Focused Microwave Thermotherapy for Preoperative Treatment of Invasive Breast Cancer: A Review of Clinical Studies*

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William C. Dooley[1], MD, Hernan I. Vargas[2], MD, Alan J. Fenn[3], PhD, Mary Beth Tomaselli[4], MD, Jay K. Harness[5], MD

[1]The University of Oklahoma, Health Sciences Center, Oklahoma City, Oklahoma
[2] Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center Torrance, California
[3]Massachusetts Institute of Technology, Lexington, Massachusetts
[4]Comprehensive Breast Center, Coral Springs, Florida
[5]St. Joseph's Hospital, Orange, California

Address correspondence to:

William C. Dooley, MD
Director, Division of Surgical Oncology
Department of Surgery
Institute for Breast Health
University of Oklahoma
920 S.L. Young Boulevard, Suite 2290
Oklahoma City, Oklahoma, USA, 73104
FAX: (405) 271-4443
E-mail: William-dooley@ouhsc.edu

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Synopsis Clinical studies of preoperative focused microwave thermotherapy for treating invasive breast cancer are reviewed. Randomized clinical studies suggest a reduction in positive margins for early-stage breast cancer as a preoperative heat-alone treatment, and a reduction in tumor volume when the heat treatment is combined with neoadjuvant chemotherapy for large tumors.

Summary

Background. Preoperative focused microwave thermotherapy (FMT) is a promising method for targeted treatment of breast cancer cells. Results of four multi-institutional clinical studies of preoperative FMT for treating invasive carcinomas in the intact breast are reviewed.

Methods. Externally applied wide-field adaptive phased array FMT has been investigated both as a preoperative heat-alone ablation treatment and as a combination treatment with preoperative anthracycline-based chemotherapy for breast tumors ranging in ultrasound-measured size from 0.8 to 7.8 cm.

Results. In phase I, eight of ten (80%) patients receiving a single low dose of FMT prior to receiving mastectomy had a partial tumor response quantified by either ultrasound measurements of tumor volume reduction or by pathologic cell kill. In phase II, the FMT thermal dose was increased to establish a threshold dose to induce 100% pathologic tumor cell kill for invasive carcinomas prior to breast conserving surgery (BCS). In a randomized study for patients with early-stage invasive breast cancer, of those patients receiving preoperative FMT at ablative temperatures, 0 of 34 (0%) patients had positive tumor margins, whereas positive margins

occurred in 4 of 41 (9.8%) of patients receiving BCS alone (*P*=0.13). In a randomized study for patients with large tumors, based on ultrasound measurements the median tumor volume reduction was 88.4% (n=14) for patients receiving FMT and neoadjuvant chemotherapy, compared to 58.8% (n=10) reduction in the neoadjuvant chemotherapy-alone arm (*P*=0.048).

Conclusions. Wide-field adaptive phased array FMT can be safely administered in a preoperative setting, and data from randomized studies suggest both a reduction in positive tumor margins as a heat-alone treatment for early-stage breast cancer and a reduction in tumor volume when used in combination with anthracycline-based chemotherapy for patients with large breast cancer tumors. Larger randomized studies are required to verify these conclusions.

Key Words: breast cancer, ablation, minimally invasive, focused microwave thermotherapy, cancer treatment

Abbreviations: cumulative equivalent minutes (CEM), ductal carcinoma in situ (DCIS), focused microwave thermotherapy (FMT), hematoxylin and eosin (H&E), magnetic resonance imaging (MRI), radiation therapy (RT)

Introduction

Current standard of care treatments for breast cancer include surgery, radiation therapy, chemotherapy, and hormonal therapy depending on the stage of the cancer.[1,2] For early-stage breast cancer, the tumor and a margin of surrounding healthy tissue are surgically excised prior to radiation therapy; however, in some cases residual cancer cells remain in the breast, which can

lead to a local recurrence. Breast surgery can involve either breast conservation, which can be performed as a lumpectomy (partial mastectomy), or it can involve a mastectomy. Overall survival at 20 years post treatment is equivalent among patients receiving mastectomy (47%), lumpectomy (46%), or lumpectomy plus radiation therapy (47%)[3]. Single institution data suggest that mastectomy rates have risen recently due to increased use of magnetic resonance imaging (MRI) of the breast.[4] To improve cosmetic outcome, chemotherapy is sometimes administered prior to surgery to downsize large breast cancer tumors.[5,6] Treatments that could increase the use of breast conservation and eliminate residual cancer cells to reduce local recurrence with limited side effects are desirable. Tumor heating (hyperthermia)[7-12] is a possible treatment technique that can kill breast cancer cells safely in the intact breast provided that surrounding healthy tissues are spared. Adaptive phased-array focused microwave thermotherapy (FMT)[13 - 15] is a technology developed for safely heating breast cancer in the intact breast. This technology has been investigated recently in clinical trials for treating both early-stage breast cancer and large breast cancer tumors.[16-20]

In a majority of cases, breast cancer can be considered to be composed of a primary invasive cancerous tumor and a diffuse component of microscopic cancer cells that invade surrounding breast tissues. Breast-conservation surgery attempts to remove all of the cancerous tumor cells in one or more excisions. During the breast-conserving surgical procedure, for example during a first incision, the excised breast tissue is submitted for intraoperative pathologic evaluation.[21] The pathology procedures determine if there are tumor cells at the surgical cut edge, which is referred

to as a positive tumor margin. If positive tumor margins are identified, additional breast tissue often will be excised in the region where the positive margin occurred. If tumor cells are found close to the surgical cut edge of the excised specimen, the surgeon can elect to perform a reexcision to provide negative margins (defined herein in this article as tumor cells >1 mm from the surgical cut edge). There is a significantly higher cancer recurrence rate when positive tumor margins occur compared to when the margins are negative.[21] If reexcision is needed to achieve negative margins, it is desirable to perform the reexcision during the first surgery, since the breast cosmetic result is inferior if a second incision is performed. In the case of a lumpectomy, the primary tumor and a 1- to 3-cm margin of healthy tissue (possibly containing microscopic cancer cells) surrounding the tumor are excised. Therefore, for a 1-cm diameter tumor, taking account of the 1- to 3-cm margin of tissue excised, the excised breast tissue diameter can range from about 3 to 7 cm in the maximum dimension. Similarly, a larger 3-cm tumor can require an excision with about a 5- to 9-cm maximum diameter to attempt to achieve negative margins. As a quantitative example, in a small multi-center clinical study for patients with early-stage invasive breast cancer that received breast conserving surgery followed by radiation therapy,[20] the ultrasound measured tumor length and corresponding pathologic tumor length for a representative number of cases (n=38) are shown in **Figure 1**. Breast conserving surgery involved a first excision of both the tumor and a margin of tissue, followed by intraoperative pathology to determine margin status, followed by any additional margin-specific excisions (reexcisions) aimed at providing negative margins during the first incision. The mean ultrasound measured tumor length at enrollment was 1.4 cm, and the first excision (during the first incision) had mean gross pathologic tumor length

7.4 cm. Taking the difference between these lengths and dividing by two, yields a mean excised margin of tissue (surgical cut edge) equal to 3.0 cm surrounding the length of the primary tumor. For these same cases, for the data shown in **Figure 2** the mean ultrasound measured tumor volume (elliptical volume = length × width × depth × 0.524) at enrollment was 1.58 cc and the mean gross pathologic tissue excision volume of the first excision was 79.05 cc, or a ratio of 50 to 1 for mean excised tissue volume relative to the mean tumor volume at enrollment. For these cases, additional excisions during the first incision were performed based on the intraoperative pathology margin status. For this example group, after the first breast conserving surgery (first incision, including all intraoperative reexcisions) was completed, based on pathology, 26.3% of patients had close or positive margins. Postoperative radiation therapy (RT) can be applied by either external or interstitial means, and RT attempts to eliminate any residual cancer cells that might be missed by the breast conserving surgical procedure.[22] For large primary breast cancer tumors, preoperative chemotherapy is sometimes used to provide local tumor shrinkage as well as to systemically treat cancer metastases.[5,6, 23-26] An additional treatment modality that could provide increased tumor cell kill (ablation) and increased tumor shrinkage would generally need to treat cancer cells distributed over large volumes of breast tissue in order to address small and large breast tumors and the tumor margins.

The subject of breast cancer tumor thermal ablation as part of a multimodality approach in the treatment of breast cancer has been reviewed by Hall-Craggs[27], Singletary[28], Noguchi[29], Agnese and Burak[30], Huston and Simmons[31], and van Esser[32]. Thermal energy treatments with

radiofrequency[33-38], interstitial laser photocoagulation[39,40], focused ultrasound[41-44], cryotherapy[45, 46], and adaptive phased array focused microwave thermotherapy[13-20] have demonstrated some success in achieving ablation of breast cancer tumors. For thermotherapy treatment of breast cancer, microwave energy is promising, because it preferentially heats and damages high-water high-ion content breast carcinomas, compared to lesser degrees of heating that occurs in lower-water, lower-ion content normal fatty breast tissues.[47-55] The measured mean water content of normal fatty breast tissue is 19.1% (n = 36), and normal mixed glandular and connective tissue has a measured mean water content of 58.5% (n = 20).[50] Breast carcinomas have a mean water content of 74.3% (n = 22), which is significantly higher than in normal breast tissues.[50] Based on these measured data for percent water content, microwave energy would heat breast carcinomas faster than normal fatty (adipose) breast tissue and mixed glandular and connective tissues. As discussed below, a majority of the breast tissue is low-water content fatty breast tissue, with a lower percentage of glandular and connective tissues.

The mean fraction by mass for the glandular content of the female breast decreases with patient age, and correspondingly the mean fraction of adipose (fatty) tissue increases.[56] For women aged 55 years and older, the mean percentage of normal fatty breast tissue is on the order of 70%. During 2000 to 2004, the median age for which breast cancer was diagnosed was 61 years.[57] It follows that in a majority of breast cancer patients that the breast consists primarily of normal fatty tissue. Based on water content, with microwave thermotherapy a preferential heating effect

for breast cancer cells compared to normal breast tissue should occur in a majority of breast cancer patients.

Methods

Focused microwave thermotherapy was performed on an outpatient basis using local anesthesia with patients in the prone position. A Food and Drug Administration IDE-approved two-channel 915 MHz adaptive phased-array focused microwave thermotherapy system (Model APA-1000; Medifocus, Inc., formerly Celsion (Canada) Limited) was used in the four clinical studies summarized in Table 1. This minimally invasive treatment system uses transcutaneous opposing microwave waveguide applicators and produces a phase-focused microwave field in the compressed breast to heat and destroy high-water high-ion content tumor tissue. The 915 MHz waveguide applicators have large rectangular aperture dimensions (6.5 cm by 13 cm), and together they produce a wide field distribution in the breast to irradiate small and large tumors and tumor cells in the margins.[20] The effective microwave radiation field encompasses an approximate 6 cm by 8 cm area in the breast tissue in a plane parallel to the breast compression plates. In the compressed breast thickness dimension, the effective microwave field extends approximately 1.5 cm on either side of the focal (target) point in the tumor. This microwave field conformation has the potential to kill high-water content tumor cells distributed over a large volume of the breast. A sensor catheter is used to monitor parameters (temperature and microwave field amplitude) in the primary tumor region during the FMT treatment. Prior to inserting the sensor catheter, a local anesthetic (1% lidocaine) is infused at the skin entry point,

and then the skin is nicked approximately 2.5 mm in length with an 11 blade. A 16-gauge (1.65 mm OD, 1.22 mm ID) closed-end plastic catheter is inserted into the tumor under ultrasound guidance, and a single-use disposable (1.12 mm OD) combination E-field focusing sensor and fiber optic temperature sensor is inserted in the catheter to adaptively phase focus the microwaves and measure the tumor temperature during thermotherapy. The combination sensor has the fiber-optic temperature sensor at the probe tip and the E-field sensor is 1.5 cm from the tip. The patient is positioned prone with the breast pendulant through a hole in the treatment table and the breast is compressed to a thickness of between about 4 and 8 cm depending both on size of the breast and on patient comfort. Cooling the breast compression plates and skin with room-temperature airflow during focused microwave thermotherapy treatments helps reduce the potential for skin-surface hot spots. Compressing the breast with the patient in a prone position, such as that used in stereotactic needle breast biopsy procedures,[58] maximizes the amount of breast tissue within the compression device and moves the breast tumor away from the chest wall. Compression immobilizes the breast tissue such that any potential patient motion complications are eliminated. The compression plates are composed of microwave-transparent acrylic plastic material with rectangular apertures that are large enough to allow an ultrasound transducer to contact the breast and localize the breast tumor for accurate sensor positioning within the tumor. Seven temperature sensors are taped (using thin sterile elastic skin closures) to the skin and nipple to monitor the surface temperatures during thermotherapy. Two microwave applicators are positioned on opposite sides of the acrylic compression plates with the tumor to be treated toward the midpoint of the applicator apertures, leaving an air gap of about 1.0 to 2.0 cm as

measured from the applicator apertures to the breast tissue to allow the desired airflow and skin surface cooling. During treatment, the amount of breast compression, focused microwave power, and air-cooling of the skin are adjusted to provide patient comfort; the microwave phase focusing is verified and adjusted adaptively under computer control. For treatment of small to large tumors, a single focal position is used for the entire therapy by means of the single microwave focusing probe positioned within the tumor throughout the treatment. During some treatments, the microwave applicators are repositioned closer to or farther from the breast tissue, or the amount of breast compression is adjusted, and the relative phase of the applicators is adjusted adaptively to maintain the focus at the microwave focusing probe. In addition to sparing low-water, low-ion content normal fatty breast tissues, FMT treatment should spare the chest wall and pleura, since the opposing microwave applicator apertures are oriented, approximately, at right angles with respect to the chest wall and pleura and would tend not to heat these regions.

