

Status of Intraductal Therapy for Ductal Carcinoma in Situ

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Abstract The intraductal approach is particularly appealing in the setting of ductal carcinoma in situ (DCIS), a preinvasive breast neoplasm that is thought to be entirely intraductal in its extent. Based on an emerging understanding of the anatomy of the ductal system as well as novel techniques to leverage the access accorded by the intraductal approach, researchers are actively exploring how ductal lavage, ductoscopy, and intraductal infusion of therapeutic agents may enhance breast cancer treatment. Both cytologic and molecular diagnostics continue to improve, and work is ongoing to identify the most effective diagnostic biomarkers for DCIS and cancer, although optimal targeting of the diseased duct remains an important consideration. Ductoscopy holds potential in detection of occult intraductal lesions, and ductoscopically guided lumpectomy could increase the likelihood of a more comprehensive surgical excision. Exciting pilot studies are in progress to determine the safety and feasibility of intraductal chemotherapy infusion. These studies are an important starting point for

future investigations of intraductal ablative therapy for DCIS, because as our knowledge and techniques evolve, it is likely that DCIS may be the target most amenable to treatment by intraductal therapy. If such studies are successful, these approaches will allow an important and meaningful transformation in treatment options for women diagnosed with DCIS.

Keywords Carcinoma in situ (DCIS) · Preinvasive cancer · Breast cancer · Intraductal therapy · Ductal lavage

Introduction

Ductal carcinoma in situ (DCIS) is the most common noninvasive lesion of the breast. It is defined as the proliferation of malignant ductal epithelial cells without penetration of the basement membrane. Greater use of screening mammography over the past few decades has led to increasing diagnosis of DCIS such that the incidence of DCIS increased from 1.87 per 100,000 women from 1973 to 1975 to 32.5 per 100,000 women in 2004 [1]. Approximately 25% of breast cancers currently diagnosed by mammography in the United States are classified as DCIS, and it has been estimated that more than 1 million women in the United States alone will be living with a diagnosis of DCIS by 2020 [1]. Despite the increasing prevalence and high mammographic detection rates, the natural history of DCIS continues to be poorly understood.

The linear model of breast carcinogenesis suggests that DCIS is an intermediate step between normal healthy breast tissue and invasive breast cancer [2]. Although a few retrospective studies evaluating misdiagnosed or biopsy only treatment of DCIS indicate that some 14% to 53% of patients will progress to invasive cancer without treatment, virtually no prospective data have been obtained regarding

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progression of untreated disease [3–6]. Substantial data indicate that recurrence rates for patients with DCIS are dramatically reduced after excision or mastectomy. Standard therapy for DCIS is surgical excision, with 31% of women treated with mastectomy, 28% with breast-conserving surgery alone, and 40% with breast-conservation therapy and radiotherapy [7]. In a study by Silverstein et al. [8] comparing local recurrence among patients with DCIS, 7-year disease-free survival rates were approximately 98% in those choosing mastectomy versus 84% in those receiving breast-conservation surgery with radiation. However, this same study, as well as others, has failed to show a survival benefit from any treatment modality used to treat DCIS [8].

Because patient perception of the risk of developing local recurrence or dying of breast cancer does not differ significantly whether diagnosed with DCIS or invasive breast cancer [9, 10], it can be inferred that patients will continue to pursue aggressive therapies that could pose considerable emotional and physical impact. Recent advances in technology and the development of new treatment modalities for DCIS have exploited the intraductal nonsystemic nature of DCIS by focusing on treatments specifically targeted to intraductal delivery. It is hoped that as these approaches are developed, patients will be provided less invasive treatment options for this disease. The intraductal approach includes procedures and technologies such as nipple aspiration, ductal lavage, ductoscopy, and most recently, the introduction of antineoplastic treatment agents directly into the ductal-alveolar unit for both diagnosis and treatment of DCIS. This review focuses on the intraductal approach to the diagnosis and treatment of breast cancer, with a specific emphasis on its role in the management of DCIS.

Breast Ductal Anatomy

The study of breast duct anatomy dates back to 1840, with Cooper's studies injecting colored wax into the ducts of over 200 breasts of women who died during lactation [11]. He described human breast tissue as organized into separate lobes consisting of one central duct, its peripheral branches, and associated glandular tissue [11]. Subsequent to this report, a considerable number of studies using histologic, ductal, and imaging approaches have been conducted in an effort to describe the number and arrangement of ducts composing the human mammary gland [12].

