A Pilot Study to Determine the Efficacy of Cryoablation for the Treatment of Invasive Breast Carcinoma Following Neoadjuvant Therapy

Study Chair:
Rosa F. Hwang, M.D.
Department of Surgical Oncology

Study Co-Chairs:
Bruno Fornage, M.D.
Department of Diagnostic Imaging

Gildy V. Babiera, M.D.
Department of Surgical Oncology

Collaborators:
Kelly K. Hunt, M.D.
Department of Surgical Oncology

H. Carisa Le-Petross, M.D.
Department of Diagnostic Imaging

Amy Zhang, M.D.
Department of Pathology

W. Fraser Symmans, M.D.
Department of Pathology

Jason Stafford, Ph.D.
Department of Imaging Physics

Caimiao Wei, M.S.
Department of Biostatistics

1 Introduction

1.1 Background—Cryoablation

1.1.1 Ablation therapies for primary malignant diseases and metastatic disease

Ablative therapy using image guidance is an established and standard therapeutic technique for treatment of metastatic hepatic tumors. The best described techniques are the applied thermal technologies, including radiofrequency (RF) ablation and cryoablation. RF ablation for hepatic metastases from colorectal metastases can achieve local tumor control [1] and 5 year survival rates as high as 30%.[2] The same techniques have been applied to renal cell carcinoma but long-term follow-up data is lacking.[3, 4] The failure of these ablative therapies to extend long-term survival is not due to any inherent failure of the ablation technique to destroy the targeted focus of cancer but rather due to the nature of hepatic metastases and the propensity for multifocal disease.

Ablation therapies are currently being investigated in the treatment of primary tumors of many organ sites including prostate, renal cell carcinoma and lung cancer. The American College of Surgeons Oncology Group (ACOSOG) protocol “A Pilot Study of Radiofrequency Ablation in High-Risk Patients with Stage 1A Non-Small Lung Cancer” is an active study designed to evaluate the role of RF ablation in the treatment of primary lung cancer.

1.1.2 Cryoablation for benign fibroadenomas

Non-operative ablation of breast lesions is also a well studied area of investigation. The clinical model for breast cancer ablative therapy exists in the current office-based practice of cryoablation for benign fibroadenomas. These tumors are detected through physical examination or mammographic screening. After a core biopsy is performed to establish the diagnosis, the cryoablation technique can be employed as definitive treatment. This involves an injection of local anesthetic into the skin followed by a small nick in the skin for placement of the cryotherapy probe. The 3 mm probe is then inserted and the probe tip is guided to the center of the tumor under ultrasound guidance. The freezing process is created by liquid nitrogen gas circulating within the probe. To achieve complete ablation the freezing process is repeated for a total of two freeze-thaw cycles. The entire cryoablation process takes about 20 minutes. Once complete, the probe is withdrawn and a bandaid is applied over the insertion site. The patients are then followed to document absorption of the fibroadenoma. Complete clinical disappearance (by physical examination, ultrasound and mammogram) of fibroadenomas is achieved within one year in 95% of patients.[5, 6] In many centers, cryoablation of fibroadenomas is the preferred practice over surgical excision for several reasons, including the convenience of using the common office-based imaging system of breast ultrasound (US). As such US-based cryoablation is available to all practitioners equipped and certified in breast ultrasound. Furthermore, cryoablation is preferred by patients as it produces less pain, requires only a local anesthetic and leaves a much smaller incision than surgical excision. Once the skin

is anesthetized, the procedure is essentially painless due to the anesthetic properties of freezing the tumor. Thus, most patients undergo the cryoablation treatments fully awake without sedation and with minimal local anesthetic. Tumors amenable to cryoablation can be visualized, localized, and treated in real-time, typically in the span of 20 minutes. Cryoablation of fibroadenomas is also cosmetically advantageous since the treatment does not require removal of normal surrounding breast tissue and there is restoration of normal or near normal breast architecture on physical examination as well as by imaging with both ultrasound and mammography.[5, 6] The same advantages would be expected for cryoablation of primary breast cancers.

1.1.3 Cryoablation for invasive breast cancer

The use of ablative technologies, including RF ablation, cryoablation, interstitial laser therapy, microwave thermotherapy, and focused ultrasound (FUS) ablation are presently being explored to treat breast cancers as an alternative to surgical resection.[7-24] Although the above described ablative therapies could be considered for study, this application focuses on cryoablation for several reasons. One of the goals of this trial is to explore non-operative, office-based options to treat primary breast cancer. Toward that end, the ablative technique should be simple, readily available and as painless as possible. As mentioned previously, cryoablation for fibroadenomas serves as a useful model as it is easily performed in the office with minimal discomfort. The availability of office-based breast ultrasound and the short time for treatment with cryoablation are advantageous over other technologies such as radiofrequency, laser, focused-US, and microwave ablation which generally require additional imaging (stereotactic mammography or breast MRI), require longer treatment times for complete ablation (up to 2 hours) and typically produce more discomfort requiring sedation and/or anesthesia support.

To replace surgical resection of primary breast tumors with cryoablation, complete tumor ablation must be performed with a high rate of success. There are technical aspects of the cryoablation procedure that are important to optimize the complete ablation. The first is the ability to reliably localize the tumor for ablation. Many breast interventionists are familiar with US localization of tumors and there are existing guidelines for US credentialing that provide a ready-made approach for protocol standardization. This protocol will use established techniques employed in the cryoablation of fibroadenomas, but these techniques will be scrutinized for improvements during this study with the goal of achieving optimal ablation success.

To date, results from cryoablation of breast cancers have been highly successful with complete cell death seen within the targeted ablation zone. A pilot study of cryoablation in 27 patients with primary breast cancer documented complete ablation in those with infiltrating ductal carcinoma measuring less than 1 cm and in those with infiltrating ductal carcinoma measuring less than or equal to 1.5 cm without an extensive intraductal component. In this report, cryoablation was performed prior to surgical resection and the zone of ablation was evaluated with standard pathologic assessment. Of these patients, 25 out of 27 had a sentinel node biopsy performed at the same setting without interference from the cryoablation. [25] While this

data is highly promising, larger multi-institutional studies are needed to explore cryoablation in the treatment of primary breast cancer. This concept is currently being explored by ACOSOG for women with T1, unifocal breast cancers (ACOSOG Z1072). Standards have been developed for all of the multidisciplinary team members and will be incorporated into the current trial.

Reports of other ablative technologies for the treatment of breast cancer have demonstrated incomplete ablation in up to 30% of cases on pathologic assessment.[7-24] MRI pre- and post-ablation was shown in one study to predict residual disease in a pilot study of RF ablation for breast cancer followed by surgical resection.[26] The ability of MRI to assess completeness of ablation following cryoablation is a component of the current trial.

