Annals of Surgical Oncology, **11**(5):542–549
DOI: 10.1245/ASO.2004.08.003

Cryoablation of Early-Stage Breast Cancer: Work-in-Progress Report of a Multi-Institutional Trial

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Background: With recent improvements in breast imaging, our ability to identify small breast tumors has markedly improved, prompting significant interest in the use of ablation without surgical excision to treat early-stage breast cancer. We conducted a multi-institutional pilot safety study of cryoablation in the treatment of primary breast carcinomas.

Methods: Twenty-nine patients with ultrasound-visible primary invasive breast cancer $\leq$2.0 cm were enrolled. Twenty-seven (93%) successfully underwent ultrasound-guided cryoablation with a tabletop argon gas–based cryoablation system with a double freeze/thaw cycle. Standard surgical resection was performed 1 to 4 weeks after cryoablation. Patients were monitored for complications, and pathology data were used to assess efficacy.

Results: Cryoablation was successfully performed in an office-based setting with only local anesthesia. There were no complications to the procedure or postprocedural pain requiring narcotic pain medications. Cryoablation successfully destroyed 100% of cancers $<$1.0 cm. For tumors between 1.0 and 1.5 cm, this success rate was achieved only in patients with invasive ductal carcinoma without a significant ductal carcinoma-in-situ (DCIS) component. For unselected tumors $>$1.5 cm, cryoablation was not reliable with this technique. Patients with noncalcified DCIS were the cause of most cryoablation failures.

Conclusions: Cryoablation is a safe and well-tolerated office-based procedure for the ablation of early-stage breast cancer. At this time, cryoablation should be limited to patients with invasive ductal carcinoma $\leq$1.5 cm and with $<$25% DCIS in the core biopsy. A multicenter phase II clinical trial is planned.

Key Words: Cryosurgery—Cryoablation—Breast cancer—Ablative therapies.

It is estimated that in 2004 there will be more than 215,000 cases of female breast cancer diagnosed in the United States, making it the most common malignant tumor in women.[1] The most striking influence on the treatment of breast cancer came after the demonstration that there was no difference in survival between breast-conservation therapy and mastectomy.[2] This shift in surgical therapy has resulted in a significant decrease in the morbidity and disfigurement associated with treatment, without a loss in survival.

A lumpectomy, however, is still an invasive procedure and leaves the patient with a scar and possible cosmetic defect in the breast. With improvements in imaging, cancers smaller than 1.0 cm are increasing in frequency.[3] Therefore, there is intense interest in the possibility of ablating small cancers within the breast without excisional surgery. This not only would improve cosmetic outcomes, but also could greatly decrease operating room and anesthesia needs, recovery times, surgical complications, and health-care costs. Several methods

Received August 8, 2003; accepted January 20, 2004.

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are presently being investigated for the in situ ablation of breast cancer, including radiofrequency ablation,[4–7] cryosurgery,[8,9] laser interstitial therapy,[10–17] high-intensity focused ultrasound,[18–25] and focused microwave thermotherapy.[26–29]

Cryosurgery has been successfully used for the ablation of tumors in the liver,[30,31] prostate,[32,33] and kidney[34,35]; the palliative treatment of locally advanced breast cancers[36,37]; and the treatment of breast fibroadenomas.[38] The ability to clearly visualize the forming ice ball with ultrasound provides outstanding precision and control for this procedure. With the recent improvements in breast imaging, our ability to visualize, take biopsy samples from, and treat breast tumors has markedly improved. This has prompted significant interest in the use of cryoablation instead of surgical excision in the treatment of small early-stage breast cancer. Preclinical studies have demonstrated that 100% tumor kill is attainable with cryosurgery and that ultrasonography can accurately guide probe placement and monitor the development of the ice ball.[8,9,39–41] On the basis of these results, we initiated a phase I multicenter trial of cryoablation followed by delayed surgical excision to determine the safety and feasibility of this treatment. Preliminary results from this trial reveal that cryoablation of early-stage breast cancer is extremely well tolerated and can be performed with minimal discomfort or morbidity. In addition, with proper patient selection, it can achieve 100% tumor ablation in most patients.

