

# Cryoablation for early-stage invasive breast cancer: pathologic and imaging outcomes from the prospective FIRST trial

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Vanessa Monteiro Sanvido, Silvio Eduardo Bromberg, Angela Flávia Logullo Waitzberg, Jackeline Oliveira Gomes, Leticia Galvão Barbante, Luis Ricardo Socolowski, Bruna Mayumi Takaki Tachibana, Antonio Rahal Junior, Tamiris Abait Miranda, Karina do Lago Negrelli, Eliana Vieira do Nascimento Martins, Renato Hideo Nakagawa Santos, Paula Martinez Vianna, Leonard Medeiros Silva, Alexandre Biasi Cavalcanti & Afonso Celso Pinto Nazário

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**Original Article****Title****Cryoablation for Early-Stage Invasive Breast Cancer: Pathologic and Imaging Outcomes from the Prospective FIRST Trial**

Authors: Vanessa Monteiro Sanvido<sup>1,2,3</sup>, Silvio Eduardo Bromberg<sup>3</sup>, Angela Flávia Logullo Waitzberg<sup>1</sup>, Jackeline Oliveira Gomes<sup>2</sup>, Leticia Galvão Barbante<sup>2</sup>, Luis Ricardo Socolowski<sup>1,3</sup>, Bruna Mayumi Takaki Tachibana<sup>1,3</sup>, Antonio Rahal Junior<sup>3</sup>, Tamiris Abait Miranda<sup>2</sup>; Karina do Lago Negrelli<sup>2</sup>; Eliana Vieira do Nascimento Martins<sup>2</sup>, Renato Hideo Nakagawa Santos<sup>2</sup>, Paula Martinez Vianna<sup>4</sup>, Leonard Medeiros da Silva<sup>5</sup>, Alexandre Biasi Cavalcanti<sup>2</sup>, Afonso Celso Pinto Nazário<sup>1,2</sup>.

**Author affiliations:**

<sup>1</sup>Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil

<sup>2</sup>Hcor Research Institute, São Paulo, SP, Brazil

<sup>3</sup>Hospital Israelita Albert Einstein, São Paulo, Brazil

<sup>4</sup>CICAP – Pathology Laboratory, São Paulo, Brazil

<sup>5</sup>Locus – OC Precision Medicine, São Paulo, SP, Brazil

**Corresponding author:** Vanessa Monteiro Sanvido

**E-mail:** vanessasanvido@gmail.com

**Institution:** Hcor Research Institute

**Address:** Desembargador Eliseu Guilherme, 200, 8th Floor, São Paulo, SP 04004-030, Brazil.

**Telephone number:** +55 11 5576-4848

**Orcid:** 0000-0003-3116-3550

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**ABSTRACT**

## Background

Cryoablation is an emerging minimally invasive ablative technique for selected patients with early-stage breast cancer. The FIRST (Freezing bReaST cancer in Brazil) trial was designed to evaluate the pathological efficacy and safety of cryoablation followed by surgery, to assess the accuracy of imaging modalities in predicting complete pathological response, and to explore technical predictors of successful tumor ablation.

## Methods

This prospective, multicenter, non-randomized, single-arm phase II study enrolled adults with unifocal invasive breast cancer  $\leq 2.5$  cm, clearly visible on ultrasound and eligible for upfront surgery. All patients underwent ultrasound-guided cryoablation using a 2.4-mm cryoprobe, followed by standard surgical resection 14–28 days later. Complete ablation was defined as the absence of residual invasive or in situ carcinoma on final pathology. Secondary outcomes included imaging–pathology correlation and the association between tumor size, ice-ball dimensions, and ablation success.

## Results

Among 48 evaluable patients, the invasive complete ablation rate was 97.9%, with 100% success in tumors  $\leq 2.0$  cm on MRI. The complete ablation rate was 89.6%, increasing to 96.9% for tumors  $\leq 2.0$  cm and 100% for tumors  $\leq 1.0$  cm. Only one patient (2.1%) had residual invasive disease (3 mm). Residual ductal carcinoma in situ was observed in 12.5% of patients, with a mean size of 2.2 mm. Cryoablation was well tolerated, with one minor skin burn (2.1%) and no serious adverse events. MRI demonstrated high predictive accuracy for complete response (negative predictive value, 93.0%), whereas ultrasound and mammography were less accurate (negative predictive values, 9.3% and 34.9%, respectively). Ice-ball

margins  $\geq 1$  cm beyond the tumor were associated with complete ablation in most cases.

## Conclusions

Cryoablation achieved high rates of complete tumor ablation with an excellent safety profile, particularly in tumors  $\leq 2.0$  cm, in carefully selected patients with early-stage invasive breast cancer. These findings provide robust pathological and imaging validation of cryoablation as an effective ablative approach; however, they do not support its use as a standalone definitive therapy or, even when combined with adjuvant treatments such as radiotherapy and endocrine therapy, as a standard of care at this stage. As a phase II study, these results are not practice-changing and warrant confirmation in randomized phase III trials to evaluate long-term oncologic outcomes and to define the potential role of cryoablation within surgical de-escalation strategies.

**Trial registration:** ClinicalTrials.gov Identifier: NCT05398497 (Registration date: May 26, 2022).

**Keywords:** Breast cancer; Cryoablation; Local therapy; Image-guided ablation; Minimally invasive treatment

## Highlights

- Cryoablation achieved a 97.9% invasive complete ablation rate in early-stage breast cancer, with 100% invasive complete ablation confirmed by pathology in tumors measuring  $\leq 2.0$  cm on MRI.
- MRI demonstrated high predictive accuracy for complete response after cryoablation (NPV 93.0%), outperforming ultrasound and mammography.
- Ice-ball margins  $\geq 1$  cm beyond the tumor correlated with high ablation success, reinforcing their role in procedural planning.

- Cryoablation was safe and well tolerated, with only one minor complication (2.1%) and no major adverse events.
- These findings support cryoablation as a promising minimally invasive approach warranting further evaluation within locoregional treatment de-escalation strategies in selected patients.

## INTRODUCTION

The locoregional management of breast cancer has evolved significantly in recent years, with a growing emphasis on less invasive approaches for both the breast and axilla [1]. Breast-conserving surgery combined with radiotherapy remains the standard treatment for early-stage breast cancer [2]. Sentinel lymph node biopsy is currently the preferred axillary staging method, even in some cases with limited metastases, and its omission is now considered feasible in selected patients—contributing to the de-escalation of locoregional therapy [3-6].

Within this context, image-guided ablative therapies such as cryoablation represent a potential milestone in the evolution of breast cancer treatment, offering less aggressive options for appropriately selected patients [7]. The omission of breast surgery in cases involving small, early-stage tumors appears both safe and feasible, potentially optimizing treatment, especially given the increasing incidence of breast cancer and the growing detection of small lesions through population-based screening programs [8,9].

Cryoablation is a non-surgical, minimally invasive technique that induces tissue necrosis through cycles of freezing and thawing. Necrosis occurs when tissue temperatures reach  $-20\text{ }^{\circ}\text{C}$  or lower for at least one minute [10]. The procedure involves the insertion of cryoprobes into the tumor under radiologic guidance. Extremely low temperatures, typically ranging from  $-40\text{ }^{\circ}\text{C}$  to  $-160\text{ }^{\circ}\text{C}$ , cause cell death through direct and

indirect mechanisms. Direct injury results from intracellular ice formation, leading to membrane rupture, and from extracellular ice formation, which creates a hyperosmolar environment that draws water out of cells. Upon thawing, rapid water re-entry leads to swelling and subsequent cell lysis. Indirect effects are mediated by vascular injury, as freezing induces vasoconstriction, ischemia, and anoxia, resulting in delayed cell death due to blood supply disruption. These cytotoxic temperatures are achieved through the expansion of argon gas (Joule-Thomson effect) or the circulation of liquid nitrogen in a closed system. In argon-based systems, thawing is actively induced using helium gas, which has opposite thermodynamic properties, whereas nitrogen-based systems rely on passive thawing by interrupting nitrogen flow and allowing gradual rewarming [10-12].