In the management of metastatic hepatic tumors, multiple ablation treatments are often required to achieve complete ablation and local control. It is possible that a similar paradigm may be applicable if cryoablation technology is applied to the treatment of locally recurrent primary breast cancers, larger primary breast cancers or to the treatment of residual foci of primary breast cancer after neoadjuvant chemotherapy.

1.1.3.1 American College of Surgeons Oncology Group (ACOSOG) Z1072

ACOSOG-Z1072 is phase II trial intended to determine if cryoablation can be successful at ablating small homogeneous tumors in a treatment-naïve breast. This is the important first step to determining whether there is feasibility of using cryoablation in any breast cancer circumstance as well as to determine the efficacy and accuracy of imaging with MRI. This trial continues to accrue patients, including women at our institution, and the results have been excellent with high rates of complete ablation and concordance between MRI findings and surgical pathology. The co-investigators and collaborators for the current trial are comprised of the same team that is participating in the ongoing ACOSOG Z1072 trial.

1.1.4 Neoadjuvant chemotherapy (NCT)

1.1.4.1 Rationale

One of the major reasons that NCT has become widely accepted as the treatment of choice for patients with locally advanced breast cancer is that it improves the surgical options for patients by downsizing the primary tumor. The frequency of mastectomies has decreased in patients receiving NCT with no significant increase in the in-breast recurrence rates.[27]

1.1.4.2 Downsizing tumors with NCT

Although there is no clear consensus regarding the optimal regimen for neoadjuvant therapy, the literature indicates that combination therapy is superior to single agent therapy. Moreover, sequential regimens, in which multiple drugs are administered in a preplanned sequence with no breaks, are associated with increased pathologic complete response (pCR) rates.[28] At our institution, the objective response rate (ORR, complete and partial responses) was 95% with

paclitaxel x 4 followed by 5-fluorouracil, epirubicin and cyclophosphamide (FEC) for 4 cycles. The pCR rate with this regimen was 26.3%. When trastuzumab is added to this regimen in patients with HER-2-positive disease, the pCR rate increased to 65.2%.[28] Correspondingly, the rate of breast conserving surgery (BCS) was also higher than would be expected in this group of patients with stage II-IIIA cancers (52.6-56.5%).[28] [27, 29]

A prospective, multicenter, randomized phase III study was conducted by the NSABP with a secondary endpoint to analyze the feasibility of breast BCS following NCT. For patients with T2 or greater invasive breast cancers treated with doxorubicin plus docetaxel or doxorubicin plus cyclophosphamide followed by docetaxel, the rate of BCS was 74%. Factors associated with a significantly higher rate of BCS included postchemotherapy tumor size ≤ 20 mm, nonlobular histology and treatment in a larger center (>10 enrolled patients).

1.1.5 Margin status with BCS after NCT

Resection of breast cancer with negative margins is an essential component of achieving optimal local control and is a significant factor in minimizing the risk of ipsilateral breast tumor recurrence[30]. Preoperative treatment of breast cancer with chemotherapy often results in downsizing with a reduction in the size of the primary tumor that can be identified on imaging, including mammogram, ultrasound and MRI. Multiple studies have demonstrated that there is no statistical difference observed for margin involvement between patients treated with NCT compared to those treated initially with surgery (21% vs. 18%, p=0.52)[31]. Resection of invasive lobular carcinomas treated with NCT was a risk factor for positive margins compared to resection of invasive ductal cancers and therefore, lobular carcinomas are excluded from the current protocol. At our institution, between 1987 and 2000, 340 patients were treated with NCT followed by BCS and 4% had positive surgical margins.[32]. Five year rates of IBTR-free and LRR-free survival were excellent at 95% and 91%, respectively. Variables that correlated with IBTR and LRR were clinical N2 or N3 disease, pathologic residual tumor greater than 2 cm, a multifocal pattern of residual disease and lymphovascular space invasion. Tumor histology was not analyzed in this series as a predictor of positive margins or locoregional recurrence-free survival.

Various imaging modalities have been evaluated for their ability to predict residual tumor after neoadjuvant therapy. MRI has been reported to have very good sensitivity, specificity and accuracy in detecting residual disease (90.5, 100, and 91.3%). In a series of 45 patients, the correlation coefficient between MRI and pathology measurements of the residual primary tumor was excellent (r^2 0.966, p<0.0001).[33] However, in a smaller series of 17 patients treated with NCT followed by surgery, MRI actually overestimated the extent of residual disease. Discordance between MRI and pathologic evaluation (<0.5-cm difference in size of residual tumor) was found in 66.6% of patients who had pathologic complete, or near-complete, response.[34] In the current protocol, FNA and/or core biopsy of the residual primary tumor site will be required to confirm residual carcinoma prior to cryoablation. Compared to physical exam, mammography and ultrasound, MRI has the highest accuracy in assessing the degree of pathologic response (19%, 26%, 35% and 71%, respectively, p<0.002).[35] [36]

1.2 Objectives

1.2.1 Primary Objective

- To determine the rate of complete tumor ablation in patients treated with cryoablation, with complete tumor ablation defined as no remaining invasive or in situ carcinoma present upon pathological examination of the targeted lesion.

1.2.2 Secondary Objectives

- To evaluate the negative predictive value of MRI in the post-ablation setting to determine residual in situ or invasive breast carcinoma
- To describe the adverse events associated with cryoablation
- To prospectively gather pain assessment data on cryoablation and surgical resection
- Explore technical variables that may affect success of cryoablation