Reports of breast and nipple duct anatomy vary depending on whether the study focused on openings on the nipple that can be cannulated *in vivo* or *in vitro* and followed into functional ducts in the breast or the number of duct profiles seen on cross-sections of the nipple. Original histologic

studies and anatomy texts describe breast anatomy as radially symmetrical lobes with 15 to 20 central ducts. Ductogram studies by Sartorius and Smith demonstrated 5 to 9 ducts per breast, whereas Teboul and Halliwell described 15 to 20 ducts converging on 5 to 8 milk pores in a series of more than 6,000 breasts studied via ultrasound and ductoscopy [13]. Moffat and Going's [14] three-dimensional (3D) computer model of a single autopsy breast traced 10 complete nonanastomosing ductal systems. Using six separate approaches, Love and Barsky [15] found that more than 90% of the nipples examined in their studies contained 5 to 9 ductal orifices. The distribution consisted of nonanastomosing central ducts that travel back toward the chest wall and peripheral ducts that drape over the central ducts in a radial fashion [15].

As a result of these and similar studies, it is now generally accepted that the ductal tree is a nonanastomosing system comprised of central ducts leading back to the chest wall and peripheral ducts draping over the central ducts. However, discrepancies remain regarding the number of ducts found on histologic cross sections and 3D image modeling versus the number of ducts visible and available for cannulation at the nipple surface. It still remains unclear whether these inconsistencies represent ductal branching close to the nipple surface or ducts with associated sebaceous glands, all of which have implications for intraductal approaches to management [16, 17].

Intraductal Lavage

The study of breast ductal fluid via nipple aspiration began in the 1950s, but the low yield of epithelial cells for cytologic analysis essentially precluded it as an efficient tool for diagnosis. In 2001, Dooley et al. [18] demonstrated significant success in producing cytologically evaluable fluid in ductal lavage specimens compared with nipple aspiration. The median epithelial yield from nipple aspiration was 120 cells per breast, whereas the yield with ductal lavage was 13,500 cells per duct [18]. Like nipple aspiration, ductal lavage is based on the principle that invasive breast cancer develops via a linear progression through intraductal precursor lesions, and that these lesions are located in the fluid-containing ducts. Accordingly, many have sought to discover whether ductal lavage may have a particular role in both diagnosis and treatment of intraductal cancers, which should be especially accessible via intraductal strategies.

A number of long-term follow-up studies have confirmed that the presence of ductal cellular atypia constitutes a risk factor for breast cancer. In a 17-year follow-up study, Page et al. [19] found that women with atypia on biopsy

had 5.3 times the risk of developing invasive disease compared with women with nonproliferative lesions on biopsy. Wrensch et al. [20, 21] associated cellular atypia in nipple aspiration fluid with a 4.9-fold higher relative risk than those women who produced no nipple fluid. Fabian et al. [22] used periareolar fine needle aspiration to assess women for the presence of atypia and found that 15% of women with an elevated Gail risk and atypia developed cancer compared with 4% of women with similar Gail risk but no atypia. Thus, the utility of ductal cytology in breast cancer risk stratification has been established, but the accuracy of ductal lavage for diagnosis of DCIS and invasive cancer remains a question. Investigators have evaluated the potential of both cytology and biomarker assessment of lavage fluid in this regard.

Cytology of Intraductal Aspirates

Because of the association of nipple aspirate fluid production and breast cancer risk, an assumption was made that fluid-yielding ducts were those most likely to harbor disease, and lavage of these ducts would yield cells with cytologic atypia corresponding to histologic abnormalities. However, multiple studies assessing the utility of cytologic evaluation have concluded that the ducts with precancerous or cancerous lesions are not necessarily more likely to yield fluid, and in addition, that the low sensitivity in detecting high-grade atypical cells makes cytologic evaluation of ductal lavage specimens a low-yield tool for assessing risk or identifying existing in situ or invasive breast cancer.

