A PHASE II STUDY OF MRI-BASED PRE-OPERATIVE ACCELERATED PARTIAL BREAST IRRADIATION

Funded by the Dr. Nancy Laning Sobczak Award for Breast Cancer

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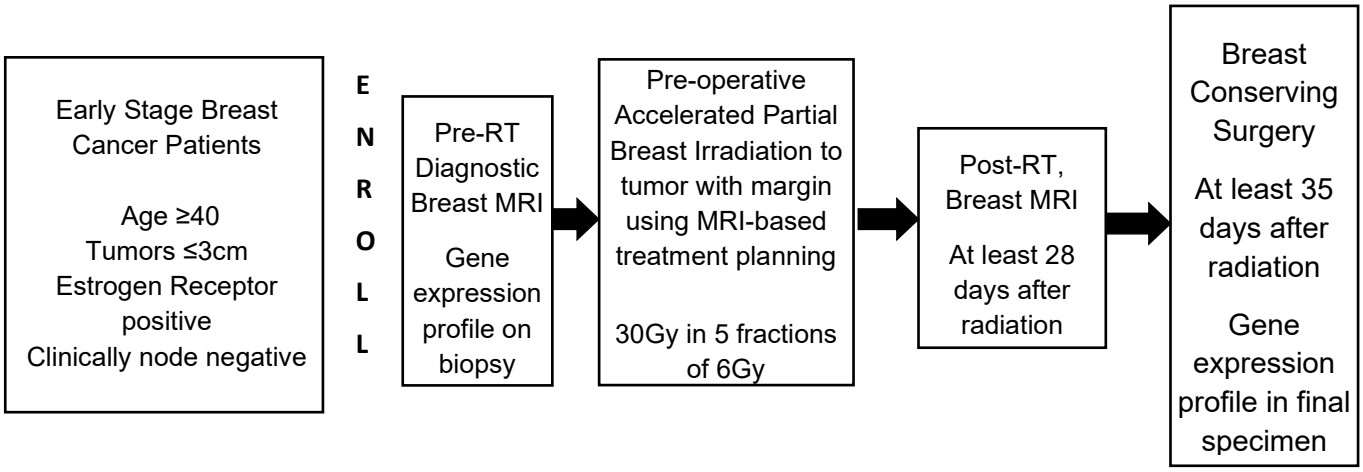
STUDY SCHEMA

A PHASE II STUDY OF MRI-BASED PRE-OPERATIVE ACCELERATED PARTIAL BREAST IRRADIATION



See Section 6.0 for details of radiation therapy

Patient Population: (See Section 3.0 for Eligibility and additional requirements
Stage I-IIA, Node negative breast cancer eligible for breast conservation.

Required Sample Size: 40

1.0 INTRODUCTION

1.1 Rationale for Pre-Operative Accelerated Partial Breast Irradiation

Each year, approximately 200,000 women are diagnosed with breast cancer, including more than 4,000 new cases in the state of Wisconsin. More than 40,000 women die of the disease each year. With the consistent use and availability of screening by mammogram and additional imaging techniques, the majority of these women are diagnosed with localized disease that is amenable to successful treatment. For these women there are two main treatment options: (1) mastectomy, removal of the breast, or (2) breast conserving therapy, consisting of lumpectomy and radiation therapy. The goals of breast conservation are to eradicate the cancer from its site of origin while maintaining a cosmetically acceptable breast. Several large prospective randomized trials have shown equivalent overall survival and breast cancer specific survival when comparing mastectomy and lumpectomy with radiation therapy (1-7).

Traditionally, women who undergo lumpectomy then receive radiation therapy to the entire breast for three to six weeks. Unfortunately, this protracted treatment course has led some patients to opt for mastectomy over breast conserving therapy. However, emerging data suggests that treatment of the whole breast may not be necessary in select patient groups and that shorter, less costly treatment regimens may be possible. When radiotherapy is omitted from breast conserving therapy, most in-breast recurrences occur near the initial site of disease at the lumpectomy cavity. Accelerated partial breast irradiation (APBI) is a technique that delivers radiation to that volume of breast tissue at the highest risk of recurrence. Because a smaller volume of tissue is treated, it is possible to deliver a higher radiation dose with each treatment and the treatment course is reduced to five days (8-10).

The largest reported series of APBI with the most mature follow-up have utilized brachytherapy as the method of radiation delivery (54). This involves surgically implanting a device in the breast and then placing a radioactive source inside that device for a period of time to deliver dose. The invasive nature of brachytherapy, lack of wide-spread expertise in its use for breast cancer, as well as improved imaging technology has led to the development of APBI using external beam radiotherapy. While initial phase II data has been promising (26), more recent data has raised concerns regarding the toxicity profile of this technique (25,27,28). One prospective phase III study comparing conventional whole breast irradiation with APBI using external beam radiotherapy reported comparable rates of local control, but increased rates of breast fibrosis, breast pain, and adverse cosmetic result for those receiving APBI (55). This has led many to question external beam-based APBI as a viable treatment alternative to more conventional radiation treatment schedules. Improving the side-effect profile of this treatment might lead to more patients selecting breast conservation with external beam-based APBI as the radiation treatment method, Some estimates have reported that more widespread use of APBI in appropriate patients could result in more patients selecting breast conservation and savings in health care costs that exceed $7.5 million per 1000 patients treated.

The current standard practice of delivering radiation therapy ***post-operatively*** has had two major drawbacks: (1) inaccurate targeting, and (2) inability to measure the radiation response in the primary tumor. The target volume for post-operative APBI has been the tissue surrounding the lumpectomy cavity. However, this cavity may not necessarily direct the radiation toward the highest risk area of the breast around the tumor as tumors can be peripherally located in the resection specimen (see Figure 1). This results in the need for a large margin of normal breast tissue (e.g., 1.5 cm) to be treated. In order to take into account the area at risk for microscopic residual disease, this volume must then be expanded. One must also account for patient motion during treatment, as well as variation in patient positioning from day to day. This requires additional expansion of the

target volume (13). Furthermore, due to the variations in daily patient setup on the treatment table and respiratory motion during radiation, an additional margin around the lumpectomy cavity has to be used (e.g., 1.0 cm for a total of 2.5 cm). This results in more normal tissue irradiated to high dose than might be necessary (13). It is well-documented that larger the area of breast tissue that receives definitive doses of radiation, the higher the risk for fibrosis of the tissue (11-12). These large margins also limit the patient eligibility for APBI - if the size of the breast is small in relation to this large target volume, APBI cannot be done safely (29,30).

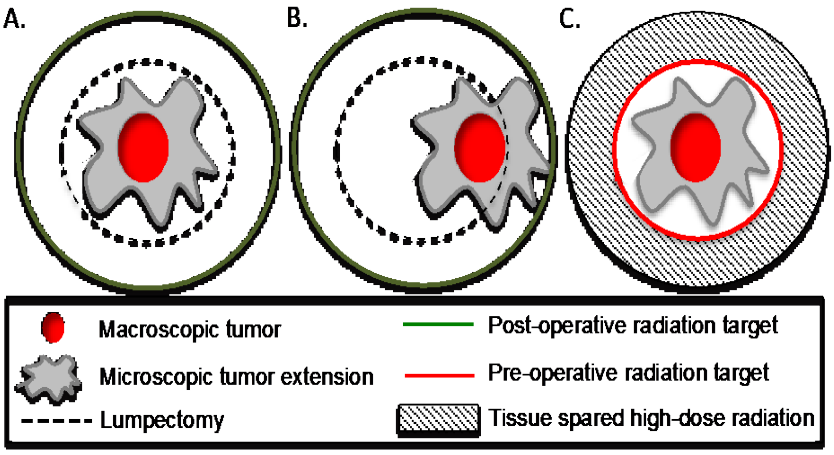


Figure 1. Schematic Demonstrating Advantages of Pre-operative Radiation Therapy. In (A), the gross tumor and microscopic disease are centered within the lumpectomy cavity and all are removed with surgery. A more common scenario is shown in (B), with the tumor near the lumpectomy margin and microscopic disease left behind. Because identification of microscopic disease is not possible, post-operative radiation is given to a radial margin from the surgical site in all directions (outer circle). Figure (C) demonstrates the volume irradiated pre-operatively. The margin extends radially from the gross tumor. Much of the high dose area is removed during the lumpectomy and the tissue spared high dose radiation is illustrated.

The current clinical practice of breast conserving therapy, including APBI, has not taken advantage of the significant advances in breast imaging to improve its geometric accuracy. In the current study, we propose treating well-selected patients with APBI *pre-operatively*. ***Pre-operative*** RT would target more precisely the area at risk for microscopic disease, and also result in less breast tissue receiving RT (Figure 1C).

Other potential benefits to pre-operative radiation include improved rates of margin negative resection and a reduced need for re-excision. Finally, with radiotherapy delivered prior to surgery, the tissue receiving the highest dose of radiation will then be surgically removed. Pre-operative radiation has been used in other tumor sites such as sarcoma, rectal, pancreatic, and lung cancers and has been shown to improve the rates of margin negative resections (16-22). In some of these disease sites, this has decreased late morbidity compared to post-operative treatment (16,18). We anticipate similar results in this population of early stage breast cancer as the portion of the breast receiving the highest dose of radiation will be removed surgically. It is anticipated that this factor will lead to additional reductions in breast fibrosis and improved cosmetic outcomes.

Pre-operative treatment also allows for assessment of tumor response to radiotherapy ***in vivo***, something that has not been studied to any extent as most breast radiotherapy is delivered in the post-operative setting. Response to pre-operative chemotherapy has been shown to be predictive of patient outcome, and it is possible that tumor response to radiation may offer similar prognostic information.

1.2 Rationale for MRI-based Pre-operative Accelerated Partial Breast Irradiation and other Advanced Imaging Techniques

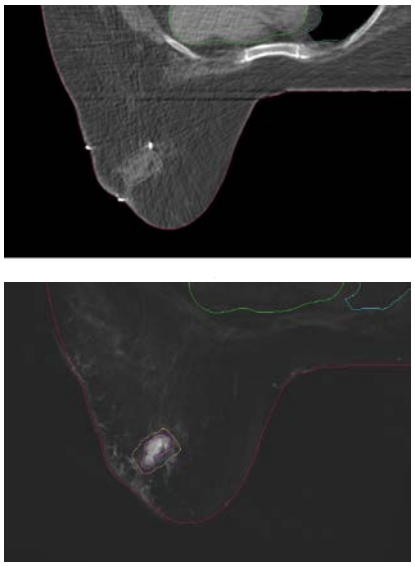


Fig. 2: Post-lumpectomy prone CT images (top). The brown contour demonstrates the lumpectomy cavity. MRI (STIR) image registered with the CT (bottom) shows the lumpectomy cavity contour (magenta) demonstrating that the volume is smaller than the same target defined on CT (brown contour). As shown, treatment of the CT-based volumes would treat a larger volume of breast tissue than is necessary.

Traditionally, most external-beam based APBI has been delivered using CT-based treatment planning. However, on traditional CT imaging, the precise size and shape of the surgical cavity can be difficult to delineate. This is exacerbated by the fact that at times, normal breast tissue can have an appearance similar to post-surgical changes.

The use of MRI will ensure the tumor can be well visualized and targeted with the radiation, resulting in smaller volume of breast tissue to be treated (14-15). It has been well-documented that MRI has a higher sensitivity than mammography in detecting and characterizing breast cancer (56,57). In particular, breast MRI is an excellent tool for determining gross tumor extent and for detecting small tumor in dense breasts (14,15). Furthermore, MRI can provide functional and biological information, further improving target definition and opening doors for novel biologically conformal RT strategies. MRI has high sensitivity for detecting cancer and for assessing the extent of disease in the breast prior to surgery. Therefore, radiation should be more ideally targeted based on MRI-defined pre-operative disease location versus surgical cavity location. In CT-based treatment planning, the target volume typically includes the seroma cavity and any clips left by the breast surgeon. When used for radiation delivery, MRI in the post-operative setting reduces the volume of normal breast tissue that is treated compared to CT-based treatment alone (35, also see Figure 2). In pre-clinical studies, MRI-based planning in the pre-operative setting has also been shown to decrease the volume of targeted breast tissue (29,30). This is significant, as series of APBI from FH/MCW and other institutions demonstrate that late toxicity of treatment and adverse cosmetic outcome are correlated with increasing volume of normal breast tissue that is irradiated (24). Consequently, reducing the volume of irradiated breast tissue by utilizing MRI identification of disease, as well as treatment in the pre-operative setting, would be expected to result in improved cosmetic outcomes and reduced late toxicity.

In traditional CT-based imaging, the contrast observed between structures is based on variations in density. Because soft tissue structures have similar densities, it can be difficult to precisely define borders of a given structure. However, subtle differences in tissue density can be better observed when the images are produced using photons with a lower energy spectrum. The result is better spatial resolution of images, and the technique is known as Dual-Energy CT. Even when MRI is used for radiation treatment planning, it is still necessary to obtain a CT simulation so that the radiation beam can be appropriately modeled. In order to optimize target definition for pre-operative APBI, patients enrolled on this protocol will have a Dual Energy CT performed as part of their treatment planning scans.

