JHM IRB - eForm A – Protocol

- **Use the section headings to write the JHM IRB eForm A, inserting the appropriate material in each. If a section is not applicable, leave heading in and insert N/A.**
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1. Abstract

Over 240,000 cases of invasive breast cancer were diagnosed in the United States in 2015 [1]. The standard of care local treatment for early invasive breast cancer is lumpectomy with sentinel node biopsy. On average, 20-30% of women diagnosed with early breast cancer require re-excision for histologically positive margins [2], with some sources citing re-excision rates as high as 70% [3]. The cost and morbidity of surgical re-excision represents a substantial burden to patients and to our health care system [4]. Therefore, other strategies to optimize local treatment of early invasive breast cancer are needed.

The primary purpose of this study is to investigate the role of non-surgical percutaneous cryoablation in the local treatment of early invasive ductal carcinoma (IDC). Cryoablation is a controlled form of freezing whereby an ultrasound-guided probe is inserted into a tumor and rapidly cooled to induce tissue necrosis. Recent literature suggests that when cryoablation is performed at the site of primary malignancy, it has the capacity to reduce both local and systemic tumor burden. The immune-potentiating effects of cryoablation have already been demonstrated in a number of different malignancies, including breast cancer. An understanding of the mechanisms underlying the cryoablation-induced immune response is critical for effectively integrating this modality into patient management [5].

We propose a pilot study that establishes the safety, feasibility, and efficacy (measured by the immune response stimulated) of pre-surgical cryoablation as a neoadjuvant treatment modality. We hypothesize that the cryoablation induced immune response has the potential to guide the development of future cryoablation-based immunotherapies.

2. Objectives

- Primary objectives:
- 1. Establish the safety and feasibility of cryoablation of early invasive breast cancer prior to lumpectomy.
- 2. Explore the immunologic biomarkers of response to cryoablation in blood, breast tissues and axillary lymph nodes.

3. Background

a. Introduction to Cryoablation: Background, Safety, and Feasibility.

Cryoablation is a non-surgical, minimally invasive freezing procedure used to destroy tumors. Typically performed by radiologists, cryoablation is an accepted treatment modality for multiple malignancies, including prostate [6, 7], lung [8, 9] and renal cancers [10] and has been performed at the Johns Hopkins Interventional Radiology Center (IRC) for over 20 years. In 2010, the FDA approved the use of cryoablation for symptomatic fibroadenomas of the breast**.**

In the breast, cryoablation is performed under ultrasound guidance. Ultrasound is used for continuous assessment of iceball size, confirmation of containment of the target lesion within the iceball, and approximation of the distance from the dermis. Due to the analgesic properties of freezing, no IV sedation or anesthesia is required. After administration of local anesthetic utilizing a 25G needle, the physician makes a 2-3 mm dermotomy and inserts one or more needle-like cryoprobes into the targeted mass, commencing a number of freeze-thaw cycles. Most published and ongoing research protocols for cryoablation of breast malignancy utilize 2 consecutive freeze-thaw cycles, as this number of freeze thaw cycles has been demonstrated to result in 100% tumor cell killing. However, the precise protocol for each cycle (including duration of each freeze and thaw cycle, freeze rate, and desired minimum temperature) depends on institutional and device-specific guidelines. After completion of the final thaw, the probe is removed, pressure is applied to the area, and a bandage is placed. The patient leaves the office without stitches and minimal, if any, pain. Any residual pain after the procedure is routinely treated with over the counter (OTC) analgesics such as acetaminophen.

The safety of breast cryoablation for benign and malignant masses has been well established by multiple studies. Major complications are unusual, but can include skin damage or hematoma formation. Skin damage is typically avoided by maintaining approximately 5mm distance between the periphery of the ice ball and the dermis or by injecting lukewarm saline between the lesion and the dermis in order to maximize distance from the skin. The more common complications are similar to those of any ultrasound-guided breast biopsy, including minor bleeding, bruising, and mild post-procedural pain. In general, cryoablation is considered a low risk procedure with excellent cosmetic outcomes when performed by an experienced physician.

b. Review of Cryoablation Trials for Breast Malignancy.