Experimental studies support the concept that tumor cell heating for 60 minutes at 43ºC is tumoricidal, and the period of time to kill tumor cells decreases by a factor of two for each one degree increase in temperature above about 43ºC.[59] During the tumor temperature increase above 43ºC, thermal dose is more rapidly accumulated in the tumor. For example, the thermal treatment time required for a heat-alone 210-minute equivalent treatment at 43ºC can be reduced to about 6.4 minutes at 48ºC, 3.2 minutes at 49ºC, or 1.6 minutes at 50ºC. The cumulative equivalent minutes (CEM) thermal dose relative to 43ºC is calculated from the measured temperatures recorded by the sensor in the tumor and also for the seven sensors on the skin and

nipple. To reach therapeutic temperature from the initial tumor temperature, the desired heating rate of the tumor is in the range of about 1°C/minute to 2°C/minute. Once the desired tumor temperature range and target thermal dose are achieved during active microwave heating, the microwave power is reduced to zero and breast compression is maintained during a 5-minute cool-down period. During the cool-down period, as a result of reduced blood flow from the breast compression and the thermal insulation of the surrounding breast tissues, particularly the low thermal conductivity of normal fatty breast tissue, the thermal dose continues to accumulate in the tumor toward the goal of the desired thermal equivalent minutes dose.

Statistical differences between thermotherapy and control groups were quantified using Student t test and Fisher's exact test (InStat, GraphPad Software, Inc.), as appropriate.[60] All tests were two-sided and P values ≤ 0.05 were considered statistically significant. Parameters were quantified by mean, range, standard deviation (SD), and 95% confidence interval (CI) as appropriate.

To determine the effectiveness of the treatment (tumor size reduction), ultrasound imaging with the patient supine and the breast and breast tumor of each patient in a consistent orientation was performed. Tumor cell kill was based on tumor necrosis and was estimated from hematoxylin and eosin (H&E) histological sections (performed at each participating site) from pre-treatment biopsy and from surgical excision of the primary breast tumor. Necrosis was estimated and expressed as a percentage of necrotic tumor areas in relation to necrotic and viable tumor areas. In the phase I

FMT study, to quantify apoptosis related cancer cell kill, M30 immunohistochemistry was used.[61] M30 staining characteristics in the pre- and post-thermotherapy tumor tissue were compared. Care was taken to avoid any areas of obvious ischemic necrosis when comparing results.

Results

Phase I Safety

The aim of the Phase I study was to determine whether a 60-minute CEM thermal dose could be used to safely heat, damage, and reduce the size of primary breast carcinomas with heat alone prior to surgery. Tumor temperatures desired in this safety study were in the range of 45 to 47ºC. Ten patients were treated with FMT prior to receiving mastectomy from December 1999 to July 2000, at Columbia Hospital in West Palm Beach, Florida and Harbor-UCLA Medical Center in Torrance, California, as part of an FDA-approved Phase I clinical feasibility and safety study with Internal Review Board Approval obtained at each hospital as discussed by Gardner.[16] After providing fully-informed written consent, female patients with breast carcinomas in the intact breast received a single thermotherapy session and 5 to 27 days later underwent mastectomy regardless of any thermotherapy effect on the tumor. Patients were enrolled in the study provided that the tumor was at least 1 cm beneath the skin surface and not attached to the chest wall.

Ten patients in the Phase I study ranging in age from 47 to 82 years (mean 58.5 years) with breast carcinomas ranging in size from 1 to 8 cm (mean 4.3 cm, maximum dimension based on clinical exam) completed the FMT treatment. The breast tissue compression thickness ranged from 4.5 to 6.5 cm (mean 5.6 cm). The peak tumor temperature, thermal dose to tumor, tumor size reduction

based on ultrasound, and tumor cell kill based on pathology (necrosis and apoptosis) are summarized in Table 2. The measured tumor CEM thermal dose ranged from 25 to 103 minutes (mean 52 minutes) and the measured peak surface CEM thermal dose ranged from 0 to 4.4 minutes (mean 0.8 minutes). The microwave treatment time ranged from 12 to 40 minutes (mean 34.7 minutes). During each treatment, the tumor temperature was raised significantly higher than that of the skin and nipple. Peak tumor temperature ranged from 43.3 to 47.7ºC (mean 44.9ºC) and the peak temperature for all surface sensors ranged from 37.2 to 42.1ºC (mean 40.7ºC); thus, the mean peak tumor temperature achieved was 4.2ºC higher than the mean peak surface temperature.

To determine the effectiveness of the heat-alone FMT treatment, the results of imaging and pathology data were analyzed. Tumor size reduction ranging from 29 to 60% (mean 41%) occurred, in 18 days or less, in 6 out of 10 (60%) patients based on ultrasound measurements. Post thermotherapy (27 days or less) mastectomy specimens showed, in 4 out of the 10 treatments (40%), ischemic tumor necrosis estimated at 40 to 60% (mean 48%) of the total tumor volume occurred, and in 6 of 8 patients tumor cell kill of 82 to 97% (mean 89.7%) based on apoptosis measurements occurred. It is possible that a longer observation time after thermotherapy could have resulted in further tumor size reduction and increased tumor cell kill.

Side effects and their presumed cause in this study were as follows. In the first three patients, limited cooling of the skin and nipple was used and peak surface temperatures of 41.9ºC (mean)

and peak surface CEM thermal dose of 2.5 minutes (mean) occurred. In the subsequent patient treatments, additional air cooling was used and helped reduce the peak temperature (mean 40.2ºC) and CEM thermal dose (mean 0.09 minutes) in the surface tissues for Patients #4 to #10. Limited (range 0.6 x 1.5 cm to 1.2 x 3.5 cm) flap necrosis occurred in the first three patients and may have been due to the combination of thin skin flaps, the peak skin temperature, skin thermal dose, and the short time (7 to 8 days) between thermotherapy and mastectomy. A small blister (approximately 1 cm diameter) occurred due to thermotherapy for Patient #3 which healed completely with no treatment required and presented no special considerations during surgery.

The pathology data of this study suggest that achieving a 60-minute thermal dose and peak temperature >45°C is correlated with the onset of significant ischemic tumor necrosis, and that a higher dose and peak temperature are required for increased ischemic tumor necrosis for breast carcinomas.

In these Phase I safety tests of a clinical focused microwave phased array thermotherapy system, ten patients were treated and a significant thermal dose was delivered to breast carcinomas 1 to 8 cm in maximum clinical dimension and located at central depth in the compressed breast. Eight of ten (80%) patients had a partial tumor response based on tumor shrinkage measured by ultrasound or tumor cell kill based on necrosis and apoptosis measurements. A higher tumor thermal dose than that used in this study is required to achieve complete tumor cell kill, as investigated in the Phase II study described in the next section.

Phase II Dose Escalation

Based on the safety data of Phase I,[16] a Phase II thermal dose escalation study for FMT was conducted at four institutions. The goal of the Phase II study was to use heat-alone prior to breast conserving surgery to determine the required threshold dose to induce 100% tumor cell kill for early-stage invasive breast carcinomas.[17] Between May 2001 and July 2002, patients with invasive breast carcinoma seen at Harbor-UCLA Medical Center in Torrance, California; The University of Oklahoma in Oklahoma City, Oklahoma; Columbia Hospital in West Palm Beach, Florida; and Martin-Luther University in Hale, Germany were invited to participate in this FDA-approved clinical trial. This study was approved and monitored by the Human Subjects Committee at each participating institution. Eligibility criteria included: 1) Karnofski performance status> 70 %, 2) core-needle biopsy proven invasive breast cancer, 3) breast-conservation treatment was planned, 4) tumor visible and measurable by ultrasound, 5) absence of involvement of the skin or pectoralis muscle. All patients were required to undergo counseling and signed a written informed consent document. Some specific exclusion criteria were pregnancy, breast-feeding, and presence of breast implants, pacemakers or defibrillators. The study was designed as an uncontrolled, prospective, multicenter, nonrandomized, thermal dose-escalation study. Escalation of thermal dose was performed in cohorts of five patients at progressively increasing cumulative equivalent minutes thermal doses of 80 CEM, 100 CEM and 120 CEM relative to 43ºC. Treatment of an additional 10 patients at the highest dose was planned if no dose limiting toxicity was reached. All toxicities were graded and reported according to dose level, and toxicities were assessed after therapy.

Physical exam of the breast and ultrasound assessment were completed before thermal therapy and prior to surgery. Complete clinical response (CR) was defined as the complete disappearance of all clinically and radiologically detectable malignant disease. Partial clinical response (PR) was defined as 50% or more decrease from baseline in the volume of the measurable breast tumor. A <50% decrease was defined as clinical stable disease. Progression of clinical disease was defined as a 25% increase in any lesion. Adverse events, vital signs, and laboratory measurements (complete blood count, blood urea nitrogen and creatinine, bilirubin, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, alkaline phosphatase, and routine chemistries) were monitored to evaluate the safety and tolerability of the thermal dose. Performance status was recorded after thermal therapy.

Statistical models to compute the percent of tumor necrosis as a function of either tumor thermal dose or peak tumor temperature achieved were developed based on linear regression to determine the best fit to the measured data.[17] With this linear regression model, a straight line is determined that minimizes the sum of the squares of the difference between the model tumor necrosis and measured tumor necrosis data. Twenty-five patients were enrolled in this study and the patient demographics are given in **Table 3**. Twenty-four of 25 (96%) patients tolerated the thermotherapy treatment. Tumoricidal temperatures (> 43^{o}C) were reached in 23 of 25 (92%) patients. The breast tissue compression thickness ranged from 3.5 to 6.5 cm and was adjusted during microwave thermotherapy as necessary to maintain patient comfort. Patients underwent breast-conserving surgery on average 17 days after thermotherapy (range: 6 – 38 days).

The mean value for ultrasound measurements of tumor size at baseline was 1.76 cm (range 0.7 – 2.5 cm). There was no demonstrable clinical or radiological change in tumor size (P = NS) with thermotherapy. Mean tumor size was 1.84 cm (range 0.7 – 3.8 cm) post-thermal therapy, prior to surgical excision. Based on the ultrasound-measured tumor volume change, 4 patients had a partial response, disease remained stable in 13 and there was clinical progression in 8. However, there was no correlation between clinical/ultrasonographic size changes and pathologic tumor response. Correlation between tumor necrosis and change in tumor volume was 0.38 (P=0.11).