Several studies have demonstrated the feasibility of cryoablation for early-stage breast cancer. The ACOSOG Z1072 trial, a non-randomized phase II study by the American College of Surgeons Oncology Group, reported a 92% complete ablation rate after excluding multifocal disease—reaching 100% for tumors <1 cm. The authors concluded that refinement in technique and patient selection could further improve outcomes, reinforcing the need for additional clinical trials to validate cryoablation as an alternative to conventional surgery [13].

Building on these findings, the ICE3 trial evaluated cryoablation as definitive local therapy in low-risk breast cancer. With a mean follow-up of 54.2 months, the ipsilateral tumor recurrence rate was 4.3%. Among 194 patients, 153 received adjuvant therapy, in whom recurrence decreased to 2.6%. Of these, 63.9% received endocrine therapy alone; 1.5% underwent radiotherapy only; 12.9% received both; and 0.5% received endocrine therapy, chemotherapy, and radiotherapy. These results suggest that cryoablation may represent a promising alternative to lumpectomy in

carefully selected patients within a multimodal treatment framework, including appropriate adjuvant therapy, particularly among elderly patients with low-risk disease [14,15].

Despite encouraging data, studies on cryoablation in early-stage invasive breast cancer have shown variable success rates due to differences in study design and patient selection. These inconsistencies continue to raise questions regarding the feasibility of omitting surgery altogether. The present phase II clinical trial — FIRST (Freezing bReaST cancer in Brazil)—was designed as a prospective before-and-after study to evaluate cryoablation as a definitive alternative to surgery for early-stage invasive breast cancer. The trial aimed to determine ablation success rates, assess imaging accuracy in predicting pathological complete response, refine patient selection criteria, and establish the optimal relationship between ice-ball margins and complete tumor ablation.

## **METHODS**

### **Study Design**

This was a prospective, multicenter, non-randomized, single-arm, before-and-after clinical trial designed to evaluate the outcomes of cryoablation followed by surgical resection in patients with early-stage breast cancer. All eligible participants underwent percutaneous cryoablation of the index tumor, followed by standard surgical resection 14 to 28 days later. Breast imaging—including mammography, ultrasound, and magnetic resonance imaging (MRI)—was performed both before and after cryoablation (Figure 1). Patient recruitment occurred from February 2020 to December 2024.

The study was reported in accordance with the CONSORT 2010 Statement, with adaptations for non-randomized, single-arm phase II clinical trials.

### **Study Population**

Eligible participants were adults of any sex with unifocal, early-stage invasive breast carcinoma measuring  $\leq 2.5$  cm on imaging, clearly visible on ultrasound, and with surgical resection indicated as first-line treatment. Eligibility was primarily determined based on ultrasound findings (lesions  $\leq 2.5$  cm and clearly visible on ultrasound), which constituted the main imaging criterion for inclusion. The upper size limit of 2.5 cm was intentionally selected to explore the technical feasibility of cryoablation beyond the  $\leq 2.0$  cm threshold used in prior studies, within a controlled setting where all patients subsequently underwent surgical resection. This approach aimed to refine patient selection criteria for future trials. Magnetic resonance imaging (MRI) was performed when feasible but was not mandatory for study inclusion.

Exclusion criteria included: tumors  $> 2.5$  cm, skin involvement, pure ductal carcinoma in situ (DCIS), multifocal or multicentric disease, clinically positive axilla, lesion  $< 5$  mm from the skin, distant metastasis, prior neoadjuvant therapy, or inability to provide informed consent due to cognitive or language barriers. The presence of DCIS associated with invasive carcinoma on biopsy did not preclude study inclusion.

### **Ethical Considerations**

The study was approved by the central Institutional Review Board (IRB) of the Universidade Federal de São Paulo (UNIFESP; approval number: 3.270.850; approval date: April 17, 2019), with additional approvals from the HCor Research Institute and Hospital Israelita Albert

Einstein prior to enrollment. The trial was registered at ClinicalTrials.gov (Identifier: NCT05398497) under the title *FIRST (Freezing bReaST cancer in Brazil): a before-after study*. All participants provided written informed consent. The study adhered to national and international ethical guidelines.

## Outcomes

The primary outcome was the rate of complete tumor ablation, defined as the absence of residual invasive or in situ carcinoma on final histopathological examination. Invasive complete ablation, a predefined pathological endpoint, was defined as the absence of residual invasive carcinoma.

Secondary outcomes included:

- The negative predictive value (NPV) of MRI, mammography, and ultrasound in predicting complete pathological response.
- The correlation between ultrasound-measured ice-ball dimensions, baseline tumor size, and pathological response.

## Sample Size

Based on a hypothesized cryoablation success rate of 92% (from ACOSOG Z1072) [13] and a minimum acceptable efficacy threshold of 70%, a sample size of 32 patients was calculated to achieve 95% statistical power and a 5% significance level. This allowed for estimation of the success rate with an 85% confidence interval and a  $\pm 7\%$  margin of error. Cryoablation was considered potentially effective if the complete ablation rate exceeded 90%, and unsatisfactory if it was 70% or lower. If the observed success rate was 96%, the 85% confidence interval would have a narrower margin of  $\pm 5\%$ . Sample size calculations were performed using R software (version 4.1.1), with the `pwr.p.test` function from the *pwr* package and `prec_prop` from the *presize* package.

## **Cryoablation Procedure**

Cryoablation was performed in a sterile environment under local anesthesia by trained breast surgeons or interventional radiologists at Hospital Israelita Albert Einstein (HIAE) or Hospital do Coração (HCor). The procedure used a 2.4-mm cryoprobe connected to a gas-based system, with argon gas for freezing and helium for active thawing (CryoCare® SL, Endocare Inc., Austin, TX, USA), guided in real-time by ultrasound.

The cryoprobe (150 mm long, 13G) was inserted through a small incision and aligned longitudinally with the tumor. Each procedure consisted of two freeze-thaw cycles: freezing (6–10 minutes) and active thawing (4–10 minutes). Duration was adjusted based on ultrasound monitoring to ensure at least a 1-cm ice margin beyond all tumor borders.

Precautions were taken to avoid complications, such as lifting the probe off the chest wall and injecting subcutaneous saline to protect the skin in patients with small breast volumes. The procedure lasted 20–40 minutes. All patients were discharged the same day without routine analgesics or antibiotics and resumed normal activities the following day.

Since cryoablation is not yet standard treatment, it did not replace surgery. The procedure was scheduled to avoid delays in definitive surgical treatment. Adjuvant therapy was based on pre-cryoablation indications and determined by the treating physician, regardless of cryoablation outcomes.

## **Statistical Analysis**

Data analyzed included demographics, tumor characteristics, imaging findings, cryoablation parameters, and pathological results. Descriptive statistics summarized baseline and outcome variables. Categorical variables were reported as frequencies and percentages; continuous variables as

means  $\pm$  standard deviation or medians with interquartile ranges, as appropriate.

The primary outcome—complete ablation—was expressed as a proportion with 95% confidence intervals (CI). Group comparisons (e.g., complete vs. incomplete ablation) used Wilcoxon rank-sum tests for continuous variables and chi-square or Fisher's exact tests for categorical variables.

NPV was calculated for each imaging modality as the proportion of true negatives (imaging showing no residual disease and pathology confirming complete response) over all negative imaging results.

All analyses were performed using R software (version 4.5.1). Statistical significance was defined as  $p < 0.05$ .

## **RESULTS**

### **Patient and Tumor Characteristics**

A total of 61 patients were enrolled; 12 were excluded due to screening failures (MRI enhancement  $>2.5$  cm or multicentric disease), and 1 patient withdrew before cryoablation. Thus, 48 patients were included in the final analysis.

The mean age was  $62.0 \pm 12.5$  years (median 61.5; IQR 53.5–71.3), and 97.9% were female. Most tumors were non-palpable (83.3%) and classified as T1 (91.7%). The mean tumor size was  $1.25 \pm 0.45$  cm (median 1.15; IQR 1.00–1.60).

Invasive carcinoma of no special type predominated (87.5%), followed by invasive lobular carcinoma (8.3%) and mucinous carcinoma (4.2%). Most tumors were hormone receptor-positive (ER 95.8% and PR 93.8%), and

10.4% were HER2-positive, with the majority classified as luminal/HER2-negative (87.5%) (Table 1).

