A Radiation Therapy Study

A PHASE II EVALUATION OF PREOPERATIVE SINGLE-FRACTION PARTIAL BREAST RADIOTHERAPY IN EARLY STAGE BREAST CANCER

DUKE CANCER INSTITUTE

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2. LIST OF ABBREVIATIONS

3D	3 Dimensional
5-FU	5 fluoro-uracil
AE	Adverse events
ANC	Absolute Neutrophil Count
AP/PA	Anterior to Posterior, Posterior to Anterior
APC	Argon Plasma Coagulation
BED	Biologically Equivalent Dose
BID	Twice Daily
CBC	Complete Blood Count
CBCT	Cone Beam Computed Tomography
CDDP	Cisplatin
Chemo	Chemotherapy
CPC	Cancer Protocol Committee
CRT	Chemoradiotherapy
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTV	Clinical Tumor Volume
D10cc	Minimum dose to the 10 milliliters of any volume receiving the highest dose
D2cc	Minimum dose to the 2 milliliters of any volume receiving the highest dose
DCI	Duke Cancer Institute
DCIS	Ductal carcinoma in situ
DLT	Dose Limiting Toxicity
Dmax	Maximum dose to any voxel within a volume
DUHS	Duke University Health System
ECOG	Eastern Cooperative Oncology Group
EFRT	Extended Field Radiotherapy
EQD2	Equivalent dose at 2 Gray per fraction
FIGO	International Federation of Gynecology and Obstetrics
G3 or G4	Grade 3 or Grade 4 toxicity
GCP	Good Clinical Practice
GOG	Gynecologic Oncology Group
GTV	Gross Tumor Volume
GU	Genitourinary
GY	Gray
HDR	High Dose Rate
ICRU	International Commission on Radiation Units and Measurement
ID	Identification
IMRT	Intensity Modulated Radiation Therapy (including Volumetric Modulated Arc Therapy)
IRB	Institutional Review Board
KPS	Karnofsky Performance Scale
LDR	Low Dose Rate

LINAC	Linear Accelerator
LRC	Loco-regional control
MRI	Magnetic Resonance Imaging.
MTD	Maximum Tolerated Dose
MV	Megavoltage
NCI	National Cancer Institute
OS	Overall Survival
PA	Para-aortic
PBI	Partial Breast Irradiation
PET	Positron Emission Tomography
PI	Primary Investigator
PTV	Planning Target Volume
NRG	National Surgical Adjuvant Breast and Bowel Project (NSABP), the Radiation Therapy Oncology Group (RTOG), and the Gynecologic Oncology Group (GOG)
SBRT	Stereotactic body radiation therapy
SOC	Safety Oversight Committee
TD5/5	Toxic dose of 5% at 5 years
V18	Partial volume receiving greater than or equal to 18 Gray
WAI	Whole abdominal irradiation
WPRT	Whole Pelvic Radiotherapy

3. PROTOCOL SYNOPSIS

3.1 Purpose:

This protocol seeks to build on the favorable results of our phase I trial (Pro00015617) by extending our findings to a larger cohort of subjects.

Primary Objective

1. Determine physician reported rates of good/excellent cosmesis at baseline and 6 months, 1, 2, and 3 years post-treatment as measured by the NRG cosmesis scale (Appendix II).

Secondary Objectives

1. Determine patient-reported rates of good/excellent cosmesis at baseline and 6 months, 1, 2, and 3 years post-treatment as measured by the NRG cosmesis scale.
2. Assess patient-reported Quality of Life (FACT-B) and patient satisfaction at the same time points.
3. Assess locoregional control in the treated breast relative to historical controls at 10 years.

Exploratory Objectives

1. Ki-67 will be assessed as a measure of tumor response.
2. Assess the impact of radiation on gene expression
3. Assess the impact of radiation on circulating cell-free DNA via serum markers and oxidative stress as measured by urinary biomarkers. Please see Appendix VI for additional detail regarding serum and urine assessment.
4. To review treatment plans, evaluate delivery techniques and assess positioning verification images

Hypotheses

In this study, we hypothesize that 21Gy can be delivered preoperatively to a large group of subjects with high rates of good/excellent cosmetic outcomes

3.2 Background and Significance:

In 1990, the National Institutes of Health concluded that breast conservation, conservative surgery followed by radiotherapy, was the preferred treatment for women with early stage breast cancer as no survival advantage was seen in women receiving a more radical and disfiguring mastectomy. As a result, breast conservation consisting of lumpectomy followed by 6 weeks of daily external beam radiotherapy became the standard of care in the United States (US). Treatment typically consisted of 4.5-5 weeks of whole breast treatment followed by a 1-1.5 week boost to the surgical resection cavity. Unfortunately, some women electing to receive breast conserving surgery struggled to complete the recommended radiotherapy. Patterns of care studies demonstrated that older women, and those living in low-income regions or far from radiotherapy centers, often omitted radiotherapy altogether[1-3]. Numerous studies have demonstrated that the local recurrence rates in these women receiving conservative surgery alone are unacceptably high[4,5].

As a result, there has been much interest in development of a more convenient alternative to standard radiotherapy. Recently, robust data has documented that a 3 week course of whole breast radiotherapy is an efficacious and safe alternative[6,7] to standard therapy. Much additional work has been done on partial breast irradiation (PBI), rapid treatment of the tumor bed plus a small margin of normal tissue, delivered in 1 to 5 days. Several techniques have evolved to deliver PBI: low-dose rate (LDR) brachytherapy, high-dose rate (HDR) brachytherapy via intracavitary applicator, external beam radiotherapy and intra-operative radiation. LDR brachytherapy has the most extensive follow-up but has not been widely adopted due to the significant technical expertise required for accurate and efficient delivery. Intracavitary and external beam radiotherapy, the two most common forms of partial breast irradiation, are widely available and relatively easily applied. However, treatment is still intensive, requiring 10 treatments delivered 6 hours apart over five days. Furthermore, though most preliminary data suggest comparable rates of local control, increasing data are accumulating that soft tissue fibrosis and cosmetic outcomes may be suboptimal with the popular external beam technique[8]. Several investigators have suggested that this may be attributable to the large volume of tissue treated to high doses in the post-operative setting[9,10].

We proposed a phase I trial evaluating pre-operative radiation as a solution to this issue. Delivery of radiation pre-operatively to the intact tumor allows more precise targeting of a much smaller volume than a large post-operative seroma. Furthermore, the highly conformal nature of stereotactic radiosurgery is ideal to minimize the volume of uninvolved breast tissue and skin receiving high-dose radiation during PBI, two advantages of the intra-operative technique that likely contribute to the acceptable outcomes seen with this single dose therapy. However, in contrast to intra-operative treatment which requires significant investment in costly intra-operative radiation equipment and a great deal of flexibility in operating room scheduling, most radiation facilities have the capability to treat with stereotactic radiosurgery. This high precision, conformal, rapid, linear accelerator based external beam partial breast irradiation captures the appeal of single-dose intra-operative treatment but in a setting that is widely accessible.