In women with known cancer undergoing mastectomy, Khan et al. [23] found that only 38% of cancer-containing ducts yielded ductal fluid for analysis, and Maddux et al. [24] determined that atypia rates were similar for both fluid-producing and dry ducts (19% vs 15%). In Khan et al.'s study [23], only 36% of the fluid-containing ducts yielded ductal lavage specimens with marked atypia or malignancy, thus making the overall sensitivity for detecting cancer approximately 13%. Brogi et al. [25] showed a similar sensitivity of 14% in their study of premastectomy ductal lavage on 26 cancer-containing breasts. Although a recent study by Wood et al. [26] found that nipple aspiration fluid was more commonly expressed in cancerous compared with unaffected breasts, overall sensitivity for detecting marked atypia or malignant cells in affected breasts was similar to previous studies, at approximately 17%.

Sartorius et al. [27] had proposed that with increasingly large invasive cancers, the ducts might become obstructed and epithelial cells with higher-grade atypia may extravasate into surrounding tissues rather than shed into the ductal system. If this were the case, early noninvasive lesions such as DCIS would be more likely to yield atypical epithelial

cells in lavage specimen. Interestingly, Chatterton et al. [28] did find that the presence of DCIS in the central ducts close to the nipple and the size of DCIS were independent predictors of atypical cytology, but most of the samples demonstrated only mild atypia on cytologic evaluation. In a follow-up study, Khan et al. [29] included only women with calcifications on mammogram, thus increasing the probability of DCIS lesions. Of the 10 women diagnosed histologically with DCIS, only one woman produced nipple fluid from a DCIS-bearing duct, and the cytology was benign [29]. These findings corroborate results of the earlier studies and suggest low overall utility for cytologic evaluation of ductal lavage, even in the setting of known DCIS.

Molecular Biomarkers in Lavage Fluid

Due to the relatively low yield of cytologic evaluation for breast cancer diagnosis, many groups have now focused on molecular biomarkers in ductal lavage fluid to identify characteristics specific to precancerous and cancerous lesions of the breast. Current studies analyzing genetic, epigenetic, and proteomic attributes of cells in ductal lavage fluid have suggested that biomarker analysis may be a more sensitive tool than cytology alone, because molecular biomarker changes often precede morphologic alterations and their detection might lead to earlier identification of cells at risk for malignant change.

Some investigators have used fluorescence in situ hybridization (FISH) to detect genomic abnormalities by identifying aneusomy of chromosomes within ductal lavage cells, as changes in copy numbers of chromosomes 1, 8, 11, and 17 have been shown to be associated with both preinvasive and invasive breast lesions. King et al. [30] described aneusomy for these chromosomes in 71% of the specimens from malignant cases and in 11% from benign cases, for a sensitivity of 71% and specificity of 89%. Krishnamurthy et al. [31] evaluated the utility of using FISH as an adjunct to cytologic evaluation. They confirmed chromosomal aneuploidy in all of the malignant and markedly atypical cases, but found aneusomy in only one case described by cytology as mildly atypical. The authors concluded that FISH-based detection of chromosomal aneuploidy could potentially be used as an adjunct to cytologic evaluation in confirming both benign and malignant diagnoses [31]. Adduci et al. [32] utilized array-based comparative genomic hybridization (CGH) in primary surgical specimens then tested matched ductal lavage specimens with region-specific FISH probes to determine whether similar chromosomal alterations were present in both specimens. Although only 11% of cytology samples were characterized as malignant, 55% of samples showed biomarker changes that were identical to those

found in the primary surgical specimen. This study clearly demonstrated the increased sensitivity of molecular probes over cytology for detection of malignancy in ductal lavage samples [32].

Genomic and epigenomic markers have also been examined. Using quantitative fluorescence image analysis, Zhang et al. [33] evaluated lavage specimens from women with both benign and malignant findings and demonstrated 100% sensitivity in detecting cancer using DNA5cER and G-actin expression. Others have shown that evaluation of methylation may also have more sensitivity for cancer detection than cytology: Krassenstein et al. [34] used a panel of six loci to detect hypermethylation of CpG islands in matched tissue and nipple aspiration fluid samples. They detected hypermethylation in all of the malignant tissue and 82% of the fluid samples [34]. Fackler et al. [35] utilized quantitative multiplex methylation-specific polymerase chain reaction (QM-PCR) to evaluate promoter methylation events in a panel of genes believed to be associated with increased breast cancer risk. In their evaluation of QM-PCR versus cytologic evaluation, QM-PCR had double the rate of detection of cancer cells.