Multiple human trials have been performed to evaluate cryoablation as a minimally invasive alternative to lumpectomy for local treatment of early invasive breast cancer [11-16]. The majority of these trials have been performed with an ablate and resect study design. With this design, cryoablation is followed by lumpectomy in order to evaluate cryoablative margins, i.e. the boundaries of the tissue frozen by the ice ball. When performed prior to lumpectomy, cryoablative margins are contained within the boundaries of the surgical margins. From these trials, it is well established that pre-surgical cryoablation does not prevent accurate assessment of lumpectomy margins [17, 18].

Early ablate and resect trials established the safety and feasibility of cryoablation for breast malignancies, but demonstrated mixed efficacy results. The most common reasons for failure of cryoablation to completely ablate a tumor were: (1) *large tumor size* exceeding the boundaries of the cryoablation zone, (2) *presence of satellite lesions including DCIS* not identified prior to surgery, and (3) *inadequate cryoablation zone* due to use of too few probes for the size of the lesion, inadequate ice ball size, and insufficiently low temperature [13, 19]. Later trials optimized the protocol by incorporating more appropriate tumor size selection criteria (ultrasound visible IDC ≥1.5 cm in diameter), creating a larger ice ball (≥ 5 mm around the ultrasound-visible margin of the tumor), and setting temperature goals of at least – 40 °C [11, 12, 15, 16, 20, 21]. These protocol modifications improved efficacy, with some trials reporting 100% tumor ablation for tumors up to 1.5 cm in size without significant associated DCIS

[11].

c. Review of Cryoablation as Immunotherapy.

Cryoablation has been demonstrated to affect both local and systemic tumor control: the former is achieved directly by temperature-induced necrosis, while the latter may be achieved by engagement of immunologic memory [5, 22]. Induction of systemic immunity has primarily been explored in murine models. In these studies, tumor specific T cells were found in higher proportions within the excised sentinel node when cryoablation was performed prior to surgery. Murine models have also demonstrated relatively increased levels of paracortical and germinal center hyperplasia in tumor draining nodes of mice treated with cryoablation prior to surgery compared with those treated with surgery alone [23], supplying additional evidence for a cryoablation-induced immune response. Other studies have demonstrated an increased number of CD3+ cells (T-cells) and an increased proportion of CD8+ (cytotoxic) over CD4+ (helper) T cells in mice undergoing cryoablation compared with surgery alone [22, 24, 25]. The immune potentiating effects of cryoablation also have been demonstrated in a number of different human malignancies, but biomarker data for human breast cancer remains limited [5].

Numerous tumor immune markers correlate well with breast cancer prognosis and treatment response [26, 27]. The relative proportion of CD4+ helper T cells, CD8+cytotoxic T cells, and CD20+ B cells within a cryoablated tumor has a prognostic relevance for chemotherapeutic response in multiple cancers [28-31]. CTLA-4 and PD-1 cell surface receptors on host T cells bind ligands such as B7-1/B7-2 and PD- L1/PD-L2, attenuating unchecked immune responses. Cancer cells overexpress these attenuator proteins as a means of evading the host immune response [32]. Moreover, expression of these attenuators has been associated with higher tumoral grade in breast cancer patients [33]. Ki-67 is a nuclear protein associated with cellular proliferation and confers both increased risk of recurrence and decreased survival rate in patients with early stage breast cancer [34, 35]. Cell populations such as CD4+ CD25+ regulatory T cells, as marked by FoxP3 transcription factor, are an immunosuppressive cell population which hinder the host anti-tumoral immune response [31]. Previous studies have linked a decrease in tumor-infiltrating FoxP3+ regulatory T cells with a more favorable disease prognosis, including complete pathologic response to neoadjuvant chemotherapy [31, 36, 37]. To our knowledge, no studies have examined this specific battery of parameters in human subjects after breast cancer cryoablation.

d. Significance and Innovation of Our Study Design

Many previous studies have presented cryoablation of breast cancer as an alternative to surgery with the primary endpoint of evaluating cryoablation margins. Ours will be the first study to present cryoablation as a form of neoadjuvant therapy to be performed concomitantly with lumpectomy with the primary endpoints of evaluating *immune response.* We believe that evaluating cryoablation as neoadjuvant (rather than primary) therapy best exploits the potential benefits of cryoablation while minimizing risk to patients, as all patients ultimately will be receiving standard surgical care.