There was evidence of pathologic necrosis in 17 of 25 (68 %) patients. There was complete ablation of invasive carcinoma in two cases. However, both patients had residual in-situ breast cancer. There was one additional patient with a single cluster of residual tumor cells (tumor necrosis estimated at 99.9%). The extent of necrosis in the other cases ranged from 25 to 90%. One of 25 (4%) of patients had positive tumor margins after breast conservation surgery. Six of the 25 subjects were excluded from the efficacy analysis of thermal energy-induced pathologic necrosis due to inaccurate tumor temperature recordings in four cases and delivery of suboptimal thermal dose in two cases. For the data of the 19 included patients, eight patients received a cumulative thermal dose in the range of 158.9 to 206 minutes and all had significant tumor necrosis (range 60 to 100%, mean 84%). Nine patients received a thermal dose in the range of 107.8 to 147.8 minutes and six of nine (67%) exhibited tumor necrosis (for all nine patients the necrosis had range 0 to 95%, mean 40%). Two patients received a thermal dose in the range of

82.8 to 97.2 minutes and one of two (50%) exhibited tumor necrosis (range 0 to 50%, mean 25%). Fourteen of fifteen (93.3%) patients receiving a peak tumor temperature ≥46.9°C had tumor necrosis (range 25 to 100%, mean 72.1%). As summarized in **Table 4**, the univariate linear regression model predicts that a thermal dose of 140 CEM is predictive of 50% tumor necrosis and 210 CEM is predictive of 100% tumor necrosis (P=0.003). The univariate linear regression model also predicts that a peak tumor temperature of 47.4°C is predictive of 50% tumor necrosis and a peak tumor temperature of 49.7°C is predictive of 100% tumor necrosis. In this study, focused microwave thermotherapy did not impair the ability to perform sentinel lymph node mapping.[18]

Side effects were as follows. In the first cohort of patients scheduled to receive 80 CEM, three patients had one treatment and two patients were given two treatments. Thermotherapy was well tolerated. Short-lived erythema developed in the skin of the treated breast in two (40%) patients. In patients assigned to the 100 CEM thermal dose, three (60%) developed short-lived erythema, two (40%) experienced mild pain during treatment and one (20%) developed a first-degree burn in the treated skin that completely healed and presented no special considerations during surgery.

In the last cohort of patients, scheduled to receive 120 CEM, one patient (6.6%) reported severe pain in the first four minutes of thermotherapy and the treatment was stopped - the highest skin temperature was 37.2ºC and the tumor temperature was 39.2ºC at the time the microwave energy was turned off. Seven (47%) experienced pain, four (27%) developed short-lived skin erythema,

five (33%) developed edema of the breast or areola and two patients (13.3%) developed skin thermal burns (first and third degree). The third-degree burn occurred over a small area (8-mm diameter) enclosing the focusing probe skin entry point, which was within the microwave field in proximity to one of the microwave applicators.

This study has established that both thermal dose and peak temperature show statistically significant association with the presence of tumor necrosis in the wide local excision specimen. Based on this statistical model, 100% tumor cell death is expected when a thermal dose of 209.8 CEM and a peak tumor temperature of 49.7°C are achieved. Furthermore, the therapeutic window of FMT takes place at a point where no significant toxicity is seen. Thermal ablation with FMT was well tolerated and no significant complications were recorded. Based on the results of this study, a randomized study was conducted as described in the next section.

Randomized Study for Early-Stage Breast Cancer

Between November 2002 and May 2004 patients with primary invasive early-stage breast carcinomas seen at (1) University of Oklahoma, Oklahoma City; (2) Harbor-UCLA Medical Center, Torrance, California; (3) Comprehensive Breast Center, Coral Springs, Florida; (4) Mroz-Baier Breast Care Center, Memphis, Tennessee; (5) Pearl Place, Tacoma, Washington; (6) St. Joseph's Hospital, Orange, California; (7) Royal Bolton Hospital, Bolton, UK; (8) Breast Care Specialists, Norfolk, Virginia; (9) Breast Care, Las Vegas, Nevada; and (10) Carolina Surgery,

Gastonia, North Carolina were invited to participate in an FDA-approved thermal dose safety and efficacy randomized clinical trial investigating the use of FMT prior to breast conserving surgery.[20] This study was approved and monitored by the Human Subjects Committee at each participating institution. Other eligibility criteria included: (1) Karnofski performance status >70%, (2) core-needle biopsy-proven invasive breast cancer with clinical classification T1 (0 to 2 cm clinical diameter, with the further designations T1a (>0.1 cm to 0.5 cm), T1b (>0.5 cm to 1.0 cm), T1c (>1.0 cm to 2.0 cm)) or T2 (>2 cm to 5 cm clinical diameter), (3) planned breast-conservation treatment by partial mastectomy or lumpectomy followed by radiation therapy, (4) visible tumor measurable by ultrasound, and (5) absence of involvement of the skin or pectoralis muscle. All patients were required to undergo counseling and to sign written informed consent. Some specific exclusion criteria were (1) pregnancy, (2) breast-feeding, and (3) presence of breast implants, (4) clinically significant heart disease, (5) pacemakers or defibrillators, (6) unable to tolerate prone position or breast compression, (7) diagnosis of cancer made by lumpectomy or incisional biopsy, (8) presence of any factor or condition, other than tumor size, which would preclude lumpectomy including multicentric (multifocal) disease or prior history of collagen vascular disease, or (9) breast cancer with a high probability of extensive intra-ductal in situ disease.

This study was designed as a prospective, multi-center randomized study with two treatment arms (thermotherapy plus surgery, and surgery alone as the control arm). The planned study population was 222 patients allowing for 10% patient attrition. An interim analysis was planned to be

performed when approximately 50% of the patients had all of the necessary data collected. The targeted thermal dose was 140 to 180 equivalent minutes during active microwave heating. With 5-minute cool down and maintaining breast compression, the overall targeted thermal dose was 210 thermal cumulative equivalent minutes relative to 43°C, as determined from the phase II thermal dose escalation study described in the previous section.[17] The target tumor temperature was in the range of 48 to 52°C. Breast conservation surgery was to be performed within 60 days after focused microwave thermotherapy. Outcomes measured in this study were pathologic margin status, surgical reexcision (intraoperative and second incision) rates, excised tissue volume, pathologic tumor necrosis, and focused microwave thermotherapy-related side effects. Margins for the primary excision were assessed relative to multi-color inking to identify the medial, lateral, superior, inferior, anterior, and posterior aspects of the breast tissue. In this study, tumor margins were assessed as follows: Positive margin refers to breast tumor cells located at the surgical margin (cut edge), close margin refers to breast tumor cells located at a distance 1 mm or less from the surgical margin, and negative margin refers to breast tumor cells located at a distance greater than 1 mm from the surgical margin. Tumor cell kill was based on tumor necrosis and was estimated from H&E histological sections from wide local excision of the primary breast tumor. If viable tumor cells were found either close (1 mm or less from the inked margin) or at the inked margin, intraoperative reexcision was deemed appropriate. Adverse events, vital signs, and laboratory measurements (complete blood count, blood urea nitrogen and creatinine, bilirubin, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, alkaline phosphatase, and routine chemistries) were monitored to evaluate the

safety and tolerability of the thermal dose. Cases excluded from the statistical analysis included patients that did not receive FMT treatment, patients with multifocal tumors, patients with extensive DCIS determined by pathological evaluation of the excised breast tissue, patients discontinued or withdrawn from the study, patients that received an excisional biopsy, or patients with tumors not T1 or T2.

Patient characteristics were as follows. A total of 92 patients were enrolled (46 in each arm), and 17 cases were excluded from the interim statistical analysis, based on the criteria discussed above, providing a study group of 75 patients for analysis (patient demographics are given in **Table 5**). In the study group, 34 patients (mean age: 59.4 years) were treated with thermotherapy prior to surgery and 41 patients (mean age 58.0 years) received surgery alone. At enrollment, mean tumor maximum diameter based on ultrasound was 1.7 cm (range 0.7 to 3.6 cm) in the thermotherapy arm versus 1.6 cm (range 0.7 to 2.7 cm) in the surgery alone arm (P=0.6). Clinically in the thermotherapy arm there were 64.7% T1 and 35.3% T2 tumors, and in the surgery alone arm there were 75.6% T1 and 24.4% T2 tumors. Clinically, at enrollment in the thermotherapy arm 93.9% of patients were node negative and 6.1% were node positive, and in the surgery alone arm 87.8% of patients were node negative and 12.2% were node positive. Based on pathologic final diagnosis, 32 of 34 (94%) patients in the thermotherapy arm had invasive ductal carcinomas compared with 37 of 41 (90%) patients in the surgery alone arm. DCIS was present in 12 of 34 (35.3%) patients in the thermotherapy arm compared to 25 of 41 (61%) in the surgery alone arm (P=0.04) at final diagnosis.

In the thermotherapy arm, 79.4% of patients were postmenopausal compared to 73.2% in the surgery-alone arm. In the thermotherapy arm, 76.5% of patients were estrogen receptor (ER) positive, 50% were progesterone receptor (PR) positive, and 21.2% were HER-2/neu positive. In the surgery-alone arm, 82.8% of patients were ER positive, 75.6% were PR positive, and 34.1% were HER-2/neu positive. In the study group, breast compression at the start of thermotherapy had a mean value of 5.3 cm (range 3.0 to 9.2 cm). Microwave treatment time had a mean value of 26.6 minutes (range 5.0 to 60 minutes). The cumulative equivalent thermal dose had mean value182.0 minutes (range 0 to 645.0 minutes). Microwave treatment energy dose had mean value 150.7 kilojoules (range 28.8 to 350.3 kilojoules). Tumoricidal temperatures (> 43^{o}C) were reached in 31 of 34 (91.2%) patients. The desired target tumor temperature range of 48 to 52^{o}C was achieved in 15 of 34 (44.1%) patients. The targeted thermal dose of 140 to 180 equivalent minutes during active microwave heating was achieved in 20 of 34 (58.8%) patients. With the additional tumor heating that occurred during the cool-down phase, the minimum desired thermal dose of 210 minutes was achieved in 17 of 34 (50%) patients. Patients underwent breast-conserving surgery on average 19.6 days after thermotherapy (range: 7 – 60 days, SD=14.1 days, 95% CI 14.2 to 25.1 days).).

Mean pathologic tumor necrosis by volume was 29.8% (range 0 to 100%) in the thermotherapy arm and 0.1% in the surgery-alone arm (P=0.0001). In the group of 17 patients that received the minimum desired thermal dose of 210 equivalent minutes, the minimum targeted temperature of

48°C was achieved in 15 of 17 (88.2%) cases and the mean tumor necrosis by volume for these 17 patients was 38.0% with two of the patients having 100% tumor necrosis. The desired target tumor temperature of (48°C or greater) was achieved in 15 of 34 (44.1%) patients. In the group of 15 patients that received the minimum desired target temperature of 48°C, the minimum thermal dose of 210 equivalent minutes was achieved in 15 of 15 (100%) cases and the mean tumor necrosis by volume for these 15 patients was 36.3% with two of the patients having 100% tumor necrosis. Therefore, the cases receiving the target temperature and target thermal dose were highly overlapping. The most consistent high degree of tumor necrosis occurred when all of the following parameters occurred together: 1) cumulative thermal dose greater than 210 minutes was achieved in the tumor, 2) tumor temperature was maintained above 48°C for greater than 2.0 minutes, 3) microwave treatment time was greater than 10 minutes, and 4) microwave energy dose was greater than 50 kJ.

Excised tissue and margin status are as follows. Predicted and actual excised breast tissue volumes for the thermotherapy arm and control arm are given in **Table 6**. At enrollment, based on ultrasound tumor measurements in three dimensions, the calculated mean tumor volume (elliptical volume = length × width × depth × 0.524) was 2.5 cc in the thermotherapy arm and 1.5 cc in the surgery-alone arm (P=0.14). Assuming a 2 cm surgical margin surrounding the ellipsoidal tumor, the predicted mean volume of excised breast tissue was 88.7 cc in the thermotherapy arm and 80.4 cc in the surgery-alone arm (P=0.18). The mean volume of excised breast tissue in the first surgery, including any intraoperative reexcisions, was 115.0 cc (range

24.3 to 363.1 cc, 95% CI 91.2 to 139.0 cc)) in the thermotherapy arm and 90.7 cc (range 4.4 to 282.0 cc, 95% CI 71.4 to 110.0 cc)) in the surgery alone arm (P=0.11). The rate of intraoperative reexcisions during the first surgery was 0.74 per patient in the thermotherapy arm and 0.71 per patient in the surgery-alone arm (P=0.88).