1.3 Use of Additional Advanced Radiation Delivery Techniques

Previous studies have demonstrated that prone APBI in the post-operative setting utilizing daily CT-image guided radiation therapy allows for reduction in the target volume, due to the decrease in daily positioning uncertainty provided by the daily CT image guidance (24). CT-guided adaptive

treatment delivery will be used to account for changes during the course of treatment (61). Patients treated on this protocol will preferentially be treated in the prone position if the size of the breast allows. One of the inherent challenges in using MRI for breast cancer therapy has been extrapolating MRI findings attained in the prone position to the surgery and/or RT typically carried out in the supine position. We have previously developed both whole breast irradiation and ABPI in the prone position and have reported that the target coverage, normal-tissue sparing, and clinical outcomes with prone treatment are comparable to those treated supine (24,64). In addition, it has been shown by us and others (24, 62-64) that the prone set up can (1) increase dose homogeneity in the breast for large, pendulous, breasts, (2) reduce respiratory motion, and (3) improve sparing of lung and heart. Combining the accuracy of MRI in assessing the extent of tumor, the precision of daily CT-guided targeting, and treatment in the prone position, will allow the proposed pre-operative APBI to deliver effective dose to the gross tumor while minimizing the irradiation of healthy tissues.

1.4 Changes in Tumor Gene Expression As a Result of Radiotherapy

Progress in elucidating the molecular basis of breast cancer, including the delineation of 'molecular subtypes,' has allowed for such treatment breakthroughs as anti-estrogen and Her2 targeted therapies. Molecular biology is also shaping the current approach to both surgical (90) and systemic therapy (91). The use of molecular information has become commonplace in directing systemic therapy decisions in breast cancer using gene expression profile scores (78), such as OncotypeDX (79), marking a significant advancement in medical oncology. Unfortunately, no similar use of molecular information has been utilized to better direct the use of *radiation* therapy for invasive breast cancer. Rather, improvements in RT have been chiefly technological, with more accurate targeting and delivery (74). Recent progress has been made with the development of a gene expression model of tumor radiosensitivity based upon *in vitro* data, and though it has not been fully validated, this approach has proved useful in evaluating outcomes in retrospective datasets, including two breast cancer studies (75-77). While significant, this approach is still limited in that it does not account for tumor behavior *in vivo* and has been limited to retrospective analysis of post-operative radiation. Recent data from a Phase I pre-operative single-fraction APBI study showed expression of genes regulating immunity and cell death were seen in response to radiation (94). We hypothesize that altered gene expression patterns will be seen in patients who have enhanced radiation responses.

1.5 Imaging Changes after Radiation Therapy

MRI has been an important tool for measuring the extent of disease prior to pre-operative chemotherapy and the amount of response afterwards. In contrast, information on MRI changes associated with radiation therapy for breast cancer is lacking. Diffusion-weighted MRI holds promise for use as a cancer treatment response biomarker as it is sensitive to macromolecular and microstructural changes occurring at the cellular level that can be detected earlier than anatomic changes. These imaging changes have been studied as predictors of response to radiation in other disease sites and as a response to pre-operative chemotherapy in breast cancer, studies on radiation-induced changes for breast cancer is lacking. (97) A phase I study of high dose single fraction APBI demonstrated evidence of dose-dependent changes in vascular permeability in response to radiation (94). Studying the MRI changes that occur after radiation treatment will lay the important groundwork for future studies in which MRI can be used to tailor pre-operative radiation dose delivery based on response and predict for patients whose disease has responded well to treatment. Patients enrolled on this study will be required to undergo diagnostic MRI prior to enrollment. A second diagnostic scan will be performed at least four weeks after radiation so comparisons can be made to pre-treatment scans. Specifically, changes

in diffusion weighted imaging and dynamic-contrast enhancement will be studied and correlated with pathologic response and local control.

1.6 Post-Operative Complications and Study Endpoints

One potential disadvantage of pre-operative radiation therapy is the possibility of an increased rate of post-operative complications. With the use of pre-operative radiation, a small, but acceptable increase in post-operative complications was observed in phase III studies comparing pre- versus post-op radiation in patients with rectal cancer and soft-tissue sarcoma (16, 18). Consequently, the aim of this phase II study will be to demonstrate safety of pre-operative APBI and the primary endpoint will be a comparison of post-operative complications with historical controls. The complications that will be tracked will include infection, wound dehiscence, hematoma, symptomatic seroma requiring intervention, and need for hospitalization or a second breast surgery (excluding those done for inadequate surgical margins).

In this study, it is anticipated that the most common post-operative complications will be infection and formation of a symptomatic seroma. This is based on a Phase I study from Horton et.al. that used a single large fraction of radiation therapy prior to breast conserving surgery. In that trial, the most common post-operative complications were infection (6%) and seroma formation. (32%) (94). Often times, these two complications can be seen in the same patient. While the Horton study did not report on individual patients who may have had both a seroma and infection, the number reported for seroma (32%) will be used as the historical control on this study.

Historically, rates of post-operative infections for breast conserving surgery in the absence of pre-operative radiation therapy have ranged from 1-5%. Post-operative seroma formation is somewhat more difficult to quantify because these are often asymptomatic. In a series by Boostrom et.al. including 167 patients treated with breast conserving surgery, the rate of clinically significant seroma formation (i.e. requiring drainage or some other intervention) was 6% (102). Hence, we anticipate that the rates of post-operative complications will exceed those rates, but with the long-term benefits described in previous sections of this introduction.

We anticipate that the rate of post-operative complications on this study will be more favorable than those reported by Horton et.al. On this study, the radiation dose and fractionation schedule will be much different than that used by Horton et.al. This regimen has been shown to be safe and effective in a recent phase III study in the post-operative setting. (95). Fractionating the dose allows normal tissues time to repair sublethal radiation injury. This would likely result in a lower rate of post-operative complications.

On the other hand, there are factors on this trial that may result in a higher rate of post-operative complications than anticipated. On this study, the interval between radiation and surgery is longer on this study than that reported on the Horton study (surgery within 10 days of radiation versus at least 35 days after radiation). This longer interval was selected based on recent data in the literature for pre-operative treatment for rectal cancer. In this disease site, waiting longer for surgery allows for improved tumor regression, improving the rate of margin negative resection, and allowing for a smaller resection. In the case of rectal cancer, this translates to sphincter preservation but may translate to an improved cosmetic result in the case of breast conserving surgery. These benefits have also translated to an improvement in disease-free survival for patients with rectal cancer (101). However, the longer interval from radiation to surgery may also lead to a slight, but tolerable increase in post-operative complications. It is anticipated that the reasons for a lower post-operative complication rate will dominate on this study. But given the potential for a higher rate than expected, the rate of the most common complication reported on the Horton study will be used as the historical control.

1.7 Conclusions

Pre-operative APBI utilizing MRI-based treatment planning could improve the toxicity profile of breast conservation therapy while maintaining excellent rates of local control and low rates of post-operative complications. This strategy also has the potential to increase the proportion of women who are eligible for APBI and to allow determinants of radiation responses in breast cancer to be elucidated. If this study meets the endpoints outlined with respect to safety, it could serve as the blueprint for future studies utilizing pre-operative MRI-based APBI. The translational component of this study will serve as a baseline to guide future studies in pre-operative radiation therapy. It will form a basis for testing biologic agents and immunomodulators that could potentiate the effects of radiation and further improve pathologic response rates. The imaging correlates of this study will also aid in the development of future treatment protocols. By better understanding the imaging changes after radiotherapy, it will be possible to design radiation treatment regimens that are based on tumor response during a treatment course, a type of treatment delivery possible using MRI-based radiation delivery. With FH/MCW having recently been selected as one of 7 sites in the world to obtain an MR-Linear Accelerator, this proposal will lay the groundwork to optimize the clinical application of this technology and will further position the institution as a leader in the provision of such treatment.

2.0 OBJECTIVES

2.1 Primary

Determine post-operative complication rates following pre-operative APBI in patients with early stage breast cancer and compare these with historical controls, thus demonstrating the safety of this treatment modality.

2.2 Secondary

2.2.1 Measure rates of breast fibrosis and pain for patients treated with APBI and compare with historical controls of patients treated with post-operative APBI.

2.2.2 Collect data on cosmetic outcomes for patients treated with pre-operative APBI

2.2.4 Measure rates of need for re-excision because of a close or positive margin after pre-operative APBI.

2.2.5 Record long term outcomes of local recurrence rates and overall survival of patients treated with this modality.

2.2.6 Demonstrate the feasibility of MRI-based treatment planning for pre-operative APBI and compare dosimetric data from treatment planning with patients treated on a previous institutional post-op APBI protocol.

2.2.7 Study changes in MRI imaging from pre-treatment evaluation, treatment planning, and pre-surgical imaging and correlate those changes to pathological response.

2.2.8 Measure changes in tumor gene expression and immune response to radiation therapy and correlate this with pathologic response.

3.0 PATIENT SELECTION

3.1 Conditions for Patient Eligibility

3.1.1 Pathologically proven diagnosis of invasive breast cancer, clinically stage I-II, planning for breast conserving surgery

3.1.2 The patient must be female

3.1.3 The patient must meet the following criteria

- Age ≥40 years
- Tumor size no greater than 3.0cm by ultrasound
- Estrogen receptor positive
- Patients with both her2 positive and her2 negative tumors are eligible
- Modified Bloom-Richardson Grade 1-3 is allowed
- Unifocal disease
- Invasive carcinoma diagnosed by core needle biopsy (including invasive ductal, papillary, tubular, mammary or medullary carcinoma) within 90 days prior to enrollment
- Clinically node negative both by physical exam and by ultrasound or MRI. All enlarged or abnormal appearing lymph nodes must be biopsied.
- Zubrod/ECOG performance status 0-2 or Karnofsky 70-100

3.1.4 Study entry must be within 90 days from initial diagnosis of breast cancer

3.1.5 Appropriate stage and pre-treatment evaluation for protocol entry, including no clinical evidence for distant metastases, based upon the following minimum diagnostic workup:

- History/Physical examination, including breast exam (inspection and palpation of the breasts) with documentation of weight and Zubrod/ECOG Performance Status of 0-2 or Karnofsky Performance Status 70-100 within 60 days prior to study entry
- Complete Metabolic Panel within 60 days prior to study entry
- Right and left mammography within 60 days of diagnostic biopsy establishing diagnosis. A breast MRI may also substitute for the mammogram if necessary.
- Evaluation of the axilla by ultrasound or MRI and biopsy of all enlarged or abnormal appearing lymph nodes within 42 days prior to study entry
- Clip placed within the biopsy proven breast cancer, with verification of placement by mammogram 60 days prior to study entry

3.1.6 Patients must have had estrogen and progesterone receptor analysis performed on the biopsy specimen prior to study entry according to current ASCO/CAP Guideline Recommendations for hormone receptor testing. Testing for her2 neu expression must also be performed and recorded prior to study entry.

3.1.7 CBC/differential obtained within 60 days prior to study entry, with adequate bone marrow function defined as follows:

- Absolute neutrophil count $\geq$1,800 cells/mm^3
- Platelets ≥ 75,000 cells/mm^3
- Hemoglobin ≥8.0g/dl.

3.1.8 Women of childbearing potential must be non-pregnant and non-lactating and willing to use medically acceptable forms of contraception during radiation therapy

3.1.9 Patient must provide study specific informed consent prior to study entry

3.1.10 Prior breast augmentation, including breast implants, is allowed

3.1.11 Patients with a prior history of contralateral breast cancer will be considered eligible if they completed all treatment (including anti-endocrine therapy) more than 2 years prior to registration

3.1.12 Patients must not have a prior treatment of malignancy diagnosed or treated within the past two years, with the exception of non-melanomatous skin cancer, carcinoma in situ of the cervix and contralateral breast cancer as described in 3.1.11.

3.1.13 Interested patients must meet with a medical oncologist prior to study entry to determine if a genomic profiling tests (such as Oncotype or Mammaprint) is recommended. If recommended and patient is amenable to the possibility of receiving chemotherapy, there must be adequate biopsy tissue for testing. If adequate tissue is not available for the testing, patients who are very interested in participation may undergo additional biopsies. If a patient plans to refuse chemotherapy regardless of a high profiling results and elects to forgo the test, they will still be eligible for enrollment.

3.2 Conditions for Patient Ineligibility

3.2.1 AJCC clinical T3, N1-3, M1, stage IIB, stage III or stage IV breast cancer

3.2.2 Prior invasive non-breast malignancy (exceptions include non-melanomatous skin cancer, carcinoma in situ of the cervix, or prior contralateral breast cancer as described in 3.1.11) unless disease free and off treatment for a minimum of 5 years prior to study entry.

3.2.3 Multifocal breast cancer

3.2.4 Estrogen receptor negative disease

3.2.5 Lymphovascular space invasion noted on biopsy

3.2.6 Invasive lobular carcinoma

3.2.7 Purely non-invasive breast cancer (i.e. ductal carcinoma in situ (DCIS), lobular carcinoma in situ). Note: DCIS is often associated with invasive ductal carcinoma. Associated DCIS does NOT preclude eligibility as long as there is invasive carcinoma on the biopsy specimen.

3.2.8 Non-epithelial breast malignancies such as sarcoma or lymphoma

3.2.9 Paget's disease of the nipple

3.2.10 Male breast cancer

3.2.11 Prior history of radiation therapy to the chest in the region of the ipsilateral breast that would result in overlap of radiation fields.