We hypothesize that cryoablation is attributable to release of tumor antigens that prime the immune response. To date, no studies have attempted directly to correlate immune parameters with cryoablation of breast cancer in humans. By engaging the host's native anti- tumor immunity, cryoablation serves as a highly personalized form of neoadjuvant immunotherapy that can be integrated with the standard of care to improve the success of lumpectomy and decrease rates of recurrence. We believe that the data obtained in this pilot study will position us to understand how to harness and modulate this immune response in future trials, with the ultimate goal of creating an in situ breast cancer vaccine that could decrease local and distant cancer recurrence.

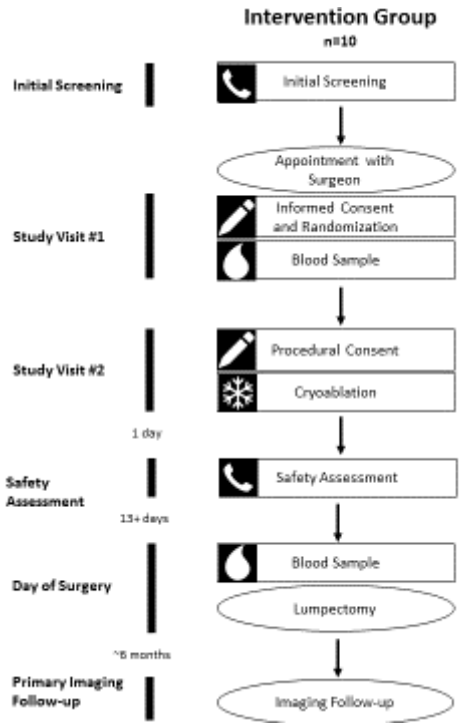
Study Procedures

a. Study Design (Fig. 1)

Recruitment, Informed Consent, and Randomization (all participants):

- This pilot study will enroll 22 patients with newly diagnosed, early stage breast cancer. The study population will be recruited from Sibley Memorial Hospital Sullivan Breast Center.
- All patients will be informed of their breast cancer diagnosis by breast imagers at Sibley and referred for surgical consultation at the time of receiving their diagnosis, according to standard management. Delivery of the final pathology diagnosis obtained after core needle biopsy typically occurs via telephone discussion with the breast imager but may also be performed in the office if the patient is present. The tissue obtained from biopsy will be available for study on all the patients enrolled.
- If the patient is referred to the Sibley Memorial Hospital Sullivan Breast Center after a diagnosis of breast cancer from an outside institution the images will be reviewed by the breast imaging team at Sibley and by pathology at Sibley as well to confirm inclusion or exclusion criteria.
- At the time of biopsy, patients will be asked if they would like to be contacted regarding any clinical trials they may be eligible for. Those who agree to be contacted will have their contact information collected and forwarded to a member of the study team.
- A member of the research team will contact interested patients utilizing a telephone script (see uploaded "Initial Telephone Screen" document) to a) determine their eligibility according to inclusion and exclusion criteria and b) explain the basic premise and obligations of the study.
- Once eligibility is established by telephone screening and review of the clinical record, the study team member will send a copy of the informed consent by mail, fax or email in order for the patient to review prior to their surgical consultation. Patients will schedule their routine surgical consultation according to standard practice. After their surgical consultation, interested patients will be formally consented by a member of the research team. In addition, the medical oncology and radiation oncology teams at Sibley will assess the patient to ensure that treatment planning will not be impacted by patients participating in the pilot trial.
- After consent, participants will be randomized into two groups by following the randomization table generated by a statistician.
- Physician/mid-level provider consent will be obtained either on the same day as initial consent or on the day the participant is scheduled for cryoablation but before the procedure is performed. A qualified physician or mid-level provider will discuss the alternatives, ensure participant understanding, and answer any questions the participant has. Physician/mid-level provider consent will not be required for participants on the control arm, as these participants will follow standard of care treatment.