Based on gross pathology, the maximum dimension of excised breast tumor had mean value 1.87 cm in the thermotherapy arm and 1.85 cm in the surgery-alone arm (P=0.94). The gross maximum dimension of the initial excised breast tissue (prior to any re-excisions) had mean value 8.3 cm in the thermotherapy arm and 7.4 cm in the surgery-alone arm (P=0.07). The mean volume of excised breast carcinoma (residual) from gross pathology in the first surgery was 3.24 cc in the thermotherapy arm and 3.05 cc in the surgery-alone arm (P=0.82). The mean volume of excised breast tissue including first excision and all reexcisions was 117.8 cc in the thermotherapy arm and 94.7 cc in the surgery-alone arm (P=0.13).

The pathologic margins and second incision results are given in **Table 7**. In the thermotherapy arm 29 of 34 (85.3%) patients had negative margins, and in the surgery-alone arm 30 of 41 (73.2%) had negative margins (P=0.26). In the thermotherapy arm, 5 of 34 (14.7%) patients had close margins, and in the surgery-alone arm 7 of 41 (17.1%) had close margins (P=0.81). In the thermotherapy arm 0 of 34 (0%) patients had positive margins and in the surgery-alone arm 4 of 41 (9.8%) patients had positive margins (P=0.13). In the thermotherapy arm, 2 of 34 (5.9%) patients received a second incision, and in the surgery-alone arm 4 of 41 (9.8%) patients received

a second incision (P=0.68).

Side effects for the 34 patients in the interim-analysis group receiving thermotherapy prior to surgery are now described. In this study, 3 of 34 (8.5%) patients receiving thermotherapy had a skin burn less than 3 cm in size. With respect to the overall thermotherapy treatment, 15 of 34 (44.1%) of patients had no discomfort during thermotherapy, 11 of 34 (32.4%) had mild discomfort, 3 of 34 (8.8%) had moderate discomfort, and 5 of 34 (14.7%) had intolerable discomfort. During thermotherapy treatment 3 of 34 (8.8%) patients experienced nausea. Skin reddening (erythema) occurred in 4 of 34 (11.8%) patients as a result of mechanical compression of the skin or from microwave treatment. Edema was reported in 3 of 34 (8.8%) cases. Moderate ecchymosis possibly related to thermotherapy occurred in 1 of 34 (2.9%) patients; the event resolved within 9 days. Mild nipple retraction possibly related to thermotherapy occurred in 1 of 34 (2.9%) patients. Mild subcutaneous fibrosis occurred in 1 of 34 (2.9%) patients. Moderate abscess caused by necrotic tissue surrounding the tumor occurred in 1 of 34 (2.9%) patients; the patient was given antibiotics and the wound was irrigated, and the event was resolved within 29 days. Hematoma / seroma was reported in 6 of 34 (17.6%) thermotherapy patients; 3 cases were mild, 2 cases were mild/moderate, and 1 case was moderate severity.

The main objective of this study was to determine whether focused microwave phased-array thermotherapy could decrease the rate of positive margins, without clinically significant toxicity in a multi-center randomized setting. The results of this study are suggestive of a reduction in

positive margins in the preoperative thermotherapy plus breast-conserving surgery arm compared to surgery alone (0% versus 9.8%, *P*=0.13). A larger randomized study is required to verify this conclusion.

Randomized Study for Large Breast Cancer Tumors

In this randomized study, it is hypothesized that, for patients with large breast cancer tumors, the combination of focused microwave thermotherapy and neoadjuvant chemotherapy could improve tumor response rates and may contribute to increased breast-conservation rates in a population thought to require mastectomy at the time of initial clinical presentation. Between November 2002 and May 2004, patients with primary invasive T2 (>2 cm to 5 cm clinical diameter), or T3 (> 5 cm clinical diameter) breast carcinomas seen at (1) University of Oklahoma, Oklahoma City; (2) Harbor-UCLA Medical Center, Torrance, California; (3) Comprehensive Breast Center, Coral Springs, Florida; (4) Mroz-Baier Breast Care Center, Memphis, Tennessee; (5) Pearl Place, Tacoma, Washington; (6) St. Joseph's Hospital, Orange, California; (7) Breast Care Specialists, Norfolk, Virginia; (8) Breast Care, Las Vegas, Nevada; and (9) Carolina Surgery, Gastonia, North Carolina were invited to participate in this FDA-approved prospective randomized open clinical study of the use of focused microwave thermotherapy in combination with neoadjuvant anthracycline-based chemotherapy (thermochemotherapy) compared with neoadjuvant anthracycline-based chemotherapy alone.[19] This study was approved and monitored by the Human Subjects Committee at each participating institution. Eligibility criteria included: (1)

Karnofski performance status > 70%, (2) core needle biopsy-proven invasive breast cancer, (3) patient was a candidate for mastectomy and was eligible for neoadjuvant chemotherapy treatment, (4) visible tumor measurable by clinical exam and by ultrasound, (5) absence of involvement of the skin or pectoralis muscle. All patients were required to undergo counseling and sign written, informed consent. Some specific exclusion criteria were pregnancy, breast-feeding, and presence of breast implants, pacemakers or defibrillators.

Patients randomized to thermochemotherapy were scheduled to receive four cycles (every 21 days) of AC chemotherapy (doxorubicin (anthracycline) at 60 mg/m^2 and cyclophosphamide at 600 mg/m^2) and two treatments of focused microwave thermotherapy concomitantly with the first two cycles of AC chemotherapy; thermotherapy was to be administered starting within one to four hours, but no later than 36 hours, after chemotherapy was infused. The maximum allowed thermotherapy treatment time was 60 minutes in each of two treatments in this study. The desired tumor thermal dose during active microwave heating for this study was in the range of 80 to 120 CEM_{43^oC} in each of two treatments, at tumor temperatures in the range of 44 to 46ºC. Based on two such thermotherapy treatments, the desired cumulative thermal dose would range between 160 to 240 CEM_{43^oC} and should provide a significant tumor response, because a heat-alone thermal dose of 210 minutes or greater (relative to 43ºC) is predictive of 100% necrosis for invasive breast carcinomas.[17] Breast tumor response was quantified by clinical exam in two dimensions (area = product of two diameters) and by ultrasound measurements in three dimensions (elliptical volume = length × width × depth × 0.524).

A total of 34 adult female patients with T2, T3 invasive breast cancer were enrolled in this study. Seventeen (17) patients were randomized to thermochemotherapy and 17 were randomized to chemotherapy-alone. Although a larger study had been planned, due to changes in standard-of-care neoadjuvant chemotherapy that occurred at some of the participating institutions during this period of time, the trial was closed early; however, key findings are discussed. Six patients were withdrawn from the study because their chemotherapy regimen was altered to a non anthracycline-based chemotherapy regimen after enrollment, leaving a total of 28 study subjects for analysis. For the 28 study subjects, analyzable data is available for 15 of 17 patients in the thermochemotherapy arm, and 13 of 17 subjects in the chemotherapy-alone arm.

Patient clinical and tumor characteristics at the time of enrollment in the study group are presented and compared in **Table 8**. At enrollment, based on clinical examination the median value of tumor size in the thermochemotherapy arm and in the chemotherapy-alone arm were (5.13 cm and 3.5 cm, respectively, (P=0.05, Mann-Whitney two-sided test). Based on ultrasound measurements, the median value of tumor volume in the thermochemotherapy arm and the chemotherapy-alone arm were 10.31 cc and 4.13 cc respectively, (P=0.02, Mann-Whitney two-sided test). Thus, prior to treatment, tumors were significantly larger in the thermochemotherapy arm compared to the chemotherapy arm. In the thermochemotherapy arm, the mean peak tumor temperature achieved was 45.0°C (range 44.6 to 46.5°C) and the mean tumor thermal dose was 150.6 $CEM_{43^{o}C}$ (range 0 to 233.9 $CEM_{43^{o}C}$). Mean thermotherapy

treatment time was 34.8 minutes per each treatment. Tumor volume reduction following thermochemotherapy and chemotherapy alone is summarized in **Table 9**. After thermochemotherapy, but prior to surgery, the mean tumor diameter was 1.59 cm (range 0 to 3.5 cm) and mean tumor volume was 3.13 cc (range 0 to 16.43 cc) based on ultrasound; mean tumor volume reduction compared to the volume at enrollment was 69.6% with median value 88.4% (n=14). After chemotherapy alone but prior to surgery, the mean tumor diameter based on ultrasound was 1.97 cm (range 1.0 to 4.54 cm) and mean tumor volume was 2.3 cc (range 0.24 to 7.65 cc) based on ultrasound; mean tumor reduction was 50.5% with median value 58.8% (n=10) based on ultrasound-measured volume. **Figure 3** provides a comparison of the tumor volume reduction in both arms based on ultrasound measurements; the data are ordered from the greatest tumor reduction to least tumor reduction with negative values indicating tumor growth. The median value of absolute tumor volume reduction for preoperative thermochemotherapy was statistically significant relative to preoperative chemotherapy alone (88.4% vs. 58.8% respectively, P=0.048, Mann-Whitney two-sided test). The percent of patients with tumor volume reduction greater than 75% was 11 of 14 (78.6%) in the thermochemotherapy arm compared to 2 of 10 (20%) in the chemotherapy alone arm (P=0.011). As a result of the thermochemotherapy treatments, 14 of 15 (93.3%) patients had sufficient tumor volume reduction in the breast to become eligible for breast conservation based on cosmetic considerations by the judgment of the surgeon, and 11 of 15 (73.3%) patients actually received breast conservation surgery. By comparison, in the chemotherapy-alone arm, 12 of 13 (92.3%) of patients were eligible and actually received breast conservation surgery (P = 0.33, Fisher's exact test). Mean

pathologic tumor necrosis by volume was 70.5% (range 0% to 100%, n=14) with two patients having a complete pathologic response among those receiving thermochemotherapy, and in the chemotherapy-alone arm mean pathologic tumor necrosis by volume was 45.7% (range 0% to 100%, n=7), *P*=0.41.

Side effects caused by thermotherapy are as follows. Erythema occurred in 6 of 26 (23.1%) treatments. A skin burn (less than 1.5 cm diameter) in the microwave treatment field occurred in 5 of 26 (19.2%) treatments; in each case the peak skin temperature exceeded 40°C (range 40.4 to 42.5°C). The subjects´ level of discomfort with thermotherapy reported for 22 treatments was 5 of 22 (22.7%) no discomfort, 11 of 22 (50.0%) mild discomfort, 5 of 22 (22.7%) moderate discomfort, and 1 of 22 (4.5%) intolerable discomfort.

In summary, in this randomized clinical study of patients with T2, T3 tumors judged to require mastectomy as the local surgical treatment for breast cancer, preoperative focused microwave phased-array thermotherapy in combination with preoperative AC chemotherapy appears to be effective and safe. The combined use of thermotherapy and AC chemotherapy in the overall group provided a greater tumor response in terms of tumor volume reduction; median tumor size reduction based on ultrasound-measured volume was 88.4% in the thermochemotherapy arm compared to 58.8% in the chemotherapy-alone control arm, and was statistically significant (*P*=0.048).