3.2.12 Patients having received or having planned neoadjuvant chemotherapy or concurrent chemotherapy. A recommendation for adjuvant chemotherapy will not preclude eligibility. However, if a patient has an Oncotype score that would lead to a recommendation for systemic chemotherapy (see section 3.1.13), and chemotherapy is planned to be given in the neoadjuvant setting, the patient would then be ineligible for enrollment.

3.2.13 Patients who are unable to undergo MRI. This could include patients with a severe allergy to gadolinium contrast or patients with renal function insufficient to receive contrast (GFR less than 30). Patients who have a minor allergy (for example, skin rash or hives) to gadolinium contrast may still be considered for enrollment. These patients would have to receive prophylactic prednisone and diphenhydramine per MCW department of radiology protocol. Such cases should be reviewed with the principal investigator and radiology co-chair prior to enrollment.

3.2.14 History of connective tissue disorder, including lupus, dermatomyositis and scleroderma

3.2.15 Known BRCA mutation

3.2.16 Severe, active co-morbidity, defined as follows:

- Unstable angina and/or congestive heart failure requiring hospitalization within the last 6 months
- Transmural myocardial infarction within the last 6 months
- Acute bacterial or fungal infection requiring intravenous antibiotics at the time of registration;
- Chronic Obstructive Pulmonary Disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy within 30 days before registration;
- Hepatic insufficiency resulting in clinical jaundice and/or coagulation defects; note, however, that laboratory tests for liver function and coagulation parameters are not required for entry into this protocol
- Acquired Immune Deficiency Syndrome (AIDS) based upon current CDC definition; note, however, that HIV testing is not required for entry into this protocol. The need to exclude patients with AIDS from this protocol is necessary because the treatments involved in this protocol may be significantly immunosuppressive

3.2.17 Medical, psychiatric or other condition that would prevent the patient from receiving the protocol therapy or providing informed consent

3.2.18 Patients, who under the best estimates of the treating radiation oncologist, have a life expectancy of 10 years or less

3.2.19 Patients who are pregnant

4.0 Pre- and Post-Treatment Evaluation and Management

Note: This section lists baseline evaluations needed before the initiation of protocol treatment that do not affect eligibility. For specific studies that affect eligibility, please see section 3.0.

4.1 Required Pre-Treatment Evaluations/Management

4.1.1 Patients must complete forms and have photos taken for cosmetic evaluation on the study

4.1.2 Bone scan or any other staging work-up for patients when warranted based on symptoms or at the discretion of the treating physicians should be completed prior to enrollment. Similarly, work up of abnormal labs or physical exam findings should all be completed prior to enrollment.

4.2 Required Post-Treatment Evaluations/Management

4.2.1 Patients will be seen one month after radiation treatment and evaluated for acute side effects of treatment. Breast exam from this visit will be documented.

4.2.2 A post-radiation MRI scan will be obtained no sooner than 28 days after completion of radiation therapy. This scan must be obtained prior to surgery.

4.2.3 Advanced MRI imaging sequences performed for simulation will be obtained on the post-treatment scan. These are described in more detail in section 6.1.9 below.

4.2.4 Tumor response will be assessed using the RECIST and mRECIST criteria (64). Also, changes in the MRI sequences detailed in section 6.1.9 will also be assessed. The baseline measurements for this analysis will be taken from the MR

sim and not from any diagnostic MRI the patient may have had prior to radiation planning.

4.2.5 Blood draw for future studies of immune markers (see section 9.1.3). These will be done prior to radiation treatment, at the end of radiation (+/- 1 treatment) and then one month after radiation treatment but prior to surgery.

5.0 Registration Procedures

5.1 Registration and Monitoring of Patients

5.1.1 Patients will be screened for eligibility and registered by a Clinical Research Associate/Clinical Research Coordinator (CRC/CRA) in the Clinical Trials Office of the Froedtert and Medical College of Wisconsin Cancer Center.

5.1.2 Patients will be registered in the institutional OnCore web-based system under the CRA console.

5.1.3 Registration of patients with consenting, eligibility review, on-study status, treatment status, and follow-up will monitored using the OnCore calendar system.

5.1.4 Serious adverse events (SAEs) and protocol deviations will be reported through the OnCore reporting system.

5.1.5 Questions regarding OnCore systems and data reporting should be reported to OnCore data management.

5.2 Questions Regarding Patient Eligibility

5.2.1 Questions regarding patient eligibility and registration procedures should be directed to the principal investigator, Adam Currey, M.D. (acurrey@mcw.edu, pager (414) 557-2221, office (414) 805-4462).

5.3 Subject Withdrawal

5.3.1 Given the short nature of the treatment regimen (radiation is given over 1.5-2 weeks), and very low rates of toxicity during radiation treatment, it is unlikely that patients will withdraw from the study once radiation treatment has begun. However, subjects have the right to withdraw from the study at any time.

5.3.2 Subjects may be withdrawn from the study if a subjects status declines and in the opinion of the treating physicians it is in the subject's best interest to be withdrawn. Subjects may also be withdrawn if there is a violation of the protocol inclusion and exclusion criteria as deemed relevant by the treating physician and the principal investigator.

5.3.3 It is anticipated that the most likely reason for a subject withdrawal will be for those not suitable for APBI based on radiation treatment planning (see Section 6.4 and 6.4.2 below for further details). As these patients would be withdrawn prior to delivery of study related treatment, they will then be replaced on the study and not counted toward the total accrual goal of 40 patients. Similarly, patients withdrawing for any other reason prior to delivery of treatment will be replaced on the study and not counted toward the total accrual goal.

5.3.4 In the rare case that a patient withdraws from the protocol after the initiation of treatment, only data collected up until the date of withdrawal will be used in analysis. If the withdrawal occurs during radiation therapy, data will only be included in analysis of the primary study endpoint if a post-operative complication occurs.

5.3.5 Patients who withdraw from the study should be treated in accordance with normal standards of care. Follow-up will be per the discretion of the patient's treating physicians, but at a minimum be in accordance with NCCN guidelines.

Management of disease recurrence or any unforeseen late morbidity of treatment will be managed by the treating physicians.

6.0 Radiation Therapy

6.1 Localization, Simulation and Immobilization

6.1.1 Treatment in the prone position is preferred. Treatment in the supine position will be allowed if the patient cannot tolerate that position, or the breast size is not suitable for prone RT as determined by the treating radiation oncologist.

6.1.2 Patients should be optimally positioned with alpha cradle casts and breast boards. Note that all immobilization devices must be MRI compatible

6.1.3 Methods to minimize cardiac exposure and target volume motion such as gating and deep-inspiration breath hold are allowed

6.1.4 A treatment planning CT scan in the treatment position will be required to define target volumes and for beam modeling. Dual-Energy CT will also be performed to enhance target delineation.

6.1.4.1 Radio-opaque markers may be placed on external landmarks at the acquisition of the CT scan to facilitate contouring segmentation of the CT data-set.

6.1.4.2 The CT should extend cephalad to the mandible and extend sufficiently caudally to encompass the entirety of the breast tissue and the lung volume.

6.1.4.3 A CT scan image thickness of 5mm or less should be used

6.1.5 External skin localization marks which may include permanent tattoos are recommended for daily localization and set-up accuracy.

6.1.6 MRI simulation will also be performed in the treatment position and same immobilization device created during the CT simulation.

6.1.7 MRI simulation will be performed on the 3.0T large bore MRI unit (MAGNETOM Verio, Siemens) with gadolinium contrast per Froedtert Hospital guidelines and guidelines in the department of radiation oncology.

6.1.8 Patients will be scanned with commercially available and custom built RF transmitter/receiver coils.

6.1.9 In addition to the standard imaging MR sequences, additional advanced imaging sequences will be obtained:

6.1.9.1 *Diffusion Weighted Imaging (DWI) and Apparent Diffusion Coefficient (ADC) Map.* A DWI sequence of about four minutes duration will be acquired. Sequences will be acquired during free-breathing. For patients scanned in the prone position, these scans will not be gated (it is anticipated that this will be the majority of patients). For patients scanned in the supine positon, free breathing will again be used, but the images will be gated. B-values of 0-10000 in 6-25 directions will be used in these scans. B-values of 50, 400 and 800 will be used to derive the ADC map.

6.1.9.2 *Perfusion Weighted Imaging (PWI) Dynamic Contrast-Enhanced (DCE)* will be used for perfusion imaging. *A* spoiled gradient echo sequence will be executed in repetition for about eight minutes while a contrast agent is administered intravenously to the patient. An FDA approved adult dose (0.05 mmol/kg) of MRI gadolinium contrast agent will be used. This contrast agent injection will be used to define the macroscopic boundaries of the tumor for T1-weighted post-contrast images, which are **the standard of care**. The acquisition of PWI scans will not necessitate any additional amount of contrast agent injection to the patient, because both the standard T1-weighted post-contrast images and the PWI images will be acquired with the same amount of contrast injection. In case the patient will

not be administered contrast agent for the T1-weighted images, as part of the standard simulation, then PWI scans will not be performed for that patient.

6.2 Treatment Planning – Target Volumes and Organs at Risk

6.2.1 The definitions for the CTV, PTV and normal structures used in this protocol generally conform to the RTOG-endorsed consensus guidelines for delineation of target and normal structures for breast cancer. (http://www.rtog.org/CoreLab/ContouringAtlases/BreastCancerAtlas.aspx)

6.2.2 The target volume will be defined by fusing image data sets in the planning system from both CT and MRI scans obtained in the treatment position. Fusion will be performed with preference given to breast alignment.

6.2.3 Target Volumes

6.2.3.1 *Gross Tumor Volume (GTV)* will be defined as all gross tumor extent as seen on the MRI scan.

6.2.3.2 *Clinical Target Volume 1 (CTV1)* will be defined as a 0.5cm expansion around the GTV, limited to 5mm within the skin surface and to normal barriers to tumor spread such as the muscle and bone of the chest wall. This volume is designed to correspond to the tissue that would be removed during a typical lumpectomy.

6.2.3.3 *Clinical Target Volume 2 (CTV*2) will be defined as a 1.0cm expansion around CTV1, again limited to 5mm within the skin surface and to normal barriers to tumor spread such as the muscle and bone of the chest wall. This volume is similar to the lumpectomy to CTV expansion used in most post-operative APBI protocols. This total expansion of 1.5cm (CTV1+CTV2) also correlates with data linking MRI delineation of tumor volume with pathological extent of disease (103).

6.2.3.4 *Planning Target Volume (PTV)* will be an expansion to account for daily set-up uncertainty and motion occurring during treatment. If daily kilovoltage CT imaging is used for image guidance treatment delivery, a PTV expansion of 0.3 cm will be used. A PTV of 0.5 cm will be used if daily megavoltage CT imaging is used. The PTV should not be limited inside the skin or at the chest wall. This volume will be used for aperture generation, but not for analysis of the dose volume histogram.

6.2.3.5 *Planning Target Volume Eval (PTV_Eval)* will be based on the PTV, but limited to 0.5cm within the skin. It may extend in to the muscle of the chestwall, but should not extend deep/posteriorly to the anterior surface of the ribs.

6.2.4 Normal Structures

6.2.4.1 Breast volume – Includes the apparent glandular breast tissue as defined on CT and MRI. Consensus anatomical borders as defined in the RTOG atlas should be used. The breast is limited anteriorly within 0.5cm from the skin and posteriorly to the anterior surface of the pectoralis and serratous anterior muscles and should exclude the chest wall.

6.2.4.2 Contralateral Breast – Includes the apparent glandular breast tissue as defined on CT and MRI. Given the fusion alignment based on the ipsilateral breast, MRI data may be less useful in defining this volume. Otherwise, the contralateral breast will be defined similarly to the ipsilateral breast as described above.

6.2.4.3 Ipsilateral Lung – may be contoured with auto-segmentation with manual verification

6.2.4.4 Contralateral Lung – may be contoured with auto-segmentation with manual verification

6.2.4.5 Heart – This is to be contoured in all cases, not just left sided cases. The heart should be contoured beginning just inferior to the level in which the pulmonary trunk branches into the left and right pulmonary arteries (PA). Above the PA, none of the heart's 4 chambers are present and no contours should appear above that level. The heart should be contoured inferiorly on every slice inferior to that until its inferior extent near the diaphragm. It is not necessary to include pericardial fat in the heart contour.

6.2.4.6 Thyroid – all lobes of the thyroid should be contoured

6.3 Treatment Planning – Plan Generation

6.3.1 3D conformal radiation therapy (3DCRT) or intensity modulated radiation therapy (IMRT) will be allowed. If 3D planning is used, dose will be delivered through at least 3 beams.

6.3.2 The PTV (not PTV_Eval) will be used for aperture generation

6.3.3 The dose prescription will be consistent with recently reported phase III study of APBI delivered in the post-operative setting. Patients will receive 30Gy in 5 fractions of 6Gy, given on non-consecutive days (95). Total treatment duration should be no longer than 18 days.

6.3.4 Skin bolus is not allowed

6.4 Treatment Planning – Dose Constraints

6.4.1 PTV

6.4.1.1 *Ideal* – 95% of the PTV will be covered by the prescription dose of 30Gy
Acceptable Variation – 95% of the PTV will be covered by 95% of the prescription dose

6.4.2 Breast Volume

6.4.2.1 *Ideal* – Less than 25% of the total breast volume should receive the prescription dose of 30Gy
Acceptable Variation – Less than 35% of the total breast volume should receive the prescription dose of 30Gy

6.4.2.2 *Ideal* – Less than 45% of the total breast volume should receive 50% of the prescription dose (15Gy)
Acceptable Variation – Less than 50% of the total breast volume should receive 50% of the prescription dose (15Gy)

Note: Failure to meet the above constraints may constitute a patient who is not a candidate for APBI. If both these constraints are not met, the patient should be taken off study and treated per standard of care.