MATERIALS AND METHODS

Cryoablative Procedure

A tabletop argon gas–based cryoablation system (Visica Cryoablation System; Sanarus Medical, Pleasanton, CA), designed to create probe temperatures of −160°C, was used to treat all tumors in an outpatient setting (Fig. 1). Ultrasound guidance facilitates accurate placement of the probe in the center of the tumor. The cryoablation system creates an ice ball that rapidly engulfs the tumor plus a margin of apparently healthy tissue. In all cases, patients underwent a double freeze/thaw cycle: a freeze followed by a passive thaw and then another freeze. This allows for osmotic shifts to occur at the cellular level that lead to enhanced destruction of the targeted tissue.[42] An active thaw with helium gas warms the cryoprobe after the second freeze, permitting its rapid removal after the last cycle. Over the course of the trial, the freeze time was altered and the cryoprobe was modified. The initial probe was a 2.4-mm-diameter, air gap–insulated, sharp-tipped probe (11 cases). The next-generation cryoprobe was introduced into the study for cases

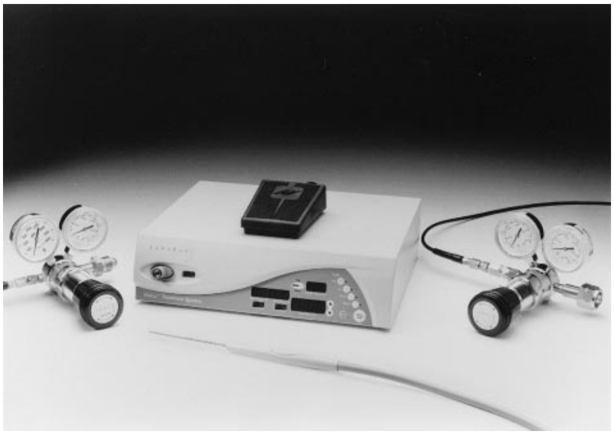

FIG. 1. Tabletop argon gas–based cryoablation system.

12 to 29. It was a 2.7-mm-diameter, vacuum-insulated, trocar-tipped instrument that allowed cooling to occur only at its distal 4 cm. The improved insulation on this probe allowed for elimination of skin protection techniques other than the injection of sterile saline between the skin and growing ice ball for lesions close to the skin. Before this probe was introduced, dripped sterile saline, moist gauze placed between the skin and probe, skin temperature monitoring, and an introducing trocar with cannula were additionally used for skin protection.

For the first 11 patients, the freeze time was 10 minutes at a 100% duty cycle (argon gas flowing 100% of the time for maximum cooling) regardless of tumor size. On the basis of experience treating fibroadenomas,[38] this was subsequently altered so that freeze time was based on the maximum tumor diameter, as assessed by ultrasound, to avoid excessive freezing of uninvolved tissue around smaller lesions (Table 1). Each freeze cycle was split between a high freeze and a low freeze. During high freeze, the system operated at a 100% duty cycle (argon flowed continuously), whereas during low freeze, it operated at a 10% duty cycle (argon gas flows for 1 second and is off for 9 seconds of every 10-second period). This 10% duty cycle maintains cold temperatures within the ice ball while slowing the ice ball's overall growth. A passive thaw lasting 10 to 12 minutes (depending on the

TABLE 1. *Cancer cryoablation*

Longest imaged cancer diameter (cm)	Freeze parameters (minutes at high freeze/minutes at low freeze[a])
<1.0	6/4
1.0–1.3	8/2
1.4–1.8	10/2

[a] Total treatment = high/low freeze + passive thaw for total time of high/low freeze + repeat high/low freeze.

cycle used) was interposed between the two freeze cycles, and an active thaw with helium gas was performed after the second freeze, thereby facilitating probe removal.

Clinical Study

Between March 2000 and February 2003, patients with primary invasive breast cancer at several participating institutions were offered an opportunity to participate in a clinical trial involving cryoablation followed by delayed surgical resection. To be considered for this trial, all patients had to have ultrasonographic evidence of a solitary breast mass measuring ≤2.0 cm in greatest dimension. The diagnosis of invasive breast cancer was made on large-core needle biopsy (LCNB). All standard histological parameters and immunohistochemical assessment of estrogen and progesterone receptors and HER-2/neu were established from the core biopsy before ablation. All participating patients signed written, informed consent that was approved by the institutional review board of their respective facilities.