Discussion

There is significant interest in the use of minimally invasive ablative techniques in the treatment of breast cancer in the intact breast.[27-32] Local tumor regression and long-term local control of breast cancer with minimal damage to the surrounding normal breast tissues and skin are the objectives in using minimally invasive treatment techniques. Furthermore, the objective of the ideal treatment is to kill the entire primary tumor as well as to kill all cancer cells in the margins of healthy tissue surrounding the primary tumor. Other characteristics of the ideal treatment are that it must be an outpatient procedure applied percutaneously or transcutaneously without the need of sedation or general anesthesia. Morbidity and local complications must be minimal. Thermal ablation such as freezing can be achieved through cryoablation[45,46], whereas heat energy can be generated by the use of interstitial laser photocoagulation[39,40], radiofrequency induced coagulation[33-38], focused ultrasound[41-44], or wide-field focused microwave thermotherapy[13-20]. The cytotoxic effects of thermotherapy on cancer cells using temperatures in the range of 45 to 53ºC have been demonstrated on cancer cells in vitro.[62] Clinical trials have demonstrated improved efficacy of tumor heating with temperatures in the range of about 42 to 46ºC when used with radiation therapy and chemotherapy.[7-12] Focused microwave thermotherapy has been explored in clinical studies using mild tumor temperatures in the range of 42 to 46ºC when used in combination with neoadjuvant chemotherapy for large (T2, T3) tumors,[19] and using higher tumor temperatures of 48 to 52ºC when used as a heat-alone treatment for early-stage (T1, T2) breast cancers.[20] Microwave energy is promising because it can preferentially heat and kill high-water content breast carcinomas, compared to lesser degrees of heating that occur in lower-water

content adipose tissues in the breast.[47-55] The coherent microwave radiation from the two applicators used in the wide-field focused microwave thermotherapy system oscillates at a rate of 915 MHz, and the timing (or relative phase) of the two waves emanating from the applicators is adjusted to achieve peak radiation at the focal point defined by the E-field probe located in the tumor. The fast oscillation of the microwave energy combined with the high electrical conductivity of breast carcinomas[47-55] produces molecular friction in the breast carcinoma cells that generates heat and rapidly elevates the temperature of the tumor.

Herein we review clinical studies of focused microwave thermotherapy delivered as a wide-field treatment of the intact breast, which has potential clinical applications for ablation of early-stage breast cancer and for neoadjuvant thermochemotherapy tumor volume reduction of large breast cancer tumors. Four clinical studies including phase I safety, phase II dose escalation, and two randomized studies performed to date indicate that focused microwave thermotherapy is feasible and safe for treatment of breast cancer.

The main objective of the randomized study for treatment of early-stage breast cancer was to determine whether preoperative focused microwave phased array thermotherapy could decrease the rate of positive margins, without clinically significant toxicity in a multi-center randomized setting.[20] The target cumulative thermal dose of 210 equivalent minutes was achieved in 17 of 34 (50%) patients and the target tumor temperature of 48^{o}C or greater was achieved in 15 of 34 (44.1%) patients. Based on H&E staining, complete (100%) necrosis of breast cancer was

achieved in 2 of 17 (11.8%) cases receiving the targeted cumulative thermal dose; in both of these cases there was no pathologic evidence of viable invasive carcinoma or DCIS cells. To achieve a high degree of necrosis of breast cancer, in addition to the target cumulative thermal dose of 210 equivalent minutes, this study indicates that the target tumor temperature should also be maintained above 48°C for greater than 2.0 minutes. Tumor cell kill measured by other pathologic testing such as nicotinamide adenine dinucleotide-diaphorase (NADH-d) and immunohistochemistry, as used in other breast tumor ablation studies,[16,31] were not evaluated in this study. At the interim analysis, an apparent reduction in the incidence of positive margins was suggested in the thermotherapy arm; however, both consistent delivery of the targeted tumor thermal dose and consistent tumor necrosis were not achieved in this preoperative heat-alone treatment and the study was closed early. Some of the cases in which the desired thermal treatment was not achieved might be a result of the learning curve associated with using a new treatment technology in a multi-institutional study setting. Thermal ablation with focused microwave thermotherapy was tolerated in a majority of cases and no significant complications were recorded. A higher thermal dose than used in this study would be required for increased tumor necrosis. The results of this study are suggestive of a reduction in positive margins in the preoperative FMT plus breast-conserving surgery arm, and a larger study will be required to confirm this conclusion.

An observation arising from the randomized study of preoperative heat-alone treatment for early-stage breast cancer[20] is the 0% rate of positive margins in the thermotherapy arm in comparison to

9.8% positive margins in the surgery alone arm and reported incidences of positive margins after breast conserving surgery in the literature of 4% to 44%.[63-66] However, this difference in positive margins did not reach statistical significance and might be the result of a type 2 statistical error requiring a larger clinical trial to demonstrate a statistical difference. Therefore, the hypothesis that focused microwave phased array thermotherapy treatment prior to breast conservation surgery provides significant tumor cell kill in the primary tumor and in the margins and is responsible for a low incidence of positive margins requires further critical study. Two other factors might be responsible for this finding regarding positive margins: (1) A smaller volume of breast tissue on average was excised in the surgery-alone arm and might have contributed to a higher rate of positive margins in the surgery-alone arm compared to preoperative thermotherapy; however, this volume difference was not statistically significant. Possible explanations for the larger volume of breast tissue excised in the thermotherapy arm might include increased firmness (induration) of the breast cancer mass as a result of thermotherapy effects leading to better resection, or investigator bias because this was not a blinded study. Future studies of focused microwave thermotherapy could investigate whether induration of the breast tumor occurs more frequently in the thermotherapy arm compared to surgery alone. Future studies should investigate whether thermal effects on tissue such as induration and coagulative necrosis have any impact on hemostasis in surgery. (2) At final pathologic diagnosis, a higher rate of ductal carcinoma in-situ (61.0% in the surgery-alone arm versus 35.3% in the thermotherapy arm (P=0.04), based on final pathology) might have been a contributing factor to the higher rate of positive margins in the surgery-alone arm compared to thermotherapy – two of the four cases in the surgery-alone arm had positive margins for DCIS. Elements of intraductal carcinoma

might extend outside the tumor mass and can be difficult to detect preoperatively with mammography.[67] This characteristic of extensive DCIS is a potential contraindication for minimally invasive ablative approaches. In addition, microwave-heating properties of DCIS lesions need to be evaluated in future studies to determine whether DCIS is high-water high-ion content similar to invasive breast carcinomas. If DCIS cells are high-water high-ion content similar to invasive breast carcinoma, they might be successfully treated with wide-field focused microwaves for targeted tumor cell heating in the breast while sparing the normal healthy breast tissue. Studies demonstrate that the differentiation of DCIS is correlated with the grade of the associated invasive ductal carcinoma indicating a pathological similarity between DCIS and invasive ductal carcinoma cells.[68,69] If microwave thermotherapy does not preferentially heat DCIS compared to normal breast tissues, then DCIS might be a potential exclusion criterion. In this study, patients were determined to have DCIS at the final pathohistological assessment (post surgery) from the excised tissue. Future studies of thermal ablation must consider selecting patients with invasive carcinoma with lower risk of an intraductal carcinoma component based on pre-treatment mammography and percutaneous biopsy results.[69,70] Future studies could determine whether wide-field focused microwave thermotherapy has an ablative effect on DCIS.[14] In this clinical trial, five patients with multifocal tumors were excluded from the study group, and each patient had positive margins after the first surgery was completed.[20] Future studies of focused microwave thermal ablation should consider using magnetic resonance imaging to better identify patients with multifocal tumors or DCIS to possibly exclude these patients prior to enrollment.[41,71] If focused microwave thermal ablation is to be studied in a clinical trial setting including patients that have multifocal tumors, to achieve negative margins multiple ablation

treatments (one for each focal tumor site) should be considered. The ability of focused microwave phased array thermotherapy to consistently deliver a specified therapeutic thermal dose and minimum temperature to ablate breast cancer tumors and achieve consistent 100% pathologic tumor cell kill might depend on a number of factors, including (1) breast compression thickness which impacts blood flow in the tumor and in surrounding tissues, and the required penetration depth for the microwaves, (2) tumor size, (3) tumor histology, (4) tumor location, (5) accuracy, number and positioning of tumor temperature probes, (6) initial temperature of the tumor, (7) magnitude of skin surface cooling, (8) patient tolerance, (9) length of time between thermotherapy and surgery, and (10) method of pathologic evaluation of tumor cell kill. It would be desirable in future clinical studies of pre-surgical heat-alone focused microwave phased array breast thermotherapy to explore a more consistent delivery of a minimum 210-minute thermal dose (with a corresponding tumor temperature in the range of about 48 to 50ºC), and higher thermal doses for more effective and consistent tumor cell kill. In this study, the wide-field focused microwave thermotherapy system uses single sensor temperature measurements, which provides limited monitoring and control of tumor thermal dose. Future studies of FMT could explore measuring the tumor blood perfusion rate with Doppler ultrasound when the breast is compressed prior to the start of thermotherapy, to assess the ability to overcome perfusion effects and more effectively heat the tumor; these perfusion measurements could be used as a guide to determine the desired breast compression thickness prior to the start of thermotherapy. In this study, in the focused microwave thermotherapy arm, 2 of 34 (5.9%) patients received a second incision, and in the surgery-alone arm 4 of 41 (9.8%) patients received a second incision; this difference was not statistically significant (P=0.68). Larger studies conducted at more consistent and/or higher

therapeutic doses of FMT will provide meaningful information regarding the success of this therapy for heat-alone treatment of breast cancer based on pathological response and second incision rates. Comparison with other means of percutaneous thermal ablation of early-stage breast cancer will then be possible.

The main objective of the randomized study for large breast cancer tumors was to determine whether focused microwave phased array thermotherapy at temperatures in the range of 44 to 46°C combined with neoadjuvant anthracycline-based AC chemotherapy could increase the reduction of volume of large breast carcinomas, without clinically significant toxicity, in a multi-center randomized setting. The median value of tumor volume reduction for preoperative thermochemotherapy was statistically significant relative to preoperative chemotherapy alone (volume reduction 88.4% (n=14) vs. 58.8% (n=10) respectively, P=0.048). This small study serves as proof of the principle for this neoadjuvant combined approach of focused microwave thermotherapy and chemotherapy. As of this date, other than in a clinical trial setting, neoadjuvant thermochemotherapy can not be recommended, because the objective responses have not yet translated into a clinically measurable improved patient outcome, such as increased use of breast conservation or decreased recurrence rates and further randomized clinical studies are needed.[72] Potential weaknesses in the study design may be responsible for a lack of clinical efficacy and may be related to the absence of very well defined or strict criteria for the selection of breast conservation surgery vs. mastectomy, as this was based solely on experienced surgeons' judgment. Another possible explanation is that tumor size was such that it required only reduction

of a small volume to permit breast conservation surgery, particularly since there is an apparent difference in pretreatment tumor size between the experimental and control arms, with tumors being larger in the experimental thermotherapy arm. There is no significantly increased morbidity with the addition of thermotherapy to the standard systemic chemotherapy. The treatment was well tolerated in the vast majority of patients. Self-limited erythema occurred commonly after thermochemotherapy and only in a small number of patients was followed by clinical evidence of a burn area in the skin. Skin burn in most patients was treated with topical treatments, but required excision of the small burn area in two cases. Close attention to the technique of delivery of thermotherapy with particular interest in the thermal dose delivered to the skin and to patient symptomatology during therapy are essential to avoid significant heating-related side effects. The risk of local recurrence is an important factor that warrants consideration. A shortcoming of our study is the limited follow up of the patients in this series. Larger studies and long-term follow up is required to accurately estimate the incidence of local recurrence after breast conservation surgery following neoadjuvant thermochemotherapy. In this study, neoadjuvant thermochemotherapy is more effective than neoadjuvant chemotherapy in effectively reducing tumor burden.

In summary, based on the four clinical studies reviewed herein, externally applied wide-field focused microwave thermotherapy has been administered safely for breast cancer patients with breast tumor sizes ranging from 0.8 cm to 7.8 cm based on ultrasound measurements. In the small randomized study using heat-alone focused microwave thermotherapy for early-stage breast

cancer, a 0% rate of positive tumor margins was observed in the preoperative FMT treatment arm compared to 9.8% in the surgery-alone arm (P=0.13). In the small randomized study using heat in combination with preoperative chemotherapy, median tumor volume reduction was 88.4% in the FMT combined with chemotherapy treatment arm compared to 58.8% in the preoperative chemotherapy alone arm (P=0.048). Focused microwave thermotherapy warrants further investigation in larger randomized clinical studies both as a preoperative heat-alone treatment to reduce positive margins for early-stage breast cancer, and as a preoperative combination heat and chemotherapy treatment to reduce tumor volume for large breast cancer tumors to improve breast conservation.