6.4.2.3 *Ideal* – Max dose within the breast should be less than 108% of the prescription dose (32.4Gy)
Acceptable Variation – Max dose within the breast should be less than 110% of the prescription dose (33Gy)

6.4.3 Contralateral Breast

6.4.3.1 *Ideal* – Less than 5% of the contralateral breast should receive 5% of the prescription dose (1.5Gy).
Acceptable Variation – Less than 5% of contralateral breast should receive 8% of the prescription dose (2.4Gy).

6.4.4 Ipsilateral Lung

6.4.4.1 *Ideal* – Less than 10% of the ipsilateral lung should receive 30% of the prescription dose (9Gy).
Acceptable Variation – Less than 15% of the ipsilateral lung should receive 30% of the prescription dose (9Gy).

6.4.4.2 *Ideal* – Less than 30% of the ipsilateral lung should receive 10% of the prescription dose (3Gy).
Acceptable Variation – Less than 35% of the ipsilateral lung should receive 10% of the prescription dose (3Gy).

6.4.5 Contralateral Lung

6.4.5.1 *Ideal* – Less than 10% of the contralateral lung should receive 10% of the prescription dose (3Gy).
Acceptable Variation – Less than 15% of the contralateral lung should receive 10% of the prescription dose (3Gy).

6.4.6 Heart

6.4.6.1 For Left Sided Cases: *Ideal* – Less than 10% of heart should receive 5% of the prescription dose (1.5Gy).
Acceptable Variation – Less than 10% of the heart should receive 8% of the prescription dose (2.4Gy).
Ideal – Mean heart dose should not exceed 200cGy
Acceptable Variation – Mean heart dose should not exceed 320cGy

6.4.6.2 For Right Sided Cases: *Ideal* – Less than 5% of heart should receive 5% of the prescription dose (1.5Gy).
Acceptable Variation – Less than 5% of the heart should receive 8% of the prescription dose (2.4Gy).
Ideal – Mean heart dose should not exceed 200cGy
Acceptable Variation – Mean heart dose should not exceed 320cGy

6.5 Radiation Treatment Delivery

6.5.1 Treatment should generally begin within 60 days of enrollment. At least one day will elapse between each fraction so that treatments are not delivered on consecutive days.

6.5.2 A pair of orthogonal portal images will be obtained at time of the second simulation and will be reviewed by a radiation oncologist prior to the first treatment.

6.5.3 Daily CT or MRI-based image guided radiotherapy will be used for table shifts and field placement at the time of second simulation and with each treatment fraction. Alignment to the biopsy clip, breast position, and tumor if visible on CT/MRI will be used.

6.5.4 On-line adaptive replanning will be allowed for patients treated with daily kilovoltage-CT or MRI based image guided radiotherapy.

6.5.5 Treatment should ideally be given over no more than 14 elapsed days, but up to 18 days will be allowed. Extenuating circumstances (e.g. patient unable to come for treatment, prolonged down-time on treatment machine) resulting in treatment interruption will be clearly documented.

6.5.6 Patients will be seen once during the treatment course for weekly review as per standard protocols in the radiation oncology department. Ideally, one of those visits should occur on the last day of treatment. Breast exam during that visit and documentation of skin reactions will be documented.

7.0 Systemic Therapy

7.1 Chemotherapy

Chemotherapy may be given and chemotherapeutic agents used are at the discretion of the patient's medical oncologist. The use of chemotherapeutic agents before or during radiation therapy is not allowed and is permitted only as an adjuvant to surgery as is the current standard for patients treated with post-operative APBI.

For the patient population for this study, resected tumor specimens often are tested with a commercially available quantitative reverse transcriptase polymerase chain reaction (RT-PCR) analysis of 21 individual genes. The results of this assay are then used to develop a recurrence score to guide decisions regarding systemic chemotherapy (OncotypeDx). Because this test is usually performed on the final surgical specimen, there is concern that any tumor regression occurring as a result of pre-operative radiation therapy might lead to a loss of important information that can guide treatment decisions. However, if there is adequate tumor present in the core biopsy used to diagnose breast cancer, the 21-gene assay may be performed on the biopsy specimen instead of the final surgical specimen. Furthermore, data presented at the San Antonio Breast Cancer Symposium in 2011 demonstrated 92% concordance between Oncotype performed on core biopsies and surgical specimens (93). This level of concordance is comparable to that of negative sentinel lymph node biopsy predicting no axillary lymph node involvement. In a recent analysis of the 548 reportable Oncotype DX tests performed at Froedtert Hospital from 2010-mid 2015, only 9% had a high recurrence score (Data from Oncotype reporting, prepared by Denise Keeler). Furthermore, the patients on this protocol are generally considered low risk for a high Oncotype Score.

Included in the eligibility criteria for this protocol is that the biopsy specimen contain adequate tissue to perform the OncotypeDx assay. The high concordance of biopsy OncotypeDx score with final surgical specimens coupled with the very low probability of a high Oncotype recurrence score in this patient population make it extremely unlikely that a patient with a high score will somehow be missed and hence not receive systemic chemotherapy as a result of receiving pre-operative radiation therapy.

7.2 Hormonal Therapy

As patients are required to have ER positive disease, all patients should be treated with anti-endocrine therapy per current guidelines. Treatment should be given at a minimum of 5 years post-surgery. The choice of anti-endocrine agent is at the discretion of the patient's medical oncologist, and dose and schedule should be consistent with the instructions in the drug package inserts. Hormone therapy cannot be initiated until after surgery. While patients should be strongly encouraged to complete a minimum of 5 years of anti-endocrine therapy, failure to do so for whatever reason will not result in a protocol deviation or be the cause of study withdrawal.

7.3 Trastuzumab

Trastuzumab or other anti-HER2 agents are permitted at the discretions of the patient's medical oncologist for patients whose tumors are HER2 positive. The dose and schedule of these agents should be per standard treatment protocol, but cannot be given until after surgery.

8.0 Surgery and Post-Operative Treatment

8.1 Breast conserving surgery

Breast conserving surgery (lumpectomy) and sentinel node biopsy will occur no sooner than 5 weeks following completion of RT, but should occur no later than 8 weeks. In patients with rectal cancer, this interval has been shown to produce optimal response to pre-operative radiation while maintaining low rates of post-operative complications (101). Surgical techniques such as needle localization will be performed at the discretion of the treating surgeon and in concordance with current standard of care. Oncoplastic procedures are discouraged, but will not be counted as a protocol violation or removal from the study.

8.2 Sentinel lymph node biopsy and management of the axilla

Sentinel lymph node biopsy should be performed at the time of lumpectomy. But at the discretion of the treating physicians, it may be performed prior to the initiation of radiation therapy. Dual tracer with radiocolloid together with blue dye is required. If a sentinel lymph node is positive for metastatic disease with any focus >0.2 mm (i.e. stage N1mic or higher), axillary node dissection may be performed at the discretion of the treating physicians. In the unlikely event that there are 4 or more positive lymph nodes, patients will require RT to the regional lymph nodes. Given the patient selection criteria and pre-treatment evaluation, this scenario would be extremely unlikely.

8.3 Management of a positive margin

The need for surgical re-excision after the initial lumpectomy because of close or positive margins will be determined by the treating surgeon and radiation oncologist. For any patient with a positive margin, re-excision is required unless technically not possible (i.e. poor anesthesia risk for the patient, inability to resect additional breast tissue at the site of the positive margin).

8.4 Post-operative Radiation Therapy

It is conceivable that patients deemed appropriate for APBI and are study eligible may have findings on final pathology that would make them inappropriate candidates for APBI. Careful selection criteria for this study make this scenario very unlikely. However, if a patient has a nodal metastasis >2mm (N1 or higher), post-operative whole breast irradiation should be strongly considered. Similarly, whole breast irradiation should also be considered for patients with persistently positive resection margins and then refuse mastectomy or with an extensive intraductal component. Regional nodal irradiation will be added to the whole breast irradiation and required for patients with 4 or more positive nodes, and should be considered for those with 1-3 positive nodes. Dose and fractionation of the subsequent treatment will be left to the discretion of the treating radiation oncologist. A boost to the surgical bed in the breast should be discouraged.

This approach is patterned after that used on the TARGIT-A trial. In that study, intra-operative APBI was administered to patients before the final results of surgical pathology were available. Patients who were found to have high risk features and not deemed appropriate for APBI alone after final pathology received whole breast irradiation without significant adverse effect (96).

9.0 Pathologic Evaluation and Genetic Evaluation of Tissue Specimens

9.1 Pathological Evaluation and Immune Biomarkers

9.1.1 The surgical specimens will be subject to routine pathological analysis. Remaining tissue from the pre-treatment biopsy, as well as any residual tumor tissue post-RT, will be stored in accordance with MCW department of Pathology guidelines. Routine hematoxyline and eosin (H&E) stain will be used for morphological analysis and for the determination of immune cell infiltrates. The samples will be assessed by the breast pathologist as previously described (65,66). One H&E slide and 20 unstained slides will be reserved for Gene Expression Profiling (described in section 9.2 below).

9.1.2 Immune cell infiltrates have previously been shown to correlate with both breast cancer-specific outcomes and response to neoadjuvant treatment (67). We anticipate that enhanced immune cell infiltration (determined as described above in 8.1.1, or with additional immunostains if needed and tissue available allows) will correlate with pathologic responses to RT.

9.1.3 Blood specimens (50mL, collected in 10mL lavender top EDTA tubes) will be obtained at the time of routine blood draws: once pre- and once post-radiation, (last day and up to one week after +/- 1 treatment) for future studies of immune markers/peripheral blood cells and other cancer and treatment related biomarkers that will attempt to predict radiation side effects and potentially treatment responses. These samples will be obtained at Froedtert Hospital, and in order to spare patient additional travel, those receiving radiation treatment outside Froedtert may opt out of the blood draw at the end of radiation. These samples will also be stored on the MCW Campus and analysis will be performed on campus. The process for handling specimens will be as follows:

9.1.3.1 After collection, invert tube(s) multiple times to ensure adequate mixing of EDTA. ***Call the co-PI's laboratory (Dr. Bergom) at 414-955-8629 to let them know they need to come pick up the PBMCs. If no answer, call the co-PI at 262-510-6488. The plasma will be processed in the laboratory performing the blood draw, and the PBMCs will be collected by the PI's laboratory member for the remainder of the processing outlined below.**

9.1.3.2 **Performed by the blood draw laboratory:** Centrifuge specimen(s) within one hour of collection in a standard clinical centrifuge at ~2500 RPM for 10 minutes at 4°C (preferred). If unable to process the samples at 4°C then spinning at room temperature is acceptable if done within 2 hours of draw. If the interval between specimen collection and processing is anticipated to be more than one hour, keep specimen on ice until centrifuging is performed.

9.1.3.3 **Performed by the blood draw laboratory:** Carefully pipette and aliquot 0.5mL plasma into 8 cryovials labeled with case numbers, collection date/time, time point collected and clearly mark specimen as "plasma". Avoid pipetting up the buffy coat layer.

9.1.3.4 Place cryovials into biohazard bag and immediately freeze at -70 to -90°C.

9.1.3.5 Store frozen plasma until ready for analysis

9.1.3.6 **Performed by the PI's laboratory:** PBMCs should be separated using sterile and endotoxin free Ficoll density gradient solution. The peripheral blood mononuclear cells (PBMCs) should be removed and pooled. The PBMC layer should be resuspended

in three times the volume of cold sterile PBS. Cells should be counted using a hemocytometer and the total cell number should be noted. The expected yield is 1-2 million cells per ml of blood.

9.1.3.7 **Performed by the PI's laboratory:** The PBMCs collected from 3 lavender top tubes will be divided into three aliquots. Roughly two-thirds of the cells should be cryopreserved for future analysis (see next item). Roughly one-third of the cells will be placed in RNAlater (see instructions below) and snap frozen with liquid nitrogen and stored at -70 to -80°C for future RNA extraction. If there are more than 15E6 viable cells the RNA later aliquot should be limited to a total of 5E6 cells and the additional cells should be cryopreserved at -70 to -90 °C, thus deviating from the 2:1 aliquoting.

9.1.3.8 **Performed by the PI's laboratory:** Cryopreservation; After counting, PBS washed PBMCs should be divided into aliquots of 5 million cells and spun down at approximately 1,000-1,500 rpm (400 x g) for 10 minutes. Each aliquot should be resuspended in 1ml of cold sterile CryoStor CS10 freeze media (catalog number 07930, STEMCELL technologies) which contains 10% DMSO. Each 1ml aliquot should be placed in a cryopreservation tube and the lid should be tightly secured. Tubes should be placed into a "Mr. Frosty" or other slow freeze container and placed into a -70 to -80°C freezer for 12-24 hours. Tubes should then be transferred to and stored in vapor phase liquid nitrogen (-135°C) until analyzed in batches.

9.1.3.9 **Performed by the PI's laboratory:** RNA later: After counting, PBS washed PBMCs should be spun down at approximately 1,000-1,500 rpm (400 x g) for 10 minutes. The cell pellet should be re-suspended in 1ml of RNAlater solution snap frozen in liquid nitrogen and stored at -70 to -80°C until analyzed in a batched manner.