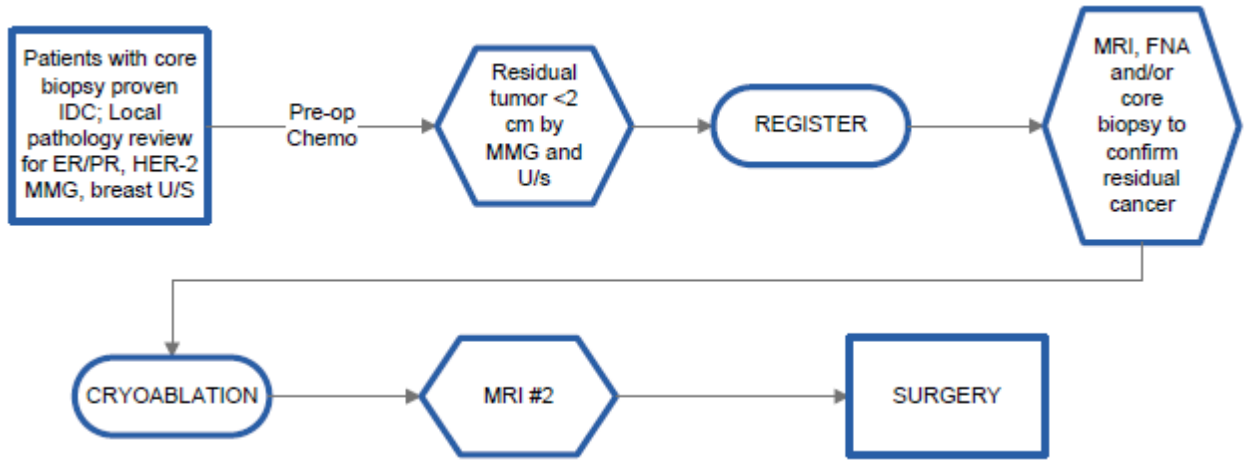
1.3 Study Design

This is a phase II non-randomized pilot study. Following neoadjuvant chemotherapy, all fully eligible and registered patients will undergo imaging by mammography, ultrasound, and breast MRI. Patients will then be treated with cryoablation therapy followed by re-imaging with breast MRI. Patients will then undergo complete surgical resection of the primary tumor.
Excised tissue will be evaluated pathologically for residual invasive disease in the targeted lesion. All patients will have standard surgical staging of the axilla with sentinel node dissection or axillary dissection and will have adjuvant therapy (hormonal therapy, chemotherapy and radiation therapy) as appropriate based on the initial stage of the disease.

1.4 Accrual Goal

A total of 10 eligible and evaluable patients are required for analysis. If results of the pilot study are encouraging and indicate that the study is feasible (see Statistical Considerations, section 12), we will plan a larger study to definitively evaluate the role of cryoablation.

1.5 Schema



2 Patient Selection

Each eligibility criterion must be evaluated and documented in the patient's medical record. Patient eligibility must be determined by the investigator.

2.1 Eligibility Criteria

A patient will be eligible for inclusion in this study only if ALL of the following criteria apply.

1. Unifocal primary invasive ductal breast carcinoma diagnosed by core needle biopsy. **NOTE:** Patients with lobular carcinoma, multifocal and/or multicentric ipsilateral breast cancer, multifocal calcifications, or DCIS with microinvasion are NOT eligible. Patients with contralateral disease will remain eligible.
2. No history of open surgical biopsy and/or lumpectomy for diagnosis/treatment of the index breast cancer. Note: Prior rotational and/or vacuum-assisted core biopsies are permitted if no significant distortion is seen on imaging that could obscure visualization and detection of residual disease on MRI, or visualization of cancer on ultrasound for cryoablation procedure.
3. Neoadjuvant chemotherapy or hormonal therapy for the index tumor is required.
4. Residual tumor size $\leq$ 2.0 cm in greatest diameter. Specifically, the tumor must measure $\leq$ 2.0 cm in the axis parallel to the treatment probe and $\leq$ 1.5 cm in the axis anti-parallel to the treatment probe. Largest size measured by mammogram, ultrasound or MRI will be used to determine eligibility.
5. Tumor enhancement on pre- registration MRI.
6. Tumor with <25% intraductal components in the aggregate.
7. Adequate breast size for safe cryoablation. Male breast cancer patients and female breast cancer patients with breasts too small to allow safe cryoablation are not eligible as the minimal thickness of the breast tissue does not lend itself to cryoablation.
8. Patients with prior in-situ or invasive breast carcinomas are eligible if the prior carcinomas occurred in the contralateral breast. Patients with prior in-situ or invasive carcinomas of the ipsilateral breast are not eligible.
9. Non-pregnant and non-lactating. Patients of childbearing potential must have a negative serum or urine pregnancy test. NOTE: Peri-menopausal women must be amenorrheic for > 12 months to be considered not of childbearing potential.
10. Patient has no contra-indication to an MRI examination, such as clips/prostheses/ implants that are not MRI compatible, or compromised renal function, with a measured or calculated glomerular filtration rate of 60ml/min/1.73m^2.
11. Patients less than 18 years of age will not be included in this study.

2.2 Staging Criteria

Patients will be staged according to the staging criteria adapted from the American Joint Committee on Cancer (AJCC) Cancer Staging Data Forms of the AJCC Cancer Staging Manual, 7th Edition, 2010.

2.3 Study Calendar

	Pre-neo adjuvant chemo therapy	Near Completion of chemo therapy	Post Chemo	Prior to Cryoablation*	Within 30 days post registration	Within 14-28 days post-cryo	Within 28 days post-cryo	Within 14 days post-surgery
History and Physical			INFORMED CONSENT/ REGISTRATION	X	CRYOABLATION	X	SURGERY	X
Pregnancy Test				X[1]				
Ipsilateral mammogram		X						
Hematology				X[2]				
Ipsilateral breast ultrasound		X		X[3]				
Breast MRI				X[4]		X		
Core biopsy	X[5]							
U/S guided FNA/Core biopsy of residual tumor				X[6]				
Pathology assessment				X				X

* Within 45 days prior to registration unless otherwise specified.

1. For women of childbearing potential only.
2. ANC ≥1500/uL and platelets > 50,000/uL
3. For Ultrasound-guided FNA and/or core biopsy.
4. Baseline MRI must be done within 14 days prior to registration.
5. Core biopsies for tissue diagnosis and tumor markers including ER, PR, and HER-2/neu, will be performed prior to registration. (Standard of Care)
6. FNA and/or core biopsy of residual tumor must confirm diagnosis of carcinoma.

Note: All procedures before informed Consent and the first MRI are Standard of Care.

2.4 Testing Guidelines

2.4.1 Core biopsy (pre-neoadjuvant chemotherapy)

The patient must have a prechemotherapy biopsy confirming the diagnosis of invasive ductal breast cancer. **Results of ER, PR and HER-2/neu must be obtained on the core biopsy material.**

The presence of *in situ* and invasive ductal carcinoma and approximate relative proportion of *in situ* component must be recorded. The Nottingham Grade Scheme incorporating the degree of tubule formation, nuclear grade and mitotic activity, should be used for invasive carcinoma. The *in situ* tumor should be described in terms of architectural type (solid, cribiform, etc.) nuclear grade and presence of luminal necrosis, and calcifications.

2.4.2 Fine needle aspiration (FNA) and/or Core biopsy (pre-registration)

Prior to registration, an ultrasound-guided FNA and/or core biopsy of the residual area of disease seen on imaging studies after neoadjuvant therapy must be performed to confirm carcinoma. If no residual carcinoma can be confirmed, then the patient is not eligible for the study.