Intervention Group:



Study Visit #1:

- Approximate time: 30-45 minutes
- Location: Sibley Memorial Hospital Sullivan Breast Center
- Immediately after informed consent is obtained, a baseline peripheral blood sample will be obtained, labeled, and sent to the Jaffee lab. Participants will be assisted in scheduling an appointment for cryoablation at least two weeks prior to surgery. Biopsy tissue samples from the standard of care biopsy the patient has already undergone will be requested and sent to the Jaffee Lab for study-related immunohistochemistry.

Study Visit #2:

- Approximate time: 4 hours
- Location: Sibley Memorial Hospital, Suburban Hospital, or Johns Hopkins Hospital Interventional Radiology Center (IRC)
- Staff involved in the cryoablation procedure will obtain procedural consent in the IRC pre-operative area, according to the department's usual protocol.
- Cryoablation procedure: This will take place in an IR suite with the breast imaging and IR teams working together so that expertise in breast imaging and cryoablation are combined. After the lesion is identified by ultrasound, the patient will be prepped and draped in sterile fashion. The physician will begin by administering 10-30 cc of 1% lidocaine for local anesthesia. An

approximately 2-3 mm dermotomy will be made, through which the cryoprobe will be inserted. Depending on the size of the lesion, more than one probe may be necessary to achieve the targeted iceball margin of 5mm surrounding the tumor in all dimensions. Ultrasound guidance will be used to confirm satisfactory position of the probe within the mass, and two freeze-thaw cycles will commence (see Table 1). Ultrasound will be utilized for real time assessment of iceball margins, appropriate containment of the tumor within the ice ball, and distance from the skin. After the final thaw cycle, the probe will be removed and pressure will be applied to the area to minimize bleeding or hematoma formation. A bandage will be placed over the skin entry site. The procedural time, including preparation and bandage placement is estimated to require about 60 minutes.

We will be ulitizing an argon based cryoablation system (either Galil or Cryocare), both of which are FDA approved for multiple indications including ablation of malignant tissue (see uploaded "FDA 510K – Approval"). These systems are commonly used in our interventional radiology department and were selected due to the experience of our attending physicians with this equipment and for its ability to achieve the necessary temperatures and target ice ball size. According to device specifications and industry-sponsored research protocols for treatment of breast malignancy (see uploaded "Vendor Research Protocol" document), two rapid rate freeze-thaw cycles (9:00/8:00/9:00) will be performed on the primary tumor to form an ice ball extending at least 5 mm beyond the ultrasound-visible margin to a minimum temperature of – 50-60 °C (5,9). These parameters have been demonstrated to result in 100% tumor cell death for tumors <2.0 cm in size without associated DCIS [11].

Cryoablation Parameters
2 freeze-thaw (f/t) cycles
9:00/8:00/9:00 (f/t/f minutes)
Minimum temperature: 50-60 °C
5 mm ice ball rim around lesion

Table 1.

- The participant will be escorted to the post-procedure area, observed to monitor for any post-procedural complications, and subsequently discharged according to standard protocol.

Telephone Safety Screen:

- Approximate time: 15 minutes
- The participant will receive a phone call from a member of the study team 1 day after their procedure. At that time, a safety checklist will be performed in order to assess for delayed procedure-related complications (see uploaded "Safety Checklist" document). Treatment-related adverse events will be graded using the Common Terminology Criteria for Adverse Events, V4.03. Any adverse events will be promptly reported both to the PI and to the IRB. The PI will be responsible for referring the participant for any required treatment or medical consultation.

Day of Surgery:

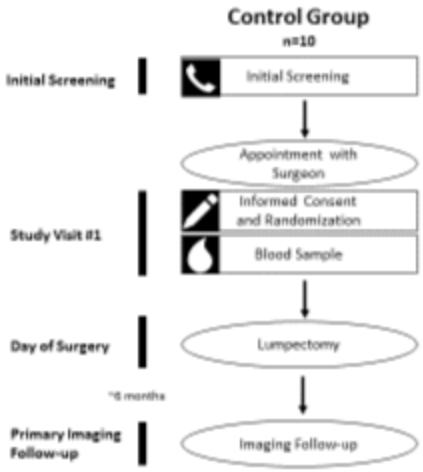
- Approximate time: 10 minutes
- Location: Sibley Memorial Hospital
- Prior to lumpectomy, a study blood specimen will be drawn in the pre-operative area from a routinely placed pre-operative IV. Routine lumpectomy and sentinel node biopsy will take place. Lumpectomy and sentinel node samples will be sent to the Sibley pathology lab for routine margin

analysis as well as study-related immunohistochemistry (see "Data Collection and Analysis" below). Transport will be arranged to the Jaffee lab and further analysis for immune response will be performed.