9.1.4 Peripheral Blood Analysis

9.1.4.1 Plasma: Plasma samples will be stored at Dr. Bergom's Laboratory for batched analysis. Plasma will be interrogated for chemokine and cytokine levels using a multiplex platform from Eve Technologies (https://www.evetechnologies.com).

9.1.4.2 Cryopreserved samples: Cryopreserved samples will be stored Dr. Bergom's Laboratory for batched analysis. Samples will be thawed and stained with fluorophore-conjugated antibodies against one or more of the following: CD4, CD8, CD25, CD62L, CD45RA, CD127, ICOS, PD-1, PD-L1, FoxP3, CD3, CD56, CD16, CD83, TIM-3, Ki-67, CD19, CD20, CD33, CD15, CD11b, HLA-DR and others. Stained cells will be interrogated by flow cytometry and results analyzed using FlowJo or similar software.

9.1.4.3 RNAlater samples: Cells in RNA later will be stored Dr. Bergom's Laboratory for batched analysis. Samples will be thawed and RNA will be extracted. The transcriptome will be analyzed by RNA deep sequencing (RNAseq) using the Illumina HiSeq platform. Targeted RT-PCR will be used to validate genes of interest identified by RNAseq. TCR deep sequencing data may also be obtained from the RNAseq.

9.1.5 The evaluating pathologist will assess the percentage of the surgical specimen that contains live tumor.

9.1.6 Sections will be subject to immunohistochemical evaluation of the marker Ki-67 pre- and post-operatively to estimate the tumor cell population capable of proliferation after treatment (68). Since the monoclonal antibody Ki-67 detects a nuclear antigen expressed in all phases of the cell cycle except G0, this offers a unique means to assess response to radiotherapy. This approach has never before possible with standard breast radiation treatment, because standardly treatment is delivered after all measurable tumor has been removed.

9.2 Gene Expression Profiling and Advanced Imaging:

9.2.1 RNA will be procured using the Tissue Bank quality control measures fixed paraffin embedded tissues pre-radiation (from biopsy) and post-radiation (surgical specimen).

9.2.2 We will perform global gene expression profiling using RNAseq to identify differential expression patterns that correlate with response to radiation. We will utilize the MCW Human and Molecular Genetics Center Sequencing Core to perform RNAseq and analysis. Total RNA will be extracted using Trizol per the MCW Tissue Bank's standard procedure, and the total RNA will be poly-A purified, transcribed, and chemically fragmented using Illumina's TruSeq RNA library kit. Libraries will be prepared for each sample, indexed for multiplexing, and then sequenced on an Illumina HiSeq 2000. Briefly, we will plan to multiplex 4 libraries per lane x 8 lanes per flow cell, enabling us to test 32 samples per flowcell, a setup which has routinely yielded us 150-180 million reads (87.5% mapped reads) per lane. For data analysis, sequencing reads will be demultiplexed and compressed into FASTQ files using CASAVA 1.8 or equivalent software, aligned to the human genome by TopHat (69), and analyzed for transcript abundance using Cufflinks (70). Cufflinks produces FPKM (fragments per Kb of exon per million fragments mapped) values for all transcript isoforms. The CuffDiff2 program (part of Cufflinks package) then statistically analyzes differences in transcript abundance, taking into account variance between biological replicates and correction for false discovery rate (71).

9.2.3 Expression of genes from patients with pathologic complete response versus non-complete responders will be compared using an unpaired t test on all probes, with a false-discovery rate (FDR) of less than 0.05. If <15% complete pathologic responses are seen, then partial responses versus no response will be compared.

9.2.4 Expression of genes from patients with pre- and post-radiation will be compared, with a false-discovery rate (FDR) of less than 0.05.

9.2.5 Gene expression data from this patient group will be compared to previously published gene expression signatures for radiation sensitivity, and OncotypeDx, a metric that predicts chemotherapy response (79,89,90).

9.2.6 Correlation of MR parameters and gene expression differences will be performed as described previously (90). Correlation of MRI parameters and CT-imaging with pathologic responses will also be determined.

9.2.7 Additional focus will be placed on genes with a statistically significant change in expression (with $p<0.05$). Pathway analysis will be used to identify likely putative genes which modulate radiation response. Targets identified as potentially leading to enhanced responsiveness will be used to generate a novel gene signature and tested and validated as prognostic factors in future studies.

10.0 Patient Assessments

10.1 Study Parameters

10.1.1 See Appendix 1 for a summary of assessments and time frames

10.1.2 Breast assessments will be conducted during radiation therapy with one ideally occurring on the last day of treatment

10.1.3 A breast exam must be conducted by a Physician, Physician Assistant or Nurse Practitioner at clinic visits and documented 42 days prior to study entry, 4-6 weeks post radiation, prior to surgery, no later than one month after the last surgery (post-operative visit), six months after surgery, 12 months after surgery (+/- 2 months) and then annually until 5 years after treatment is completed. The annual follow-up interval of 12 months +/-2 months will be calculated from the actual date of the last follow-up visit.

10.1.4 Post-operative complications (the primary endpoint of the study) will be defined as any complication occurring within 3 months of surgery. These may include but are not limited to: post-operative infection requiring oral/IV antibiotics (CTCAE grade 2 or higher), need for hospital re-admission due to surgical complications, wound dehiscence (CTCAE grade 2 or higher), significant hematoma at the operative site requiring drainage (CTCAE grade 2 or higher), and persistent symptomatic seroma formation that will be defined as need for seroma drainage or need for pain medications because of seroma formation (CTCAE grade 2 or 3), or any need for re-operation excluding re-excision for concerns related to resection margin.

10.1.5 At follow-up visits (see Appendix I for schedule), documentation will be made of disease status (no evidence of disease, local recurrence, regional recurrence, or distant recurrence) and toxicity. Toxicity will be graded according to CTCAE v4.0.

10.2 Cosmetic Outcomes

10.2.1 Physician reported cosmetic outcome has been consistently reported from prospective studies evaluating new methods for breast radiation. It is important to document physician reported cosmetic outcomes with this novel treatment method as well.

10.2.2 Physician assessed cosmetic outcome will be assessed prior to radiation, at the end of radiation, one month following radiation prior to surgery, one month after the last surgery, six months after surgery, 12 months after surgery and then annually until 5 years after treatment is completed.

10.2.3 Cosmetic assessments will be assessed using a 4 point scale. The form contained in Appendix III will be used for data collection.

10.2.4 Digital photographs will be taken of the treated and untreated breasts using an RTOG established protocol familiar to the research associates at Froedtert Hospital. These images will be taken prior to treatment, one month after surgery, then 6 months, 12 months, 24 months and 36 months after surgery. Two digital images will be taken at each of these assessment points. One will be a close up of the treated breast alone in order to provide detailed information regarding the treatment effects. The second digital image will be a straight frontal view of both breasts taken in either a standing or seated position with the patient's hands symmetrically placed on her hips. Care will be taken to exclude the face and framing and focusing will be done to include both the treated and untreated breast to allow optimal comparison for symmetry.

10.2.5 Photos will then be saved in MCW's secure I: drive with the following naming convention:

Save photo as: "Case #, View, Time Point" (ex. 7 Single_6mo; or 12 Both_1)

Breast View: Single = Treated breast; Both = Both breasts

Time Point: B=Baseline prior to radiation; 1mo = 1 month post-surgery; 6mo = 6months post-surgery; 1 = 1 year post-surgery; 2 = 2 years post- surgery; 3 = 3 years post-surgery

10.2.6 The digital images will later be evaluated for cosmetic results by the investigators using the criteria established in previous trials (again see Appendix III). It is of interest and important to obtain multiple measures of cosmetic outcome. With this protocol, three methods will be undertaken – physician reported outcomes assessed at time of follow-up, photographs to be evaluated by the investigators, and patient reported outcomes as described in the next section.

10.3 Quality of Life Outcomes and Patient Reported Outcomes

10.3.1 The Breast Cancer Treatment Outcome Scale (BCTOS) is a 22 item measure of perceived aesthetic (e.g. breast shape) and functional status (e.g. pain and mobility) that has been used after breast conserving therapy and radiation (Appendix IV)

10.3.2 The BCTOS has high reliability and validity and has been used in a variety of previous studies on recovery from breast cancer treatment, including studies of post-operative APBI.

10.3.3 The BCTOS focuses on symptoms that are specifically relevant to radiation therapy (e.g. skin problems, breast tenderness, fibrosis)

10.3.4 These forms will be completed prior to treatment, at the end of treatment, one month after radiation, one month after surgery, then 6 months after surgery, then 12 months, 24 months, 36 months, 48 months and 60 months after surgery or chemotherapy (if given), whichever is later. These may be collected +/- 2 months from the specified time points.

10.3.5 The BCTOS will ideally be completed at clinic visits, but may be completed by mail or over the phone by research associates.

10.3.6 As the BCTOS has been used on previous studies of radiation therapy, the data collected from this study can then be compared to historical controls of patients treated with more traditional post-operative radiation therapy regimens including post-operative APBI.

10.4 Patient Safety Monitoring and Confidentiality

10.4.1 This study will be reviewed by the Medical College of Wisconsin Cancer Center Data and Safety Monitoring Committee (MCWCC DSMC). A summary of the MCWCC DSMC activities are as follows:

10.4.1.1 Review the clinical trial for data integrity and safety

10.4.1.2 Review all unexpected grade 3, and all grade 4, and 5 adverse events, as well as any others requiring expedited reporting as defined in this protocol. (Grades 4 & 5 events must be reported to the DSMC within 5 calendar days of study staff's knowledge.)

10.4.1.3 Review all Data and Safety Monitoring reports.

10.4.1.4 Submit a summary of any recommendations related to study content.

10.4.1.5 Terminate the study if deemed unsafe for patients.

10.4.2 A copy of the MCWCC Data and Safety Monitoring Plan and membership roster will be maintained in the study research file and updated as membership changes. The committee will review reports from the study PI twice annually (or more

frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary.

10.4.3 Any available DSMC letters will be submitted to the IRB of record as required.

10.4.4 Data on patient information and follow-up information will be collected and entered into the password protected OnCore system. Data will be accessible only by the research team and DSMC.

10.4.5 Hard copy forms (see Appendix III and IV) will be kept in a locked file in offices of the CTO. The forms will be linked to patients by a unique identifier and patient initials in the event that the identifier is transcribed incorrectly. Access to these forms will be limited to the CRA and PI only as the data collected will be entered in to OnCore.

11.0 Statistical Considerations

11.1 Sample Size, Power Calculations, and Comparison of Post-Operative Complications to Historical Controls

The primary endpoint of this study will be post-operative complications (as defined in section 10.1.4). The complication rate measured on this study will be compared to a historical control of 32% of patients, as outlined in section 1.6. This will be measured on an individual patient level. Hence a surgical complication rate that exceeds 31% will be considered unacceptable and result in a negative study. A post-op complication rate of 14% or less will be considered a successful demonstration of the safety of this treatment. This number was selected because it is roughly half the complication rate observed on the study by Horton et.al. (94) and represents an increase in complication rate of only 8% over that reported by Boostrom et.al. (102). To demonstrate this, a Simon's two stage design (104) will be used for this study. The value of this approach is that in stage 1 it can be demonstrated that pre-operative APBI using this dose and fractionation scheme is not excessively risky. Once that condition is satisfied, stage 2 is then used to demonstrate that it meets a better standard of complications compared to the Horton study with only a modest increase in complications when compared with historical series of complications observed without pre-operative radiation therapy.

For this analysis we assume as a null hypothesis that the true complication rate is 0.31. This will be tested against a one-sided alternative. In stage one of accrual, 17 patients will be enrolled. If there are 5 or more complications in these 17 patients, enrollment will be suspended and the study will be evaluated for possible closure. This will serve as the stopping rules for the study under the null hypothesis of a 31% complication rate. Otherwise, 22 additional patients will be accrued (total 39) in stage two, and the null hypothesis will be rejected if there are 7 or fewer complications among the 39 total. This design yields a type I error rate of approximately 0.05 and power of 0.80 when the true complication rate is 14%. With this design, the probability of stopping early is 0.6453. To allow for possible withdrawal prior start of treatment or other unforeseen problem with enrollment, total enrollment will be 40 patients.

Sample size for Simon's Two-Stage Design was calculated using a web-based calculator (retrieved February 11, 2016) (106).

In 2012-2013, 586 new cases of invasive breast cancer were seen at FH. This included 424 patients who had stage I and IIA disease, the population targeted in this proposal. We estimate that 60-70% of these patients would be eligible for screening, with 30-40% eligible for the study. This will allow for an adequate pool of patients for the targeted enrollment of 40 patients.

11.2 Secondary Endpoints

Secondary endpoints of the study will include (1) local control, (2) overall survival, (3) need for surgical re-excision due to inadequate margin, (4) Soft tissue fibrosis of the breast, (5) Breast pain, (6) radiologic and pathologic response, and (7) cosmetic outcome. The re-excision rate and rate of partial/complete response with be estimated with exact confidence intervals, and compared to historical controls (28 and 95) using Chi-Square or Fisher's exact tests. Kaplan-Meier survival estimates or estimate of the cumulative incidence rate will be used where censoring or competing risks occur (local control, cosmetic outcomes, fibrosis, and pain).