### **Pre-treatment Imaging Findings**

All tumors were detected by ultrasound and MRI, while mammography identified 83.3%. Mean lesion size was 1.25 cm on ultrasound, 1.27 cm on mammography, and slightly larger on MRI ( $1.57 \pm 0.59$  cm). Ultrasound measurements were, on average, smaller than those obtained by MRI (mean difference approximately 0.3 cm), reflecting differences between imaging modalities rather than true underestimation or overestimation when compared to pathological size (Table 2; Supplementary Figure 1).

### **Cryoablation Procedure and Technical Performance**

All procedures used a 2.4-mm argon-based cryoprobe under ultrasound guidance with two freeze-thaw cycles. The median maximum ice-ball diameter was 4.2 cm (IQR 3.9–5.2), approximately 3.6 times the median tumor size, ensuring adequate coverage in most cases (Table 3).

The procedure demonstrated high reproducibility and a favorable safety profile, with only one minor skin burn (2.1%) and no serious adverse events.

### **Ablation Success**

The overall complete ablation rate was 89.6% (43/48; 95% CI 76.6–96.1), and the invasive complete ablation rate was 97.9% (47/48; 95% CI 87.5–99.9).

For tumors  $\leq 2.0$  cm on MRI, the complete ablation rate was 96.9% (31/32; 95% CI 82.0–99.8) and the invasive complete ablation rate reached

100% (32/32; 95% CI 86.7–100.0). For lesions  $\leq 1.0$  cm, both rates were 100% (8/8; 95% CI: 59.8–100.0%). Results were consistent using ultrasound measurements (Table 4).

### **Pathological Outcomes**

Only one patient (2.1%) had residual invasive carcinoma (3 mm). Residual DCIS was observed in 12.5% (6/48), with a mean size of 2.2 mm. Pathological staging showed pT0 in 89.6%, pTis in 8.3%, and pT1a in 2.1%, with pN0 in 91.7% (Table 5).

Of the four cases with residual in situ disease, MRI could not be performed in two patients due to clinical contraindications to the examination, while in the remaining two patients, MRI demonstrated lesion enhancement  $> 2$  cm (Table 6). These patients were included based on ultrasound eligibility criteria (lesions  $\leq 2.5$  cm and clearly visible on ultrasound) and multidisciplinary clinical assessment at the time of study enrollment. This finding highlights the potential added value of MRI in refining patient selection.

None of the cases with incomplete invasive ablation corresponded to invasive lobular carcinoma.

### **Predictive Value of Imaging**

MRI demonstrated high accuracy in predicting complete response, with a negative predictive value (NPV) of 93.0% (95% CI 80.9–98.5). In contrast, ultrasound and mammography showed substantially lower accuracy (NPVs 9.3% and 34.9%, respectively).

These findings should be interpreted with caution, as all patients underwent surgical excision, precluding definitive conclusions regarding MRI as a standalone post-ablation assessment tool.

## Ice Ball

Median ice-ball diameter was similar between cases of complete and incomplete ablation (4.2 cm in both;  $p=0.906$ ). Larger tumors were observed in the incomplete group (median 1.7 vs. 1.1 cm on ultrasound;  $p=0.03$ ) (Table 7).

Most patients achieved margins between 2–4 cm (Figure 2). Notably, the only invasive ablation failure occurred in a case in which the intended  $\geq 1$  cm ice-ball margin beyond the tumor was not achieved, likely due to technical or anatomical constraints.

These results confirm the excellent short-term oncologic performance and safety of cryoablation for early-stage breast cancer, particularly for tumors  $\leq 2$  cm, and support further investigation in randomized clinical trials.

## DISCUSSION

This prospective study demonstrates that cryoablation is a technically effective and safe ablative technique in carefully selected patients with early-stage invasive breast cancer, achieving an invasive complete ablation rate of 97.9%, with 100% success in tumors  $\leq 2.0$  cm on MRI. These findings are consistent with previously published studies and further strengthen the evidence supporting cryoablation as a minimally invasive local ablative approach in selected patients [13–16]. Our findings should be interpreted within the context of multimodal treatment strategies, as in trials such as ICE3 and FROST, where adjuvant therapies—particularly radiotherapy and endocrine therapy—likely contributed to favorable outcomes.

The overall complete ablation rate, defined as the absence of both residual invasive and in situ carcinoma, was 89.6%, increasing to 96.9% for

tumors  $\leq 2.0$  cm and 100% for tumors  $\leq 1.0$  cm, based on MRI measurements.

The observed rates of complete ablation were highest in tumors measuring  $\leq 2.0$  cm, reinforcing tumor size as a key determinant of technical success. Notably, the only case of residual invasive carcinoma occurred in a lesion exceeding 2.0 cm on MRI, underscoring the importance of careful patient selection. Residual ductal carcinoma in situ (DCIS) was identified in a subset of patients, with a small mean lesion size; however, its presence highlights an important limitation of cryoablation when applied to invasive tumors with associated in situ components. Interestingly, there was no clear correlation between the presence of DCIS on pre-treatment biopsy and residual in situ disease after cryoablation, suggesting limited predictive value of biopsy findings for this outcome. While the clinical significance of small-volume residual DCIS following ablation remains uncertain, its potential implications for local recurrence cannot be fully excluded. These findings emphasize that imaging-based assessment, including MRI, may not reliably detect residual in situ disease and should be interpreted with caution. Consequently, the role of cryoablation in non-surgical treatment strategies must be evaluated within prospective trials incorporating long-term follow-up, predefined criteria for surveillance and rescue surgery, and careful monitoring of locoregional outcomes.

It is noteworthy that small residual foci such as those observed in this study might have been adequately managed with adjuvant radiotherapy, similar to the standard approach following breast-conserving surgery (BCS) [17,18]. Moreover, BCS itself is associated with a well-recognized risk of re-excision due to positive or close surgical margins, reported in approximately 20–25% of cases in some series [19,20]. In contrast, cryoablation allows real-time visualization of the ablation zone under ultrasound guidance, enabling intraprocedural assessment of treatment margins [21].

In addition, active surveillance strategies for carefully selected patients with low-risk ductal carcinoma in situ (DCIS) have shown encouraging early results. In trials such as COMET, LORIS, and LORD, patients randomized to active monitoring did not demonstrate higher rates of ipsilateral invasive breast cancer at two years compared with those managed according to guideline-concordant treatment [22–24]. Although follow-up remains limited, these findings suggest that small-volume residual in situ disease may not invariably translate into early invasive recurrence.

Taken together, these data support the continued investigation of cryoablation as a minimally invasive local ablative approach in highly selected patients with small, early-stage breast cancer, particularly when integrated with adjuvant therapies and structured follow-up. Importantly, these observations should be interpreted cautiously and do not establish equivalence or superiority over surgery, underscoring the need for randomized clinical trials with long-term oncologic outcomes.

These findings are consistent with those reported by Roca Navarro et al., who observed successful cryoablation in more than 97% of purely infiltrating tumors  $\leq 2$  cm. Their multivariate analysis identified associated DCIS and tumor size as predictors of incomplete ablation, with odds ratios of 7.43 for DCIS and 1.27 for tumor size, highlighting the importance of patient selection and tumor characteristics when considering ablative strategies [25].

Our results reinforce tumor size as a key determinant of cryoablation success. Smaller lesions were consistently associated with higher rates of complete ablation, in agreement with previous studies evaluating percutaneous ablative techniques for breast cancer [13–16,26–28]. These findings highlight the importance of careful patient selection, particularly with respect to tumor size, when considering cryoablation as a local ablative approach. In our cohort, the complete ablation rate was 96.9% for lesions

$\leq 2.0$  cm on MRI and 94.1% for lesions  $\leq 1.5$  cm on ultrasound, exceeding the predefined success threshold of  $>90\%$  established in the study design. These results underscore the complementary role of MRI and ultrasound in accurately assessing tumor size and informing appropriate case selection.