Therefore, we set out to determine the maximally tolerated dose of pre-operative radiation in our previous phase I trial[11]. Our target dose was 21Gy, the dose established as effective in intraoperative partial breast trials[12,13]. Our results have been positive thus far, with only one locoregional recurrence and no dose-limiting acute toxicities despite escalation to 21Gy. Long-term toxicities at a median follow-up of 3 years are similar to expected toxicities with standard therapy. As expected, the volume of breast receiving prescription dose was vastly decreased compared to post-operative treatment. In this study, we hypothesize that 21Gy can be delivered preoperatively to a larger group of subjects with high rates of good/excellent cosmetic outcomes.

3.3 Rationale:

Rationale for single-fraction preoperative technique
We propose in this trial to build on the favorable results of our phase I trial by extending our findings to a larger cohort of subjects. The preoperative approach has several advantages:

1) a small intact breast tumor results in significantly less uninvolved breast tissue receiving high radiation doses which likely decreases toxicity;
2) more accurate targeting of the high-risk areas of subclinical disease surrounding the tumor is possible,
3) smaller treatment volumes are amenable to dose escalation which can further accelerate treatment and improve accessibility for subjects,
4) this technical approach is widely utilized in other tumor sites and can be delivered at most radiation facilities
5) the pre-operative approach provides a novel opportunity to study breast cancer radiation response.

A. Rationale for magnetic resonance imaging (MRI) treatment planning

Target delineation has always been highly subjective in the post-lumpectomy setting. The tumor bed is currently defined as the area of architectural distortion plus surgical clips. Defining this area is subject to inherent variability depending on the bias of the treating physician. Pre-operative assessment of the tumor should certainly improve delineation of the target and the surrounding area at risk. However, the typical tools used for defining breast tumors, mammography and ultrasound, cannot currently be used in conjunction with radiation planning software. CT is commonly used for radiation treatment planning but, as we noted in our phase I trial, does not facilitate clear target delineation. MRI, on the other hand, clearly identifies the tumor and can be used in conjunction with radiation treatment planning software.

B. Rationale for patient selection

Numerous trials have demonstrated that local recurrence rates are unacceptably high after lumpectomy alone in all but the elderly population with small, node negative, estrogen-receptor positive tumors[14]. Published data establishing partial breast techniques have demonstrated low local recurrence rates in conjunction with careful patient selection. Those subjects felt to be suitable for partial breast treatment outside the setting of a clinical trial, as defined by the ASTRO consensus statement[15,16] will be eligible for this study

C. Rationale for endpoints

Clinical data suggests that ER+ breast cancers have a larger proportional benefit from radiation than their ER- counterparts. However, clinical assessment of radiation response (aside from long-term follow-up of local control) is rarely observed as radiation is typically delivered in the post-operative setting after resection of gross disease. As a result, tumor and normal tissue radiation response remain relatively poorly understood. Markers capable of predicting radiation response are rare indeed. Therefore, if biologic markers of radiation response could be identified in a subset of subjects, these clinical markers could be used in future clinical trials to explore the impact of alternative total doses, dose per fraction or concurrent therapy.

In addition, two large randomized trials have documented less toxicity with intraoperative radiation at a single dose of 21Gy[12,13] as compared to standard whole breast radiotherapy. However, this technique does have the critical advantage of delivering radiation to the subcutaneous tissue and minimizing dose to the overlying skin. Pre-operative treatment must necessarily be given with the skin intact. Fibrosis and skin thickening in this area could contribute to suboptimal cosmesis. In our phase I trial, there were

no dose-limiting acute toxicities. In addition, all 29 subjects treated with only 21Gy of radiation (3 subjects received post-operative radiation for high-risk features) had good or excellent outcomes. However, these promising early outcomes must be confirmed in a larger group of subjects with diverse tumor locations and radiation plans. We would expect a rate of good/excellent cosmetic outcomes at 3 years comparable to those seen in the clinical trials evaluating short-course whole breast radiation (75-80% at 3 years)[17]. Local control will also be carefully collected and gathered as a secondary endpoint.

D. 10/01/2025 Amendment Summary and Rationale:

The classification of several study endpoints is being revised. Due to the limited number of subjects enrolled, the small amount of tissue available for analysis, and the absence of prior data to inform statistical assumptions, current secondary endpoints 1 (Ki-67), 4 (gene expression), 5 (circulating DNA) and 6 (treatment planning) will now be considered exploratory in nature.

The small sample size and restricted biospecimen availability preclude adequately powered, confirmatory analyses. Furthermore, without pre-existing data to guide selection of specific genes, biomarkers, or serum markers, it is not scientifically appropriate to designate these two endpoints as definitive or hypothesis-testing. Reframing these study endpoints as exploratory allows for hypothesis generation and identification of potential signals of interest, while ensuring that study results are interpreted within an appropriate methodological context.

a. Amendment Impact on Risk/Benefit Profile:

This amendment does not alter the study design, subject participation, or risk/benefit profile. No additional procedures will be performed. The change pertains only to the classification and interpretation of study endpoints

3.4 Design and Procedure:

Prospective, single arm study in subjects with newly diagnosed ductal carcinoma in situ or invasive carcinoma of the breast

3.5 Selection of Subjects and Sample Size:

Women ages 60 or older with clinically node-negative, 2cm or less, biopsy proven ductal carcinoma in situ or invasive carcinoma will be considered for this trial. Women age 50-59 with a cT1N0 tumor and a low Oncotype score will also be eligible.

The final sample size is 100 subjects. Up to 150 subjects may be consented in order to enroll the target goal of 100. Given the rapid accrual of our previous trial with similar eligibility (approximately 30/32 subjects over 2 years), we anticipate completion of this trial in 5 years.

3.6 Duration of Study:

Subjects will be on study for approximately 3 years. Thereafter subjects will continue to be followed by the treating medical oncologist and/or radiation oncologist/mid level provider as per standard of care for follow up care.

4. SUBJECT ELIGIBILITY

4.1 Inclusion Criteria:

1. Women with a biopsy proven diagnosis of ductal carcinoma in situ or invasive carcinoma of the breast

   a. Biopsy tissue (either slides or block) from outside institutions will be reviewed to confirm diagnosis.
2. Breast preservation candidates (no prior breast or nodal radiotherapy, no imaging evidence of multicentric or multifocal disease, no pregnant women, and no comorbid conditions precluding surgery)
3. Clinical T1N0M0 invasive carcinoma or DCIS < or equal to 2cm
4. 60 years of age or older or 50-59 with a low (0-17) Oncotype score . Oncotype is not required for women diagnosed with DCIS.
5. ER+, HER2- (HER2 status is not required for women diagnosed with DCIS)
6. Women of child-bearing potential must consent to use adequate contraception during the course of the study. Female subjects must agree to use a medically acceptable contraceptives including: (1) surgical sterilization (such as a tubal ligation or hysterectomy), (2) approved hormonal contraceptives (such as birth control pills, patches, implants or injections), (3) barrier methods (such as a condom or diaphragm) used with a spermicide, or (4) an intrauterine device (IUD). Contraceptive measures such as Plan B (TM), sold for emergency use after unprotected sex, are not acceptable methods for routine use.
7. WBC > 3000, Hgb > 9, platelets >100000 within 45 days of consent
8. MRI is required for radiation treatment planning on this study. A diagnostic MRI performed within 60 days of obtaining consent is acceptable and will not be repeated. Subjects who have not had a diagnostic MRI will be required to have a research treatment planning MRI with contrast ordered by a radiation oncologist; these subjects must have a GFR≥ 60 ml/min.
9. Outside breast imaging will be reviewed at Duke to confirm findings are consistent with trial eligibility.