11.3 Statistical Analysis of Translational Studies

A description of the statistical methods for the measurement of the changes in gene expression is also contained in section 9.2.2 through 9.2.4. Analysis will be performed using the CuffDiff2 program, part of a commercially available Cufflinks package that will be used in the analysis of the tissue samples. The software models how variability in measurements of a RNA transcript's fragment count is dependent on both its expression and splicing structure. It fits the observed variance in fragment counts as a function of the mean across replicates. It then estimates the number of fragments that originated from each transcript. The algorithm estimates uncertainty by calculating the confidence that each fragment is correctly assigned to the transcript that generated it. The uncertainty in the fragment's count is then mapped as a beta distribution and the overdispersion in RNA sequences as a negative binomial. The algorithm then mixes the distributions together. The result is a beta negative binomial distribution that reflects both the sources of variability in an isoform's measured expression level. This method is well published and further detail can be found in reference 71.

Expression of genes from patients with pre- and post-radiation will be compared, with a false-discovery rate (FDR) of less than 0.05.

Expression of genes from patients with pathologic complete response versus non-complete responders will be compared using an unpaired t test on all probes, with a false-discovery rate (FDR) of less than 0.05. If <15% complete pathologic responses are seen, then partial responses versus no response will be compared in a similar manner.

REFERENCES

1. 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(16):1233–1241.

2. Veronesi U, Cascinelli N, Mariani L, et al. Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer. *N Engl J Med* 2002; 347(16):1227–1232.

3. Veronesi U, Zucali R, Luini A. Local control and survival in early breast cancer: the Milan trial. *Int J Radiat Oncol Biol Phys* 1986;12(5):717–720.

4. van Dongen JA, Bartelink H, Fentiman IS, et al. Randomized clinical trial to assess the value of breast-conserving therapy in stage I and II breast cancer, EORTC 10801 trial. *J Natl Cancer Inst Monogr* 1992 (11):15–18.

5. Blichert-Toft M, Rose C, Andersen JA, et al. Danish randomized trial comparing breast conservation therapy with mastectomy: six years of life-table -analysis. Danish Breast Cancer Cooperative Group. *J Natl Cancer Inst Monogr* 1992(11):19–25.

6. Sarrazin D, Le MG, Arriagada R, et al. Ten-year results of a randomized trial comparing a conservative treatment to mastectomy in early breast cancer. *Radiother Oncol* 1989;14(3):177–184.

7. Poggi MM, Danforth DN, Sciuto LC, et al. Eighteen-year results in the treatment of early breast carcinoma with mastectomy versus breast conservation therapy: the National Cancer Institute Randomized Trial. *Cancer* 2003;98(4):697–702.

8. Fisher B, Anderson S, Fisher ER, et al. Significance of ipsilateral breast tumour recurrence after lumpectomy. *Lancet* 1991;338(8763):327–331.

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

10. Smith BD, Arthur DW, Buchholz TA, et al. Accelerated partial breast irradiation consensus statement from the American Society for Radiation Oncology (ASTRO). *Int J Radiat Oncol Biol Phys* 2009;74(4):987–1001.

11. Mukesh M, Harris E, Jena R, Evans P, Coles C. Relationship between irradiated breast volume and late normal tissue complications: a systematic review. *Radiother Oncol.* 2012 Jul;104(1):1-10.

12. Vrieling C, Collette L, Fourquet A, et al. The influence of patient, tumor and treatment factors on the cosmetic results after breast-conserving therapy in the EORTC “boost vs. no boost” trial. EORTC Radiotherapy and Breast Cancer Cooperative Groups. *Radiother Oncol* 2000;55(3):219–232.

13. http://www.rtog.org/CoreLab/ContouringAtlases/BreastCancerAtlas.aspx

14. Giezen M, Kouwenhoven E, Scholten AN, *et al*. MRI- versus CT-based volume delineation of lumpectomy cavity in supine position in breast-conserving therapy: an exploratory study. *Int J Radiat Oncol Biol Phys.* 2012 Mar 15;82(4):1332-40.

15. Jacobson G, Zamba G, Betts V, Muruganandham M, Buechler-Price J. Image-Based Treatment Planning of the Post-Lumpectomy Breast Utilizing CT and 3TMRI. *Int J Breast Cancer.* 2011

16. O’Sullivan B, Davis AM, Turcotte R, et al. Preoperative versus postoperative radiotherapy in soft-tissue sarcoma of the limbs: a randomised trial. *Lancet* 2002;359(9325):2235–2241.

17. Swedish Rectal Cancer Trial. Improved survival with preoperative radiotherapy in resectable rectal cancer. *N Engl J Med* 1997;336(14):980–987.

18. van Gijn W, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12-year follow-up of the multicentre, randomised controlled TME trial. *Lancet Oncol* 2011;12(6):575– 582.

19. Pisters PW, et al. Rapid-fractionation preoperative chemoradiation, pancreaticoduodenectomy, and intraoperative radiation therapy for resectable pancreatic adenocarcinoma. *J Clin Onc* 1998;16(12):3843–3850.

20. Evans DB, et al. Preoperative gemcitabine-based chemoradiation for patients with resectable adenocarcinoma of the pancreatic head. *J Clin Oncol* 2008;26(21):3496–3502.

21. Le Scodan R, et al. Preoperative chemoradiation in potentially resectable pancreatic adenocarcinoma: feasibility, treatment effect evaluation and prognostic factors, analysis of the SFRO-FFCD 9704 trial and literature review. *Ann Oncol* 2009;20(8):1387–1396.

22. Albain KS, Rusch VW, Crowley JJ, et al. Concurrent cisplatin/etoposide plus chest radiotherapy followed by surgery for stages IIIA (N2) and IIIB non-small-cell lung cancer: mature results of Southwest Oncology Group phase II study 8805. *J Clin Oncol* 1995;13(8):1880–1892.

23. Mohammed ZM, Going JJ, Edwards J, et al. The relationship between components of tumour inflammatory cell infiltrate and clinicopathological factors and survival in patients with primary operable invasive ductal breast cancer. *Br J Cancer* 2012;107(5):864-73.

24. Bergom C, Prior P, Kainz K, Morrow NV, Ahunbay EE, Walker A, Li XA, White J. A phase I/II study piloting accelerated partial breast irradiation using CT-guided intensity modulated radiation therapy in the prone position. *Radiotherapy and Oncology,* 2013-08-01, Volume 108, Issue 2, Pages 215-219

25. Chafe S, Moughan J, McCormick B, *et al*. Late toxicity and patient self-assessment of breast appearance/satisfaction on RTOG 0319: a phase 2 trial of 3-dimensional conformal radiation therapy-accelerated partial breast irradiation following lumpectomy for stages I and II breast cancer. *Int J Radiat Oncol Biol Phys.* 2013 Aug 1;86(5):854-9

26. Vicini F, Winter K, Wong J*,et al.* Initial efficacy results of RTOG 0319: three-dimensional conformal radiation therapy (3D-CRT) confined to the region of the lumpectomy cavity for stage I/ II breast carcinoma. *Int J Radiat Oncol Biol Phys.* 2010 Jul 15;77(4):1120-7.

27. Vicini F, Winter K, Straube W, *et al.* A phase I/II trial to evaluate three--dimensional conformal radiation therapy confined to the region of the lumpectomy cavity for stage I/II breast carcinoma: initial report of feasibility and reproducibility of Radiation Therapy Oncology Group (RTOG) Study 0319. *Int J Radiat Oncol Biol Phys* 2005;63(5):1531–1537.

28. Olivotto IA, Whelan TJ, Parpia S, *et al*. Interim cosmetic and toxicity results from RAPID: a randomized trial of accelerated partial breast irradiation using three-dimensional conformal external beam radiation therapy. *J Clin Oncol.* 2013 Nov 10;31(32):4038-45.

29. Nichols EM, Feigenberg SJ, Marter K,*et al*. Preoperative radiation therapy significantly increases patient eligibility for accelerated partial breast irradiation using 3D-conformal radiotherapy.*Am J Clin Oncol.* 2013 Jun;36(3):232-8.

30. Nichols EM, Dhople AA, Mohiuddin MM, Flannery TW, Yu CX, Regine WF. Comparative analysis of the post-lumpectomy target volume versus the use of pre-lumpectomy tumor volume for early-stage breast cancer: implications for the future. *Int J Radiat Oncol Biol Phys.* 2010 May 1;77(1):197-202.

31. Polgár C, Van Limbergen E, Pötter R, *et al.;* GEC-ESTRO breast cancer working group. Patient selection for accelerated partial-breast irradiation (APBI) after breast-conserving surgery: recommendations of the Groupe Européen de Curiethérapie-European Society for Therapeutic Radiology and Oncology (GEC-ESTRO) breast cancer working group based on clinical evidence (2009). *Radiother Oncol.* 2010 Mar;94(3):264-73.

32. Belkacemi Y, Bourgier C, Kramar A, *et al.* SHARE: a French multicenter phase III trial comparing accelerated partial irradiation versus standard or hypofractionated whole breast irradiation in breast cancer patients at low risk of local recurrence. *Clin Adv Hematol Oncol.* 2013 Feb;11(2):76-83.

33. Vaidya JS, Wenz F, Bulsara M, *et al.*; TARGIT trialists' group. Risk-adapted targeted intraoperative radiotherapy versus whole-breast radiotherapy for breast cancer: 5-year results for local control and overall survival from the TARGIT-A randomised trial. *Lancet.* 2014 Feb 15;383(9917):603-13.

34. http://www.rtog.org/ClinicalTrials/ProtocolTable/StudyDetails.aspx?study=0413

35. van der Leij F, Elkhuizen PH, Janssen TM, *et al*. Target volume delineation in external beam partial breast irradiation: less inter-observer variation with preoperative- compared to postoperative delineation. *Radiother Oncol.* 2014 Mar;110(3):467-70.

36. van Heijst TC, den Hartogh MD, Lagendijk JJ, van den Bongard HJ, van Asselen B. MR-guided breast radiotherapy: feasibility and magnetic-field impact on skin dose. *Phys Med Biol.* 2013 Sep 7;58(17):5917-30

37. Formenti SC, Volm M, Skinner KA, *et al*. Preoperative twice-weekly paclitaxel with concurrent radiation therapy followed by surgery and postoperative doxorubicin-based chemotherapy in locally advanced breast cancer: a phase I/II trial. *J Clin Oncol*. 2003;21(5):864–870.

38. Adams S, Chakravarthy AB, Donach M,*et al.* Preoperative concurrent paclitaxel-radiation in locally advanced breast cancer: pathologic response correlates with five-year overall survival. *Breast Cancer Res Treat*. 2010 Dec;124(3):723-32.

39. Bollet MA, Belin L, Reyal F, *et al.* Preoperative radio-chemotherapy in early breast cancer patients: Longterm results of a phase II trial. *Radiother Oncol.* 2012 Jan; 102(1):82-8.

40. McCormick B, Yahalom J, Cox L, *et al*. The patients perception of her breast following radiation and limited surgery. *Int J Radiat Oncol Biol Phys* 1989;17(6):1299–1302.

41. Darby SC, McGale P, Taylor CW, Peto R. Long-term mortality from heart disease and lung cancer after radiotherapy for early breast cancer: prospective cohort study of about 300,000 women in US SEER cancer registries. *Lancet Oncol* 2005;6(8):557–565.

42. Darby SC1, Ewertz M, McGale P, *et al*. Risk of ischemic heart disease in women after radiotherapy for breast cancer. *N Engl J Med.* 2013 Mar 14;368(11):987-98.

43. Rutqvist LE, Liedberg A, Hammar N, *et al.* Myocardial infarction among women with early-stage breast cancer treated with conservative surgery and breast irradiation. *Int J Rad Oncol Biol Phys* 1998;40(2):359–363.

44. Fowble B, Hanlon A, Freedman G, *et al*. Second cancers after conservative surgery and radiation for stages I–II breast cancer: identifying a subset of women at increased risk. *Int J Radiat Oncol Biol Phys* 2001;51(3):679–690.

45. Clark RM, Wilkinson RH, Miceli PN, *et al.* Breast cancer. Experiences with conservation therapy. *Am J Clin Oncol* 1987;10(6):461–468.

46. Bartelink H, Horiot JC, Poortmans P, *et al*. Recurrence rates after treatment of breast cancer with standard radiotherapy with or without additional radiation. *N Engl J Med* 2001;345(19):1378–1387

47. Arthur DW, Cuttino LW, Neuschatz AC, *et al*. Tumor bed boost omission after negative re-excision in breast-conservation treatment. *Ann Surg Oncol* 2006;13(6):794–801.

48. Romestaing P, Lehingue Y, Carrie C, *et al.* Role of a 10-Gy boost in the conservative treatment of early breast cancer: results of a randomized clinical trial in Lyon, France. *J Clin Oncol* 1997;15(3):963–968.

49. Bartelink H, Horiot JC, Poortmans PM, *et al*. Impact of a higher radiation dose on local control and survival in breast-conserving therapy of early breast cancer: 10-year results of the randomized boost versus no boost EORTC 22881–10882 trial. *J Clin Oncol* 2007;25(22):3259–3265.

50. Fowble B, Solin LJ, Schultz DJ, *et al*. Breast recurrence following conservative surgery and radiation: patterns of failure, prognosis, and pathologic findings from mastectomy specimens with implications for treatment. *Int J Radiat Oncol Biol Phys* 1990;19(4):833–842.