However, the present study was not designed or powered to evaluate outcomes according to molecular or histological subtype (including invasive lobular carcinoma and special subtypes), given their limited representation in the cohort.

From a technical perspective, cryoablation demonstrated high reproducibility, with a consistent relationship between ice-ball dimensions and tumor size. The median ice-ball diameter was approximately 3.6 times the median tumor diameter, a factor that appeared critical for achieving complete ablation. This observation is consistent with prior cryoablation studies, including ACOSOG Z1072 [13] and ICE3 [14,15], which identified adequate ice-ball margins as a key predictor of successful local tumor control. A visible ice margin extending at least 1 cm beyond all tumor borders has been widely recommended to ensure complete tumor coverage and minimize the risk of residual disease [21,29].

Post-cryoablation imaging performance was evaluated against histopathological findings from post-ablation surgical specimens. MRI demonstrated a high negative predictive value (93.0%) for complete pathological response, outperforming ultrasound (NPV 9.3%) and conventional mammography (NPV 34.9%). Contrast-enhanced mammography, an important functional imaging modality, was not systematically performed in our cohort and therefore could not be formally assessed. Accordingly, our conclusions regarding imaging performance are limited to the modalities evaluated in this study. The role of MRI in guiding non-surgical management remains investigational and should be validated in prospective trials with long-term follow-up [13].

Cryoablation was well tolerated, with only one minor skin freeze burn (2.1%), which resolved without clinical sequelae. No serious adverse events, including infection, bleeding, or delayed wound healing, were observed. This favorable safety profile is consistent with the low complication rates reported in previous cryoablation studies in breast cancer and other solid tumors [14,27].

Collectively, these findings contribute to the growing body of evidence supporting cryoablation as a technically effective and safe local ablative approach in carefully selected patients with early-stage breast cancer. Importantly, the cohort represents a highly selected population, predominantly composed of hormone receptor-positive, low-grade, small tumors, which limits the generalizability of these results. Caution is therefore warranted when extrapolating these findings to broader clinical practice, particularly to younger patients and those with biologically aggressive tumor subtypes. The potential advantages of cryoablation—including reduced procedural morbidity, favorable cosmetic outcomes, outpatient feasibility, and avoidance of general anesthesia—support its continued evaluation within structured clinical trials designed to assess long-term oncologic outcomes and its role in treatment de-escalation strategies [30-36].

### **Limitations and Findings Supported by Current Data**

This study has several limitations. The small number of patients with residual disease limited the power for subgroup analyses, particularly regarding predictors of incomplete ablation. In addition, all patients underwent surgical excision following cryoablation, which precluded assessment of long-term oncologic outcomes in patients treated with cryoablation alone.

A key strength of this study lies in its strict patient selection criteria, which resulted in high rates of invasive complete ablation. The systematic correlation between imaging findings and final pathology provides robust validation of cryoablation as a technically effective and safe ablative approach in carefully selected patients. In this context, MRI demonstrated high accuracy in identifying complete pathological response; however, its performance should be interpreted as hypothesis-generating and complementary to pathological assessment, rather than practice-changing.

### **Future Directions**

Given the favorable pathological outcomes observed—particularly in tumors  $\leq 2.0$  cm—and the excellent safety profile, further investigation in randomized controlled trials is warranted. Importantly, because all patients in the present study underwent early surgical excision, late post-ablation symptomatic or imaging sequelae could not be evaluated. This limitation underscores the need for structured long-term assessment in future studies.

Accordingly, our group has initiated a prospective randomized clinical trial comparing cryoablation with standard surgical treatment in patients with invasive breast carcinoma  $\leq 2.0$  cm. In this trial, all patients will receive guideline-concordant adjuvant therapy, including radiotherapy and endocrine therapy when indicated. Axillary management will follow standard clinical practice; in selected patients with clinically and sonographically negative axilla, omission of sentinel lymph node biopsy will be considered, in line with the principles of the SOUND trial [5]. The study incorporates predefined long-term imaging surveillance, systematic symptom assessment, and clear criteria for biopsy or surgical rescue to address concerns related to delayed post-ablation findings. The trial is registered at ClinicalTrials.gov (NCT06839001) under the acronym CRYSTAL-SIX (CRYoablation for Small Tumors As Local treatment - SIX Trial) and is designed to evaluate long-term oncologic safety, locoregional

control, and the potential role of cryoablation within surgical de-escalation strategies.

## Conclusion

These results demonstrate that cryoablation is a technically effective and minimally invasive local ablative approach in carefully selected patients with small, early-stage breast cancer. Within the context of ongoing efforts to tailor locoregional treatment, cryoablation represents a promising strategy warranting further evaluation as part of surgical de-escalation paradigms. However, confirmation of long-term oncologic safety and clinical equivalence to surgery will require robust evidence from randomized controlled trials comparing cryoablation with standard breast-conserving surgery.

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## LIST OF ABBREVIATIONS

ACOSOG: American College of Surgeons Oncology Group

BCS: Breast-conserving surgery

CI: Confidence interval

COMET: Comparison of Operative to Monitoring and Endocrine Therapy

CONSORT: Consolidated Standards of Reporting Trials

CRYSTAL-SIX: CRYoablation for Small Tumors As Local treatment - SIX

DCIS: Ductal carcinoma in situ

ER: Estrogen receptor

FIRST: Freezing bReaST cancer in Brazil

HER2: Human epidermal growth factor receptor 2

Hcor: Hospital do coração

HIAE: Hospital Israelita Albert Einstein

IRB: Institutional review board

IHC: ImmunoHistoChemistry

IQR: Interquartile range

LORD: Low Risk DCIS

LORIS: Low Risk DCIS

MMG: Mammography

MRI: Magnetic resonance imaging

NPV: Negative predictive value

NST: No special type (invasive carcinoma, NST)

OR: Odds ratio

PR: Progesterone receptor

RCT: Randomized controlled trial

SD: Standard deviation

UNIFESP: Universidade Federal de São Paulo

US: Ultrasound

## **DECLARATIONS**

### **Ethics approval and consent to participate**

This study was approved by the Institutional Review Board (IRB) of the Universidade Federal de São Paulo (UNIFESP; approval number: 3.270.850;

approval date: April 17, 2019), with additional approvals from the Hcor Research Institute and Hospital Israelita Albert Einstein prior to enrollment. All participants provided written informed consent before inclusion in the trial. The study adhered to national and international ethical standards, including the Declaration of Helsinki and Good Clinical Practice guidelines.

### **Consent for publication**

Not applicable. No identifiable patient information is included in this manuscript.

### **Availability of data and materials**

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. Due to privacy and ethical restrictions, individual patient data are not publicly available.

### **Competing interests**

The authors declare that they have no competing interests.

### **Funding**

This study did not receive external funding for research execution. Institutional support for procedures was provided by Hcor Research Institute and Hospital Israelita Albert Einstein. The sponsors had no role in study design, data collection, analysis, interpretation, or manuscript writing.

### **Authors' contributions**

VMS, SEB, and ACPN conceived and designed the study.

VMS, JOG, and RHNS performed the statistical analysis.

VMS, SEB, LRS, BMTT, ARJ, and ACPN contributed to patient procedures and/or data acquisition.

VMS, JOG, LGB, TAM, KLN, EVNM, RHNS, ABC, and ACPN contributed to site coordination and project conduction.

AFLW, PV, and LMS performed the pathological evaluation and contributed to pathology-related manuscript content.

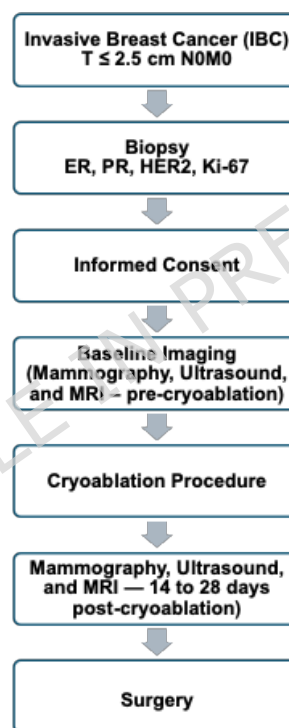
VMS drafted the manuscript with input from SEB and ACPN.