Four early cryoablation cases were performed in outpatient surgery by using general anesthesia[1] or intravenous sedation.[3] All subsequent cases[25] were performed in an office-based setting without sedation, with the patient awake and alert throughout the procedure. The breast was prepared and draped in typical sterile fashion, and the breast tumor was identified by ultrasonography. Xylocaine was injected in the skin and along the projected path toward the center of the tumor. An 11-blade scalpel was used to make a 3-mm skin incision to allow placement of the cryoprobe (Fig. 2).

All procedures were performed under real-time ultrasound guidance. The probe was inserted through the center of the cancer, and the tip was advanced beyond the distal edge of the tumor to ensure equal treatment on both sides of the tumor in the longitudinal plane of the freeze zone (Fig. 3). Transverse ultrasound examination confirmed the central position of the cryoprobe within the tumor. Failure to adequately place the cryoprobe was considered a protocol violation. After central placement within the tumor was confirmed, the cryoablation procedure was performed as described previously. Skin appearance, ice ball size, and patient comfort were closely monitored during the procedure. Sterile normal saline was injected between the forming ice ball and skin whenever they were within 5 mm of each other (Fig. 4). After the second freeze cycle, the probe was actively thawed and removed, a sterile dressing was applied, and pressure was maintained according to the institution's standard for LCNB. Patients were instructed to look for transient ecchymosis, tenderness, edema, or a palpable mass in the region of cryoablation and were asked to report all post-procedural symptoms.

FIG. 2. Cryoablation of breast cancer. Ultrasound is used to monitor the creation of an ice ball, which engulfs the cancer, destroying the targeted area.

Within 7 to 30 days of the cryoablation procedure, patients returned for their planned surgical treatment: either lumpectomy or mastectomy. Sentinel lymph node biopsy, when clinically indicated, was performed at the time of surgical resection. The excised breast specimen was sent for pathologic evaluation, where the margins were inked. The tumor was bisected centrally to grossly determine the dimensions of the region exposed to cryosurgery. Radial sections were then submitted for fixation and histopathological preparation with hematoxylin and

FIG. 3. Ultrasound image of the cryoprobe advanced through the center of the cancer.

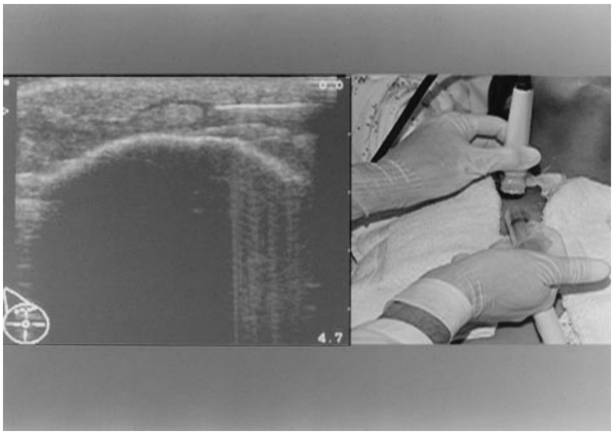

FIG. 4. Injection of saline between the ice ball and skin for skin protection during cryoablation.

eosin staining and were evaluated according to the routine of the individual institution. These sections were also assessed to determine the extent of microscopic destruction of the tumor. A successful ablation was defined as a complete (100%) ablation of the clinically detectable tumor, including both invasive and in situ components.

RESULTS

Twenty-nine women participated in the study. The median age was 52.5 years (range, 34–77 years). The clinical characteristics of the patients are listed in Table 2. The mean greatest dimension of breast tumors was 1.2 ± .5 cm (range, .6–2.0 cm). Distance to the skin ranged from .2 to 2.0 cm (average, 1.1 cm). The probe was successfully placed under ultrasound guidance in the center of the tumor in 27 (93%) of 29 patients. In two cases, the cryoprobe could not be accurately placed within the tumor. Of the 27 women successfully treated, the first 10 patients were treated with 10-minute high-freeze cycles regardless of tumor size. The next 17 patients underwent tailored ablation with the high/low freezes previously described. Nine patients with tumors >1.0 cm had two freeze cycles consisting of an 8- or 10-minute high freeze and a 2-minute low freeze. Eight patients with tumors <1.0 cm underwent two cycles consisting of a 6-minute high freeze and a 4-minute low freeze.