Primary Imaging Follow-Up:

- Approximate time: No additional time.
- Participants will undergo routine post-lumpectomy imaging follow-up at approximately 6 months after surgery. The results from this appointment be retrospectively reviewed at the time of data collection and analysis (see below). Participating in the study will have no effect on the scheduling or the type of imaging performed at this visit.

Control Group



Study Visit #1:

- Approximate time: 30-45 minutes
- Location: Sibley Memorial Hospital Sullivan Breast Center
- Immediately after informed consent is obtained, a baseline peripheral blood sample will be obtained, labeled, and sent to the Jaffee lab.

Day of Surgery:

- Approximate time: No additional time.
- Location: Sibley Memorial Hospital Participants in the control group will undergo standard lumpectomy and sentinel node biopsy as scheduled. Lumpectomy and sentinal node samples will be sent to the Sibley pathology lab for routine margin analysis as well as study-related immunohistochemistry in the Jaffee lab. (see "Data Collection and Analysis" below).

Primary Imaging Follow-Up:

- Approximate time: No additional time.
- Participants will undergo routine post-lumpectomy imaging follow- up which is usually scheduled at approximately 6 months after surgery. The results from this appointment will be retrospectively

reviewed. Participation in the study will have no effect on the scheduling or type of imaging performed at this visit.

Data Collection and Analysis

- **Immunohistochemistry:** Tissue samples collected at the time of surgery will undergo the standard evaluation for margin status, after which the pathology lab and the Jaffee lab will perform immunohistochemistry (IHC) analyses on tissue samples obtained from three different sites:
    - On the excised tumors, we will stain for Ki-67, CD4, CD8, FoxP3, and CD20 in order to determine the relative quantities of each cell type, as well as the CD8/FoxP3 ratio. We will also stain resected tumor tissue for PD-L1. For pre- and post-treatment comparison, these analyses will also be performed on tissue obtained during the participant's prior standard of care biopsy.
    - On the sentinel node(s), we will stain for Ki-67, CD4, CD8, FoxP3, CD20. We will also evaluate for a panel of immune checkpoints (including CTLA-4, PD-1, and PD-L1) using IHC. Sentinel nodes will also be evaluated for differences in germinal center and paracortical hyperplasia as reflective of T- and B-cell activity.
    - On peripheral blood samples obtained both at baseline (before cryoablation) and at surgery (after cryoablation), we will evaluate circulating lymphocytes for the above biomarkers. The above analyses will be performed on both the treatment and control arms of the study, with the latter providing a critical parallel control. With the exception of the peripheral blood sample, which can be obtained by a simple blood draw, all of these analyses can be performed without any additional risk to the participant, as the necessary tissue samples will be obtained at the time of standard surgery or biopsy.

- **Retrospective review of participant's medical record to determine:**
    - Participant age and personal or family history of malignancy
    - Size of lesion at detection as determined by routine diagnostic ultrasound and mammography
    - Receptor status of the lesion as determined by routine initial core biopsy
    - Distance from the skin and/or nipple areola complex on saved diagnostic ultrasound images
    - Diagnostic pre-procedural imaging
    - Date of detection, cryoablation, and surgery
    - Weight and volume of resected tissue
    - Results from sentinel node assessment
    - Operative note to evaluate for any deviations from the standard surgical procedure due to cryoablation
    - Surgical pathology note to evaluate for any deviations from the standard margin assessment due to cryoablation
    - Surgical margin status
    - 6-month diagnostic imaging follow up
    - Clinical notes pertaining to breast-related care during the time of the study

- **Surveys:**
    - Safety checklist: brief telephone questionnaire administered to the intervention group the

day after cryoablation to assess for any possible side effects or complications

- Delay in surgery survey: participants who delay their originally scheduled date of surgery will be contacted by telephone to inquire about the reason for delay (see uploaded "Delay in Surgery" document).

b. Study duration and number of study visits required of research participants.