All authors contributed to data interpretation, critically revised the manuscript for important intellectual content, approved the final version of the manuscript, and agree to be accountable for all aspects of the work.

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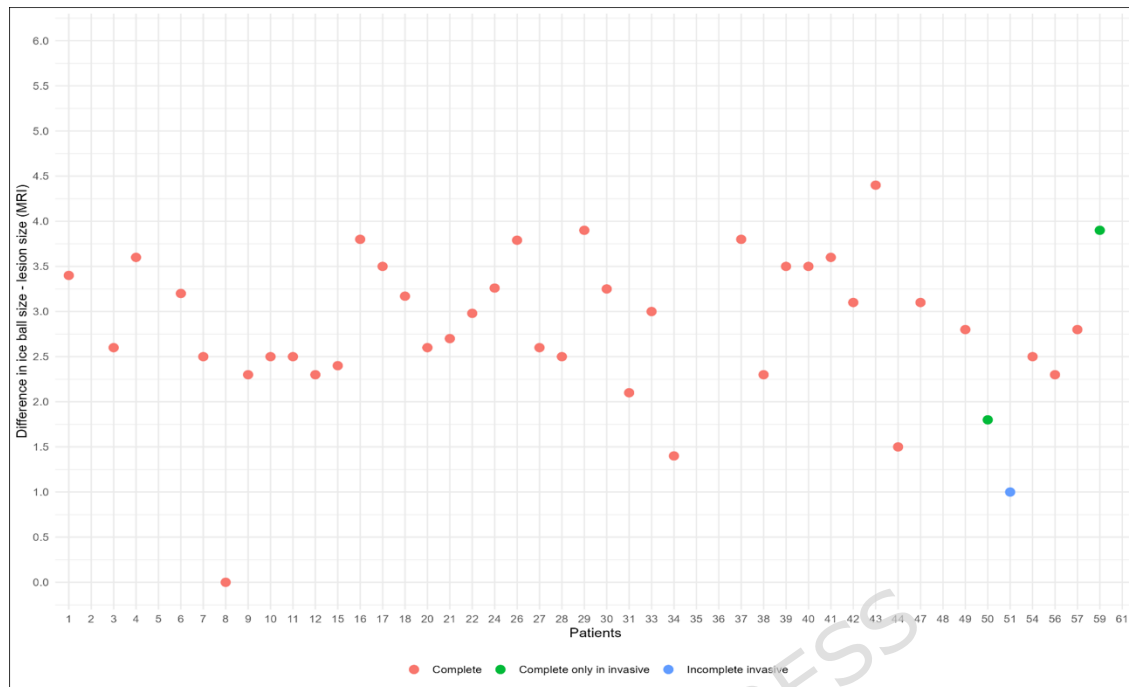
Figure 1. Study flowchart



Eligibility included invasive breast cancer  $\leq 2.5$  cm, clinically node-negative (cN0), M0 disease. After biopsy confirmation and immunohistochemical evaluation (ER, PR, HER2, Ki-67), informed consent was obtained. Baseline breast imaging (mammography, ultrasound, MRI) was performed before cryoablation. Post-cryoablation imaging findings were correlated with pathological results obtained from mandatory surgical excision performed 14–28 days after cryoablation, not from clinical or imaging surveillance.

*ER* estrogen receptor, *PR* progesterone receptor, *HER2* human epidermal growth factor receptor 2, *MMG* mammography, *US* ultrasound, *MRI* magnetic resonance imaging.

Figure 2. Ice-ball margin versus lesion size on MRI



Scatter plot illustrating the difference between maximum ice-ball diameter and MRI-measured lesion size for each patient. Imaging findings were correlated with histopathological results obtained from post-ablation surgical specimens. Red points indicate complete ablation; green points indicate invasive complete ablation with residual DCIS; blue points indicate incomplete invasive ablation. Most patients achieved 2–4 cm margins, while the only invasive failure occurred in a case with minimal margin.

Table 1. Clinical characteristics of the study population

Characteristics	Patients
<b>Patients characteristics</b>	
Age, years	
Mean $\pm$ SD	62.02 $\pm$ 12.47 (n = 48)
Median [Q1; Q3]	61.50 [53.50; 71.25] (n = 48)
Menopausal status	
Premenopausal	10/48 (20.9%)
postmenopausal	38/48 (79.1%)
Sex	

women	47/48 (97.9%)
men	1/48 (2.1%)

### Tumor characteristics

Histotype	
No special type	42/48 (87.5%)
Lobular	4/48 (8.3%)
Mucinous	2/48 (4.2%)

### Nottingham tumor score (combine histologic grading)

Low: 1 (range 3-5)	20/48 (41.6%)
Intermediate: 2 (range 6-7)	28/48 (58.4%)
High (8-9)	0/48 (0%)

### Ductal carcinoma in situ associated with biopsy results

yes	9/48 (18.7%)
no	39/48 (81.2%)

### Tumor size (cm) 1

Mean $\pm$ SD	1.25 $\pm$ 0.45 (n = 48)
Median [Q1; Q3]	1.15 [1.00; 1.60] (n = 48)

### Clinical tumor staging (TNM)

T1	44/48 (91.7%)
T2	4/48 (8.3%)

### Palpable lesion

Non-palpable	40/48 (83.3%)
Palpable	8/48 (16.7%)

### Receptor status

#### Estrogen Receptor (ER)

Positive	46/48 (95.8%)
Negative	2/48 (4.2%)

#### Progesterone Receptor (PR)

Positive	45/48 (93.8%)
Negative	3/48 (6.2%)

### HER2 2

Not overexpressed	43/48 (89.6%)
Overexpressed	5/48 (10.4%)
ki-67	
Mean $\pm$ SD	15.75 $\pm$ 11.32 (n = 48)
Median [Q1; Q3]	10.50 [10.00; 20.00] (n = 48)
Immunoistochemical subtype	
Luminal ERBB2-negative	42/48 (87.5%)
ERBB2-enriched	5/48 (10.4%)
Triple-negative	1/48 (2.1%)

*SD standard deviation; ER estrogen receptor; PR progesterone receptor; HER2 human epidermal growth factor receptor 2; FISH fluorescence in situ hybridization.*

<sup>1</sup> Tumor size measured by ultrasound.

<sup>2</sup> Equivocal HER2 results confirmed by FISH.

Table 2. Pre-treatment imaging findings

<b>Pre-treatment imaging</b>	<b>number</b>
<b>Mammography</b>	
Type of Lesion	
Asymmetry	5/42 (11.9%)
Distortion	2/42 (4.8%)
Nodule	35/42 (83.3%)
Lesion Size	
Mean $\pm$ SD (cm)	1.24 $\pm$ 0.64 (n=48) 1.25 [0.97; 1.63]
Median [Q1; Q3] (cm)	(n=48)
<b>Ultrasound</b>	
Type of Lesion	
Nodule	48/48 (100.0%)
Lesion Size	
Mean $\pm$ SD (cm)	1.25 $\pm$ 0.45 (n=48) 1.15 [1.00; 1.60]
Median [Q1; Q3] (cm)	(n=48)
<b>MRI</b>	
Type of Lesion	
Distortion	0/41 (0.0%)
Nodule	41/41 (100.0%)

Lesion Size	
Mean $\pm$ SD (cm)	1.57 $\pm$ 0.59 (n=42)
	1.50 [1.20; 1.98]
Median [Q1; Q3] (cm)	(n=42)

Number of lesions visualized on each modality and lesion size measurements by ultrasound, mammography, and MRI.

*SD standard deviation, MRI Magnetic Resonance Imaging*

Table 3. Cryoablation Parameters

Cryoablation	
Largest Ice-ball Diameter (cm):	
Mean $\pm$ SD	4.46 $\pm$ 0.84 (n = 48)
	4.20 [3.90; 5.12] (n =
Median [Q1; Q3]	48)
Two Cycles	48/48 (100.0%)
Cryoablation	
Duration:	
6x4 min	38/46 (82.6%)
8x8 min	7/46 (15.2%)
10x10 min	1/46 (2.2%)

Cryoprobe specifications, freeze-thaw protocol, and ice-ball size achieved.