TABLE 2. *Tumor characteristics*

Characteristic	No. of patients (%)
Tumor size (greatest dimension; cm)	
0–.5	0 (0%)
.51–1.00	11 (41%)
1.01–1.50	9 (33%)
1.51–2.0	7 (26%)
Histology	
Invasive ductal carcinoma	16 (59%)
Invasive ductal carcinoma with DCIS	5 (19%)
Invasive lobular carcinoma	3 (11%)
Colloid carcinoma	2 (7%)
Medullary carcinoma	1 (3%)

DCIS, ductal carcinoma-in-situ.

Continuous ultrasound monitoring documented the size of the ice ball forming around the cryoprobe. The mean dimensions (length × width × depth) of the ice balls after high-freeze cycles of 8 or 10 minutes was 4. 8 ± .7 cm × 3.38 ± .6 cm × 3.31 ± .69 cm. For high-freeze cycles of 6 minutes, the dimensions were 3.8 ± .5 cm × 2.7 ± .5 cm × 2.7 ± .4 cm. The mean volume of the ice ball was 29.5 ± 13.4 mm^3 and 15.2 ± 5.7 mm^3, respectively.

Cryoablation was very well tolerated in all patients. Early procedures were performed in the operating room, but 25 procedures were performed in an office-based setting with only local anesthesia. These patients were awake and comfortable throughout the freeze-thaw-freeze cycle, which takes approximately 30 minutes. No patients had postprocedural pain requiring narcotic pain medications, and there were no significant complications from the procedure.

All 27 successfully treated patients underwent lumpectomy an average of 14 days after the cryoablation (range, 6 to 30 days). Twenty-five of 27 patients had axillary staging by intraoperative lymph node mapping and sentinel lymph node biopsy performed at the same time. Four (16%) of the 25 patients had a positive sentinel lymph node. The cryoablation had no effect on the subsequent interpretation of the sentinel lymph node.

Microscopic evaluation showed no viable invasive cancer in 23 (85%) of the 27 patients who had successful cryoablation. An additional four patients had ductal carcinoma-in-situ (DCIS) present within the normal tissue surrounding the cryozone. In two cases this was adjacent to the treatment zone, whereas in two cases this represented multifocal disease in the healthy tissue surrounding the ablation. The likelihood of success of cryoablation to leave no residual cancer cells was correlated with tumor size and histology (Table 3). Looking at size alone, of the 11 patients with tumors <1.0 cm in size whose tumors were successfully ablated, all 11 had complete ablation of their cancer, with no residual invasive or intraductal carcinoma. Of the 16 patients with tumors >1.0 cm, 10 (63%) had neither residual invasive carcinoma or DCIS.

TABLE 3. *Correlation of tumor size and histology with successful cryoablation*

Treatment group	No. Patients	No residual invasive or DCIS[a]
All patients	27	21 (78%)
Tumors <1.0 cm (all histology)	11	11 (100%)
Tumors >1.0 cm (all histology)	16	10 (63%)
Any size lobular or colloid	5	2 (40%)
Any size ductal with EIC	5	3 (60%)
Any size ductal or medullary, no EIC	17	15 (88%)
Tumors <1.5 cm; ductal or medullary, no EIC	10	10 (100%)
Tumors >1.5 cm; ductal or medullary, no EIC	7	5 (71%)

DCIS, ductal carcinoma-in-situ; EIC, extensive intraductal component.
[a] Excludes foci of DCIS found in healthy tissue surrounding the treatment zone.

Looking at histology, three patients with invasive lobular carcinoma and two with colloid carcinoma were treated. Three of these five had residual invasive cancer, and the final pathology demonstrated the tumor size to be significantly larger than predicted by pretreatment ultrasound. Five patients had invasive cancer and DCIS on core biopsy. Two of these patients had residual disease after cryoablation: DCIS alone located outside of the cryozone in one case and DCIS and invasive cancer at the treatment edge in the second case. There were 16 patients with invasive ductal carcinoma and 1 with medullary carcinoma. Of these 17 patients, 10 had tumors <1.5 cm. All 10 had complete ablation of their invasive cancer. One had a residual small focus of DCIS in the healthy tissue away from the cryozone. Of the seven patients with invasive ductal carcinoma >1.5 cm, five (71%) had complete ablation of their cancer, one had residual invasive disease, and one had residual DCIS at the periphery.