2.5 Magnetic Resonance Imaging

A standard-of-care bilateral breast MRI examination will be performed pre-registration to evaluate the extent of the tumor and multifocality, and to compare areas of segmental or regional enhancement in each breast.

2.5.1 Required Imaging Sequences

The examination protocol of both breasts should contain at a minimum the following sequences:

- Bilateral pre-contrast axial T1-weighted 3D fast spoiled gradient echo pulse images with fat saturation (≤ 2 mm slice thickness, no gap),
- Bilateral three post-contrast series of axial T1-weighted 3D fast spoiled gradient echo images with fat saturation (≤ 2 mm slice thickness, no gap) with the first post-contrast series obtained immediately after intravenous injection of gadolinium (at T0)
- A delayed sagittal T1-weighted 3D fast spoiled gradient-echo pulse sequence with fat suppression (2-4mm slice thickness, no gap).
- A diffusion-weighted imaging sequence.

2.5.2 Contrast Medium

The intravenous dose gadolinium contrast media should be 0.1 mmol per kilogram of body weight, given via a power injector at a rate of 3 mL/sec, followed by a 20-mL saline flush.

2.5.3 Image Interpretation

Interpretation of the MRI exams will be according to the categories established in the American College of Radiology (ACR) Breast Imaging Reporting and Data System (BI-RADS) for MR Imaging. (American College of Radiology. ACR breast imaging reporting and data system (BI-RADS): breast imaging atlas. Reston, Va: American College of Radiology, 2003).

Areas of enhancement classified as suspicious for malignancy not contiguous with the main tumor on preregistration scan must be found to be benign by histology for the patient to be eligible for the study. Assessment by ultrasound-guided or MRI-guided biopsy is acceptable with concordant results.

3 Patient Registration

- The patient or the patient's legally acceptable representative must provide a signed and dated informed consent prior to registration and prior to beginning any study–related procedure or intervention.

4 Interventions

4.1 Tumor Cryoablation

All registered patients will receive cryoablation of the index breast cancer within 4 weeks after the last dose of chemotherapy. Only the devices and procedures described in the sections below may be used for patients registered to this study.

4.1.1 Cryoablation Device

The cryoablation will be performed using the commercially available Visica 2™ Treatment System. The Visica 2™ Treatment System (Sanarus Technologies, Inc.) consists of a Visica 2 Console, Visica 2 ICE Probe(s) and associated liquid nitrogen (cryogen) Case Dewars. The Visica 2 Console is a self-contained unit, which features an easy-to-use interface for complete control and monitoring of the cryoablation procedure. The Console operates off of standard 120 VAC (60Hz) power. The Visica 2 ICE Probe is a single-use, disposable probe designed for use with the Visica 2 Treatment System. The primary components of the Visica 2 ICE Probe consist of a surgical stainless steel probe with an integrated T-Type Thermocouple for internal temperature measurements. There are two configurations of the Probe – the Visica 2 ICE Probe and the Visica 2 ICE Probe SL. Only the Visica 2 ICE Probe will be used in this study.

In accordance with its FDA market clearance, the Visica 2 Treatment System is indicated for use in general surgery, gynecology and oncology. The system is designed to destroy tissue by the application of extremely cold temperatures. In addition, the system is intended for use in the following indications:

General Surgery
- Ablation of breast fibroadenoma
- Localization of breast lesions

Gynecology
- Ablation of malignant neoplasia or benign dysplasia of the female genitalia

Oncology
- Ablation of cancerous or malignant tissue
- Ablation of benign tumors
- Palliative intervention

Although this study will evaluate the cryoablation of cancerous tissues, it is important to understand that ablation will be followed by surgical resection in all cases. As such, cryoablation will not serve as a definitive therapy. Definitive cancer therapy, inclusive of surgical resection and further treatment (e.g. radiation therapy and adjuvant hormonal or chemotherapy), are at the treating physician's discretion, per the standard of care.

4.1.2 Device Specifications

Visica 2™ ICE Probe	
Overall length	8 ft. 6 in. nominal
Shaft Diameter	3.4 mm (0.134 in.)
Shaft Length	13 cm nominal
Shaft Active Freeze Zone	Distal 4.5 cm of Probe Shaft
Shaft Insulation	Proximal portion vacuum insulated

Visica 2™ Console	
Height	60 in. nominal
Width	20 in. nominal
Depth	27 in. nominal
Weight	250 lbs.
Electrical	120 VAC (60 Hz) 6 Amps

4.1.3 Device Operation

The Visica 2™ Treatment System uses a closed system to circulate liquid nitrogen within the probe tip creating sub-freezing temperatures that result in precision cryoablation of the intended target tissue. After the target lesion has been appropriately identified, the Probe is placed under ultrasound guidance into the center of the lesion and cryoablated according to a predetermined freeze algorithm consisting of a freeze cycle followed by a thaw cycle, followed by a final freeze cycle. The probe is then warmed by an internal electrical resistance heater and removed from the patient.

For the purposes of this study, the Visica 2™ Treatment System will be operated in accordance with the Visica 2™ Treatment System Operator's Manual and Visica™ 2 ICE Probes Instructions for Use except for:

- the collection of specific clinical and device operational information and
- the use of a treatment freeze algorithm designed to ablate the target lesion and a margin of tissue, analogous to the surgical margins of a lumpectomy.

4.1.4 Cryoablation Procedure

The cryoablation procedure will be performed in accordance with the instructions outlined in the Visica 2™ ICE Probe Instructions for Use except for:

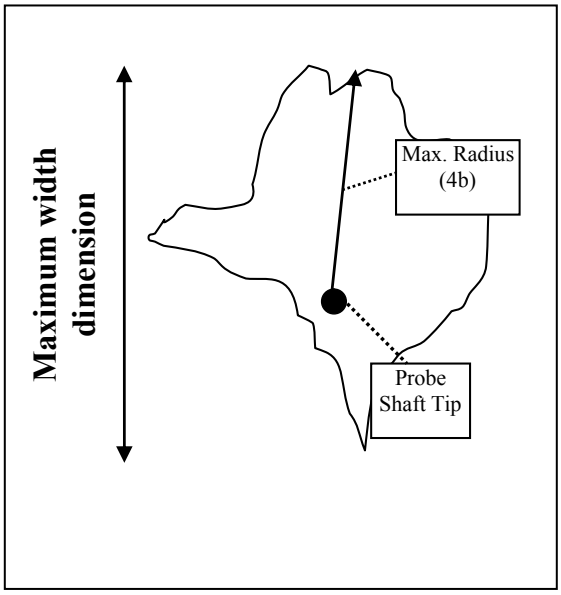
- the collection of specific clinical and device operational information, and
- the use of the treatment freeze algorithm in Table 1.