4.2 Exclusion Criteria:

1. Neoadjuvant chemotherapy
2. Breast implant in the breast to be treated with SBRT
3. Medical conditions that may increase risk for poor cosmetic outcome (i.e. Lupus, rheumatoid arthritis, scleroderma)
4. Subjects unable to receive study treatment planning secondary to body habitus or inability to lie flat on the stomach at length
5. HER-2+ ((HER2 status is not required for women diagnosed with DCIS)
6. Positive serum pregnancy test
7. Insufficient breast imaging to judge clinical stage

8. Subjects without placement of a biopsy clip at the diagnostic procedure who are unwilling to undergo clip placement.
9. Subjects in whom treatment planning constraints cannot be met

4.3 Inclusion of Women and Minorities

Only women will be eligible for this study. Minority groups will also be eligible and accrual is expected to reflect the population of subjects seen in the Duke Multi-disciplinary Breast Cancer Clinic.

5. TREATMENT PLAN

5.1 Surgery:

Surgical tumor resection will be completed within 10 days of RT delivery. Subjects will undergo intraoperative lymphatic mapping and sentinel lymphadenectomy as is standard at our institution. Standard axillary nodal dissection is acceptable if there is a contraindication to the sentinel node procedure or if felt to be clinically indicated based on nodal involvement. Omission of nodal assessment is also acceptable if clinically indicated and will not be performed in patient's diagnosed with DCIS. Resection of the tumor will include a 2mm margin of normal tissue. In general, re-excision is required for histologic margins <2mm. There may be exceptions, however, when a close margin occurs at an anatomic boundary (typically chest wall/skin) or is nearly 2mm or only focally close. Those margins are generally felt to be less significant prognostically and the role for re-excision in these cases will be made by the treating surgeon and radiation oncologist. Typically, surgical clips marking the site of tumor excision will be placed to allow for localization of the tumor bed on follow-up imaging. Standard pathologic assessment will follow.

5.2 Radiation Therapy:

Target Volume:

Prior to treatment, a biopsy clip fiducial marker will be placed in the region of the tumor to provide localization for radiotherapy delivery. Kilovoltage on-board-imaging (OBI) and/or cone beam CT (CBCT) will be used for localization as described in the section on target localization.

When required per protocol the patient will then undergo magnetic resonance (MR) imaging. The patient will be placed on the MR table with the breast pendant in a dedicated breast surface coil with arms raised overhead. Alternatively, the patient will be placed supine and specialized thoracic coils will be utilized in order to keep the breast in the same shape for the MR, CT, and radiation treatment to the extent possible. MR/CT compatible surface markers will be used to assist in this effort. T1-weighted imaging, T2-weighted imaging, inversion-recovery imaging, diffusion weighted imaging (DWI), and Dynamic contrast enhanced (DCE) MRI of the breasts will be performed. More specifically, T1 weighted MRI will be acquired with a 3D gradient-echo with spoiler (SPGR) sequence to achieve spatial resolution of sub-millimeter; T2 weighted MRI will be acquired with a 2D fast spin-echo (FSE) sequence; inversion recovery MRI will be acquired by a 2D axial short-T1 inversion-recovery (STIR) sequence; DCE-MRI will be

acquired with a 3D SPGR sequence. Before DCE-MRI acquisition, a MedRad Power Injector will be used to administer weight appropriate dose of gadolinium at 2cc/second after a 30 second inject delay.

Axial T1, axial short-T1 inversion-recovery (STIR), and dynamic VIBRANT images will be required for the delineation of the enhancing tumor, the gross tumor volume (GTV). A planning CT scan will also be acquired in order to generate dose calculations.

Treatment Planning and Dose:

The MR images will be used to define the GTV. A 1.5 cm uniform expansion will be applied around the GTV to create the clinical target volume (CTV). MRI images will be fused to the CT images for further identification of normal structures and target volumes. The first 5mm of subcutaneous tissue and any chest wall (pectoralis muscle and deeper) if >1 cm from the GTV will be excluded from this volume. The first 5mm of tissue is in the photon "build-up" region and to include this area would skew the dose calculations. The chest wall is considered an anatomic boundary to tumor that is separate from the chest wall. An additional 0.5 cm margin will be applied to the GTV and CTV to create two distinct PTV volumes (PTV-GTV and PTV-CTV). PTV-GTVeval and PTV-CTVeval, which includes the same volumes minus the skin, will be utilized for analysis of dose coverage.. Subjects will receive a single fraction of 21Gy to the PTV-GTV and 15Gy to the PTV-CTV. This will allow for high dose to the area at greatest risk with a more moderate dose to the intermediate risk area and associated reduction in dose to the skin.

The skin, ipsilateral and contralateral lung, ipsilateral and contralateral breast tissue, spinal cord and heart will be segmented as per institutional standard and analyzed for dose. The skin will be defined as a 3 mm layer from the external body surface. The ipsilateral and contralateral breast tissue will be segmented utilizing the guidelines from ongoing phase III NRG trials. In short, the breast tissue typically included in standard breast tangents minus the lungs and chest wall will be designated the breast volume with hand modification as needed by the principal investigator/or designee.

The skin, spinal cord, and lungs will be auto-contoured in standard fashion. The heart should be contoured from the apex to the root of the great vessels. The thyroid and brachial plexus will also be contoured as appropriate depending on tumor location. The thyroid should be easily visible and contoured at the base of the neck. The brachial plexus should be visible on MRI and will be contoured with the assistance of radiology as indicated. Alternatively, the axillary vessels will be used as a surrogate for the brachial plexus[18].

A treatment plan utilizing arc therapy, or multiple conformal beams, or intensity-modulated therapy, or a combination of these techniques will then be designed. The beam arrangement can be organized in any fashion provided that the dose constraints listed below are met. The dose will be normalized to provide a desired coverage (95% to 98%) to the CTV.

Dose constraints are based on our phase I experience, as well as that of our Dutch collegues[19] (including a joint analysis of Duke/Utrecht data pending presentation/publication) and will be considered appropriate as follows:

Target volumes:

(CTV): prescribed dose (15Gy) covers >95% of the CTV without exceeding maximum dose of 110% of prescribed dose

PTV-GTVeval: ≥95% of the prescribed dose (21Gy) covers >95% volume without exceeding 110%

PTV(CTV)eval: ≥95% prescribed dose (15Gy) covers >90% of the PTV-CTV eval without exceeding 110% of prescription dose

Normal breast: <30% of the whole breast reference volume should receive 50% or more of the prescribed dose and <15% of the whole breast reference volume should receive the prescribed dose

- Optimal: ipsilateral breast V100% <7%; V50<21%

Contralateral breast: the contralateral whole breast reference volume should receive <10% of the prescribed dose to any point

- Optimal: Dmax ≤1.6Gy

Lungs: 1) Mean lung dose <3.6Gy 2) <37% of lung volume should receive 8Gy, 2) <1500cc to 7Gy, 3) <1000cc 7.6Gy

- Optimal: Mean ≤0.7Gy

Heart: 1) Mean should not exceed 1.5Gy; 2) Point dose < 5Gy.

- Optimal: Mean ≤0.6Gy, Dmax ≤3.4Gy

Chest wall: D20cc <16.3Gy.