DISCUSSION

The use of freezing temperatures to treat breast cancer is not a new concept. More than a century ago, irrigation devices were designed to bring iced saline solutions in contact with advanced breast tumors, resulting in the amelioration of pain and bleeding and a reduction in the size of the tumor.[43–45] Since then there have been several advances in technology that expanded the applicability of cryosurgery in the treatment of solid tumors. In 1961, modern cryosurgery was made possible by the introduction of automated cryosurgical units that pump liquid nitrogen through the tip of an instrument, allowing the cryoablation of tumors at diverse sites, including the breast. Tanaka[37] treated 49 patients with advanced or recurrent breast cancer with cryosurgery, reporting not only alleviation of pain, control of hemorrhage, and reduction of tumor bulk, but also a 5-year survival of 44.4% in this group of "incurable" patients. Rand et al.[41,46] examined the use of cryotherapy performed in conjunction with surgical excision (cryolumpectomy) for early-stage cancer. Preclinical studies demonstrated that this reduced local recurrence, presumably by preventing the spillage or dissemination of tumor cells as a result of surgical manipulation. Staren et al.[8] explored the concept of using cryoablation instead of lumpectomy to treat early-stage breast cancer. Both small- and large-animal studies demonstrated this to be both feasible and effective. One patient who opted for cryoablation instead of surgery remains recurrence free 7 years later.[8,47]

These results, and the potential benefit of cryosurgery in the treatment of early-stage breast cancer and development of an office-based treatment system, prompted a phase I multicenter trial of cryoablation followed by delayed surgical excision. The preliminary results from this multicenter trial highlight several issues. It is clear that cryoablation not only is clinically feasible, but also has minimal pain and morbidity. The subzero temperatures of the ice ball are naturally analgesic, so only local anesthesia is required for probe placement. Postprocedure discomfort was limited to mild edema, ecchymosis, and tenderness and was well controlled with over-the-counter medications. Tumor proximity to the skin is not a contraindication to cryoablation, particularly since the introduction of the 2.7-mm vacuum-insulated cryoprobe. The ice ball is extremely well visualized by ultrasound, and the injection of sterile normal saline easily increases its distance from the skin. Hyperthermic ablation techniques, in contrast, require a skin distance of at least 1 cm to avoid the risk of skin injury,[7,15] (AN Mirza et al., unpublished data) because inadvertent burns have complicated such techniques.[5,14]

In this study, all patients with tumors <1.0 cm had complete (100%) ablation of all breast cancer. All patients with tumors <1.5 cm, excluding those with invasive lobular or colloid carcinoma or significant DCIS, also had complete ablation (100%) of all breast cancer. For tumors >1.5 cm, successful cryoablation was not predictable with these preoperative imaging techniques. These findings are similar to those described by Pfleiderer et al.[9] in their report of 15 patients with primary breast tumors treated with cryosurgery. A 1.5-cm size limit has also been recommended for the use of laser interstitial therapy and radiofrequency ablation.[7,14]

These results highlight that the key to ablative techniques is the ability to preoperatively identify truly unifocal lesions while being able to visualize both the tumor and the zone of destruction. With the limitations of mammography and ultrasonography, tumors without well-demarcated boundaries should not be treated by in situ ablation. Invasive lobular carcinomas are often multifocal or poorly defined on mammography and ultrasound, and the extent of the tumor may be substantially underestimated.[49–51] Likewise, mucinous (colloid) carcinoma is often poorly defined on preoperative imaging.[52,53] In this series, of the five patients with either lobular or mucinous cancer, three had residual invasive cancer after cryoablation.

On the basis of these results, we recommend that inclusion for a phase II study of cryoablation without surgical excision should be limited to patients with invasive ductal carcinoma $\leq$1.5 cm and with $<$25% DCIS in the aggregate LCNBs. Although tumors $>$1.5 cm may eventually be treated with cryoablation, perhaps by either placing more than one cryoprobe or repositioning the cryoprobe and performing a second ablation, it will be necessary to first define a population of patients who can be treated with simple central placement of a single cryoprobe. It is also possible that advances in the imaging techniques available today or those in development might be necessary for the preoperative selection of patients with more complicated breast cancer in the future.