Proteomic analyses using the surface-enhanced laser desorption and ionization-time of light/mass spectrometry (SELDI-TOF-MS) technique have also been conducted on lavage fluid with success. Sauter et al. [36] prospectively collected nipple aspiration fluid from women scheduled for diagnostic breast surgery and identified three protein peaks associated with the pathologic categories of atypical ductal hyperplasia, DCIS, and invasive disease. With identification of additional proteins, this method may become a new tool for tumor detection.

At this time, the overall utility of nipple fluid as a tool for risk assessment and early detection of breast cancer remains controversial. Technical limitations of cannulation include duct perforation and lavage of non-cancer-containing ducts. Analysis of ductal fluid by cytology has been shown to have low sensitivity, with ambiguous predictive value associated with findings of mild atypia. However, the potential use of molecular biomarkers as a comprehensive panel or as an adjunct to cytologic analysis

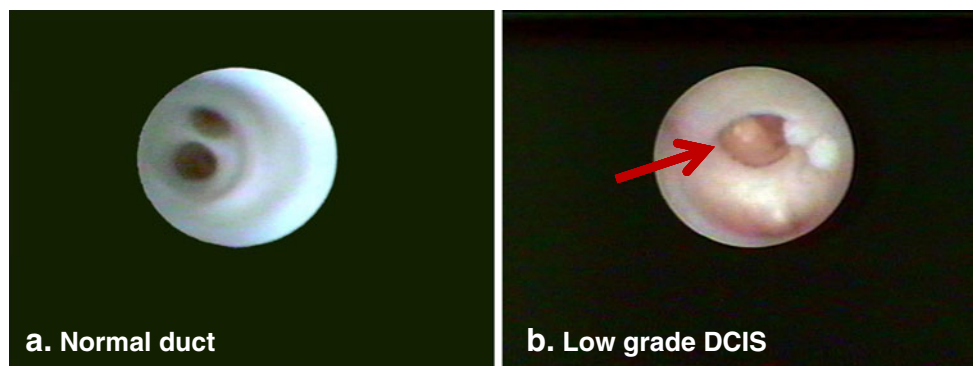
will continue to improve the diagnostic utility of ductal lavage as a minimally invasive method for risk assessment and early detection of breast cancer lesions.

Ductoscopy

Clearly, another way to exploit the access provided by the ductal system is through direct endoscopic visualization of the ductal tree, which may afford a novel approach to intraductal lesions such as DCIS. Although mammary ductoscopy (MD) has not yet garnered widespread use, tremendous improvements in endoscope technology in the past decade have allowed for unprecedented visualization and access to the intraluminal duct (Fig. 1). Both in the United States and Asia, MD has had the greatest utility in the setting of pathologic nipple discharge. Currently available endoscopes measure 0.55 to 0.9 cm in external diameter, often with a working side port that allows for snaring and extraction of ductal lesions. Investigators have shown that MD-guided extraction is effective for benign intraductal papillomas, but less effective in removing malignant lesions, including DCIS. Uchida et al. [37] have suggested two reasons for this observation, including a stiff and noncompliant ductal wall that is resistant to forceps biopsy, and the distal location of malignant lesions, many of which are located in the peripheral terminal ductal-lobular units.

In evaluation of abnormal nipple discharge, ductoscopy has been shown to be of benefit by avoiding the need for preoperative ductogram. One series reported that 61% of ducts with discharge had abnormal endoscopic findings that were surgically excised with ductoscopic guidance [38••]. Of the six cancers diagnosed, four had had negative preoperative radiographic work-up. In patients with known DCIS or breast cancer, ductoscopic guidance has been shown to improve reexcision rates. Dooley et al. [39••] have had extensive experience with this technique, and in their hands, lumpectomy for early-stage cancers guided by ductoscopy had a markedly reduced likelihood of local failure compared with standard lumpectomy techniques. As scope technology continues to improve, ductoscopy may

Fig. 1 Intraductal endoscopy. Current endoscopic technology has allowed high resolution visualization of intraductal anatomy and intraluminal abnormalities. **a** Normal duct, demonstrating smooth ductal walls and ductal bifurcation. **b** Exophytic lesion seen on ductoscopy of a duct with pathologic nipple discharge. Pathology of the excised lesion showed low-grade ductal carcinoma in situ (DCIS)



prove to be a useful localization option to guide resection of nonpalpable intraductal cancers.

Intraductal Therapy

Because most breast cancers arise from ductal epithelial cells, enormous opportunities exist in targeting therapy directly into the ductal system. Additionally, intraductal therapy is less likely to cause systemic toxicity and adverse effects. Currently, there are a number of phase 1 feasibility and safety trials evaluating the intraductal approach to breast cancer prevention and treatment.