- Optimal: D20cc ≤10.2Gy

Thyroid: maximum point dose < 10% of the prescribed dose

Brachial plexus: no point in the brachial plexus should receive more than 10% (2.1Gy maximum) of the prescribed dose.

Skin dose: 1) Maximum dose will not exceed maximum prescription dose (21Gy). 2) Dose to 1cc: <14Gy 3) dose to 10cc <9Gy

Cord: maximum dose to 1 cc should be 1 Gy.

In addition, a low dose survey will be performed to ensure no unanticipated regions are receiving inappropriate dose.

Target Localization and Treatment Delivery:

Subjects will be taken to the treatment machine and positioned comfortably as they were at the time of CT simulation. Image-guidance will be used to localize the treatment volume, including on-board radiographic imaging and cone-beam CT when possible. Positioning correction will be performed as necessary, verified and documented. Implanted marker may be considered as surrogates for effective target localization. The target localization procedure will be documented and adjustments will be made as appropriate. Treatment will be delivered to the target volume in one fraction. Each treatment plan will be checked for quality assurance in both calculation and dosimetry. All treatment will be recorded and verified through ARIA information system.

Post-operative Treatment:

Within 10 days, subjects will proceed to surgical resection. Pathologic assessment will be completed and reviewed per standard of care. Ki-67 will be assessed on core biopsy and definitive resection tumor (as per institutional standard) as a measure of tumor response.

Systemic therapy will only be delivered if indicated. The role for adjuvant radiation will be discussed for any patient whose pathologic assessment reveals an unanticipated high risk feature. Features judged by the multi-disciplinary treatment team to be of comparable clinical risk relative to the presenting

diagnosis (ex. a 1.8cm tumor on imaging that is just over 2cm at resection) will not necessarily require post-operative radiation. Additional radiation will be recommended to individual patients whose locoregional extent of disease is significantly greater than expected at the time of surgery (ex. nodal involvement).
Treatment volumes and prescribed doses will follow institutional standards. Hypofractionated breast treatment will be considered an acceptable alternative to standard therapy (46-50Gy) when clinically appropriate.

Subjects requiring mastectomy will be included in the correlative portion of the trial. However, their cosmesis, recurrence and late RT toxicities outcomes will be gathered and recorded separately. It would not be accurate to compare the secondary endpoints of these subjects to those receiving breast conservation.

5.3 Supportive Care:
Standard supportive therapies at the discretion of the treating radiation oncologist.

6. PATIENT ASSESSMENTS

Assessment	**Pre-Tx.**	**Follow-up**
Consent	X	
History/Physical	X	X[a]
Complete Blood Count (no differential)/CMP	X	
Breast imaging (mammogram minimum)	X	X[a]
Core Biopsy/Fiducial Markers	X[b]	
Planning MRI	X	
Surgical Excision with Tissue Procurement		X[c]
Acute Toxicity Evaluation		X[d]
Cosmetic Evaluation Form/Digital Photographs/QOL	X[e]	X[e]
Clinical Outcomes		X[a]
Serum collection	X[f]	X[f]
Urine collection	X[g]	X[g]
BRPC Consent prior to surgery		X [h]

a. Physical exam by member of oncology team recommended every 4-6 months for 2 years, every 6 months through year 5 and annually years 5-10. Bilateral mammogram recommended at least annually.

b. Verify placement of a biopsy clip at diagnostic procedure and availability of pre-treatment tissue for correlative science.

c. Within 10 days of radiation.

d. Appointment with the Radiation Oncologist/mid level provider 1-2 months after surgery.

e. At baseline (prior to treatment), six months and one year after treatment and then annually for 2 additional years. Cosmetic assessment to be filled out by both patient and physician/mid level provider. Digital photographs at the same time points and at investigator's discretion.

f. 2 lavender tubes drawn prior to radiation therapy and post radiation therapy (before surgery)

g. Urine collected prior to radiation therapy and post radiation therapy (before surgery)

h. Subjects will be consented to the existing Biospecimen Repository and Processing Core (BRPC)tissue collection protocol which includes safeguards to ensure adequate tissue for diagnostic evaluation and biomarker assessment

6.1 Screening Examination

The screening examination can take place up to 45 days before study treatment. An informed consent must be signed by the patient before any screening procedure takes place. If however, standard of care evaluation procedures have been obtained and are within the screening evaluation time points, the SOC procedures do not need to be repeated and may be included in the screening examination. Subject data to be collected includes a complete history and physical including signed informed consent, basic clinical laboratory studies, staging and a biopsy demonstrating ductal carcinoma in situ or invasive breast carcinoma.

Assessment of the tumor by physical exam and mammogram (further imaging at radiologist/physician discretion) should include tumor measurements. Each patient will complete a baseline cosmetic evaluation as will her physician/mid level provider. QOL measures will also be assessed. Prior to study treatment, placement of a biopsy clip at the time of diagnostic biopsy will be verified as this will serve as a fiducial marker (described in section 6.2) to assist in radiotherapy localization. MRI and CT of the affected breast will then be completed for radiation treatment planning

6.2 Correlative Assessment

Blood and urine will be collected at two time points, once before radiation therapy is delivered and once after radiation therapy is delivered. See Appendix VI for additional details regarding serum and urine processing and assessment. Specimens will be stored in a -80° freezer in locked facilities in the Department of Radiation Oncology for future study and analysis. The specimens may be used specifically

for this study and potentially for unspecified research. The reason, biomarkers and assays are changing rapidly. This study is expected to enroll over 5 years, during that time it is possible a new biomarker or assay could be developed that is relevant to the study of breast cancer. A study amendment would be submitted in the event of this occurring.

6.3 Treatment Period:

Lumpectomy will be completed within 10 days of RT delivery. Tissue will be processed as described below in section 8.1. A second biopsy will be collected for gene expression profiling as described in Appendix 1. After preoperative treatment, the patient will be assessed 1-2 months after surgery by a Radiation Oncology faculty member/mid level provider to evaluate for acute toxicity. Subjects will go on to systemic therapy or conventional radiation if a high risk feature not anticipated pre-operatively is noted at the time of surgery. If indicated, subjects will be seen weekly during conventional treatment as is standard procedure in our department. .

6.4 Follow-up Period:

Follow-up after completion of therapy will follow national guidelines and includes a history and physical every 4-6 months for the 1st 2 years, every 6 months to a year through year 5 and annually through year 10. Mammograms will be obtained at least annually. Other laboratory or radiographic studies will be obtained as directed by patient symptoms or physical exam. Quality of life and cosmetic assessments, physician/mid level provider and patient, will be performed at 6 month, 1 year, and then annually through 3 years of follow-up as will digital photographs. Loco regional recurrence will be monitored on protocol at each follow-up visit.

6.5 Early Withdrawal of Subject(s):

Study treatment is a one-day event thus it is not foreseeable that subjects will withdraw from protocol therapy. Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health and/or the patient no longer wishes to continue on the study long-term, the patient shall be removed from the protocol follow-up. In this event the reason(s) for discontinuation of study participation will be noted by the PI in the Radiation Oncology record.

6.5.1 Criteria for Early Withdrawal

Subjects may voluntarily withdraw from the study at any time. The PI may also withdraw a subject from the study at any time based on his/her discretion. Reasons for PI-initiated withdrawal may include, but is not limited to the following:

- Adverse events
- Abnormal laboratory values
- Abnormal test procedure results
- Protocol deviation
- Administrative issues
- Disease progression
- Pregnancy

6.5.2 Follow-up Requirements for Early Withdrawal

Standard of care follow-up will be adhered to.