Completion of the 6 month post-operative imaging follow-up will mark the end of study participation for both groups. A summary of the total number of encounters required of each participant is summarized in the table below (see Table 2).

Visit or Screen	Intervention Group	Control Group
Initial Screening Telephone	X	X
Study Visit #1: Informed Consent + Blood Sample	X	X
Study Visit #2: Cryoablation	X	
Safety Assessment Telephone	X	
Lumpectomy +/- Pre-operative Blood Sample	X	X
Post-Op Imaging Follow-Up	X	X

Table 2.

c. Blinding (including justification for blinding).

This is an unblinded study.

d. Justification of why participants will not receive routine care.

All participants will receive routine standard of care.

e. Justification of inclusion of a placebo or non-treatment group.

The control arm serves as a comparison group for the effects of cryoablation on surgical margin status, as well as the cryoablation-induced immune response. The presence of a control arm is critical given the lack of standard values for comparison for our proposed biomarker analysis, which is exploratory in nature.

f. Definition of treatment failure or participant removal criteria.

1. Inability to complete cryoablation according to the specified technical parameters
2. Inability to complete lumpectomy or sentinel node sampling
3. Voluntary desire for removal from the study by the participant
4. Significant delay (> 6 months) between initial diagnosis and treatment (cryoablation + lumpectomy) or lumpectomy alone. The six month interval is based on the established interval for follow up according to the BIRADS Classification System. In this system, a BIRADS category 3 lesion merits 6 month follow up. Implicit in this is an acknowledgement that after 6 months a previously diagnosed breast cancer may have changed and requires re-assessment.
5. Participant becoming pregnant prior to cryoablation procedure (this is self-reported as part of the procedural consent for cryoablation).

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

We will stop following the patients for research purposes after primary post-surgical imaging is obtained, usually around 6 months after lumpectomy. After that, patients will continue to undergo routine clinical and imaging follow up. Patients who prematurely leave the study will continue to receive standard of care imaging and clinical follow up.

5. Inclusion and Exclusion Criteria.

Our *inclusion* criteria include:

- Ultrasound visible IDC diagnosed by core needle biopsy
- Lesion visible by ultrasound at time of treatment
- Unifocal primary disease
- Tumor $\geq$ 0.5 cm in size
- Tumor size $\leq$ 2.0 cm in greatest diameter (as measured on ultrasound)
- No neoadjuvant chemotherapy
- Depth $\geq$ 0.5 cm from the skin or nipple-areola complex
- Female $\geq$ 18 years of age
- Non-pregnant patients

Our *exclusion* criteria include:

- Multifocal or metastatic disease
- Tumor ≥ 2.0 cm or ≤ 0.5 cm in size
- Planned neoadjuvant chemotherapy or radiation
- Extensive DCIS (>25% DCIS component) either diagnosed on core biopsy or strongly suggested by imaging
- Proximity of < 0.5 cm to the skin or nipple-areola complex.
- Known allergy to both lidocaine and benzocaine
- Females < 18 years of age
- Pregnant patients

We have devised these criteria in order to a) minimize the confounding effects of neoadjuvant chemotherapy b) maximize the technical success of cryoablation, which more thoroughly freezes its target at sizes ≤2.0 cm (5), c) minimize the likelihood of skin-related complications ranging from minor burns to necrosis, d) reduce any additional foreseeable risk to the participants and e) ensure adequate tissue is present from the core biopsy by requiring lesions to be ≥ 0.5 cm.

6. Drugs/ Substances/ Devices

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7. Study Statistics

a. Primary Outcome Variables

1). Safety and feasibility of pre-surgical cryoablation in patients with early stage breast cancer will be evaluated as per the National cancer Institute Common Terminology Criteria for Adverse Events, V4.03 as well as assessment for delays in surgical treatment. If no more than 2 of the first 5 patients in the intervention group experience any significant adverse events or delays in surgery due to any of the uncommon risks associated with cryoablation (see the Risks section of this document), the procedure will be considered safe and feasible and the final 5 patients will be recruited. These uncommon risks include skin necrosis or thermal damage, wound infection, significant bleeding, allergic reaction, and collapsed lung. As part of the feasibility assessment, we will be taking into account the volumes of tissue removed at lumpectomy in both groups in order to assess for any potentially confounding Hawthorne effect (given that the study is unblinded). When less tissue is removed, the cosmetic outcome is frequently improved. The surgeons will subjectively assess postoperative cosmetic results. The surgeon and pathologist will also be asked to report any deviations from the standard protocol, which may potentially result in a biased result. Exploratory immunologic analysis will be performed on peripheral blood, lumpectomy, and sentinel lymph node samples obtained from all participants, with the control group serving as a parallel control.