Current clinical trials are based on intriguing initial animal studies by Murata et al. [40] that involved intraductal administration of the active tamoxifen metabolite 4-hydroxytamoxifen (4-OHT) and pegylated liposomal doxorubicin (PLD) into transgenic mouse models of breast cancer. Results from the study demonstrated that not only was the intraductal administration of chemopreventive or chemotherapeutic drugs significantly more effective in preventing tumor development and promoting tumor regression, but there were also lower circulating levels of the agents and, as a result, no evidence of systemic toxicity. 4-OHT was found to be as effective as subcutaneous tamoxifen in the prevention of tumors, and intraductal administration of PLD caused complete regression in 24 of 25 tumors with a tumor-free 3-month follow-up period. PLD was also found to be protective against tumor formation. Toxicity studies showed no myelosuppression and peak levels of drug were significantly lower for intraductal versus intravenous injection.

As a result of the encouraging efficacy and safety data from these animal studies, a number of clinical trials are

being conducted to explore the possibility of intraductal therapy in humans, primarily in the setting of DCIS or early-stage invasive cancer. Stearns et al. [41] at Johns Hopkins University School of Medicine have completed a phase 1 trial to determine the feasibility, safety, and maximum tolerated dose of PLD administered into one duct of women awaiting mastectomy. Preliminary data reveal that doxorubicin concentrations were not detectable in plasma, nipple aspirate fluid, or breast tissue [41]. Although histologic findings have not yet been reported, the safety of this intervention in women awaiting surgery for cancer appears promising.

In a similar Phase 1 study, Love and colleagues [42, 43] conducted a dose-escalation study in Beijing, China to determine safety and evaluate histopathologic response to either intraductal carboplatin or PLD instilled into 5 to 8 ducts in 32 women 2 to 7 days prior to mastectomy for breast cancer. Three dose levels were used, with the highest level approaching the dose used intravenously. At the highest doses of PLD, patients experienced tenderness and mild erythema and breast swelling, but no serious adverse events were noted. In the carboplatin group, both inflammatory responses and epithelial changes increased in a dose-dependent fashion. In the PLD group, no inflammatory changes were seen, but there was a marked increase of epithelial response to PLD treatment compared with the carboplatin-treated patients, including epithelial attenuation in the terminal ductal-lobular units (Fig. 2).

In a study directly targeted to test the practicality and efficacy of intraductal PLD treatment for DCIS, Love and Mahoney [44••] are testing intraductal PLD in the neoadjuvant setting. In patients with core biopsy-proven DCIS, 20 mg of PLD (10 cm³) is injected intraductally followed

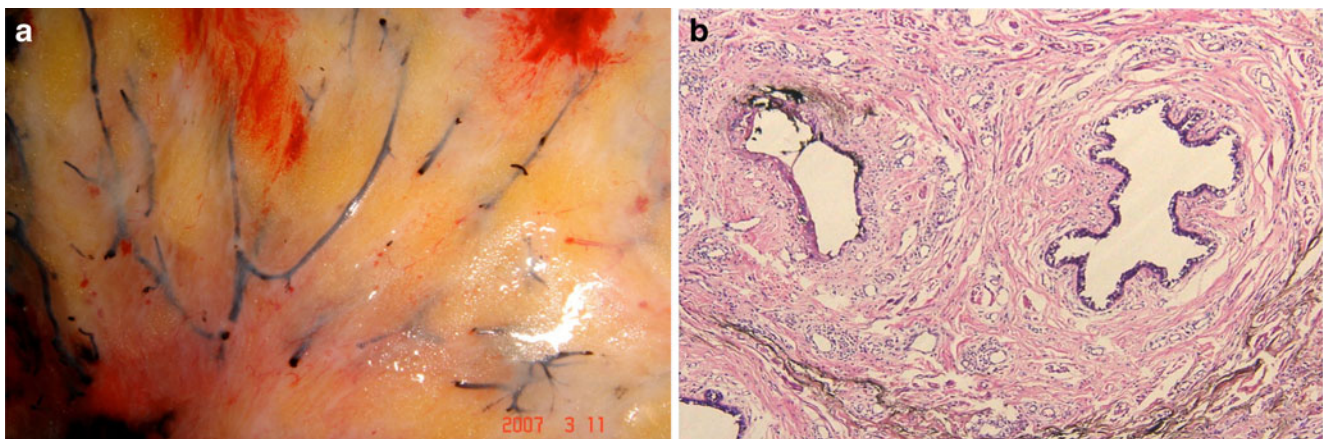


Fig. 2 Mastectomy specimen in a patient treated with intraductal pegylated liposomal doxorubicin (PLD). All patients in the study underwent surgery 2 to 5 days following PLD administration. Cannulated ducts were concurrently injected with dye to enable identification of treated ducts on pathologic evaluation. **a** Surgical