6.5.3 Replacement of Early Withdrawal(s)

Subjects who prematurely withdraw will not be replaced unless radiation has not been initiated.

7. STATISTICAL METHODS AND DATA ANALYSIS

7.1. Amendment to the Statistical Section

The original sample size of this trial was 40 patients, and the original primary objective was to estimate the pCR rate and test the null hypothesis that the true rate was less than or equal to 0.10. The trial has been amended so that the primary objective is now the estimation of the proportion of patients with good/excellent cosmesis. Pathologic response is no longer an endpoint in this trial. These changes were necessary for two reasons. First, it was determined during the course of the trial that long-term data on cosmesis with the partial breast approach are needed regardless of the pathologic response rate. Second, since an increase in post-operative toxicity (seroma/delayed wound healing) was seen when surgery was completed 6 weeks after radiation, the amendment includes a return to surgery within 10 days of radiotherapy (as in our Phase I trial with minimal toxicity); this eliminates the opportunity to observe pathologic response. The sample size is now being increased to 100 in order to obtain a more precise estimate of the good/excellent cosmesis rate.

7.2. Statistical Analysis of Cosmetic Outcome

The rate of *physician-reported* good/excellent cosmesis will be estimated at baseline and at 6, 12, 24, and 36 months. Assuming an observed good/excellent cosmesis rate of about 0.75, an increase in the sample size from 40 to 100 will allow the width of the 95% confidence interval (CI) of the rate to be decreased by 0.10 units. Specifically, a rate of 0.75 with samples sizes of 40 and 100 has 95% CI's of 0.62 — 0.88 and 0.67 — 0.83, respectively.

A true rate of good/excellent cosmesis of ≥ 0.80 would be considered very good, while a rate of ≤0.60 would be unacceptable. The table below gives a rough guideline for monitoring the 12 month cosmesis rate (the rates at the other time points will not be monitored).

Sample size	Review data if the number of patients with good/excellent cosmetic outcome is:
30	≤ 21 (21/30 = 0.70)
60	≤ 42 (42/60 = 0.70)
100	≤ 70 (70/100 = 0.70)

Simply said, any evidence that the observed cosmesis rate would likely be ≤0.70 would be cause for review. For example, suppose 30 of the first 50 patients (60%) had good/excellent cosmesis. Then even if the next 10 patients had acceptable cosmesis (40/60), this would not be enough to avoid a review of the data. Therefore, a review would have to be begun when N=50. The monitoring rules above have high probability (i.e., 0.97) of initiating a review of the cosmetic data if the true probability of having

good/excellent cosmetic outcome is ≤ 0.60 and a low probability (i.e., 0.14) of initiating a review if the true probability of a good/excellent cosmetic endpoint is ≥0.80.

7.3 Statistical Analyses of the Secondary Objectives

(a) The mean of *patient-reported* rate of good/excellent cosmesis, and its 95% CI, will be estimated at baseline and at 6, 12, 24, and 36 months.

(b) Quality of life (FACT-B) and patient satisfaction will be measured at baseline and at 6, 12, 24, and 36 months. For each scale, the mean and its 95% CI's will be estimated at each time point; boxplots will be used to estimate the distribution of these scales at each time point.

(c) Time to local or regional failure will be defined as the time from start of treatment to date of local or regional failure, whichever comes first; distant failures will be ignored; deaths will be censored. Time to distant failure will be defined as the time from start of treatment to date of distant failure; local and regional failures will be ignored; deaths will be censored. The Kaplan Meier method will be used to estimate both of these distributions.

7.4 Statistical Analyses of the Exploratory Objectives

(a) Rates of ki-67 at core biopsy and 5-7 weeks post-RT will be estimated with 95% CI's.

(b) The analysis of the impact of radiation on gene expression is described in Appendix 1.

(c)To review treatment plans, evaluate delivery techniques and assess positioning verification images

8. SAFETY MONITORING AND REPORTING

The PI is responsible for the identification and documentation of adverse events and serious adverse events, as defined below. At each study visit, the PI or designee must assess, through non-suggestive inquiries of the subject or evaluation of study assessments, whether an AE or SAE has occurred.

Previously, an interim analysis of the first 17 patients was completed. Delayed wound healing, and an increase in grade 2 seromas, relative to our Phase I experience, were observed. The PI and study team believe that there is an interaction between the radiation and surgery (when completed 6 weeks after protocol treatment) that was not seen with our previous practice of 10 days after protocol treatment. Therefore, with the current amendment, we are returning to the practice established from our phase 1 study (Pro00015617) where minimal toxicities were observed. Whilst adverse events will continue to be monitored vigorously, it is not anticipated that that those toxicities will be seen in future enrolled subjects given our prior experience. Therefore, there is no plan to complete an additional interim analysis for this study.

8.1 Adverse Events:

An adverse event (AE) is any untoward medical occurrence in a subject receiving study therapy and which does not necessarily have a causal relationship with this treatment. For this protocol, the definition of AE also includes worsening of any pre-existing medical condition. An AE can therefore be any unfavorable and unintended or worsening sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of radiation therapy, whether or not related to use of the radiation therapy. Abnormal laboratory findings without clinical significance (based on the

PI's judgment) should not be recorded as AEs. But laboratory value changes that require therapy or adjustment in prior therapy are considered adverse events.

From the time the subject signs the informed consent form through the End of Study visit, all AEs must be recorded in the subject medical record and adverse events case report form.

AEs will be assessed according to the CTCAE version 4.0. If CTCAE grading does not exist for an AE, the severity of the AE will be graded as mild (1), moderate (2), severe (3), life-threatening (4), or fatal (5).

Attribution of AEs will be indicated as follows:

- Definite: The AE is clearly related to the study therapy
- Probably: The AE is likely related to the study therapy
- Possible: The AE may be related to the study therapy
- Unlikely: The AE is doubtfully related to the study therapy
- Unrelated: The AE is clearly NOT related to the study therapy

8.1.1 AEs of Special Interest

All possible treatment related adverse events/toxicities reported or observed, the information should be recorded in the patient's medical record and on the study's Toxicity Evaluation Form or in the study's toxicity database. This should include a description of the event, its severity grade, the relationship to the study treatment and the onset date.

8.1.2 Reporting of AEs

All grade 4 or higher unexpected and possibly related toxicity will be reviewed by the PI and study team and reported to the Duke University Health System IRB via the eIRB system.

8.2 Serious Adverse Events:

An AE is considered "serious" if in the opinion of the investigator it is one of the following outcomes:

- Fatal
- Life-threatening
- Constitutes a congenital anomaly or birth defect
- A medically significant condition (defined as an event that compromises subject safety or may require medical or surgical intervention to prevent one of the three outcomes above)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption to conduct normal life functions

8.3 Safety Oversight Committee (SOC):

The Duke Cancer Institute SOC is responsible for annual data and safety monitoring of DUHS sponsor-investigator phase I and II, therapeutic interventional studies that do not have an independent Data Safety Monitoring Board (DSMB). The primary focus of the SOC is review of safety data, toxicities and new information that may affect subject safety or efficacy. Annual safety reviews includes but may not be limited to review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor-investigator. The SOC in concert with the DCI Monitoring Team (see Section 12.1 for Monitoring Team description) oversees the conduct of DUHS cancer-related, sponsor-investigator greater-than-minimal-risk intervention studies that do not have an external monitoring plan, ensuring subject safety and that the protocol is conducted, recorded and reported in accordance with the protocol, standing operating procedures (SOPs), Good Clinical

Practice (GCP), and applicable regulatory requirements. The DCI Safety Oversight Committee (SOC) will perform annual reviews on findings from the DCI Monitoring Team visit and additional safety and toxicity data submitted by the Principal Investigator.