Representative sonographic images from the cryoablation procedure will be submitted for review by the study radiologist.

4.1.5 Probe Positioning Procedure -Locate the target lesion to be ablated using high resolution real-time ultrasound in both the radial and antiradial projections.

1) Determine the maximum diameter of the lesion in both projections. Record maximum lesion diameter on the Case Report Form (CRF). *Maximum lesion diameter must be ≤ 2.0 cm and the maximum lesion width dimension must be ≤ 1.5 cm to be eligible for inclusion into the study.*

2) Utilizing sterile technique and local anesthesia, a cryoprobe will be inserted percutaneously through a small skin incision (approximately 3-5mm). The probe will be manipulated under real-time ultrasound guidance and the probe tip advanced through the center of the malignant lesion such that the length of the probe shaft penetrates and is centered in the longest imaged lesion plane, as measured above. Proper probe placement will be documented with longitudinal and transverse sonographic imaging.



3) Visualize the lesion in the anti-parallel axis relative to probe shaft (i.e., viewed in cross section "end on"). Document probe position within the target lesion with an image printout including:

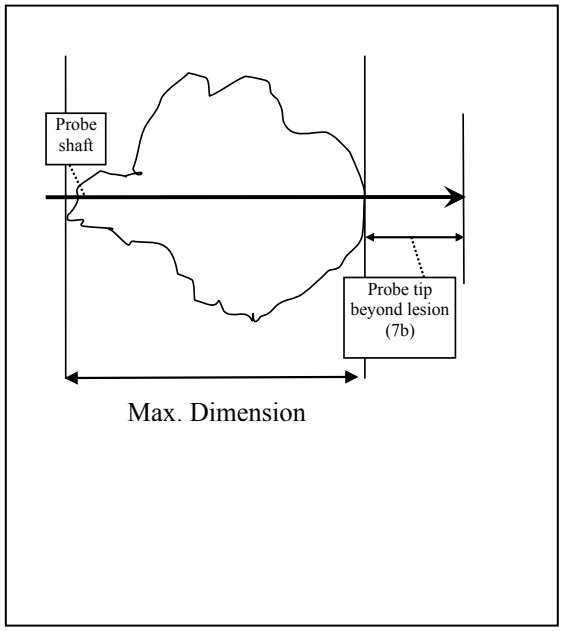
- The maximum width dimension shown in that plane. The tumor must measure 1.5 cm or less in the anti-parallel axis relative to the probe shaft. Record this measurement on the CRF.
- Maximum radius (4b) from the probe shaft to the outermost edge (farthest edge from probe tip) of the target lesion. This measurement should be less than 0.75 cm. Record this measurement on the CRF.
- Print out an image showing both recorded width dimensions above. One or two prints may be produced, but both measurements must show on the print(s).

4) Calculate the maximum lesion width dimension to be ablated as 2x the maximum radius as measured above. If the calculated maximum lesion width dimension to be treated (anti-parallel to probe within the target lesion) is greater than 1.5 cm, the probe must be relocated more central to the middle of the lesion.

5) Use that calculated lesion width dimension to apply the treatment algorithm in Table I if the calculated maximum lesion width dimension is ≥ the largest imaged pre-biopsy lesion width on either mammogram, ultrasound or MRI. If the largest imaged pre-biopsy lesion width on either mammogram, ultrasound or MRI is larger than the calculated lesion width dimension imaged at the time of the cryoablation, use the pre-biopsy lesion width to determine which algorithm should be used.

6) When the probe has been centered adequately, view the lesion in the long axis plane, parallel to the probe shaft and measure the following:

- The maximum lesion dimension shown in that plane. This dimension should not exceed 2.0 cm in the axis parallel to the probe shaft. Record this measurement on the CRF.
- The distance of the probe tip beyond the distal lesion edge. Enter the maximum lesion dimension into the V2 Console. The Console will calculate the distance of the probe tip beyond the lesion (7b) to ensure that the probe is properly positioned.
- Record this measurement on the CRF and print out an image of this measurement.
- Print out an image showing both recorded dimensions above. One or two prints may be produced, but both measurements must show on the print(s).



7) Initiate freeze cycles. Treatment Algorithm selected in Table 1. Look at the time listed in the second Freeze cycle in order to confirm that the freeze time is set to either 6 or 8 minutes as referenced in the algorithm.

8) Record Treatment Algorithm on the CRF.

9) Monitor ice ball growth using ultrasound visualization.

10) At the end of HI freeze 1, document maximum ice ball length and width with a printout (including measurements on the print). Record these dimensions on the CRF. Ensure that the ice ball diameter exceeds the calculated maximum lesion width dimension by 10 mm on all sides as determined via ultrasound by extending the HI freeze time if necessary. Record additional HI freeze time and document maximum ice ball length and width with a print out (including measurements on the print) if additional freeze time is required.

11) At the end of HI freeze 2, document maximum ice ball length and width with a printout (including measurements on the print). Record these dimensions on the CRF. Ensure that the ice ball diameter exceeds the calculated maximum lesion width dimension by 10 mm on all sides as determined via ultrasound by extending the HI freeze time if necessary. Record additional HI freeze time and document maximum ice ball length and width with a print out (including measurements on the print) if additional freeze time is required.

12) After completion of the cryoablation, the probe will be warmed and removed from the breast.

Table 1: Treatment Algorithm		
Max. Width Diameter	**< 1.0 cm**	**1.0-1.5 cm**
1st Freeze	HI 6 min US measure and ensure 10 mm margin	HI 8 min US measure and ensure 10 mm margin
THAW	10 min	10 min
2nd Freeze	HI 6 min US measure and ensure 10 mm margin	HI 8 min US measure and ensure 10 mm margin

5 Magnetic Resonance Imaging (Post-cryoablation)

A research breast MRI examination will be performed within 14 to 28 days post-ablation to determine whether any residual tumor can be defined within the targeted lesion.

The cost of the post-ablation MRI will be reimbursed using research funds.

Required Imaging Sequences

- Bilateral pre-contrast axial T1-weighted 3D fast spoiled gradient echo pulse images with fat saturation (≤ 2 mm slice thickness, no gap),
- Bilateral three post-contrast series of axial T1-weighted 3D fast spoiled gradient echo images with fat saturation (≤ 2 mm slice thickness, no gap) with the first post-contrast series obtained immediately after intravenous injection of gadolinium (at T0)
- A delayed sagittal T1-weighted 3D fast spoiled gradient-echo pulse sequence with fat suppression (2-4mm slice thickness, no gap).
- A diffusion-weighted imaging sequence.