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References

1. Newman LA and Kuerer HM. Advances in breast conservation therapy. *J Clin Oncol*. 2005;23:1685-1697.

2. Carlson RW, et al. The NCCN invasive breast cancer clinical practice guidelines

in oncology. *J Nat Comprehensive Cancer Network*. 2007;5(3):246-312.

3. Fisher B, Anderson S, Bryant J, et al. Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. *N Engl J Med.* 2002;347:1233–1241.

4. Katipamula R, Hoskin TL, Boughey JC, et al. Trends in mastectomy rates at the Mayo Clinic Rochester: Effect of surgical year and preoperative MRI. *J Clin Oncol.* (Meeting Abstracts). 2008;26(15S):509

5. Fisher B, Brown A, Mamounas E, et al. Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from the National Surgical Adjuvant Breast and Bowel Project B-18. *J Clin Oncol***.** 1997**;**15, 2483-2493.

6. Fisher B, Bryant J, Wolmark N, et al. Effect of preoperative chemotherapy in the outcome of women with operable breast cancer. *J Clin Oncol*. 1998;16(8):2672-2685.

7. Falk MH and Issels RD. Hyperthermia and Oncology. *Int J. Hyperthermia.* 2001;17(1):1-18.

8. Field SB and Hand JW, (eds). *An Introduction to the Practical Aspects of Clinical Hyperthermia*, London: Taylor & Francis; 1990:242-274.

9. Overgaard J, Gonzalez Gonzalez D, Hulshof MCCH, Arcangeli G, Dahl O, Mella O, and Bentzen SM. Hyperthermia as an adjuvant to radiation therapy of recurrent or metastatic malignant melanoma. A multicentre randomized trial by the European Society for Hyperthermic Oncology. *Int J Hyperthermia.* 1996;12(1):3-20.

.

10. Valdagni R, and Amichetti M. Report of long-term follow-up in a randomized trial comparing radiation therapy and radiation therapy plus hyperthermia to metastatic lymphnodes in stage IV head and neck patients. *Int J Radiat Oncol Biol Phys.* 1993;28:163-169.

11. van der Zee, J, Gonzalez Gonzalez D, van Rhoon GC, van Dijk JDP, van Putten WLJ, and Hart AAM**.** Comparison of radiotherapy alone with radiotherapy plus hyperthermia in locally advanced pelvic tumors: a prospective, randomised, multicentre trial. *Lancet.* 2000;355:1119-1125.

12. Vernon CC, Hand JW, Field SB, et al. Radiotherapy with or without hyperthermia in the treatment of superficial localized breast cancer: results from five randomized controlled trials. *Int J Radiat Oncol Biol Phys.* 1996;35:731-744.

13. Fenn AJ, Wolf GL, and Fogle RM. An adaptive phased array for targeted heating of deep tumors in intact breast: animal study results. *Int J Hyperthermia.* 1999;15(1):45-61.

14. Fenn AJ. *Breast Cancer Treatment by Focused Microwave Thermotherapy*. Sudbury, Massachusetts: Jones and Bartlett; 2007.

15. Fenn AJ (2009) *Adaptive Phased Array Thermotherapy for Cancer*. Norwood, Massachusetts: Artech House; 2009.

16. Gardner RA, Vargas HI, Block JB, Vogel CL, Fenn AJ, Kuehl GV, Doval M. Focused microwave phased array thermotherapy for primary breast cancer. *Ann Surg Oncol*. 2002;9(4):326-332.

17. Vargas HI, Dooley WC, Gardner RA, Gonzalez KD, Venegas R, Heywang-Kobrunner SH, Fenn AJ. Focused microwave phased array thermotherapy for ablation of early-stage breast cancer: Results of thermal dose escalation. *Ann Surg Oncol*. 2004;11(2):139-46.

18. Vargas HI, Dooley WC, Gardner RA, Gonzalez KD, Heywang-Kobrunner SH, Fenn AJ. Success of sentinel lymph node mapping after breast cancer ablation with focused microwave phased array thermotherapy. *Am J Surg*. 2003;186:330-332.

19. Vargas HI, Dooley WC, Fenn AJ, Tomaselli MB, and Harness JK. Study of preoperative focused microwave phased array thermotherapy in combination with neoadjuvant anthracycline-based chemotherapy for large breast carcinomas. *Cancer Therapy*. 2007;5B:401-408. Available;

http://www.cancer-therapy.org/CT5B/pdf/45._Vargas_et_al,_401-408.pdf. Accessed November 25, 2007.

20. Dooley WC, Vargas, HI, Fenn AJ, Tomaselli MB, and Harness JK. Randomized study of preoperative focused microwave phased array thermotherapy for early-stage invasive breast cancer. *Cancer Therapy*. 2008;6B:395-408. Available; http://www.cancer-therapy.org/CT6B/pdf/43._Dooley_et_al,_395-408.pdf. Accessed August 25, 2008.

21. Singletary SE. Surgical margins in patients with early-stage breast cancer treated with breast conservation therapy. *Am J Surgery*. 2002;184:383-93.

22. Arthur DW, and Vicini FA. Accelerated partial breast irradiation as a part of breast conservation therapy. *J Clin Oncol*. 2005;23(8):1726-1735.

23. Hamilton A, and Hortobagyi G. Chemotherapy: What progress in the last 5 years?" *J Clin Oncol*. 2005;23:1760-1775.

24. Kaufmann M, Hortobagyi G, Goldhirsch A, Scholl S, Makris A, 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):1940-1949.

25. Kaufmann, P., C.E. Dauphine, M.P. Vargas, M.L. Burla, N.M. Isaac, et al. Success of neoadjuvant chemotherapy in conversion of mastectomy to breast conservation surgery. *Am Surgeon*. 2006;72(10):935-938.

26. Wolmark, N., J. Wang, E. Mamounas, J. Bryant, and B. Fisher, Preoperative chemotherapy in patients with operable breast cancer: Nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18. *J Natl Cancer Inst Monographs*. 2001;No. 30:96-102.

27. Hall-Craggs MA. Interventional MRI of the breast: minimally invasive therapy. *Eur Radiol.* 2000;10(1):59-62.

28. Singletary SE. Minimally invasive techniques in breast cancer treatment. *Seminars in Surg Onc.* 2001;20:246-50.

29. Noguchi M. Minimally invasive surgery for small breast cancer, *J Surg Oncol*. 2003;84:94-101.

30. Agnese DM, Burak WE. Ablative approaches to the minimally invasive treatment of breast cancer. *Cancer J.* 2005;11:77–82.

31. Huston TL and Simmons RM. Ablative therapies for the treatment of malignant diseases of

the breast. *Am J Surg.* 2005;189:694-701.

32. van Esser S, Maurice van den Bosch AAJ, van Diest PJ, Mali WTM, Borel Rinkes IHM, van Hillegersberg R. Minimally invasive ablative therapies for invasive breast carcinomas: an overview of current literature. *World J Surg***.** 2007;31(12):2284-2292.

33. Jeffrey, SS, Birdwell RL, Ikeda DM, et al. Radiofrequency ablation of breast cancer: first report of an emerging technology. *Arch Surg.* 1999;134(10):1064-1068.

34. Izzo F, Thomas R, Delrio P, Rinaldo M, et al. Radiofrequency ablation in patients with primary breast carcinoma. a pilot study of 26 patients. *Cancer.* 2001;92(8):2036-2044.

35. Singletary SE, Fornage BL, Sneige N, et al. Radiofrequency ablation of early-stage invasive breast tumors: an overview. *Cancer J.* 2002;8:177-180.

36. Burak, WE, Agnese DM, Povoski SP, et al. Radiofrequency ablation of invasive breast carcinoma followed by delayed surgical excision. *Cancer*. 2003;98:1369-1376.

37. Fornage, B.D., Nour S, N, Ross MI, et al. Small (≤2-cm) breast cancer treated with US-guided radiofrequency ablation: feasibility study. *Radiology*. 2004;231(1):215-226.

38. Ross MI and Fornage BD. Radiofrequency Ablation of Early-Stage Breast Cancer. In: Chapter 9 of *Radiofrequency Ablation for Cancer: Current Indications, Techniques, and Outcomes*. Ellis LM, Curley SA, and Tanabe KK, (eds.), New York: Springer-Verlag; 2004:137-158.

39. Dowlatshashi K, Francescatti DS, Bloom KJ. Laser therapy for small breast cancers. *Am J Surgery*. 2002;184:359-363.

40. Dowlatshashi K, Dieschbourg JJ, Bloom KJ. Laser therapy of breast cancer with 3-year follow-up. *The Breast Journal*. 2004;10(3):240-243.

41. Huber PE, Jenne JW, Rastert R, et al. A new noninvasive approach in breast cancer therapy using magnetic resonance imaging-guided focused ultrasound surgery. *Cancer Res*. 2001;61:8441-8447.

42. Wu F, Zhi-Wang ZB, Zhu H, et al. Extracorporeal high intensity focused ultrasound treatment for patients with breast cancer. *Breast Cancer Res Treat*. 2005;92:51-60.

43. Lu X-Q, Burdette EC, Bornstein BA, Hansen JL, and Svensson GK. Design of an ultrasonic therapy system for breast cancer treatment. *Int J Hyperthermia*. 1996;12(3):375-399.

44. Hynynen K. Focused ultrasound surgery guided by MRI. *Science & Medicine*. 1996;3(5):62-71.

45. Pfleiderer, SO, Freesmeyer MG, Marx C, Kuhne-Heid R, Schneider A, and Kaiser WA. Cryotherapy of breast cancer under ultrasound guidance: initial results and limitations. *Eur Radiol.* 2002;12:3009-3014.

46. Sabel MS, Kaufman CS, Whitworth P, Chang H, Stocks LH, Simmons R, and Schultz M. Cryoablation of early-stage breast cancer: work-in-progress report of a multi-institutional trial. *Ann Surg Oncol*. 2004;11(5):542-549.

47. Chaudhary SS, Mishra RK, Swarup A, and Thomas JM. Dielectric properties of normal and malignant human breast tissue at radiowave and microwave frequencies. *Indian J of Biochemistry and Biophysics*. 1984;21:76-79.

48. Joines WT, Zhang Y, Li C, and Jirtle RL. The measured electrical properties of normal and malignant human tissues from 50 to 900 MHz. *Med Phys*. 1994;21(4):547-550.

49. Surowiec AJ, Stuchly SS, Barr JR, and Swarup A. Dielectric properties of breast carcinoma and the surrounding tissues. *IEEE Trans Biomed Eng*. 1988;35(4):257-263.

50. Campbell AM, and Land DV. Dielectric properties of female human breast tissue measured in vitro at 3.2 GHz. *Phys Med Biol*. 1992;37(1):193-210.

51. Burdette EC. Electromagnetic and Acoustical Properties of Tissue. In *Physical Aspects of Hyperthermia*. Nussbaum GH, (ed.), AAPM Medical Physics Monographs, No. 8, 1982:105-130.

52. Lazebnik M, Popovic D, McCartney L, et al. A large-scale study of the ultrawideband microwave dielectric properties of normal, benign, and malignant breast tissues obtained from cancer surgeries. *Phys Med Biol*. 2007;52:6093-6115.

53. Lazebnik M, McCartney L, Popovic D, et al. A large-scale study of the ultrawideband microwave dielectric properties of normal breast tissue obtained from reduction surgeries. *Phys Med Biol*. Vol. 52, 2007:2637-2656.

54. Sha L, Ward ER, Story B. A review of dielectric properties of normal and malignant breast tissue. *Proc IEEE SoutheastCon*. 2002:457-462.

55. Gabriel S, Lau RW, and Gabriel C. The dielectric properties of biological tissues: part III. parametric models for the dielectric spectrum of tissues. *Phys Med Biol*. Vol. 41, 1996, pp. 2271-2293. Available; http://niremf.ifac.cnr.it/tissprop. Accessed May 2, 2009.

56. Klein R., Aichinger H, Dierker J, Jansen JTM, Joite-Barfuß S, et al. Determination of average glandular dose with modern mammography units for two large groups of patients. *Phys Med Biol*. 1997;42:651-671.