The most challenging issue facing all ablative techniques is multifocal noncalcified DCIS. Every attempt should be made to exclude patients who may have a significant intraductal component. This includes evidence on LCNB and the presence of microcalcifications on mammography.[54] The extent of microcalcifications on mammography often underestimates the histological size of DCIS,[55,56] so all tumors with more than just the most minimal of calcifications present should also be excluded. However, there were four patients with no evidence of DCIS on core biopsy or diagnostic imaging who had DCIS in the periphery of the cryoablated zone. In two of these cases, this seemed to represent foci of DCIS in the healthy tissue away from the ablated cancer. These were detected on wide excision but would not have been identified if these cancers had been ablated without surgical excision. Today's gold standard for breast-conserving surgery requires the ability to assess the lumpectomy specimen margins, and, of course, tumor-free margins are required for subsequent optimal radiotherapy. Before in situ breast cancer ablation can become a mainstream therapy, the question of margin assessment will need to be addressed. With no specimen, how will margin clearance be ensured? Accurate and predictable preoperative imaging will be necessary to identify the full extent of disease.

Ultrasound today is unfortunately not accurate enough to predict the presence and extent of an intraductal component.[57] Magnetic resonance imaging (MRI) has been to reported to more accurately diagnose the extent of an intraductal component than either mammogram or ultrasound.[58] MRI has been shown to accurately define the extent of disease in breast cancer, including invasive lobular tumors,[51,58] and has been used to guide ablative breast procedures.[17,21,59] MRI may, therefore, play a future role in selecting patients appropriate for in situ ablation and/or assessing the efficacy of treatment. Further research will be necessary to address this issue.

Although there are several obstacles to overcome, given the potential benefits, there is significant motivation to identify whether cryoablation can replace lumpectomy in selected patients for the local treatment of small breast cancers. With the expanding use of screening mammography, the size of tumors at presentation is decreasing. Almost two thirds of women who present with breast cancer today will present with disease localized to the breast,[1] and it has been predicted that by the year 2010, 50% of newly diagnosed breast cancers may be $<$1 cm.[60] That would represent 90,000 patients for whom a lumpectomy will be necessary, with the associated operating room time, anesthesia, cost, and cosmetic and psychological impact. Compared with lumpectomy, cryoablation can be performed in an office-based setting with no IV sedation. Cosmetically, cryosurgery has great esthetic appeal. It has long been known to cause minimal scarring when used in the treatment of cancers of the face, mouth, nose, and cervix.[61] Cryoablation of benign fibroadenomas in the breast is performed with minimal scarring, outstanding cosmesis, and excellent patient satisfaction.[38] Several months after cryosurgery, the cryotreatment site cannot be identified by ultrasound, mammogram, or MRI, and this has important consequences for surveillance.[39,62]

Finally, a unique aspect of cryosurgery that makes it particularly attractive for in situ ablation is the potential immunological benefit and the possibility that an immune response generated by the residual tumor protein material after ablation may be able to inhibit the growth of tumor foci distant from the primary tumor.[63] The suggestion that a cryoimmunological benefit may exist initially came from clinical observations that patients being treated with cryosurgery showed evidence for an effect distant from the primary tumor.[36,64–66] These observations prompted several laboratory and clinical studies that support the notion that cryosurgical destruction can augment the host's immune response to tumor tissue.[36,67–77] Ongoing research is examining whether cryoablation of early-stage breast cancer will stimulate a clinically relevant immune response.

In conclusion, cryoablation can safely and efficiently treat small primary breast cancers. It can be performed in an office-based setting with only local anesthesia and with minimal side effects or discomfort. Ultrasound provides excellent visualization and control of the ablation and allows for adequate skin protection so that even tumors close to the skin can be treated without complications. Proper patient selection and probe placement is crucial to successful ablation. Patients with invasive breast cancer <1.5 cm without invasive lobular or colloid cancers or an extensive intraductal component are good candidates for cryoablation. Assessing margin status and the presence of noncalcified DCIS in the healthy-appearing tissue surrounding the tumor remains a dilemma for all ablative technologies. Further research is necessary to evaluate the most appropriate clinical use of cryoablation in the treatment of early-stage breast cancer.

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