A 1.5 Tesla strength or higher magnet and dedicated breast coil should be used.

5.1 Contrast Medium

The intravenous dose gadolinium contrast media should be 0.1 mmol per kilogram of body weight, given via a power injector at a rate of 3 mL/sec, followed by a 20-mL saline flush.

5.2 Review of Ultrasound and MRI Images

The pre-registration and post-cryoablation MRI images of the affected breast and ultrasound images from the cryoablation procedure will be transmitted to the study radiologist after the post-cryoablation visit.

5.2.1 Image Submission

The following materials will be submitted to the study radiologist.

- Pre-registration and post-cryoablation MRI images of the affected breast .
- Ultrasound images from the cryoablation procedure, including images of probe placement parallel and anti-parallel to the probe, and images of the largest dimension of the ice ball at the end of each freeze cycle, representing the ablation zone.
- Copies of corresponding radiology reports.

5.2.2 Final Imaging Review

The sonographic images of all cases will be compared to the MRI images by radiologist HL at the conclusion of the recruitment. The following data from the pre-registration MRI images will be recorded:

- Index cancer shows enhancement (yes/no)
- Maximal size of lesion (cm)
- Location of lesion (o'clock, cm from nipple)
- Other areas of suspicious enhancement (yes/no, number)
- Maximal size of lesion (cm)
- Location of lesion (o'clock, cm from nipple)

The following data from the post-cryoablation MRI images will be recorded:

- Cryoablated lesion shows enhancement (yes/no)
- Suspicious for residual disease (yes/no)
- Consistent with post-treatment change (yes/no)
- Indeterminate for residual disease (yes/no)

Each MRI study (pre-registration and post-cryoablation) and its interpretation will be reviewed and evaluated by the designated study radiologist, who will be blinded to the surgical and pathologic information.

Any enhancement in the ablated target lesion on the post-ablation scan similar to that seen on the pre-ablation scan will be considered as residual disease for purposes of interpreting the MRI exam.

6 Surgical Resection

At the time of surgical resection the surgeon will perform sentinel lymph node dissection and/or axillary dissection as per standard of care.

The surgeon will excise the ablation zone with standard technique. Additional surgical resection (i.e., re-excision or mastectomy) and further treatment (e.g., radiation therapy and adjuvant hormonal or chemotherapy) are at the treating surgeon's discretion as per the standard of care.

6.1 Pathology of Surgical Specimen

The surgical resection specimen, following cryoablation, will be oriented by the surgeon in a manner that facilitates the study of all six margins (lateral, medial, anterior, posterior, superior and inferior). The pathologist will ink the specimen margins and serially section the specimen at 2-3 mm intervals in the coronal or sagittal plane (as appropriate for the particular specimen) as is standard for breast specimens at our institution. The entire "cryolesion" will be subjected to histological examination. The remainder of the breast tissue will be thoroughly examined, and histological sections will be obtained from any grossly abnormal areas as well as from random fibrous areas.

Reporting of the histological evaluation of the surgical resection biopsy specimen should be similar to that of the diagnostic core biopsy specimen (See Testing Guidelines section). In addition, the effects of cryoablation (coagulative necrosis, fibrosis, organizing hemorrhage, etc.) will be recorded. The presence of any remaining in situ or invasive carcinoma in the target lesion will be categorized by the pathologist. This study will explore possible methods for quantification and development of an objective scoring system for any residual cancer after cryoablation for breast cancer. Possible methods that may be incorporated or modified include an existing classification model for pathologic assessment of tumor response after neoadjuvant chemotherapy. This includes the Symmans method (J Clin Oncol 2007).

7 Follow-up

7.1 Post-operative Follow-up

Patients will be seen by the surgical team within 14 days after surgical resection. Patients will undergo a physical exam. No further follow-up after the 14-day post-surgery visit will be required for this study.

8 Evaluation of Outcomes

The rate of complete tumor ablation in the target lesion will be evaluated by pathologic review of the surgical specimen.

The negative predictive value of MRI in the post-ablation setting to determine residual in situ or invasive breast carcinoma will be evaluated by radiologic review of pre- and post-ablation MRI imaging.

All patients will be evaluated for adverse events (including pain) associated with cryoablation using history and physical examinations.

The technical variables that may affect success of cryoablation will be reviewed and evaluated in procedure notes and on data forms.

9 Adverse Event (AE) and Serious Adverse Event (SAE) Reporting

The prompt reporting of adverse events is the responsibility of each investigator engaged in clinical research, as required by Federal Regulations.

Toxicities/adverse events must be described and graded using the terminology and grading categories defined in the most current version of the NCI's Common Toxicity Criteria (CTCAE) version 4.0. The CTCAE is available at http://ctep.cancer.gov/reporting/ctc.html.

Attribution to protocol treatment for each adverse event must be determined by the investigator and reported on the required forms, using the codes provided.

10 Routine Adverse Event Reporting

All expected or unexpected adverse events, regardless of grade or treatment attribution, must be recorded on AE case report forms (CRFs).

10.1 Expedited AE and SAE reporting definitions

The investigator must initially report the AE within 5 calendar days of learning of the event. If death occurs that is unexpected and definitely, probably or possibly related to study intervention during and within 30 days after the last day of active study intervention, the SAE must be reported within 1 working day (24 hours) from the time the research team becomes aware of the event. All SAEs that do not fall under the prompt reporting requirements will be reported during continuing review using the Internal SAE Log or database printout.

10.2 IRB Policy

We will follow the University of Texas M.D. Anderson Cancer Center IRB policies and procedures in submitting adverse events.

10.3 Expected Adverse Events

Expected AEs from MRI Contrast		
More Likely	**Less Likely**	**Rare but Serious**
Heat sensation	Hypotension due to vasodilatation	Hypotensive shock
Dizziness	Mild allergic reaction	Anaphylactic reactions
Nausea	Local pain and tenderness at the injection site	
Headache		

Expected AEs from Cryoablation		
More Likely	**Less Likely**	**Rare but Serious**
Pain at site of needle placement	Edema at site of treatment	Hypothermic damage to the skin of the breast
Bruising	Hematoma	Hematoma requiring intervention
Bleeding at site of needle placement	Bleeding requiring suture	Bleeding requiring surgical intervention
Infection	Infection treated with oral antibiotics	Infection requiring IV antibiotics

Expected AEs from Surgical Intervention
Cosmetic deformity from multiple procedures
Treatment delays due to complications

11 Statistical Considerations

11.1 Overview of study design

This is a phase II, one-stage, single arm pilot study to assess the success rate of cryoablation therapy following neoadjuvant chemotherapy in the treatment of invasive breast carcinoma.