Table 4. Ablation rates by lesion size and imaging modality

Ablation Rate Description	Value
Complete Ablation Rate	43/48; 89.6%; 95% CI [76.6%; 96.1%]
Invasive Complete Ablation Rate	47/48; 97.9%; 95% CI [87.5%; 99.9%]
Lesion Size (MRI)	
Complete Ablation Rate, Lesion Size $\leq$ 2.0 cm	31/32; 96.9%; 95% CI [82.0%; 99.8%]
Invasive Complete Ablation Rate, Lesion Size $\leq$ 2.0 cm	32/32; 100.0%; 95% CI [86.7%; 100.0%]
Complete Ablation Rate, Lesion Size $\leq$ 1.5 cm	22/23; 95.7%; IC95% [76.0%; 99.8%]
Invasive Complete Ablation Rate, Lesion Size $\leq$ 1.5 cm	23/23; 100.0%; IC95% [82.2%; 100.0%]
Complete Ablation Rate, Lesion Size $\leq$ 1.0 cm	8/8; 100.0%; IC95% [59.8%; 100.0%]

Invasive Complete Ablation Rate, Lesion Size $\leq 1.0$ cm	8/8; 100.0%; IC95% [59.8%; 100.0%]
Lesion Size (US)	
Complete Ablation Rate, Lesion Size $\leq 2.0$ cm	41/46; 89.1%; 95% CI [75.6%; 95.9%]
Invasive Complete Ablation Rate, Lesion Size $\leq 2.0$ cm	45/46; 97.8%; 95% CI [87.0%; 99.9%]
Complete Ablation Rate, Lesion Size $\leq 1.5$ cm	32/34; 94.1%; IC95% [78.9%; 99.0%]
Invasive Complete Ablation Rate, Lesion Size $\leq 1.5$ cm	34/34; 100.0%; IC95% [87.4%; 100.0%]
Complete Ablation Rate, Lesion Size $\leq 1.0$ cm	15/15; 100.0%; IC95% [74.7%; 100.0%]
Invasive Complete Ablation Rate, Lesion Size $\leq 1.0$ cm	15/15; 100.0%; IC95% [74.7%; 100.0%]

Complete and invasive complete ablation rates are presented with their respective 95% confidence intervals, stratified by lesion size ( $\leq 2.0$  cm,  $\leq 1.5$  cm, and  $\leq 1.0$  cm) based on pre-treatment MRI and ultrasound measurements.

Table 5. Pathological findings from surgical specimens after cryoablation

<b>Surgical Results - Pathological Anatomy</b>	
Residual invasive carcinoma	
No	47/48 (97.9%)
Yes	1/48 (2.1%)
Residual ductal carcinoma in situ	
No	42/48 (87.5%)
Yes	6/48 (12.5%)
Pathological staging (TNM):	
pT0	43/48 (89.6%)
pTis	4/48 (8.3%)
pT1	1/48 (2.1%)
pN0	44/48 (91.7%)
pN1	4/48 (8.3%)
M0	48/48 (100.0%)

Rates of residual invasive carcinoma, DCIS, pathological staging (TNM), and nodal status.

Table 6. Clinicopathological characteristics of patients with residual disease following cryoablation

Patients	1	2	3	4	5
Response status	Complete response in invasive component only	Complete response in invasive component only	Complete response in invasive component only	Complete response in invasive component only	Incomplete response in invasive component
Age, years	89	83	49	72	52
Histotype	Invasive carcinoma of no special type	Invasive carcinoma of no special type	Invasive carcinoma of no special type	Invasive carcinoma of no special type	Invasive carcinoma of no special type
DCIS associated with biopsy results	No	No	Yes	No	No
Nottingham tumor score	Grade 1	Grade 2	Grade 1	Grade 1	Grade 2
Tumor size (cm) on ultrasound at the day of cryoablation	1.3	1.3	1.9	1.7	1.8
Tumor size (cm) on MRI at the day of cryoablation	Not performed	Not performed	2.1	1.3	2.5
Ice-ball diameter (cm)	4.2	5.8	3.9	5.2	3.5
Residual invasive carcinoma	No	No	No	No	Yes
DCIS	Yes	Yes	Yes	Yes	Yes
Residual lesion size /	DCIS / 0.25	DCIS / 0.15	DCIS / 0.20	DCIS / 0.3	Invasive carcinoma of no

tumor size (cm)					special type / 0.3
Chemotherapy	No	No	No	No	Yes
Radiotherapy	Ultrahypofractionated	Moderately hypofractionated	Moderately hypofractionated	Ultrahypofractionated	Moderately hypofractionated
Endocrine therapy	Yes	Yes	Yes	Yes	No
Anti-HER2 therapy	No	No	No	No	Yes

Clinicopathologic features and post-cryoablation therapy among cases with residual invasive carcinoma or DCIS.