9. QUALITY CONTROL AND QUALITY ASSURANCE

9.1 Monitoring:

This clinical research study will be monitored both internally by the PI and institutionally by the Duke Cancer Institute (DCI). In terms of internal review the PI will continuously monitor and tabulate adverse events. Appropriate reporting to the Duke University Medical Center IRB will be made. If an unexpected frequency of Grade III or IV events occur, depending on their nature, action appropriate to the nature and frequency of these adverse events will be taken. This may require a protocol amendment, dose de-escalation, or potentially closure of the study. The PI of this study will also continuously monitor the conduct, data, and safety of this study to ensure that:

Stopping rules for toxicity and/or response are met;

Risk/benefit ratio is not altered to the detriment of the subjects;

Appropriate internal monitoring of AEs and outcomes is done;

Over-accrual does not occur;

Under-accrual is addressed with appropriate amendments or actions;

Data are being appropriately collected in a reasonably timely manner.

DCI review and monitoring of this protocol occurs in accordance with the NCI-approved Data and Safety Monitoring Plan. Briefly, protocol review begins with an initial review by the Cancer Protocol Committee (CPC), which assesses the ethics and safety of the protocol. Documentation of these assessments will be maintained. Formal, independent monitoring will be conducted by the DCI Monitoring Team after the first 3 subjects are enrolled, followed by annual monitoring of 1-3 subjects until the study is closed to enrollment and subjects are no longer receiving study interventions that are more than minimal risk. DCI Monitoring Team reports and additional data/safety/toxicity reports submitted by the PI will be reviewed by the Safety Oversight Committee (SOC) on an annual basis. Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns. Monitoring visits may also be initiated upon request by DUHS and DCI Leadership, CPC, SOC, a sponsor, an investigator, or the IRB.

9.2 Audits:

The Duke School of Medicine Clinical Trials Quality Assurance (CTQA) office may conduct confidential audits to evaluate compliance with the protocol and the principles of GCP. The PI agrees to allow the CTQA auditor(s) direct access to all relevant documents and to allocate his/her

time and the time of the study team to the CTQA auditor(s) in order to discuss findings and any relevant issues.

CTQA audits are designed to protect the rights and well-being of human research subjects. CTQA audits may be routine or directed (for cause). Routine audits are selected based upon risk metrics generally geared towards high subject enrollment, studies with limited oversight or monitoring, Investigator initiated Investigational Drugs or Devices, federally-funded studies, high degree of risk (based upon adverse events, type of study, or vulnerable populations), Phase I studies, or studies that involve Medicare populations. Directed audits occur at the directive of the IRB or an authorized Institutional Official.

CTQA audits examine research studies/clinical trials methodology, processes and systems to assess whether the research is conducted according to the protocol approved by the DUHS IRB. The primary purpose of the audit/review is to verify that the standards for safety of human subjects in clinical trials and the quality of data produced by the clinical trial research are met. The audit/review will serve as a quality assurance measure, internal to the institution. Additional goals of such audits are to detect both random and systemic errors occurring during the conduct of clinical research and to emphasize “best practices” in the research/clinical trials environment.

10. ADMINISTRATIVE AND ETHICAL CONSIDERATIONS

10.1 Regulatory and Ethical Compliance:

This protocol was designed and will be conducted and reported in accordance with the International Conference on Harmonization (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice, the Declaration of Helsinki, and applicable federal, state, and local regulations.

10.2 DUHS Institutional Review Board and DCI Cancer Protocol Committee:

The protocol, informed consent form, advertising material, and additional protocol-related documents must be submitted to the DUHS Institutional Review Board (IRB) and DCI Cancer Protocol Committee (CPC) for review. The study may be initiated only after the Principal Investigator has received written and dated approval from the CPC and IRB.

The Principal Investigator must submit and obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent form. The CPC should be informed about any protocol amendments that potentially affect research design or data analysis (i.e. amendments affecting subject population, inclusion/exclusion criteria, agent administration, statistical analysis, etc.).

The Principal Investigator must obtain protocol re-approval from the IRB within 1 year of the most recent IRB approval. The Principal Investigator must also obtain protocol re-approval from the CPC within 1 year of the most recent IRB approval, for as long as the protocol remains open to subject enrollment.

10.3 Informed Consent:

The informed consent form must be written in a manner that is understandable to the subject population. Prior to its use, the informed consent form must be approved by the IRB.

The Principal Investigator or authorized key personnel will discuss with the potential subject the purpose of the research, methods, potential risks and benefits, subject concerns, and other study-related matters. This discussion will occur in a location that ensures subject privacy and in a manner that minimizes the possibility of coercion. Appropriate accommodations will be made available for potential subjects who cannot read or understand English or are visually impaired. Potential subjects will have the opportunity to contact the Principal investigator or authorized key personnel with questions, and will be given as much time as needed to make an informed decision about participation in the study.

Before conducting any study-specific procedures, the Principal Investigator or authorized key personnel must obtain written informed consent from the subject or a legally acceptable representative. The original informed consent form will be stored with the subject's study records, and a copy of the informed consent form will be provided to the subject. The Principal Investigator is responsible for asking the subject whether the subject wishes to notify his/her primary care physician about participation in the study. If the subject agrees to such notification, the Principal Investigator will inform the subject's primary care physician about the subject's participation in the clinical study.

10.4 Study Documentation:

Study documentation includes but is not limited to source documents, case report forms (CRFs), monitoring logs, appointment schedules, study team correspondence with sponsors or regulatory bodies/committees, and regulatory documents that can be found in the DCI-mandated "Regulatory Binder", which includes but is not limited to signed protocol and amendments, approved and signed informed consent forms, FDA Form 1572, CAP and CLIA laboratory certifications, and clinical supplies receipts and distribution records.

Source documents are original records that contain source data, which is all information in original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source documents include but are not limited to hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial. When possible, the original record should be retained as the source document. However, a photocopy is acceptable provided that it is a clear, legible, and an exact duplication of the original document.

Electronic case report forms (eCRF) in REDCap will be the primary data collection document for the study. The CRFs will be updated within two weeks of acquisition of new source data. Only approved study staff will be permitted to make entries, changes, or corrections in the CRF. For paper CRFs, errors will crossed out with a single line, and this line will not obscure the original entry. Changes or corrections will be dated, initialed, and explained (if necessary). The Principal Investigator or authorized key personnel will maintain a record of the changes and corrections. For electronic CRFs, an audit trail will be maintained by REDCap.

10.5 Privacy, Confidentiality, and Data Storage:

The Principal Investigator will ensure that subject privacy and confidentiality of the subject's data will be maintained. Research Data Security Plans (RDSPs) will be approved by the appropriate institutional Site Based Research group.