11.2 Primary Objective

The primary objective is to determine the rate of complete ablation. Complete ablation is defined as no remaining invasive or in situ carcinoma present upon pathological examination of the targeted lesion. The rate will be computed as the number of patients with complete tumor ablation divided by the total number of eligible patients.

We will declare that the study is feasible if we observe at least 8 patients with complete ablations out of 10 patients.

11.3 Decision rule

There will be three basic decisions made at the end of this trial: recommend the treatment regimen for further trials in selected patient populations, recommend further studies to assess whether modifications to the cryoablation technique can achieve the target goal, or recommend no further studies of this treatment regimen.

If the number of patients with a complete tumor ablation rate is 8 or more (out of 10 eligible patients) we will recommend the treatment regimen for further trials in selected patient populations.

If the number of patients with complete tumor ablation is greater than or equal to 5 but less than 8 (out of 10 eligible patients), we will recommend testing modifications to the cryoablation technique to determine if these will achieve the target goal of 80% success rate or greater.

If the number of patients with a complete tumor ablation rate is less than 5 (out of 10 eligible patients), we will recommend no further studies of this treatment regimen.

11.4 Design Characteristics

The implications of the design depend upon the decision rule and the true (unknown) ablation rate. Table 2 characterizes the implications of the proposed decision rule for several different true ablation rates.

Table 2. Decision rules

Decision	Rule (n=10)	True Ablation Rate	Probability of making the Decision
Recommend for further trials	≥ 8 complete ablations	0.50	0.0525
		0.55	0.0944
		0.60	0.1683
		0.65	0.2696
		0.70	0.3816
		0.75	0.5308
		0.80	0.6722
		0.85	0.8158
		0.90	0.9246
		0.95	0.9885
Recommend testing modifications of cryoablation technique	≥ 5 and < 8 complete ablations	0.45	0.4627
		0.50	0.5732
		0.55	0.6419
		0.60	0.6686
		0.65	0.6347
		0.70	0.5705
		0.75	0.4466
		0.80	0.3214
		0.85	0.1829
		0.90	0.0753
Recommend no further studies	< 5 complete ablations	0.30	0.8465
		0.35	0.7475
		0.40	0.6303
		0.45	0.5121
		0.50	0.3743
		0.55	0.2637
		0.60	0.1631
		0.65	0.0957
		0.70	0.0479

11.5 Analysis plan

We will estimate the complete ablation with a 95% confidence interval. If we observe 8 of these patients with complete ablation, then the 95% confidence interval will be 44.4% to 97.5% (Table 3).

Table 3. The 95% exact confidence intervals of complete ablation rate if we observed x patients with complete ablation out of 10 patients.

Number of Complete Ablations Observed (out of 10)	Lower Bound of 95% CI	Upper Bound of 95% CI
3	0.0667	0.653
4	0.122	0.738
5	0.187	0.813
6	0.262	0.878
7	0.348	0.933
8	0.444	0.975
9	0.555	0.997
10	0.692	1.000

12 Protocol Monitoring Plan

Accrual, adverse event data, and efficacy data will be reviewed by the study team semi-annually. In addition, efficacy, toxicity, and administrative information for the trial will be reviewed by the study team monthly. The study team will monitor the trial for evidence of severe adverse events and feasibility and logistical problems. This includes monitoring for the progress of the study, the study and safety of participants, compliance with the consent process and assurance of data accuracy and compliance with the protocol.

REFERENCES

1. Solbiati, L., T. Livraghi, S.N. Goldberg*, et al.*, *Percutaneous radio-frequency ablation of hepatic metastases from colorectal cancer: long-term results in 117 patients.* Radiology, 2001. **221**(1): p. 159-66.
2. Gillams, A.R. and W.R. Lees, *Five-year survival in 309 patients with colorectal liver metastases treated with radiofrequency ablation.* Eur Radiol, 2009. **19**(5): p. 1206-13.
3. Gervais, D.A., F.J. McGovern, R.S. Arellano, W.S. McDougal, and P.R. Mueller, *Renal cell carcinoma: clinical experience and technical success with radio-frequency ablation of 42 tumors.* Radiology, 2003. **226**(2): p. 417-24.
4. Mayo-Smith, W.W., D.E. Dupuy, P.M. Parikh, J.A. Pezzullo, and J.J. Cronan, *Imaging-guided percutaneous radiofrequency ablation of solid renal masses: techniques and outcomes of 38 treatment sessions in 32 consecutive patients.* AJR Am J Roentgenol, 2003. **180**(6): p. 1503-8.
5. Kaufman, C.S., B. Bachman, P.J. Littrup*, et al.*, *Office-based ultrasound-guided cryoablation of breast fibroadenomas.* Am J Surg, 2002. **184**(5): p. 394-400.
6. Kaufman, C.S., P.J. Littrup, L.A. Freeman-Gibb*, et al.*, *Office-based cryoablation of breast fibroadenomas with long-term follow-up.* Breast J, 2005. **11**(5): p. 344-50.
7. Bloom, K.J., K. Dowlat, and L. Assad, *Pathologic changes after interstitial laser therapy of infiltrating breast carcinoma.* Am J Surg, 2001. **182**(4): p. 384-8.
8. Dowlatshahi, K., M. Fan, V.E. Gould, K.J. Bloom, and A. Ali, *Stereotactically guided laser therapy of occult breast tumors: work-in-progress report.* Archives of Surgery, 2000. **135**(11): p. 1345-52.
9. Dowlatshahi, K., D.S. Francescatti, and K.J. Bloom, *Laser therapy for small breast cancers.* Am J Surg, 2002. **184**(4): p. 359-63.
10. Elliott, R.L., P.B. Rice, J.A. Suits, A.J. Ostrowe, and J.F. Head, *Radiofrequency ablation of a stereotactically localized nonpalpable breast carcinoma.* American Surgeon, 2002. **68**(1): p. 1-5.
11. Fornage, B.D., N. Sneige, M.I. Ross*, et al.*, *Small (< or = 2-cm) breast cancer treated with US-guided radiofrequency ablation: feasibility study.* Radiology, 2004. **231**(1): p. 215-24.
12. Fujimoto, S., K. Kobayashi, M. Takahashi*, et al.*, *Clinical pilot studies on pre-operative hyperthermic tumour ablation for advanced breast carcinoma using an 8 MHz radiofrequency heating device.* Int J Hyperthermia, 2003. **19**(1): p. 13-22.
13. Gardner, R., S. Heywang-Kobrunner, W. Dooley*, et al.*, *Phase II clinical studies of focused microwave phased array thermotherapy for primary breast cancer: a progress report*, in *American Society of Breast Surgeons* 2002.
14. Gardner, R.A., H.I. Vargas, J.B. Block, C.L. Vogel, A.J. Fenn, G.V. Kuehl, and M. Doval, *Focused microwave phased array thermotherapy for primary breast cancer.* Ann Surg Oncol, 2002. **9**(4): p. 326-32.
15. Gianfelice, D., A. Khiat, M. Amara, A. Belblidia, and Y. Boulanger, *MR imaging-guided focused ultrasound surgery of breast cancer: correlation of dynamic contrast-enhanced MRI with histopathologic findings.* Breast Cancer Res Treat, 2003. **82**(2): p. 93-101.
16. Harms, S.E., *Percutaneous ablation of breast lesions by radiologists and surgeons.* Breast Dis, 2001. **13**: p. 67-75.
17. Harms, S.E., H. Mumtaz, B. Hyslop, S. Klimberg, K. Westbrook, and S. Kourourian, *RODEO MRI guided laser ablation of breast cancer.* Proc. SPIE, 1999. **3590**: p. 484-489.