51. Fowble B, Solin LJ, Schultz DJ, *et al*. Breast recurrence following conservative surgery and radiation: patterns of failure, prognosis, and pathologic findings from mastectomy specimens with implications for treatment. *Int J Radiat Oncol Biol Phys* 1990;19(4):833–842

52. Fisher ER, Sass R, Fisher B, *et al*. Pathologic findings from the National Surgical Adjuvant Breast Project (protocol 6). II. Relation of local breast recurrence to multicentricity. *Cancer* 1986;57(9):1717–1724.

53. Kurtz JM, Amalric R, Brandone H, *et al*. Local recurrence after breast-conserving surgery and radiotherapy. Frequency, time course, and prognosis. *Cancer* 1989;63(10):1912–1917.

54. Kuske RR, Winter K, Arthur DW, *et al*. Phase II trial of brachytherapy alone after lumpectomy for select breast cancer: toxicity analysis of RTOG 95–17. *Int J Radiat Oncol Biol Phys* 2006;65(1):45–51.

55. Leonard KL1, Hepel JT, Hiatt JR, Dipetrillo TA, Price LL, Wazer DE. The effect of dose-volume parameters and interfraction interval on cosmetic outcome and toxicity after 3-dimensional conformal accelerated partial breast irradiation. *Int J Radiat Oncol Biol Phys.* 2013 Mar 1;85(3):623-9

56. Kriege M, Brekelmans CT, Boetes C, *et al*. Efficacy of MRI and mammography for breast-cancer screening in women with a familial or genetic predisposition. *N Engl J Med* 2004;351(5):427–437.

57. Lehman CD, Blume JD, Thickman D, *et al.* Added cancer yield of MRI in screening the contralateral breast of women recently diagnosed with breast cancer: results from the International Breast Magnetic Resonance Consortium (IBMC) trial. *J Surg Oncol* 2005;92(1):9–16.

58. Hylton N. Magnetic resonance imaging of the breast: opportunities to improve breast cancer management. *J Clin Oncol* 2005;23(8):1678–1684.

59. Esserman L, Hylton N, Yassa L, *et al*. Utility of magnetic resonance imaging in the management of breast cancer: evidence for improved preoperative staging. *J Clin Oncol* 1999;17(1):110–119.

60. Buchanan CL, Morris EA, Dorn PL, *et al*. Utility of breast magnetic resonance imaging in patients with occult primary breast cancer. *Ann Surg Oncol* 2005;12(12):1045–1053.

61. Chen X, Quiao Q, Currey A, *et al.*. Online adaptive replanning for sequential boost after whole breast irradiation (abstract). *American Association of Physicist in Medicine (AAPM) 55th Annual Meeting,* August 4-8, 2013, Indianapolis, IN.

62. Stegman LD, Beal KP, Hunt MA, *et al*. Long-term clinical outcomes of whole-breast irradiation delivered in the prone position. *Int J Radiat Oncol Biol Phys* 2007;68(1):73–81.

63. Formenti SC, DeWyngaert JK, Jozsef G, Goldberg JD. Prone vs supine positioning for breast cancer radiotherapy. *JAMA*. 2012 Sep 5;308(9):861-3

64.Lymberis SC, deWyngaert JK, Parhar P*, et al.* Prospective assessment of optimal individual position (prone versus supine) for breast radiotherapy: volumetric and dosimetric correlations in 100 patients. *Int J Radiat Oncol Biol Phys*. 2012 Nov 15;84(4):902-9.

65. Mohammed ZM, Going JJ, Edwards J, McMillan DC. The role of the tumour inflammatory cell infiltrate in predicting recurrence and survival in patients with primary operable breast cancer. *Cancer Treat Rev* 2012 Dec;38(8):943-55.

66. Klintrup K1, Mäkinen JM, Kauppila S,*et al*. Inflammation and prognosis in colorectal cancer. *Eur J Cancer*. 2005 Nov;41(17):2645-54

67. Mohammed ZM, Going JJ, Edwards J, Elsberger B, Doughty JC and McMillan DC.The relationship between components of tumour inflammatory cell infiltrate and clinicopathological factors and survival in patients with primary operable invasive ductal breast cancer. *British Journal of Cancer.* 2012. 107, 864–873.

68. Brown JR, DiGiovanna MP, Killelea B, Lannin DR, Rimm DL. Quantitative assessment of Ki-67 score for predictions of response to neoadjuvant chemotherapy in breast cancer. Lab Invest. 2014 Jan 94(1):98-106.

69. Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics.* 2009 May 1;25(9):1105-11.

70. Roberts A, Trapnell C, Donaghey J, Rinn JL, Pachter L. Improving RNA-Seq expression estimates by correcting for fragment bias. *Genome Biol.* 2011;12(3):R22.

71. Trapnell C, Hendrickson DG, Sauvageau M, Goff L, Rinn JL, Pachter L. Differential analysis of gene regulation at transcript resolution with RNA-seq. *Nat Biotechnol.* 2013 Jan;31(1):46-53.

72. Morrow, M. Personalizing extent of breast cancer surgery according to molecular subtypes. *Breast Edinb. Scotl.* **22 Suppl 2,** S106–109 (2013).

73. Cadoo, K. A., Traina, T. A. & King, T. A. Advances in molecular and clinical subtyping of breast cancer and their implications for therapy. *Surg. Oncol. Clin. N. Am.* **22,** 823–840 (2013).

74. Høyer, M. *et al.* Advances in radiotherapy: from 2D to 4D. *Cancer Imaging Off. Publ. Int. Cancer Imaging Soc.* **11 Spec No A,** S147–152 (2011).

75. Eschrich, S. A. *et al.* Validation of a radiosensitivity molecular signature in breast cancer. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* **18,** 5134–5143 (2012).

76. Torres-Roca, J. F. A molecular assay of tumor radiosensitivity: a roadmap towards biology-based personalized radiation therapy. *Pers. Med.* **9,** 547–557 (2012).

77. Eschrich, S. A. *et al.* A gene expression model of intrinsic tumor radiosensitivity: prediction of response and prognosis after chemoradiation. *Int. J. Radiat. Oncol. Biol. Phys.* **75,** 489–496 (2009).

78. Ahn, S. J. *et al.* Quantitative assessment of tumor responses after radiation therapy in a DLD-1 colon cancer mouse model using serial dynamic contrast-enhanced magnetic resonance imaging. *Yonsei Med. J.* **53,** 1147–1153 (2012).

79. Carlson, J. J. & Roth, J. A. The impact of the Oncotype Dx breast cancer assay in clinical practice: a systematic review and meta-analysis. *Breast Cancer Res. Treat.* **141,** 13–22 (2013).

80. Bhide, S. A. & Nutting, C. M. Recent advances in radiotherapy. *BMC Med.* **8,** 25 (2010).

81. Lee, D. J., Ahmed, H. U., Moore, C. M., Emberton, M. & Ehdaie, B. Multiparametric magnetic resonance imaging in the management and diagnosis of prostate cancer: current applications and strategies. *Curr. Urol. Rep.* **15,** 390 (2014).

82. Bhatnagar, P., Subesinghe, M., Patel, C., Prestwich, R. & Scarsbrook, A. F. Functional imaging for radiation treatment planning, response assessment, and adaptive therapy in head and neck cancer. *Radiogr. Rev. Publ. Radiol. Soc. N. Am. Inc* **33,** 1909–1929 (2013).

83. Chen, J.-H. & Su, M.-Y. Clinical application of magnetic resonance imaging in management of breast cancer patients receiving neoadjuvant chemotherapy. *BioMed Res. Int.* **2013,** 348167 (2013).

84. Wang, C.-H., Yin, F.-F., Horton, J. & Chang, Z. Review of treatment assessment using DCE-MRI in breast cancer radiation therapy. *World J. Methodol.* **4,** 46–58 (2014).

85. Schwartz, D. L. Current progress in adaptive radiation therapy for head and neck cancer. *Curr. Oncol. Rep.* **14,** 139–147 (2012).

86. Ahn, S. J., An, C. S., Koom, W. S., Song, H.-T. & Suh, J.-S. Correlations of 3T DCE-MRI quantitative parameters with microvessel density in a human-colorectal-cancer xenograft mouse model. *Korean J. Radiol. Off. J. Korean Radiol. Soc.* **12,** 722–730 (2011).

87. Stephen, R. M. *et al.* Monitoring the development of xenograft triple-negative breast cancer models using diffusion-weighted magnetic resonance imaging. *Exp. Biol. Med. Maywood NJ* **237,** 1273–1280 (2012).

88. Zahra, M. A., Hollingsworth, K. G., Sala, E., Lomas, D. J. & Tan, L. T. Dynamic contrast-enhanced MRI as a predictor of tumour response to radiotherapy. *Lancet Oncol.* **8,** 63–74 (2007).

89. Dewhirst, M. W. & Chi, J.-T. Understanding the tumor microenvironment and radioresistance by combining functional imaging with global gene expression. *Semin. Radiat. Oncol.* **23,** 296–305 (2013).

90. Yamamoto, S., Maki, D. D., Korn, R. L. & Kuo, M. D. Radiogenomic analysis of breast cancer using MRI: a preliminary study to define the landscape. *AJR Am. J. Roentgenol.* **199,** 654–663 (2012).

91. Weichselbaum, R. R. *et al.* An interferon-related gene signature for DNA damage resistance is a predictive marker for chemotherapy and radiation for breast cancer. *Proc. Natl. Acad. Sci. U. S. A.* **105,** 18490–18495 (2008).

92. Greenup, R. A. *et. al.* Cost comparison of radiation treatment options after lumpectomy for Breast Cancer. *Annal Surg Oncol* 19:3275-3281 (2012).

93. Stull, T.S. et.al. Comparison of Oncotype Dx between surgical and core biopsy specimens in breast cancer patients. Presented at the San Antonio Breast Cancer Symposium, December 6-10, 2011.

94. Horton, J.K. et al. Preoperative single-fraction partial breast radiation therapy: A novel phase I, dose-escalation protocol with radiation response biomarkers. *Int. J. Radiat. Oncol. Biol. Phys.* 92:846-855, 2015.

95. Livi, L. *et al.* Accelerated partial breast irradiation using intensity-modulated radiotherapy versus whole breast irradiation: 5-year survival analysis of a phase 3 randomized controlled trial. *Euro J Cancer*. 51:451-463, 2015.

96. Vaidya J.S. *et al.* Risk-adapted targeted intraoperative radiotherapy versus whole-breast radiotherapy for breast cancer: 5-year results for local control and overall survival from the TARGIT-A randomized trial. *Lancet*. 383:603-613, 2014.

97. Thoeny, H.C. *et al*. Predicting and monitoring cancer treatment Response with diffusion-weighted MRI. J of Magnetic Resonance Imaging 32:2-16, 2010.

98. Formenti, S.C. *et al.* Pre-operative twice-weekly paclitaxel with concurrent radiation therapy followed by surgery and post-operative doxorubicin-based chemotherapy in locally advanced breast cancer: A phase I/II trial. *J Clin Oncol* 21:864-870, 2003.

99. Bollet M.A. *et al.* Pathological response to pre-operative concurrent chemo-radiotherapy for breast cancer: Results of a phase II study. *Euro J Cancer* 42:2286-2295, 2006.

100. Kao J. *et al.* Concomitant radiation therapy and paclitaxel for unresectable locally advanced breast cancer: results from two consecutive phase I/II trials. *Int J Radiation Oncology Biol Phys.* 61(4):1045-1053, 2005.

101. Kim J.H., *et al*. Interval between surgery and radiation therapy is an important prognostic factor in treatment of rectal cancer. *Cancer Res Treat* 44(3):187-194, 2012.

102. Boostrom S.Y. *et.al*. Incidence of clinically significant seroma after breast and axillary surgery. *J Am Coll Surg* 208(1):148-150, 2009.

103 Grimsby G.M. *et al*. Is there concordance of invasive breast cancer pathologic tumor size with magnetic resonance imaging? *Am J Surg* 198:500-504, 2009.

104. Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 10(1):1-10, 1989.

105. Ivanova A. Simon's Two-Stage design. Retrieved February 11, 2016, from http://cancer.unc.edu/biostatistics/program/ivanova/SimonsTwoStageDesign.aspx

APPENDIX 1 – Patient Assessments

Screening

Assessments	Within 90 Days of Enrollment	Within 60 Days of Enrollment
History and Physical, Performance Status and Height and Weight Documentation		X
Biopsy Proven Invasive Breast Cancer	X	
Breast Examination		X
Bilateral Mammogram		X (within 60 days of diagnosis)
Clip placed in tumor, verified by mammogram		X
CBC w/diff and ANC		X
Complete Metabolic Panel		X
Determination of hormone receptor and her2 status	Prior to enrollment	
Ultrasound or MRI of axilla with biopsy of any enlarged/abnormal lymph nodes		X (within 42 days of enrollment)
Consultation with Medical Oncologist	Prior to enrollment	
If Oncotype or Mammaprint is recommended, assessment of adequacy of biopsy specimen	Prior to enrollment	

Assessments During Treatment

Assessments	Prior to Start of RT	Last Day of RT (+/- 1 treatment)
History and Physical or weekly review, Performance Status and Weight Documentation	X	X
Breast Examination	X	X
Adverse event evaluation		X
Physician Cosmetic and QOL Questionnaire	X	X
Breast Photos	X	
Specimens for Research (blood, biopsy specimen)	X	X (blood only)*

*Optional for those treated outside Froedtert Hospital

Follow-up Assessments

Assessments	4-6 weeks After RT Completion	Within 4-6 weeks after Last Surgery	6 Months (+/-2wks) After Surgery	12 Months (+/- 1 month) After Surgery then Annually* (+/- 2 months)
History and Physical, Performance Status and Weight Documentation	X	X	X	X (until year 5)
Breast Examination	X	X	X	X (until year 5)
Adverse event evaluation	X	X	X	X (until year 5)
Specimens for Research (Blood, surgical specimen)	X (Blood)	X (surgical specimen)		
Physician Cosmetic and QOL Questionnaire	X	X	X	X (until year 5)
Breast Photos		X	X	X (until year 3)
Breast MRI	X			
Mammogram of Ipsilateral Breast			X	
Mammogram of Bilateral Breasts* (based on surgical date)				X (until year 5)**

*Follow-up and mammogram interval of every 12 months +/- 2months will be calculated from the actual date of the previous follow-up visit or mammogram.