To protect privacy, every reasonable effort will be made to prevent undue access to subjects during the course of the study. Prospective participants will be consented in an exam room where it is just the research staff, the patient and her family, if desired. For all future visits, interactions with research staff (study doctor and study coordinators) regarding research activities will take place in a private exam room. All research related interactions with the participant will be conducted by qualified research staff who are directly involved in the conduct of the research study.

To protect confidentiality, subject files in paper format will be stored in secure cabinets under lock and key accessible only by the research staff. Subjects will be identified only by a unique study number and subject initials. Electronic records of subject data will be maintained using a secure online REDCap database. Access to the REDCap database will be limited to essential study personnel. Subject data may be stored temporarily on encrypted and password-protected portable memory devices such as flash drives and external hard drives, but only when absolutely necessary. Data stored on portable memory devices will be de-identified. Subject data will be deleted from the portable memory device at the earliest opportunity. The security and viability of the IT infrastructure will be managed by the DCI and/or Duke Medicine.

Upon completion of the study, research records will be archived and handled per DUHS HRPP policy.

Subject names or identifiers will not be used in reports, presentations at scientific meetings, or publications in scientific journals.

10.6 Data and Safety Monitoring:

Data and Safety Monitoring will be performed in accordance with the DCI Data and Safety Monitoring Plan. For a more detailed description of the DSMP for this protocol, refer to Section 10 (Sections 11.6 and 11.7 in particular) along with section 12.

10.7 Protocol Amendments:

All protocol amendments must be initiated by the Principal Investigator and approved by the IRB prior to implementation. IRB approval is not required for protocol changes that occur to protect the safety of a subject from an immediate hazard. However, the Principal Investigator must inform the IRB and all other applicable regulatory agencies of such action immediately.

10.8 Records Retention:

The Principal Investigator will maintain study-related records for the longer of a period of at least six years after study completion, per Duke policy.

REFERENCES

1. Morrow M, White J, Moughan J, et al: Factors predicting the use of breast-conserving therapy in stage I and II breast carcinoma. J Clin Oncol 19:2254-62, 2001

2. Nattinger AB, Hoffmann RG, Kneusel RT, et al: Relation between appropriateness of primary therapy for early-stage breast carcinoma and increased use of breast-conserving surgery. Lancet 356:1148-53, 2000

3. Nattinger AB, Kneusel RT, Hoffmann RG, et al: Relationship of distance from a radiotherapy facility and initial breast cancer treatment. J Natl Cancer Inst 93:1344-6, 2001

4. Foley KL, Kimmick G, Camacho F, et al: Survival disadvantage among Medicaid-insured breast cancer patients treated with breast conserving surgery without radiation therapy. Breast Cancer Res Treat 101:207-14, 2007

5. Darby S, McGale P, Correa C, et al: Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. Lancet 378:1707-16, 2011

6. Whelan TJ, Pignol JP, Levine MN, et al: Long-term results of hypofractionated radiation therapy for breast cancer. N Engl J Med 362:513-20, 2010

7. Haviland J, Agrawal R, Aird E, et al: The UK START (Standardisation of Breast Radiotherapy) Trials: 10-year follow-up results. Cancer Research 72, Supplement 3 (abs), 2012

8. 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 31:4038-45, 2013

9. Jagsi R, Ben-David MA, Moran JM, et al: Unacceptable cosmesis in a protocol investigating intensity-modulated radiotherapy with active breathing control for accelerated partial-breast irradiation. Int J Radiat Oncol Biol Phys 76:71-8, 2010

10. Hepel JT, Tokita M, Macausland SG, et al: Toxicity of Three-Dimensional Conformal Radiotherapy for Accelerated Partial Breast Irradiation. Int J Radiat Oncol Biol Phys, 2009

11. Horton JK, Blitzblau RC, Yoo S, et al: Preoperative Single-Fraction Partial Breast Radiation Therapy: A Novel Phase 1, Dose-Escalation Protocol With Radiation Response Biomarkers. Int J Radiat Oncol Biol Phys 92:846-55, 2015

12. Vaidya JS, Wenz F, Bulsara M, 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 randomised trial. Lancet 383:603-13, 2014

13. Veronesi U, Orecchia R, Maisonneuve P, et al: Intraoperative radiotherapy versus external radiotherapy for early breast cancer (ELIOT): a randomised controlled equivalence trial. Lancet Oncol 14:1269-77, 2013

14. Hughes KS, Schnaper LA, Berry D, et al: Lumpectomy plus tamoxifen with or without irradiation in women 70 years of age or older with early breast cancer. N Engl J Med 351:971-7, 2004

15. 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 74:987-1001, 2009

16. Correa C, Harris EE, Leonardi MC, et al: Accelerated Partial Breast Irradiation: Executive summary for the update of an ASTRO Evidence-Based Consensus Statement. Pract Radiat Oncol, 2016

17. Whelan T, MacKenzie R, Julian J, et al: Randomized trial of breast irradiation schedules after lumpectomy for women with lymph node-negative breast cancer. J Natl Cancer Inst 94:1143-50, 2002

18. Hall WH, Guiou M, Lee NY, et al: Development and validation of a standardized method for contouring the brachial plexus: preliminary dosimetric analysis among patients treated with IMRT for head-and-neck cancer. Int J Radiat Oncol Biol Phys 72:1362-7, 2008

19. Charaghvandi KR, den Hartogh MD, van Ommen AMLN, et al: The dosimetric feasibility of MRI-guided single fraction ablative radiotherapy for early-stage breast cancer: a brachytherapy versus VMAT planning study. Radiother Oncol In Press., 2015

20. Nichols E, Kesmodel SB, Bellavance E, et al: Preoperative Accelerated Partial Breast Irradiation for Early-Stage Breast Cancer: Preliminary Results of a Prospective, Phase 2 Trial. Int J Radiat Oncol Biol Phys 97:747-753, 2017

11. APPENDICES

APPENDIX I: Tissue Studies

Tissue collected pre and post treatment will be used to explore the biologic response to radiotherapy by comparing changes in gene expression pre and post-radiotherapy:

a. Assess changes in gene expression following radiation.
Formalin-fixed paraffin-embedded tissue will be utilized for harvesting of RNA. Gene expression will be assessed using the Affymetrix HTA arrays designed for FFPE samples. We have previously successfully used an FFPE Affymetrix platform on our phase I FFPE samples.

b. Ki-67 will be assessed in the pre and post radiotherapy tissue samples

Gene Expression
Optimally tissue will be obtained from outside sources and available as formalin-fixed, paraffin-embedded (FFPE) tissue from the diagnostic biopsy prior to enrollment on the trial. FFPE was chosen as a tool based on prior experience in our phase I preoperative clinical trial. In that trial, tumors were very small and our attempts to obtain fresh-frozen core biopsies at the time of lumpectomy resulted in a low tumor yield. An approach utilizing fresh-frozen tissue also requires subjects to undergo a second core biopsy prior to radiation treatment which would likely decrease accrual. Furthermore, since we were able to successfully utilize a FFPE based gene expression platform with our previous samples, we are confident that this approach will yield meaningful data.