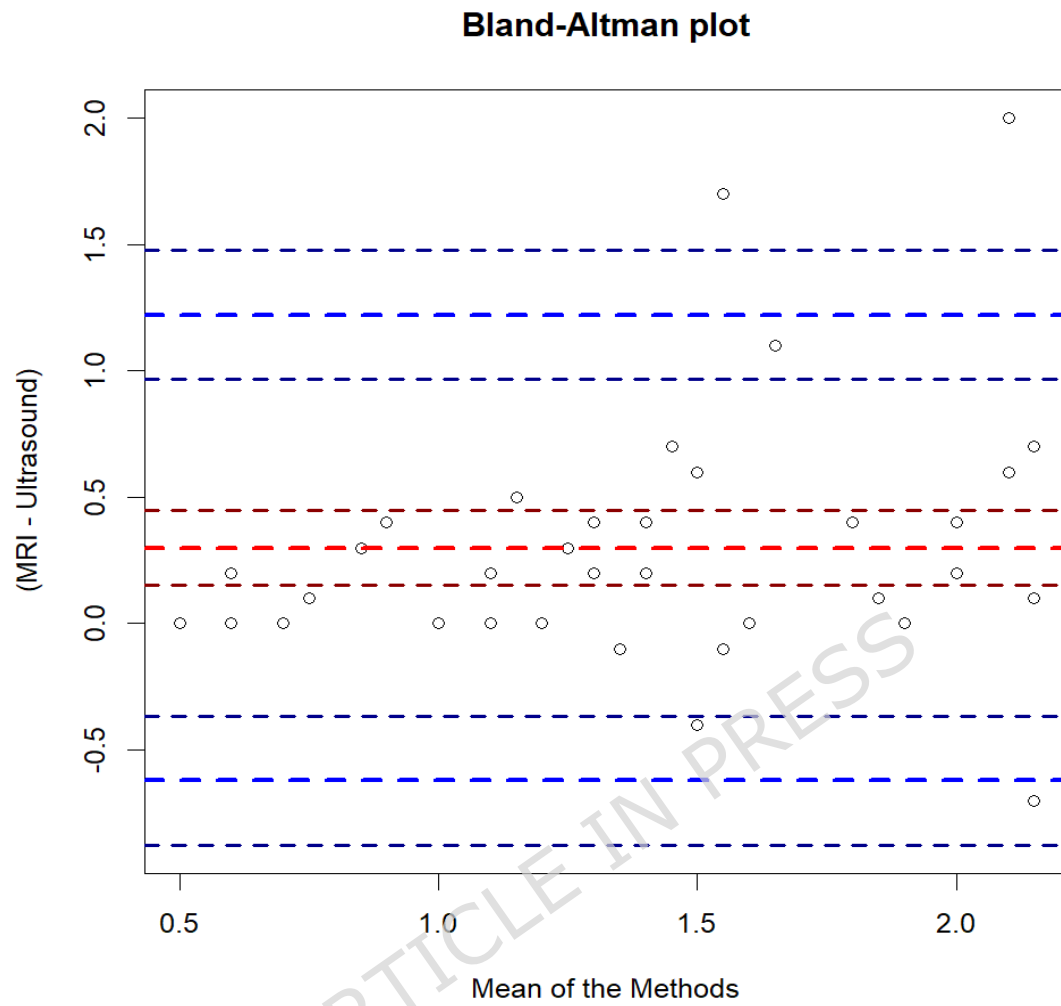
Table 7. Ice-ball margins and outcomes

<b>Complete Ablation Rate</b>	<b>No (n=5)</b>	<b>Yes (n=43)</b>	<b>Total (n=48)</b>	<b>p-value</b>
Ice-ball size; median [IQR]	4.2 [3.9-5.2]	4.2 [3.9-5.1]	4.2 [3.9-5.1]	0.906
Pre-treatment lesion size - Ultrasound; median [IQR]	1.7 [1.3-1.8]	1.1 [1.0-1.5]	1.1 [1.0-1.6]	0.03
Difference between ice-ball and pre-treatment lesion size - Ultrasound; median [IQR]	2.9 [2.0-3.5]	3.2 [2.7-3.7]	3.1 [2.7-3.7]	0.521
Pre-treatment lesion size - MRI; median [IQR]	2.1 [1.7-2.3] (n=3)	1.5 [1.1-1.9] (n=39)	1.5 [1.2-2.0] (n=42)	0.25
Difference between ice-ball and pre-treatment lesion size - MRI; median [IQR]	1.8 [1.4-2.9] (n=3)	2.8 [2.5-3.4] (n=39)	2.8 [2.4-3.5] (n=42)	0.434

p-value: Wilcoxon test

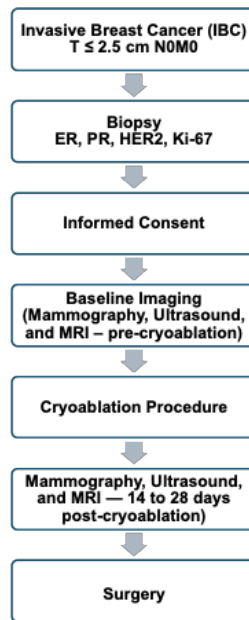
Comparison of tumor size, ice-ball diameter, and margins between complete and incomplete ablation cases.

Supplementary Figure 1. Bland-Altman plot for ultrasound and MRI measurements



Plot illustrating the agreement and systematic differences between ultrasound and MRI in pre-cryoablation lesion sizing.

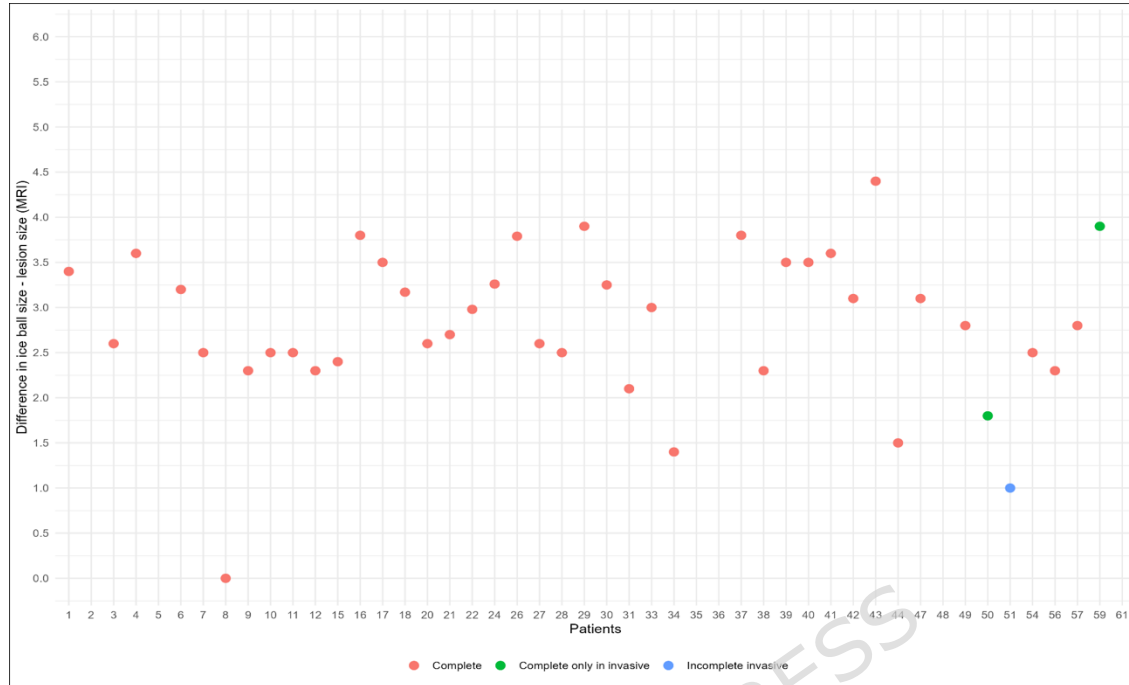
Figure 1. Study flowchart



Eligibility included invasive breast cancer  $\leq 2.5$  cm, clinically node-negative (cN0), M0 disease. After biopsy confirmation and immunohistochemical evaluation (ER, PR, HER2, Ki-67), informed consent was obtained. Baseline breast imaging (mammography, ultrasound, MRI) was performed before cryoablation. Post-cryoablation imaging findings were correlated with pathological results obtained from mandatory surgical excision performed 14–28 days after cryoablation, not from clinical or imaging surveillance.

*ER estrogen receptor, PR progesterone receptor, HER2 human epidermal growth factor receptor 2, MMG mammography, US ultrasound, MRI magnetic resonance imaging.*

Figure 2. Ice-ball margin versus lesion size on MRI



Scatter plot illustrating the difference between maximum ice-ball diameter and MRI-measured lesion size for each patient. Imaging findings were correlated with histopathological results obtained from post-ablation surgical specimens. Red points indicate complete ablation; green points indicate invasive complete ablation with residual DCIS; blue points indicate incomplete invasive ablation. Most patients achieved 2–4 cm margins, while the only invasive failure occurred in a case with minimal margin.

Table 1. Clinical characteristics of the study population

<b>Characteristics</b>	<b>Patients</b>
<b>Patients characteristics</b>	
Age, years	
Mean $\pm$ SD	62.02 $\pm$ 12.47 (n = 48)
Median [Q1; Q3]	61.50 [53.50; 71.25] (n = 48)
Menopausal status	
Premenopausal	10/48 (20.9%)
postmenopausal	38/48 (79.1%)
Sex	
women	47/48 (97.9%)
men	1/48 (2.1%)
<b>Tumor characteristics</b>	
Histotype	
No special type	42/48 (87.5%)
Lobular	4/48 (8.3%)
Mucinous	2/48 (4.2%)
Nottingham tumor score (combine histologic grading)	
Low: 1 (range 3-5)	20/48 (41.6%)
Intermediate: 2 (range 6-7)	28/48 (58.4%)
High (8-9)	0/48 (0%)
Ductal carcinoma in situ associated with biopsy results	
yes	9/48 (18.7%)
no	39/48 (81.2%)
Tumor size (cm) 1	
Mean $\pm$ SD	1.25 $\pm$ 0.45 (n = 48)
Median [Q1; Q3]	1.15 [1.00; 1.60] (n = 48)
Clinical tumor staging (TNM)	
T1	44/48 (91.7%)
T2	4/48 (8.3%)

Palpable lesion	
Non-palpable	40/48 (83.3%)
Palpable	8/48 (16.7%)
Receptor status	
Estrogen Receptor (ER)	
Positive	46/48 (95.8%)
Negative	2/48 (4.2%)
Progesterone Receptor (PR)	
Positive	45/48 (93.8%)
Negative	3/48 (6.2%)
HER2 2	
Not overexpressed	43/48 (89.6%)
Overexpressed	5/48 (10.4%)
ki-67	
Mean $\pm$ SD	15.75 $\pm$ 11.32 (n = 48)
Median [Q1; Q3]	10.50 [10.00; 20.00] (n = 48)
Immunohistochemical subtype	
Luminal ERBB2-negative	42/48 (87.5%)
ERBB2-enriched	5/48 (10.4%)
Triple-negative	1/48 (2.1%)

*SD standard deviation; ER estrogen receptor; PR progesterone receptor; HER2 human epidermal growth factor receptor 2; FISH fluorescence in situ hybridization.*

<sup>1</sup> Tumor size measured by ultrasound.