specimen with delineation of treated ducts by dye. The extent of ductal branching is clearly seen. **b** Hematoxylin and eosin (H&E)-stained section of treated duct (*left*) adjacent to untreated duct (*right*). The treated duct shows signs of marked epithelial atresia in response to PLD injection

by a 6-week observation period before scheduled surgery. The study end points are regression of the lesion on surgical pathology, decreased markers in ductal fluid, and changes on MRI before and after treatment. Thus far, four patients have received the full drug dose into the duct harboring DCIS, with two women reporting mild to moderate breast inflammatory reactions that responded to anti-inflammatory medications. Initial evaluation of histology of surgical specimens has confirmed a tissue response of inflammation, squamous metaplasia, and fat necrosis [44••]. This trial continues to accrue patients, and the investigators plan to collect data on tissue markers in the DCIS as well as in the cell pellet specimens collected at the time of PLD injection. Although women in this study will undergo surgery, the long-term implications of the trial may be to facilitate future treatment of DCIS with intraductal therapy alone. In addition, if this approach is effective in stripping cancer precursor cells from the ducts, intraductal injection of ablative agents in unaffected, high-risk women may allow for true loco-regional chemoprevention.

Ductal Lavage for Monitoring DCIS

Lavage biomarkers have been evaluated as potential indicators of response to systemic treatment, particularly in regard to analysis during repeat sampling in chemopreventive studies. Bhandare et al. [45] evaluated ductal lavage specimens from 145 high-risk women and found significant positive correlations between atypical cytology, epithelial cell number, and levels of the estrogen-related biomarkers Ki-67, estrogen receptor α , and cyclooxygenase-2. As a follow-up study, these investigators conducted a phase 2 trial assessing the utility of ductal lavage to measure biomarkers of tamoxifen action [46••]. Of 182 women with Gail risk higher than 1.7% recruited to the study, 63% underwent entry and repeat ductal lavage and 47% had sufficient cells for analysis at both the initial and the follow-up lavage; 46 women chose observation and 39 women chose tamoxifen. The expected reduction in estrogen-related biomarkers was observed for Ki-67 and estrogen receptor α , but not in cyclooxygenase-2 in the tamoxifen-treated group. Additionally, cytologic findings showed a trend toward improvement in the tamoxifen group. Although the biomarker and cytologic profiles improved during the tamoxifen treatment, and thus seem to suggest clinical utility in this method of monitoring, the attrition rate and difficulty with obtaining adequate samples on repeat lavage make it necessary to re-evaluate this method [47]. However, as DCIS treatment evolves towards less invasive management strategies, women opting for active surveillance for DCIS may in the future be offered ductal lavage to monitor for cancer progression or response to systemic treatments.

Conclusions

The intraductal approach to the breast offers a unique opportunity to diagnose, study, and treat DCIS, a highly prevalent preinvasive lesion that is entirely confined within the ductal system. Additional studies are necessary to further elucidate the ductal system of the breast as well as the common anatomic distribution of DCIS within the system. The utility of intraductal lavage as a tool in the diagnosis and treatment of DCIS will hinge upon technologic advances that will allow for easier and more specific cannulation of affected ducts and continued research to establish a panel of biomarkers in addition to cytology that can reliably indicate disease. Thus far, small pilot trials have demonstrated that intraductal therapy is feasible and can be safely administered, and we await clinical trials of intraductal therapy alone to better evaluate the potential of this new and exciting therapeutic modality. The intraductal approach is of particular interest for disease confined to the ducts that does not require systemic therapy. It is hoped that continued advancements will offer women with breast cancer a minimally invasive way to detect breast disease, as well as in future allow for an important adjunct to surgical treatment for DCIS.

Disclosure No potential conflicts of interest relevant to this article were reported.

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