Subjects will receive preoperative treatment and proceed to surgical resection, where a second tumor sample will be obtained. The Biospecimen Repository and Processing Core (BRPC), under the leadership of Dr. Shannon McCall, will assist with all aspects of tissue acquisition and processing (including storage of tissue obtained from outside sources as outlined above). Tumor tissue will be identified from the pre- and post-radiation samples and sectioned in preparation for RNA extraction using the RNeasy FFPE kit from Qiagen. The extracted RNA will be used with the Human Transcriptome Array 2.0 (HTA 2.0) and SensationPlus™ FFPE Amplification and WT Labeling Kit. The Sensation Plus Kit will enable us to prepare RNA samples for analysis using the HTA 2.0 arrays. The HTA 2.0 arrays are high-resolution arrays with >6.0 million probes that cover coding and non-coding transcripts. Quantitative PCR will be utilized to confirm our findings. Samples will be batched periodically for assessment of gene expression. Processing and analysis will proceed with the assistance of Dr. Holly Dressman, Director of the Microarray Facility. Results will then be directly transmitted to Dr. Owzar, Director of the Bioinformatics Shared Resource and lead statistician on this protocol. Dr. Owzar's team will query the clinical data directly from the REDCap database, ensuring proper chain of custody for this data.

Appendix II: NRG Cosmesis Scale

1. ____-____-_____DATE EVALUATION DONE

2. PLEASE ASSESS THE COSMETIC RESULTS OF BREAST CONSERVATION THERAPY AT THIS TIME.
(Circle the number next to the word that best describes the cosmetic results.)

1 EXCELLENT: when compared to the untreated breast or the original appearance of the treated 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 seroma within the breast but not enough to change the appearance.

2 GOOD: there is mild asymmetry between the breasts, which means that there is some acceptable difference in the size or shape of the treated breast as compared to the opposite breast or the appearance of the breast before treatment. There may be some mild reddening or darkening of the breast. The thickening or scar tissue within the breast causes a mild change in its shape or size.

3 FAIR: moderate deformity of the breast, with an obvious difference in the shape and size of the treated breast. This change involves 1/4 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 1/4 of the breast tissue. The skin change may be obvious and detract from the appearance. Severe scarring and thickening of the breast which clearly alters its appearance may be present. In retrospect, the breast may have been better treated by a mastectomy.

Please circle one (1) number for each of the following treatment effects.

	None	Yes, present but does not affect cosmesis	Yes, present and affects cosmesis
Skin telangiectasia	0	1	2
Skin atrophy	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 tx effects Specify: _____	0	1	2

APPENDIX III: Patient Self-Assessment

1. Please assess the cosmetic outcome of your treatment. (Circle the number next to the word that best describes the appearance of the breast.)

1 Excellent - when compared to the untreated breast, there is minimal or no difference in the size, shape or texture of the treated breast. There may be mild thickening or scar tissue within the breast or skin, but not enough to change the appearance.

2 Good - there is mild asymmetry in the size or shape of the treated breast as compared to the normal breast. The thickening or scar tissue within the breast causes only a mild change in the shape.

3 Fair - there is obvious difference in the size and shape of the treated breast. This change involves 1/4 or less of the breast.

4 Poor - marked change in the appearance of the treated breast involving more than ¼ of the breast tissue.

2. How satisfied with your treatment are you?

Extremely satisfied Very Satisfied Satisfied Unsatisfied

3. Would you choose this treatment again?

Yes No

12.

APPENDIX IV: Quality of Life

Below is a list of statements that other people with breast cancer have said are important to their quality of life. Please indicate the extent to which you have experienced each of the following statements during the past 7 days by circling the appropriate number using the following scale.

0	1	2	3	4
not at all	a little bit	somewhat	quite a bit	very much

During the past week:

PHYSICAL WELL-BEING

1. I have a lack of energy	0	1	2	3	4
2. I have nausea	0	1	2	3	4
3. Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
4. I have pain	0	1	2	3	4
5. I am bothered by **side effects of treatment**	0	1	2	3	4
6. I feel sick	0	1	2	3	4
7. I am forced to spend time in bed	0	1	2	3	4

SOCIAL/FAMILY WELL-BEING

8. I feel close to my friends	0	1	2	3	4
9. I get emotional support from my family	0	1	2	3	4
10. I get support from my friends	0	1	2	3	4
11. My family has accepted my illness	0	1	2	3	4
12. I am satisfied with family communication about my illness	0	1	2	3	4
13. I feel close to my partner (or the person who is my main support)	0	1	2	3	4
14. I am satisfied with my sex life	0	1	2	3	4

0	1	2	3	4
not at all	a little bit	somewhat	quite a bit	very much

EMOTIONAL WELL-BEING

15. I feel sad	0	1	2	3	4
16. I am proud of how I am coping with my illness	0	1	2	3	4
17. I am losing hope in the fight against my illness	0	1	2	3	4
18. I feel nervous	0	1	2	3	4
19. I worry about dying	0	1	2	3	4
20. I worry that my condition will get worse	0	1	2	3	4

FUNCTIONAL WELL-BEING

21. I am able to work (include work at home)	0	1	2	3	4
22. My work is fulfilling (include work at home)	0	1	2	3	4
23. I am able to enjoy life	0	1	2	3	4
24. I have accepted my illness	0	1	2	3	4
25. I am sleeping well	0	1	2	3	4
26. I am enjoying the things I usually do for fun	0	1	2	3	4
27. I am content with the quality of my life right now	0	1	2	3	4

ADDITIONAL CONCERNS

28. I have been short of breath	0	1	2	3	4
29. I am self-conscious about the way I dress	0	1	2	3	4
30. My arms are swollen or tender	0	1	2	3	4
31. I feel sexually attractive	0	1	2	3	4
32. I have been bothered by hair loss	0	1	2	3	4
33. I worry about the effect of stress on my illness	0	1	2	3	4
34. I am bothered by a change in weight	0	1	2	3	4

35. I am able to feel like a woman	0	1	2	3	4

APPENDIX V: Digital Photographs

The first photograph should be a close-up encompassing only the treated breast at a 45 degree oblique with arms elevated over the head. The second photograph should 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, taking care to exclude her face and framing or focusing on both the treated and untreated breast to allow optimal comparison of the breasts for symmetry.

Appendix VI: Blood Processing

Blood collected pre and post treatment will be used to explore the biologic response to radiotherapy by comparing changes in circulating cell-free DNA expression pre and post-radiotherapy:

cfDNA

2 EDTA tubes will be collected prior to radiation and following radiation. Following collection, plasma will be isolated with the following protocol.

1. Spin blood at 3200xg for 12 minutes at room temperature.
2. Pipette off plasma using a plastic pasteur pipette. Transfer into tube.
3. Spin NEW plasma tube at 2000xg for 10 minutes at 4°C.
4. Aliquot into 1ml aliquots in labelled cryovials.
5. Store at -80°C.

Plasma isolation will be performed in the Clinical Cancer Research Lab and stored as above for future biomarker analysis.

Urine samples will be collected before and after treatment. Samples will be stored at -80^{o}C until analyzed. Four isomers of F2-isoprostanes – iPF(2alpha)-III; iPF(2 alpha)-VI; 8,12-iso-iPF(2 alpha)-VI; and 2,3-dinor-iPF(2 alpha)-III – will be quantified by liquid chromatography-tandem mass spectrometry as funding permits. Allantoin will be quantified using ultra performance liquid chromatography-tandem mass spectrometry. Analysis will also include measuring the marker 8-hydroxydeoxyguanosine (8-OHdG) levels by HPLC