2). The response of immunologic markers in blood, breast tissues and axillary lymph nodes. For breast tissues, biomarkers will be quantified using immunohistochemical staining. For blood we will be using polychromatic flow cytometry to quantify CD8 and other biomarkers such as CD4. The details of these protocols can be found in Lutz et. al. Cancer Immunol Res. 2014; 2(7):616-31. Epub 2014/06/20.

b. Secondary Outcome Variables (none)

c. Statistical plan including sample size justification and interim data analysis

Sample size justification

Tissue and blood samples can be obtained both at baseline (diagnosis) and at surgery (lumpectomy). Node samples will only be available at the time of lumpectomy. So we will investigate the change of immunological response for tissue and blood samples, and compare post-operation immunological response of node samples.

The percentage change of immunological response is assumed to be 10% for the control arm and 50% for the treatment arm. A sample of 10 patients per group can achieve 80% power to detect a mean difference of 0.4 (0.5 vs. 0.1) with a standard deviation of 0.3, using a two-sided two-sample equal variance t test(alpha=0.05). Assuming 10% drop out rate, a total of 22 patients will be enrolled. Since this is an exploratory study, multi-comparison is not considered.

Analysis plan:

Baseline characteristics, clinical and safety parameters will be summarized with descriptive statistics. Immunological response will be summarized with descriptive statistics by arm as well. The change of immunological response of biomarkers or post-surgery immunological response will be compared between the two groups with two-sided t test.

d. Early stopping rules:

Systemic issues which may lead to early stopping of the trial include:

- A larger than expected number of serious complications from the study intervention, such as skin burn or significant bleeding
- Non diagnostic margin data following surgical resection affecting patient treatment planning

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

Cryoablation:

a. Common (> 5%):
    i. Pain with injection of local anesthetic
    ii. Pain during local anesthesia or during procedure
    iii. Mild bleeding
    iv. Swelling of the breast
    v. Bruising of the breast
b. Uncommon (< 5%):
    i. Skin necrosis or thermal damage
    ii. Wound infection
    iii. Significant bleeding
    iv. Allergic reaction to cleaning solution, local anesthetic, or wound dressing
    v. Pneumothorax (collapsed lung; very rare)

Lumpectomy:

a. Bleeding
b. Infection
c. Pain
d. Temporary swelling
e. Tenderness
f. Formation of hard scar tissue at surgical site
g. Loss of sensation in the breast
h. Change in shape and appearance of breast (particularly if a large portion is removed)
i. Fluid accumulation requiring drainage

Venipuncture:

a.. Hematoma (bruising)
b. Swelling, tenderness, and inflammation at the site
c. Persistent bleeding
d. Vasovagal response (dizziness, sweating, coldness of skin, numbness and tingling of hands and feet, nausea, vomiting, possible visual disturbance, syncope, and injury from falling or fainting)
e. Thrombosis of the vein (blood clot; rare)
f. Infection (rare)

b. Steps taken to minimize the risks.

We will be utilizing universal precautions and sterile technique for all invasive procedures. All procedures will be performed by experienced and trained personnel. We will be evaluating safety of cryoablation by recording treatment-related adverse events, which will be graded using the Common Terminology Criteria for Adverse Events, V4.03. Any adverse events will be reported to the IRB in a timely fashion.

All participant information and research data will be subject to HIPAA privacy rules and protocols as established by Johns Hopkins. Participants will be informed of the data safety precautions and will be advised that results may be published but that all data will remain anonymous. Each participant will be assigned a study ID based on order of enrollment. Any personal identifier information will be linked to this study ID in order to maintain confidentiality and facilitate anonymity of study related data and biospecimens. This will be stored in hard copy in a locked cabinet in a restricted access location. All electronic lab data will also be coded by study ID and will be stored in a password locked file on a LAN server. Access to the participants' study ID will be restricted to designated study team members. All study team members will complete HIPAA compliance training and will be briefed about the study-specific security measures to ensure data safety.

c. Plan for reporting unanticipated problems or study deviations.

