encouraging given our earlier findings are reinforced with the increased number of enrollments.
A statistically significant separation of pretreatment HbT between subjects with pCR and pIR (P = 0.01) was also observed for the first time, which suggests that DOST may have potential to differentiate the two groups even before NAC has begun with an easily measured parameter. Although P values of pretreatment cStO2 (0.07) and cH2O (0.06) did not individually yield a statistically significant separation between the pCR and pIR groups, the AUC values of 0.8 (cStO2) and 0.74 (cH2O) suggest that the two properties can add diagnostic value to pretreatment HbT at the start of treatment. For example, when we added these two properties to pretreatment HbT, the multiparametric AUC increased from 0.92 to 0.93. One solution to improving the StO2 and H2O image quality, which could also improve the accuracy of the estimated HbT values, would be to acquire more data at longer wavelengths that are known to be strongly absorbed by H2O and lipids. Previous and ongoing studies (26, 27) indicate that the addition of CW data acquired from at least three longer wavelengths and the inclusion of lipid as an additional chromophore in the image reconstruction has improved the contrast in H2O by more than 30%. As the imaging time in the present study was about 15 minutes for each breast, patient movement during an exam may contribute to noise in the images. Concurrent multilength data acquisition can accomplish complete single-plane tomographic recordings in less than 1 minute (28), which is expected to improve image quality by reducing/eliminating patient movement effects.

The observation that pretreatment HbT is predictive of response to NAC concurs with the hypothesis that functional vasculature is required for adequate blood flow to the tumor, which is necessary for adequate chemotherapy distribution and cellular uptake, and that tumors which lack sufficient vascular supply will suffer from inadequate chemotherapy delivery (29, 30). While this hypothesis was not studied explicitly, the apparently high correlation between pretreatment HbT and pathological response is consistent with the theory. Specifically, our earlier study of DOST versus pathologic immunohistochemical staining of biopsy specimens and resected surgical tissues in the same breasts showed that HbT changes were directly correlated to changes in pathologically measured CD31 reduction in response to NAC (17). Here, CD31 is a panendothelial marker for identifying preexisting blood vessel density and size. The results indicate that the current measure of HbT before NAC is an indicator of the actively perfused microvessels, and that the tumor region must be perfused to 50% above baseline to respond to the chemotherapy. The observation is potentially important both for fundamental understanding of NAC and for providing a simple prognostic indicator for managing patients.

Relative to other diffuse optical spectroscopy systems for monitoring breast tumor response to NAC (16, 19), the tomographic approach applied in this study has the advantage of providing more sensitive and spatially resolved information about tumor response, and this information is especially important when a significant amount of tumor is located deep (greater than ~3 cm) in the breast. DOSTs also have the potential to separate responses of each tumor component in cases involving multicentric disease. The pIR case presented in Fig. 2 is a good example. The DOST results localized the response in index tumor with rim-enhancing skin metastases to NAC, whereas the response in subpectoral lymph node metastasis was excluded.

As shown in Table 2, statistically significant separation of either ER or PR between subjects with pCR and pIR (P = 0.04 and 0.01, respectively) was also observed. However, analysis of the multiparametric predictive power of tumor response to NAC by combining these variables with DOST properties was not considered because of the limited number of patients enrolled in the study. Nonetheless, we expect that these variables, as well as other clinical parameters such as age and radiologic density, will add predictive power to DOST properties in future studies involving larger numbers of subjects.

The initial design of this NAC imaging study incorporated several therapeutic regimens that included 6 to 8 cycles of chemotherapy. Enrollment of women was inhibited by the requirement of breast imaging several times during the course of therapy with additional contrast MRI scans. Several women dropped out of the trial after the pretreatment imaging session because of treatment-induced fatigue from NAC or due to scheduling conflicts relative to the multiple imaging sessions that were required for participation. Imaging patients during the process of chemotherapy infusion within the oncology clinic could ease the burden of participation. Of note, a portable DOST monitor to track NAC response in subjects who are unable to return for a separately scheduled imaging session is technically feasible.

Conclusion

In this study of 19 patients with locally advanced breast cancer, pretreatment HbT inside the tumor ROI relative to the contralateral breast, and change in HbT after the first cycle of NAC were significant predictors of a pCR. Therefore, HbT of the involved and contralateral breasts measured before the start of NAC or a change in HbT in the involved breast within the first cycle of treatment could be used as prognostic indicators of neoadjuvant chemotherapeutic response. Given the importance of patient management in these complex, expensive, and time-consuming trials, validation of imaging indicators in a prospective clinical trial that can lead to wider adoption of the simple NIRST is needed. Identification of potential biomarkers that could lead to image-based surrogates for pCR, and accelerate the validation of optimal NAC regimens through future randomized clinical trials by reducing the number of patients required for enrollment and the length of time they need to be followed, is also critically important.
Disclosure of Potential Conflicts of Interest
S.P. Poplack reports receiving a commercial research grant from Hologic Inc. No potential conflicts of interest were disclosed by the other authors.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Jiang, B.W. Pogue, P.A. Kaufman, J. Gui, M. Jermy, S.P. Poplack, R. DiFiorio-Alexander, K.D. Paulsen
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References


Predicting Breast Tumor Response to Neoadjuvant Chemotherapy with Diffuse Optical Spectroscopic Tomography prior to Treatment

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