57. American Cancer Society, Breast Cancer Facts & Figures 2007-2008. Atlanta: American Cancer Society, Inc.

58. Bassett L, Winchester DP, Caplan RB, et al. Stereotactic core-needle biopsy of the breast: a report of the joint task force of the American College of Radiology, American College of Surgeons, and College of American Pathologists. *CA Cancer J Clin*. 1997;47:171-190.

59. Sapareto SA, Dewey WC. Thermal dose determination in cancer therapy. *Int J Radiat Oncol Biol Phys.* 1984;10:787-800.

60. Armitage P and Berry G. *Statistical Methods in Medical Research, Third Edition*, Oxford: Blackwell Science. 1994;156-163:283-311.

61. Leers MPG, Kolgen W, Bjorklund V, et al. Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis. *J Pathol*. 1999;187:567-572.

62. Gerhard H, Klinger HG, and Gabriel E. Short term hyperthermia: In vitro survival of different human cell lines after short exposure to extreme temperatures. in *Cancer Therapy by Hyperthermia and Radiation*, Streffer C, (ed.), Baltimore-Munich: Urban & Schwarzenberg. 1978;201-203.

63. Recht A, Come SE, Henderson IC, Gelman RS, Silver B, Hayes DF, Shulman LN, Harris JR. The sequencing of chemotherapy and radiation therapy after conservative surgery for early stage breast cancer. *N Engl J Med.* 1996;334:1356–1361.

64. Park CC, Mitsumori M, Nixon A, Recht A, Connolly J, Gelman R, Silver B, Hetelekidis S, Abner A, Harris JR, Schnitt SJ. Outcome at 8 years after breast-conserving surgery and radiation therapy for invasive breast cancer: influence of margin status and systemic therapy on local recurrence. *J Clin Oncol.* 2000;18:1668–1675.

65. Smitt MC, Nowels KW, Zdeblick MJ. The importance of lumpectomy surgical margin status in long term results of breast conservation. *Cancer.* 1995;76:259–267.

66. Ryoo MC, Kagan AT, Wollin M, Tome MA, Tedeschi MA, Rao AR, Hintz BL, Kuruvilla AM, Nussbaum H, Streeter OE Jr, et al. Prognostic factors for recurrence and cosmesis in 393 patients after radiation therapy for early mammary carcinoma. *Radiology.* 1989;172:555–559.

67. Holland R, Hendriks JH, Vebeek AL, Mravunac M, Schuurmans Stekhoven JH. Extent, distribution, and mammographic/histological correlations of breast ductal carcinoma in situ. *Lancet.* 1990;335:519–522.

68. Douglas-Jones AG, Gupta SK, Attanoos RL, Morgan JM, and Mansel RE. A critical appraisal of six modern classifications of ductal carcinoma in situ of the breast (DCIS): correlation with grade of associated invasive carcinoma. *Histopathology.* 1996;29:397-409.

69. Cadman B, Ostrowski J, Quinn C. Invasive ductal carcinoma accompanied by ductal carcinoma *in situ* (DCIS): comparison of DCIS grade with grade of invasive component. *The Breast.* 1997;6:132–137.

70. Bagnall MJ, Evans AJ, Wilson AR, Pinder SE, Denley H, Geraghty JG, Ellis IO. Predicting invasion in mammographically detected microcalcification. *Clin Radiol.* 2001; 56:828-32.

71. Saslow D, Boetes C, Burke W, et al. American Cancer Society Breast Cancer Advisory Group. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. *CA Cancer J Clin.* 2007;57:75-89.

72. Vargas HI, Dooley WC, Fenn AJ, Tomaselli MB, and Harness JK. A protocol for focused

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Table 1. Clinical trials conducted for focused microwave thermotherapy treatment for patients with breast cancer in the intact breast.

Study Type	Protocol	Number of Patients Enrolled
Phase I (safety)	TT prior to mastectomy[16]	10
Phase II (Dose escalation)	TT prior to BCS[17]	25
Randomized	TT prior to BCS[20]	92
Randomized	TCT prior to breast surgery[19]	34
TT=thermotherapy, TCT=thermochemotherapy, BCS=breast conserving surgery		

Table 2. Phase I safety study results for 10 patients receiving low-dose FMT prior to mastectomy.[16]

Phase I Patient / Tumor Histology	Tumor size* (cm)		Tumor heating parameters		Time to surgery (days)	% Tumor size reduction (US)	% Tumor cell kill	
	CE	US	Peak (°C)	Dose CEM43°C (minutes)			Necrosis	Apoptosis
1 / IDC	3.0	2.4	44.5	41.0	7	29	0	97.2
2 / IDC	8.0	4.0	43.3	24.5	8	60	0	0.0
3 / IDC	4.5	2.7	45.1	67.1	7	29	60	81.7
4 / DCIS	4.0	1.1	44.6	47.8	6	0	0	NM
5 / IDC	2.5	1.9	45.0	42.0	5	0	0	NM
6 / IDC	5.0	2.9	45.1	61.0	15	42	0	89.2
7 / IDC	5.0	1.5	47.7	100.0	13	0	40	0.0
8 / IDC	0.9	0.9	NM	NM	27	29	0	92.7
9 / IDC	3.5	1.1	46.5	63.7	18	0	50	83.5
10 / IDC	5.5	3.4	46.1	61.7	13	59	40	93.8

IDC, Invasive ductal carcinoma; DCIS, Ductal carcinoma in situ; CE, Clinical exam; US, Ultrasound; NM, Not measured; *Maximum tumor diameter

Table 3. Phase II dose escalation study demographic and tumor characteristics of the study population for patients with early-stage breast cancer.[17]

N		25
Age, years (mean)		57.2
Tumor diameter (ultrasound measurements)	Mean, cm	1.76
	Range, cm	0.7 – 2.8
Histology	Invasive ductal	80%
	Invasive lobular	8%
	Colloid	8%
	Other	4%
Tumor Grade	High Grade	25%
	Intermediate Grade	56%
	Low Grade	19%

Table 4. Phase II thermal dose escalation study results for preoperative heat treatment of early-stage breast cancer. Projected thermal dose and peak tumor temperature required for 0, 50, 85, and 100% necrosis of invasive breast carcinomas are given.

Tumor Necrosis (%)	Thermal Dose $CEM_{43^{o}C}$	Peak Tumor Temperature (^{o}C)
0	<71	< 44.5
50	140.3	47.1
85	188.9	49.0
100	>209.8	>49.7

Table 5. Randomized study for preoperative heat-alone treatment of early-stage breast cancer - demographic and tumor characteristics of the study population of 75 patients for the interim analysis.

		Thermotherapy	Surgery Alone
N		34	41
Age, Years	Mean	59.4	58.0
	Range	42 – 89	41-89
Tumor Size Based on Ultrasound Measurements at Enrollment	Mean, cm	1.7	1.6
	Range, cm	0.74 – 3.64	0.70 – 2.73
	95% Confidence Interval, cm	1.47 – 1.94	1.44 – 1.77
Clinical Tumor Classification at Enrollment	T1a	0%	2.4%
	T1b	20.6%	19.5%
	T1c	44.1%	53.6%
	T2	35.3%	24.4%
Clinical Nodal Status at Enrollment	Negative / Positive	93.9% / 6.1%	87.8% / 12.2%
Tumor Histology (final diagnosis)	Invasive Ductal Carcinoma	94%	90%
	Invasive Lobular Carcinoma	3%	7%
	Colloid	3%	3%
	DCIS Component Present	35%	61%
Tumor Grade (final diagnosis)	High Grade	40%	36%
	Intermediate Grade	43%	33%
	Low Grade	17%	31%

Source: Fenn AJ, Breast Cancer Treatment by Focused Microwave Thermotherapy, 2007: Jones and Bartlett Publishers, Sudbury, MA. www.jbpub.com. Reprinted with permission.

Table 6. Randomized study results for early-stage breast cancer comparing thermotherapy plus surgery arm with surgery alone arm. Summary of excised tissue volumes in both arms of the study for the interim analysis.

Early-Stage Randomized Study		Thermotherapy	Surgery Alone	
Number of patients per arm		34	41	*P*
Ultrasound measured maximum tumor diameter at enrollment (cm)	Mean	1.7	1.6	0.61
	Range	0.74 – 3.64	0.7 – 2.73	
	95% Confidence Interval	1.49 – 1.97	1.44 – 1.77	
Ultrasound measured ellipsoidal tumor volume at enrollment (cc)	Mean	2.5	1.5	0.14
	Range	0.13 – 14.12	0.1 – 6.7	
	95% Confidence Interval	1.33 – 3.58	1.05 – 2.03	
Predicted breast tissue excision volume based on 2 cm margin surrounding ellipsoidal tumor measured by ultrasound in three dimensions (cc)	Mean	88.7	80.4	0.18
	Range	52.4 – 182.6	50.2 – 133.8	
	95% Confidence Interval	77.9 – 99.5	73.9 – 86.8	
Number of interoperative reexcisions per patient	Mean	0.74	0.71	0.88
Pathology measured maximum tumor diameter from initial excised specimen (cm)	Mean	1.87	1.85	0.94
	Range	0.0 – 4.5	0.8 – 3.3	
	95% Confidence Interval	1.5 – 2.2	1.6 – 2.1	
Pathology measured maximum tissue diameter of initial excised specimen (cm)	Mean	8.3	7.4	0.07
	Range	4.5 – 14.0	3.0 – 11.7	
	95% Confidence Interval	7.6 – 9.0	6.7 – 8.1	
Pathology measured ellipsoidal tumor volume from excised specimens in first surgery (cc)	Mean	3.24	3.05	0.82
	Range	0 – 13.95	0.15 – 14.15	
	95% Confidence Interval	2.0 – 4.47	1.98 – 4.12	
Actual breast tissue excision ellipsoidal volume including first excision and all reexcisions including second incisions (cc)	Mean	117.8	94.7	0.13
	Range	24.3 – 363.1	4.4 – 282.0	
	95% Confidence Interval	93.7 – 141.9	75.1 – 114.2	
% Excess breast tissue (by volume) excised compared to predicted	Mean	41.7	16.5	0.18
	Range	-65 to 253	-94 to 240.9	
	95% Confidence Interval	12.0 to 71.5	-6.5 to 39.4	
DCIS = ductal carcinoma in situ P>0.05 not significant				

Source: Fenn AJ, Breast Cancer Treatment by Focused Microwave Thermotherapy, 2007: Jones and Bartlett Publishers, Sudbury, MA. www.jbpub.com. Reprinted with permission.

Table 7. Randomized study results for early-stage breast cancer comparing thermotherapy plus surgery arm with surgery alone arm. Summary of margin status and second incision rates in both arms of the study in the interim analysis.

Early-Stage Randomized Study		Thermotherapy	Surgery Alone	
Number of patients per arm		34	41	*P*
Margin status at completion of first surgery	Rate of negative margins	29/34 (85.3%)	30/41 (73.2%)	0.26
	Rate of close margins	5/34 (14.7%)	7/41 (17.1%)	0.81
	Rate of positive margins	0/34 (0%)	4 of 41 (9.8%)	0.13
	Rate of close or positive margins	5/34 (14.7%)	11/41 (26.8%)	0.26
Incidence rate for type of tumor cells involved in close or positive final margins (Invasive only, DCIS only or both invasive and DCIS) for first surgery	Invasive only	3/34 (8.8%)	7/41 (17.1%)	0.33
	DCIS only	0/34 (0%)	4/41 (9.8%)	0.13
	Both invasive and DCIS	2/34 (5.9%)	0/41 (0%)	0.20
Rate of second incisions		2/34 (5.9%)	4/41 (9.8%)	0.68
DCIS = ductal carcinoma in situ P>0.05 not significant				

Source: Fenn AJ, Breast Cancer Treatment by Focused Microwave Thermotherapy, 2007: Jones and Bartlett Publishers, Sudbury, MA. www.jbpub.com. Reprinted with permission.

Table 8. Randomized study for thermochemotherapy compared to chemotherapy alone for patients with large breast cancer tumors: demographic and tumor characteristics of the overall study population.