We plan to keep a log for protocol deviations and adverse events. Any adverse events will be promptly reported to the IRB and promptly addressed by the principal investigator. All participants will receive contact information for the PI, as well as emergency contact information for the IRC as part of their initial informed consent and at the time of procedural consent for cryoablation.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

e. Financial risks to the participants.

There is no anticipated financial risk to the participants. Our itemized budget includes funding for cryoablation, immunohistochemistry and tumor marker analysis, venipuncture, and travel and parkingassociated with the cryoablation study visit. Lumpectomy, office visits, imaging, and standard pathologic assessment of the lumpectomy specimen (i.e. determination of margin status) are considered routine care and are not covered by the study. These will be billed to the patient or their medical insurance as per standard care.

9. Benefits: Description of the probable benefits for the participant and for society.

While the possibility of a reduced re-excision rate represents a theoretical benefit to participants, strictly speaking this outcome is experimental. Hence there is no direct benefit to participating in the study. However, patients will be advised that their participation may result in future benefit to breast cancer patients.

The anticipated benefit to society includes the possibility of reducing re-excision rates both domestically and globally via integration of neoadjuvant cryoablation into standard management of invasive cancer. If effective, neoadjuvant cryoablation has the potential to spare patients the compound morbidity of a second surgery (including the physical and emotional burden of re-excision) and importantly to decrease health care costs.

With respect to our exploratory aim of identifying the immune biomarkers of response to cryoablation, the identification of such biomarkers will give us a clearer understanding of how the body mobilizes an effective immune response against breast cancer. Cryoablation effectively converts a moderately immunogenic cancer into a powerfully immunogenic substrate through the release of tumor antigens that prime the immune response. As such, it provides a unique opportunity to explore the immune response to breast cancer with the aim of developing personalized immunotherapies. Moreover, through its release of immunogenic antigens cryoablation may effectively serve as a highly personalized in situ breast cancer vaccine to reduce both local and systemic cancer recurrence potentially by the elimination of circulating tumor cells. Our hope is that the preliminary data obtained from our study will guide future cryoablation immunotherapy trials.

10. Payment and Remuneration

Patients will not receive additional remuneration for participating in this study. For patients in the intervention group, travel (including gas and parking validation or a travel voucher) will be provided. There is no proposed penalty for not completing the study. Additional costs to participants that will be covered by the study are itemized in the Table 3.

11. Cost

Consumable Supplies / Equipment	**Per Patient**	**Number of patients**	**Sub-Total**
Procedural expenses			

Cryoablation including probes, gas and other consumable supplies	$3,018	11	$33,198
O.R. time	$0	11	$0
Professional fee	$0	11	$0
Laboratory expenses			
Pathologic assessment of margins	$0	22	$0
IHC for lumpectomy specimens	$360	22	$7,920
IHC for sentinel nodes	$450	22	$9,900
Pre- and post-surgical blood draws	$720	22	$15,840
Additional lab fees			$1000
Travel and Parking			
Patient travel reimbursement	$48	11	$528
Total			**$68,386**

Table 3.

Procedural expenses: Radiology intervential room time is estimated at 25 minutes per case. Although the typical fee is $44.26/min, this fee (as well as the professional fee) will be waived when performed in the IR department. There is no sedation or anesthesia cost as all procedures will be performed with local sedation only. Supplies such as bandages, gauze, IV needles, and syringes are all considered consumable supplies.

Laboratory expenses: Margin analysis is considered routine care so no additional costs will be incurred. All IHC will be performed at The Johns Hopkins Oncology Tissue Service Immunostaining Core Facility. Cost estimates are based on the fee listings provided on the facility website. Each of our proposed markers (Ki-67, CD4, CD8, FoxP3, CD20, CTLA-4, PD-1, PD-L1) have available antibodies listed and are charged at $45 per specimen. Additional laboratory fees will go toward set-up and consultation fees.

Travel fees: Participants will be reimbursed for their travel time and parking fees when undergoing cryoablation. This applies to the intervention group only.

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