<sup>2</sup> Equivocal HER2 results confirmed by FISH.

Table 2. Pre-treatment imaging findings

<b>Pre-treatment imaging</b>	<b>number</b>
<b>Mammography</b>	
Type of Lesion	
Asymmetry	5/42 (11.9%)
Distortion	2/42 (4.8%)
Nodule	35/42 (83.3%)
Lesion Size	
Mean $\pm$ SD (cm)	1.24 $\pm$ 0.64 (n=48) 1.25 [0.97; 1.63]
Median [Q1; Q3] (cm)	(n=48)
<b>Ultrasound</b>	
Type of Lesion	
Nodule	48/48 (100.0%)
Lesion Size	
Mean $\pm$ SD (cm)	1.25 $\pm$ 0.45 (n=48) 1.15 [1.00; 1.60]
Median [Q1; Q3] (cm)	(n=48)
<b>MRI</b>	
Type of Lesion	
Distortion	0/41 (0.0%)
Nodule	41/41 (100.0%)
Lesion Size	
Mean $\pm$ SD (cm)	1.57 $\pm$ 0.59 (n=42) 1.50 [1.20; 1.98]
Median [Q1; Q3] (cm)	(n=42)

Number of lesions visualized on each modality and lesion size measurements by ultrasound, mammography, and MRI.

*SD standard deviation, MRI Magnetic Resonance Imaging*

Table 3. Cryoablation Parameters

Cryoablation	
Largest Ice-ball Diameter (cm):	
Mean $\pm$ SD	4.46 $\pm$ 0.84 (n = 48)
Median [Q1; Q3]	4.20 [3.90; 5.12] (n = 48)
Two Cycles	48/48 (100.0%)
Cryoablation Duration:	
6x4 min	38/46 (82.6%)
8x8 min	7/46 (15.2%)
10x10 min	1/46 (2.2%)

Cryoprobe specifications, freeze-thaw protocol, and ice-ball size achieved.

Table 4. Ablation rates by lesion size and imaging modality

<b>Ablation Rate Description</b>	<b>Value</b>
Complete Ablation Rate	43/48; 89.6%; 95% CI [76.6%; 96.1%]
Invasive Complete Ablation Rate	47/48; 97.9%; 95% CI [87.5%; 99.9%]
Lesion Size (MRI)	
Complete Ablation Rate, Lesion Size $\leq$ 2.0 cm	31/32; 96.9%; 95% CI [82.0%; 99.8%]
Invasive Complete Ablation Rate, Lesion Size $\leq$ 2.0 cm	32/32; 100.0%; 95% CI [86.7%; 100.0%]
Complete Ablation Rate, Lesion Size $\leq$ 1.5 cm	22/23; 95.7%; IC95% [76.0%; 99.8%]
Invasive Complete Ablation Rate, Lesion Size $\leq$ 1.5 cm	23/23; 100.0%; IC95% [82.2%; 100.0%]
Complete Ablation Rate, Lesion Size $\leq$ 1.0 cm	8/8; 100.0%; IC95% [59.8%; 100.0%]
Invasive Complete Ablation Rate, Lesion Size $\leq$ 1.0 cm	8/8; 100.0%; IC95% [59.8%; 100.0%]
Lesion Size (US)	
Complete Ablation Rate, Lesion Size $\leq$ 2.0 cm	41/46; 89.1%; 95% CI [75.6%; 95.9%]
Invasive Complete Ablation Rate, Lesion Size $\leq$ 2.0 cm	45/46; 97.8%; 95% CI [87.0%; 99.9%]
Complete Ablation Rate, Lesion Size $\leq$ 1.5 cm	32/34; 94.1%; IC95% [78.9%; 99.0%]
Invasive Complete Ablation Rate, Lesion Size $\leq$ 1.5 cm	34/34; 100.0%; IC95% [87.4%; 100.0%]
Complete Ablation Rate, Lesion Size $\leq$ 1.0 cm	15/15; 100.0%; IC95% [74.7%; 100.0%]
Invasive Complete Ablation Rate, Lesion Size $\leq$ 1.0 cm	15/15; 100.0%; IC95% [74.7%; 100.0%]

Complete and invasive complete ablation rates are presented with their respective 95% confidence intervals, stratified by lesion size ( $\leq$ 2.0 cm,  $\leq$ 1.5 cm, and  $\leq$ 1.0 cm) based on pre-treatment MRI and ultrasound measurements.

Table 5. Pathological findings from surgical specimens after cryoablation

<b>Surgical Results - Pathological Anatomy</b>	
Residual invasive carcinoma	
No	47/48 (97.9%)
Yes	1/48 (2.1%)
Residual ductal carcinoma in situ	
No	42/48 (87.5%)
Yes	6/48 (12.5%)
Pathological staging (TNM):	
pT0	43/48 (89.6%)
pTis	4/48 (8.3%)
pT1	1/48 (2.1%)
pN0	44/48 (91.7%)
pN1	4/48 (8.3%)
M0	48/48 (100.0%)

Rates of residual invasive carcinoma, DCIS, pathological staging (TNM), and nodal status

Table 6. Clinicopathological characteristics of patients with residual disease following cryoablation

Patients	1	2	3	4	5
Response status	Complete response in invasive component only	Complete response in invasive component only	Complete response in invasive component only	Complete response in invasive component only	Incomplete response in invasive component
Age, years	89	83	49	72	52
Histotype	Invasive carcinoma of no special type	Invasive carcinoma of no special type	Invasive carcinoma of no special type	Invasive carcinoma of no special type	Invasive carcinoma of no special type
DCIS associated with biopsy results	No	No	Yes	No	No
Nottingham tumor score	Grade 1	Grade 2	Grade 1	Grade 1	Grade 2
Tumor size (cm) on ultrasound at the day of cryoablation	1.3	1.3	1.9	1.7	1.8
Tumor size (cm) on MRI at the day of cryoablation	Not performed	Not performed	2.1	1.3	2.5
Ice-ball diameter (cm)	4.2	5.8	3.9	5.2	3.5
Residual invasive carcinoma	No	No	No	No	Yes
DCIS	Yes	Yes	Yes	Yes	Yes
Residual lesion size / tumor size (cm)	DCIS / 0.25	DCIS / 0.15	DCIS / 0.20	DCIS / 0.3	Invasive carcinoma of no special type / 0.3
Chemotherapy	No	No	No	No	Yes
Radiotherapy	Ultrahypofractionated	Moderately hypofractionated	Moderately hypofractionated	Ultrahypofractionated	Moderately hypofractionated

Endocrine therapy	Yes	Yes	Yes	Yes	No
Anti-HER2 therapy	No	No	No	No	Yes

Clinicopathologic features and post-cryoablation therapy among cases with residual invasive carcinoma or DCIS.

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Table 7. Ice-ball margins and outcomes

<b>Complete Ablation Rate</b>	<b>No (n=5)</b>	<b>Yes (n=43)</b>	<b>Total (n=48)</b>	<b>p-value</b>
Ice-ball size; median [IQR]	4.2 [3.9-5.2]	4.2 [3.9-5.1]	4.2 [3.9-5.1]	0.906
Pre-treatment lesion size - Ultrasound; median [IQR]	1.7 [1.3-1.8]	1.1 [1.0-1.5]	1.1 [1.0-1.6]	0.03
Difference between ice-ball and pre-treatment lesion size - Ultrasound; median [IQR]	2.9 [2.0-3.5]	3.2 [2.7-3.7]	3.1 [2.7-3.7]	0.521
Pre-treatment lesion size - MRI; median [IQR]	2.1 [1.7-2.3] (n=3)	1.5 [1.1-1.9] (n=39)	1.5 [1.2-2.0] (n=42)	0.25
Difference between ice-ball and pre-treatment lesion size - MRI; median [IQR]	1.8 [1.4-2.9] (n=3)	2.8 [2.5-3.4] (n=39)	2.8 [2.4-3.5] (n=42)	0.434

p-value: Wilcoxon test

Comparison of tumor size, ice-ball diameter, and margins between complete and incomplete ablation cases