**The mammogram to be done 12 months (+/- 1 month) after surgery is optional based on standard of care mammography schedules.

APPENDIX II

ZUBROD/ECOG PERFORMANCE SCALE

0 Fully active, able to carry on all predisease activities without restriction

1 Restricted in physically strenuous activity but ambulatory and able to carry work of a light or sedentary nature. For example, light housework, office work.

2 Ambulatory and capable of self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.

3 Capable of only limited self-care, confined to bed or chair 50% or more of waking hours

4 Completely disabled. Cannot carry on self-care. Totally confined to bed

5 Death

KARNOFSKY

100 – Normal; no complaints; no evidence of disease.

90 – Able to carry on normal activity; minor signs or symptoms of disease.

80 – Normal activity with effort; some signs or symptoms of disease.

70 – Cares for self; unable to carry on normal activity or to do active work.

60 – Requires occasional assistance, but is able to care for most of their personal needs.

50 – Requires considerable assistance and frequent medical care.

40 – Disabled; requires special care and assistance.

30 – Severely disabled; hospital admission is indicated although death not imminent.

20 – Very sick; hospital admission necessary; active supportive treatment necessary.

10 – Moribund; fatal processes progressing rapidly.

0 – Dead.

Patient Initials ☐,☐☐ *Last First Middle*

Patient Study ID ☐☐

APPENDIX III PHYSICIAN SCORED COSMETIC RESULTS

Please circle the result that best describes the breast appearance

1	**EXCELLENT**: When compared to the untreated breast or the original appearance of the breast, there is minimal or no difference in the size or shape of the treated breast. The way the breast feels (its texture) is the same or only slightly different. There may be thickening scar tissue or fluid accumulation within the breast, but not enough to change the appearance.
2	**GOOD**: There is slight difference in the size or shape of the treated breast as compared to the opposite breast or the original appearance of the treated breast. There may be some mild reddening or darkening of the breast. The thickening or scar tissue within the breast causes only a mild change in the shape or size.
3	**FAIR:** Obvious difference in the size and shape of the treated breast. This change is a quarter or less of the breast. There can be moderate thickening or scar tissue of the skin and the breast, and there may be obvious color changes.
4	**POOR**: Marked change in the appearance of the treated breast involving more than a quarter of the breast tissue. The skin changes may be obvious and detract from the appearance of the breast. Severe scarring and thickening of the breast, which clearly alters the appearance of the breast, may be found.

Please circle a number of each of the following treatment effects.

	None	Yes, present but does not affect cosmesis	Yes, present and affects cosmesis
Skin Telangectasia	0	1	2
Skin Atrophy	0	1	2
Scarring	0	1	2
Pigment Change	0	1	2
Erythema	0	1	2
Fat Necrosis	0	1	2
Fibrosis	0	1	2
Retraction or Contour Defect	0	1	2
Volume Loss	0	1	2
Other significant Treatment Effects	0	1	2
Specify:			
Comments:			

Assessment Time Point (Circle one): Pre-Treatment Last Day of RT

1 Month After RT Completion (Pre-Surgery) 1 month post-op 6 months 12 months (1yr)

24 months (2 years) 36 months (3 years) 48 months (4 years) 60 months (5 years)

Physician's Signature **Signature Date**

Patient Initials ☐ , ☐ ☐
Last *First* *Middle*

Pre-Operative Accelerated Partial Breast Irradiation Quality of Life Questionnaire

Patient Study ID ☐☐

Participants should complete this questionnaire at baseline (after consent and prior to randomization) and at end of radiation therapy, one month after radiation therapy (prior to surgery), one month after surgery, 6 months post-surgery, 12, 24, 36, 48, and 60 months after surgery or chemotherapy whichever is later. The first page is to be completed by a clinical staff member. Fill in the items listed on this page, print the patient's study ID at the top of pages 2 through 7 and give the questionnaire to the patient for completion. After the patient has completed the questionnaire, verify that the date has been recorded at the top of page 2, and submit the completed questionnaire to the CRA/CRC in the Clinical Trials Office.

Please administer the questionnaire at an office visit if possible. If that is not possible, mail the questionnaire to the patient, then call to ask for the patient's responses over the phone.

Staff Member Administering Form

Last Name	First Name	Phone

Time point for this questionnaire – Circle One

Baseline (after consent, prior to radiation)
Last Day of radiation therapy
4 weeks (1 month) after the end of radiation therapy (prior to surgery)
1 month post-surgery
6 months post-surgery
12 months after surgery (whichever is later)
2 years after surgery
3 years after surgery
4 years after surgery
5 years after surgery

This form is being filled out: (*Mark one.*)

O By participant in doctor's office
O By participant not in doctor's office
O By clinical staff, on phone with participant
O Other

Record the participant's study ID on each of the remaining pages before giving the questionnaire to the participant.

Patient Study ID ☐☐

Date this questionnaire is completed: ☐☐ ☐☐ ☐☐☐☐
Month Day Year

(For example, if you were completing the questionnaire on September 8, 2014, you would write 09 08 2014 in the boxes.)

Thank you for completing this questionnaire.
We are interested in your evaluation of your physical appearance and functioning since you have been treated for breast cancer. Please rate the following items on this four-point scale, according to your evaluation at this point in time.

	Difference between treated and untreated breast and area			
	None	**Slight**	**Moderate**	**Large**
1 Breast size	1	2	3	4
2 Breast texture (hardening)	1	2	3	4
3 Arm heaviness	1	2	3	4
4 Nipple appearance	1	2	3	4
5 Shoulder movement	1	2	3	4
6 Arm movement	1	2	3	4
7 Breast pain	1	2	3	4
8 Ability to lift objects	1	2	3	4
9 Fit of shirt sleeve	1	2	3	4
10 Breast tenderness	1	2	3	4
11 Shoulder stiffness	1	2	3	4
12 Breast shape	1	2	3	4
13 Breast elevation (how high the breast is)	1	2	3	4
14 Scar tissue	1	2	3	4
15 Shoulder pain	1	2	3	4
16 Arm pain	1	2	3	4
17 Arm swelling	1	2	3	4
18 Breast swelling	1	2	3	4
19 Arm stiffness	1	2	3	4
20 Fit of bra	1	2	3	4
21 Breast sensitivity	1	2	3	4
22 Fit of clothing	1	2	3	4

Patient Study ID ☐☐

We are interested in your personal reactions to the radiation and surgery you have received for your breast cancer. Please answer the following questions by circling one (1) number. Please note that the response options are labeled at the end-points only. However, you can and should use all of the points on the scale as appropriate to best convey your response. This page should be filled out only one month after radiation and one month after surgery

1. To what extent has your radiation/surgery **disrupted** your normal daily activities?

0	1	2	3	4	5	6	7	8	9	10
Not at all										A lot

2. To what extent has your surgery/radiation **disrupted** your normal recreational activities?

0	1	2	3	4	5	6	7	8	9	10
Not at all										A lot

3. To what extent has your radiation/surgery **disrupted** your normal activities with your family and friends?

0	1	2	3	4	5	6	7	8	9	10
Not at all										A lot

4. To what extent has your radiation/surgery **disrupted** your normal sleep pattern?

0	1	2	3	4	5	6	7	8	9	10
Not at all										A lot

5. To what extent has your radiation/surgery **reduced** your enjoyment of life?

0	1	2	3	4	5	6	7	8	9	10
Not at all										A lot

6. To what extent has your radiation/surgery **disrupted** your regular activities at work (e.g., need to take time off, not getting done as much as you'd like)? If you do not work outside the home for pay, please check this box ☐ and go to the next question.

0	1	2	3	4	5	6	7	8	9	10
Not at all										A lot

7. How **satisfied** are you with the length of time your treatment has taken to this point in time?

0	1	2	3	4	5	6	7	8	9	10
Not at all										A lot

8. How **disruptive** has your radiation/surgery been to the other important people in your life (e.g., family and close friends)?

0	1	2	3	4	5	6	7	8	9	10
Not at all										A lot

These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the past 4 weeks...

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
1. Did you feel full of life?	1	2	3	4	5
2. Did you have a lot of energy?	1	2	3	4	5
3 Did you feel worn out?	1	2	3	4	5
4. Did you feel tired?	1	2	3	4	5

5. Rate your pain at its worst in the past four weeks. (Circle one number.)

0	1	2	3	4	5	6	7	8	9	10

No pain — Pain as bad as you can imagine

6. Rate your pain at its least in the past four weeks. (Circle one number.)

0	1	2	3	4	5	6	7	8	9	10

No pain — Pain as bad as you can imagine

7. Rate your pain on average in the past four weeks. (Circle one number.)

0	1	2	3	4	5	6	7	8	9	10

No pain — Pain as bad as you can imagine

8. Rate how much pain you have right now. (Circle one number.)

0	1	2	3	4	5	6	7	8	9	10

No pain — Pain as bad as you can imagine

9. Are you currently receiving treatments or taking medications for your pain?
Circle one: Yes No

By circling one (1) number per line, please indicate how much you have been bothered by each of the following problems **in the past four weeks**.

	Not bothered at all	A little bit bothered	Some-what bothered	Bothered quite a bit	Bothered very much
Fever or shivering (shaking, chills)	0	1	2	3	4
Swelling of breast (breast feels larger)	0	1	2	3	4
Breast heaviness	0	1	2	3	4
Breast warm to touch	0	1	2	3	4
Breast skin is red	0	1	2	3	4
Breast skin is tanned	0	1	2	3	4
Breast skin or area around nipple is pale in color	0	1	2	3	4
Breast skin is flaking or peeling	0	1	2	3	4
Bleeding or fluid leakage from breast	0	1	2	3	4
Breast itching	0	1	2	3	4
Blisters on the breast (or breast skin moist and raw)	0	1	2	3	4
Coughing	0	1	2	3	4
Difficulty breathing	0	1	2	3	4
Muscle aches	0	1	2	3	4
Rib or chest wall pain	0	1	2	3	4
Infections	0	1	2	3	4
Slow healing of breast wounds	0	1	2	3	4
Visible small blood vessels (spider veins)	0	1	2	3	4
Pockmarks or puncture wounds on breast	0	1	2	3	4
Thickening of breast skin	0	1	2	3	4
Hardening of breast	0	1	2	3	4
Breast or nipple numbness	0	1	2	3	4
Sharp shooting pains or twinges in the breast	0	1	2	3	4

	Not bothered at all	A little bit bothered	Some-what bothered	Bothered quite a bit	Bothered very much
Breast aches	0	1	2	3	4
Breast tenderness	0	1	2	3	4
Decrease or lack of arousal on breast	0	1	2	3	4
Any other problems? (Specify below)	0	1	2	3	4

Specify other problems:

You have been treated with breast conserving therapy for breast cancer. As you know, a reason for choosing this treatment is to keep a breast that looks and feels as close to normal as possible. Your opinion concerning the appearance of your breast is valuable to us. Circle the number next to the word that best describes how your breast looks now.

1	**EXCELLENT:** when compared to the untreated breast or the original appearance of the breast, there is minimal or no difference in the size or shape of the treated breast. The way the breast feels (its texture) is the same or slightly different. There may be thickening, scar tissue or fluid accumulation within the breast, but not enough to change the appearance.
2	**GOOD:** there is a slight difference in the size or shape of the treated breast as compared to the opposite breast or the original appearance of the treated breast. There may be some mild reddening or darkening of the breast. The thickening or scar tissue within the breast causes only a mild change in the shape or size.
3	**FAIR**: obvious differences in the size and shape of the treated breast. This change involves a quarter or less of the breast. There can be moderate thickening or scar tissue of the skin and the breast, and there may be obvious color changes.
4	**POOR:** marked change in the appearance of the treated breast involving more than a quarter of the breast tissue. The skin changes may be obvious and detract from the appearance of the breast. Severe scarring and thickening of the breast, which clearly alters the appearance of the breast, may be found.

My satisfaction about the treatment and results is: (Select the phrase that best describes your satisfaction.)

☐	☐	☐	☐	☐
Totally satisfied	Somewhat satisfied	Neither satisfied nor dissatisfied	Somewhat dissatisfied	Totally dissatisfied

Before any treatment to your breast, the size of your breasts was: (Select the phrase that best describes your breast size prior to treatment.)

☐ Larger on left

☐ The same on both sides

☐ Larger on right

The size of your breasts now is: (Select the phrase that best describes your breast size now.)

☐ Larger on left

☐ The same on both sides

☐ Larger on right

Thank you for completing this questionnaire!