Large tumor study	Thermochemotherapy	Chemotherapy Alone	*P*-value
N	15	13	
Age, Years			
Mean	45.1	45.8	0.86
Range	26 – 72	32 - 79	
Menopausal status			1.0
Pre	11	9	
Post	4	4	
Tumor size based on ultrasound measurements at enrollment			
Mean, cm	3.65	2.69	0.10
Median, cm	3.24	2.39	0.07
Range, cm	2.0 - 7.8	1.22 - 6.5	
Tumor volume based on ultrasound measurements at enrollment			
Mean, cc	22.09	10.17	0.18
Median, cc	10.31	4.13	0.02
Range, cc	1.85 – 90.98	0.39 – 78.68	
Tumor size based on clinical measurements at enrollment			
Mean, cm	5.26	4.19	0.17
Median, cm	5.13	3.5	0.05
Range, cm	2.4 - 7.5	2.5 – 9.0	
Clinical tumor classification at enrollment			0.15
T2	6	9	
T3	9	4	
Clinical nodal status at enrollment			0.70

Negative Positive	10 5	7 6	
Tumor histology IDC ILC IMDLC	 11 3 1	 12 0 1	0.33
Estrogen-receptor status Negative Positive	 8 7	 4 9	0.28
Progesterone-receptor status Negative Positive	 10 5	 8 5	1.00
HER2 Status Negative Positive Unknown	 8 6 1	 11 2 0	0.11
IDC=Invasive ductal carcinoma, ILC=Invasive lobular carcinoma, IMDLC=Invasive mixed ductal and lobular carcinoma			

Source: Fenn AJ, Breast Cancer Treatment by Focused Microwave Thermotherapy, 2007: Jones and Bartlett Publishers, Sudbury, MA. www.jbpub.com. Reprinted with permission.

Table 9. Randomized study results for thermochemotherapy compared to chemotherapy alone for patients with large breast cancer tumors**:** percent tumor shrinkage based on ultrasound measurements prior to surgery for chemotherapy-alone arm and thermochemotherapy arm of the overall study population.

Study Parameter		Preoperative Thermochemotherapy (New Arm, n=14)	Preoperative Chemotherapy (Control Arm, n=10)	P value
Tumor shrinkage based on Ultrasound volume	Mean	69.6%	50.5%	0.358
	Median	88.4%	58.8%	0.048
	Range	–99.9 to 100	–66.7 to 99.1	

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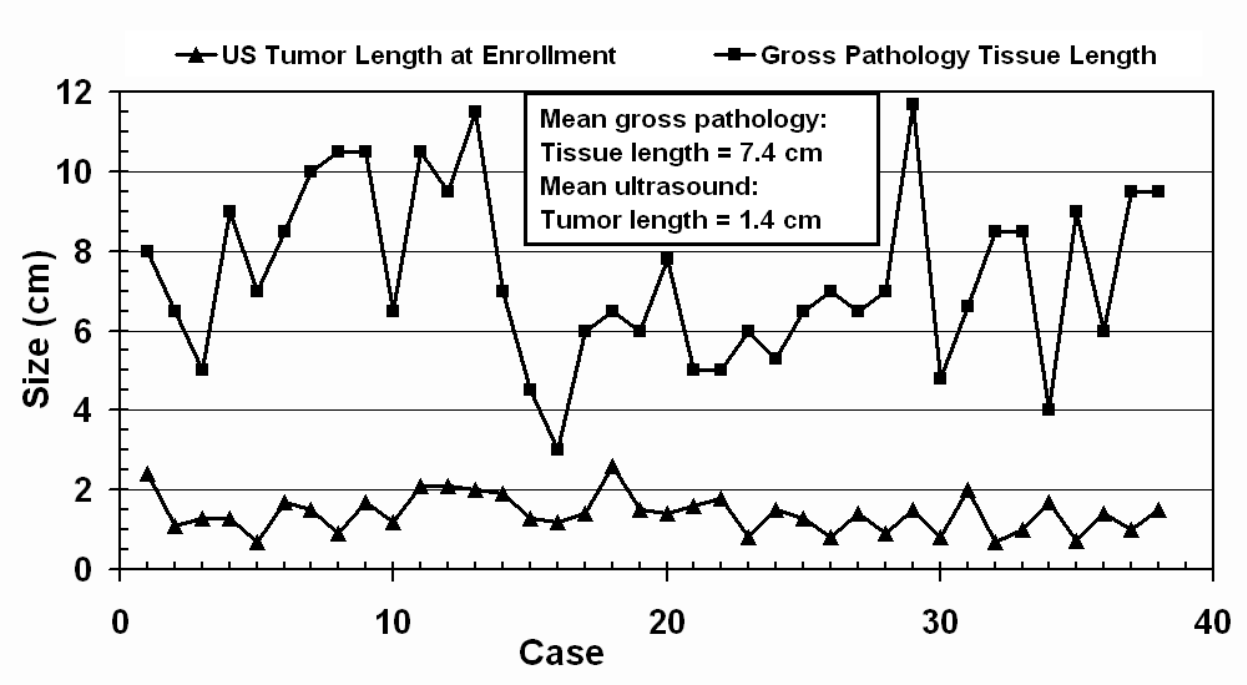

Figure 1. Gross pathology excised tissue length compared to ultrasound measured tumor length at enrollment for patients with early-stage invasive breast cancer receiving breast-conservation treatment.[20]

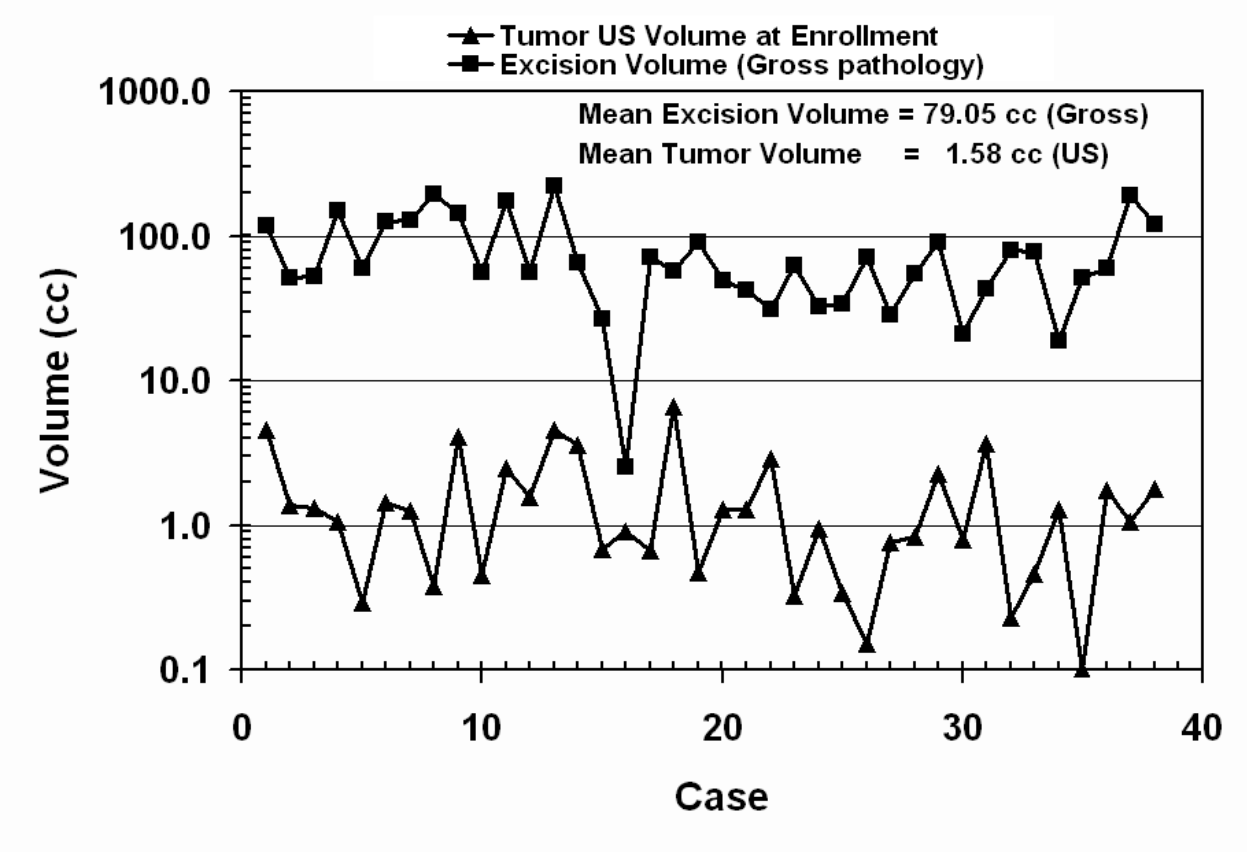

Figure 2. Gross pathology excised tissue volume compared to ultrasound measured tumor volume at enrollment for patients with early-stage invasive breast cancer receiving breast-conservation treatment.[20]

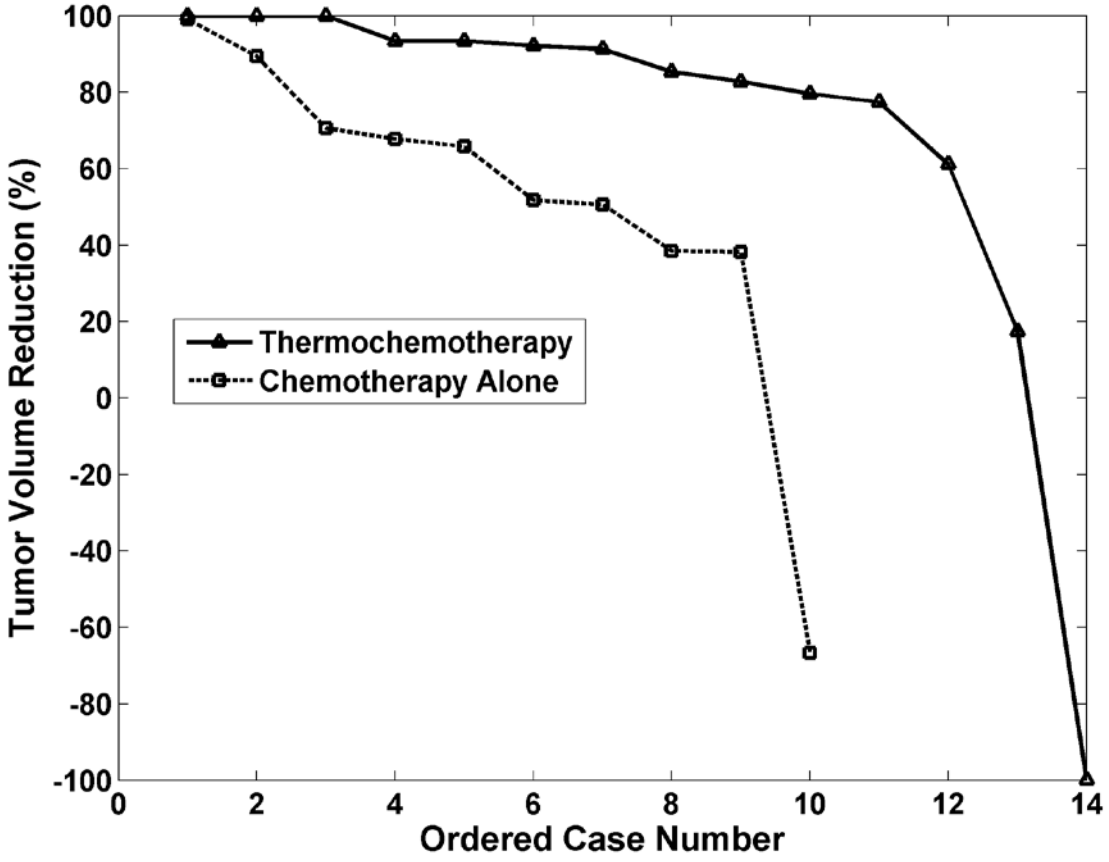

Figure 3. Comparison of breast tumor volume reduction based on ultrasound measurement for patients treated in the two arms of the randomized thermochemotherapy versus chemotherapy study. The cases have been ordered by decreasing tumor response. From Vargas[19], www.cancer-therapy.org, reprinted with permission.