18. Hayashi, A.H., S.F. Silver, N.G. van der Westhuizen*, et al.*, *Treatment of invasive breast carcinoma with ultrasound-guided radiofrequency ablation.* Am J Surg, 2003. **185**(5): p. 429-35.
19. Huber, P.E., J.W. Jenne, R. Rastert*, et al.*, *A new noninvasive approach in breast cancer therapy using magnetic resonance imaging-guided focused ultrasound surgery.* Cancer Res, 2001. **61**(23): p. 8441-7.
20. Izzo, F., R. Thomas, P. Delrio*, et al.*, *Radiofrequency ablation in patients with primary breast carcinoma: a pilot study in 26 patients.* Cancer, 2001. **92**(8): p. 2036-44.
21. Jeffrey, S.S., R.L. Birdwell, D.M. Ikeda, B.L. Daniel, K.W. Nowels, F.M. Dirbas, and S.M. Griffey, *Radiofrequency ablation of breast cancer: first report of an emerging technology.* Arch Surg, 1999. **134**(10): p. 1064-8.
22. Mirza, A.N., B.D. Fornage, N. Sneige, H.M. Kuerer, L.A. Newman, F.C. Ames, and S.E. Singletary, *Radiofrequency ablation of solid tumors.* Cancer J, 2001. **7**(2): p. 95-102.
23. Simmons, R., K. Dowlatshahi, S.E. Singletary, and E.D. Staren, *Ablative therapies for breast cancer.* Contemp Surg, 2002. **58**: p. 61-72.
24. Singletary, S.E., B.D. Fornage, N. Sneige*, et al.*, *Radiofrequency ablation of early-stage invasive breast tumors: an overview.* Cancer J, 2002. **8**(2): p. 177-80.
25. Sabel, M.S., C.S. Kaufman, P. Whitworth, H. Chang, L.H. Stocks, R. Simmons, and M. Schultz, *Cryoablation of early-stage breast cancer: work-in-progress report of a multi-institutional trial.* Ann Surg Oncol, 2004. **11**(5): p. 542-9.
26. Burak, W.E., Jr., D.M. Agnese, S.P. Povoski, T.L. Yanssens, K.J. Bloom, P.E. Wakely, and D.G. Spigos, *Radiofrequency ablation of invasive breast carcinoma followed by delayed surgical excision.* Cancer, 2003. **98**(7): p. 1369-76.
27. Kaufmann, M., G.N. Hortobagyi, A. Goldhirsch*, et al.*, *Recommendations From an International Expert Panel on the Use of Neoadjuvant (Primary) Systemic Treatment of Operable Breast Cancer: An Update.* J Clin Oncol, 2006. **24**(12): p. 1940-1949.
28. Buzdar, A.U., *Preoperative chemotherapy treatment of breast cancer—A review.* Cancer, 2007. **110**(11): p. 2394-2407.
29. von Minckwitz, G., J.U. Blohmer, G. Raab*, et al.*, *In vivo chemosensitivity-adapted preoperative chemotherapy in patients with early-stage breast cancer: the GEPARTRIO pilot study.* Ann Oncol, 2005. **16**(1): p. 56-63.
30. Cabioglu, N., K.K. Hunt, T.A. Buchholz*, et al.*, *Improving local control with breast-conserving therapy.* Cancer, 2005. **104**(1): p. 20-29.
31. Soucy, G., J. Bélanger, G. Leblanc*, et al.*, *Surgical Margins in Breast-Conservation Operations for Invasive Carcinoma: Does Neoadjuvant Chemotherapy Have an Impact?* Journal of the American College of Surgeons, 2008. **206**(6): p. 1116-1121.
32. Chen, A.M., F. Meric-Bernstam, K.K. Hunt*, et al.*, *Breast Conservation After Neoadjuvant Chemotherapy: The M.D. Anderson Cancer Center Experience.* J Clin Oncol, 2004. **22**(12): p. 2303-2312.
33. Belli, P., M. Costantini, C. Malaspina, A. Magistrelli, G. LaTorre, and L. Bonomo, *MRI accuracy in residual disease evaluation in breast cancer patients treated with neoadjuvant chemotherapy.* Clinical Radiology, 2006. **61**(11): p. 946-953.
34. Kwong, M., G. Chung, L. Horvath*, et al.*, *Postchemotherapy MRI overestimates residual disease compared with histopathology in responders to neoadjuvant therapy for locally advanced breast cancer.* Cancer J, 2006. **12**(3): p. 212-21.
35. Yeh, E., P. Slanetz, D.B. Kopans*, et al.*, *Prospective Comparison of Mammography, Sonography, and MRI in Patients Undergoing Neoadjuvant Chemotherapy for Palpable Breast Cancer.* AJR Am J Roentgenol, 2005. **184**(3): p. 868-877.
36. Pleijhuis, R., M. Graafland, J. de Vries, J. Bart, J. de Jong, and G. van Dam, *Obtaining Adequate Surgical Margins in Breast-Conserving Therapy for Patients with Early-Stage*

Breast Cancer: Current Modalities and Future Directions. Annals of Surgical Oncology, 2009. **16**(10): p. 2717-2730.