Trastuzumab not for ductal carcinoma *in situ*?

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Ductal carcinoma *in situ* (DCIS) is a preinvasive breast lesion accounting for approximately 30% of all newly detected breast cancers in the US. DCIS has been separated into two groups by architecture (comedo versus noncomedo) and nuclear grade. The expression of biological markers in DCIS, however, would reflect the true biologic potential of the lesion. Patients with estrogen receptor (ER)-negative, human epidermal growth factor-2 (HER-2)-positive DCIS pose a treatment challenge. They are not candidates for tamoxifen; trastuzumab has an undetermined role in DCIS. In this report, we present a case of a 45-year-old woman diagnosed with invasive breast cancer and ER-negative/HER-2-positive DCIS who developed recurrence and progression of DCIS as manifested by a new palpable mass while receiving trastuzumab as part of adjuvant treatment for invasive breast cancer. The potential clinical implications are discussed. *Anti-Cancer Drugs* 18:1231–1235 © 2007 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2007, **18**:1231–1235

Keywords: ductal carcinoma *in situ*, trastuzumab

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Received 22 February 2007 **Revised form accepted** 30 May 2007

Introduction

Ductal carcinoma *in situ* (DCIS) is a preinvasive breast lesion. It represents a significant percentage of newly diagnosed breast cancer cases, largely as a result of earlier diagnoses through mammographic screening [1,2]. Common clinicopathologic factors that increase the recurrence risk for DCIS include lesions of high nuclear grade, involved surgical excision margins, presence of comedo necrosis and a younger age at diagnosis [2–4]. High-grade DCIS is more aggressive and is more likely to be associated with invasive cancer than the nonhigh-grade type. High-grade DCIS treated conservatively is also more likely to recur locally than is nonhigh-grade DCIS [5,6]. Traditionally, DCIS has been separated into two groups by architecture (comedo versus noncomedo) and nuclear grade. This separation is an oversimplification and might not reflect the true biologic potential of the lesion. Recently, studies have focused on the expression of biological markers in DCIS, including hormone receptors, oncogenes, tumor suppressor genes, cell cycle regulators and markers of angiogenesis [7–10]. Information regarding biomarker expression in DCIS has improved our understanding of its biological background, but has not impacted upon patient management decisions. An exception is the relationship between estrogen receptor (ER) expression in DCIS and the use of tamoxifen [11]. Results from the NSAB-B24 study, assessing the relationship between ER expression in DCIS and local recurrence in patients who had been treated by lumpectomy and radiation therapy with or without tamoxifen, showed a significant reduction of local recurrences with the use of tamoxifen in patients whose DCIS was ER-positive [11]. Patients with ER-negative DCIS are not candidates for tamoxifen.

The human epidermal growth factor receptor-2 gene (*HER-2*), also known as c-*erb*B2 or *neu*, is amplified in approximately 25% of invasive carcinomas [12,13]. Overexpression of the amplified gene can be detected by immunohistochemistry (IHC) available as a standard technique in pathology laboratories [14]. In DCIS, *HER-2* overexpression is reported in about 50% of cases and is predominantly associated with high-grade lesions [7,15]. Trastuzumab, a recombinant DNA-derived humanized monoclonal antibody directed against HER-2, has represented a major improvement in the treatment of breast cancers with this genetic abnormality [16,17]. Recent randomized trials evaluating over 10 000 women have shown that the addition of trastuzumab to traditional adjuvant chemotherapy results in a 50% reduction in the risk of recurrence in early-stage invasive breast cancer overexpressing HER-2 [18,19]. The benefit of trastuzumab in DCIS has not been determined. In this report, we present a case of a 45-year-old woman diagnosed with invasive breast cancer and ER-negative/HER-2-positive DCIS, who developed recurrence of DCIS manifested by a new palpable mass while receiving trastuzumab as part of adjuvant treatment for invasive breast cancer. The potential clinical implications are discussed.

Case presentation

A relatively healthy 45-year-old, premenopausal, African American woman presented with a palpable lump in the

in the upper outer quadrant of the left breast. Subsequent workup led to the diagnosis of a T1cN1M0, stage IIA left-breast-infiltrating ductal carcinoma. Histologically, the tumor consisted of DCIS (high-grade comedo type) and areas of invasive ductal carcinoma. The in-situ component constituted 70% of the total tumor and was present in association with, and away from, the invasive component. The invasive component showed poor tubule formation, marked variation in nuclear size, prominent nucleoli, chromatin clumping and 0–5 mitoses/10 high power fields (a total score of 8 points, grade III). The invasive tumor revealed 0% expression of ERs and progesterone receptors (PR). Evaluation of HER-2 expression revealed a score of *2+* by IHC using the chromavision 'ACIS' system (ChromaVision Medical Systems, San Juan Capistrano, California, USA). The HER-2 *neu* gene was amplified by fluorescence in-situ hybridization. The DCIS component showed negative ER and PR expression (Fig. 1a). It, however, showed 3+ HER-2 overexpression by IHC (Fig. 2a). The patient underwent a partial mastectomy, sentinel lymph node dissection (one of three sentinel lymph nodes was positive for metastasis, confirmed with pankeratin immunostain) and subsequent axillary lymph node dissection; a total of two out of 13 lymph nodes were involved with metastatic adenocarcinoma. The margins of resection were not involved by either invasive or the in-situ components. The patient received adjuvant chemotherapy with dose-dense doxorubicin and cyclophosphamide (four doses), followed by paclitaxel (three doses, fourth dose omitted as per the patient's wish owing to grade 2 neuropathy) and radiation therapy. After a period of hesitation due to potential toxicity, the patient finally agreed to receive trastuzumab; this was administered on a 3-week basis at a dose of 8 mg/kg bolus, on a 3-week basis at a dose of 6 mg/kg every 3 weeks, with a plan to continue this for 1 year. The time frame of starting trastuzumab was approximately 1 year from primary diagnosis and surgery (partial mastectomy), i.e. 14 weeks after the completion of chemotherapy and 6 weeks after completion of the radiation therapy. Around 16 weeks after starting trastuzumab, having received six doses, the patient presented with a new palpable left breast mass in the upper outer quadrant at the site of the earlier partial mastectomy. Core biopsy revealed two microscopic foci of high-grade DCIS, no invasive tumor was seen Figs 1b and 2b. She subsequently underwent several lumpectomy cavity reexcisions with residual DCIS being present in each reexcision. This led to mastectomy of the left breast, and trimming of the left inferior and superior skin flaps. Histologically, microscopic foci of DCIS, high-grade comedo type were also detected in the lower outer quadrant of the resected breast. Margins of resection were free of tumor. No invasive component was identified. Following mastectomy, the patient resumed treatment with trastuzumab.

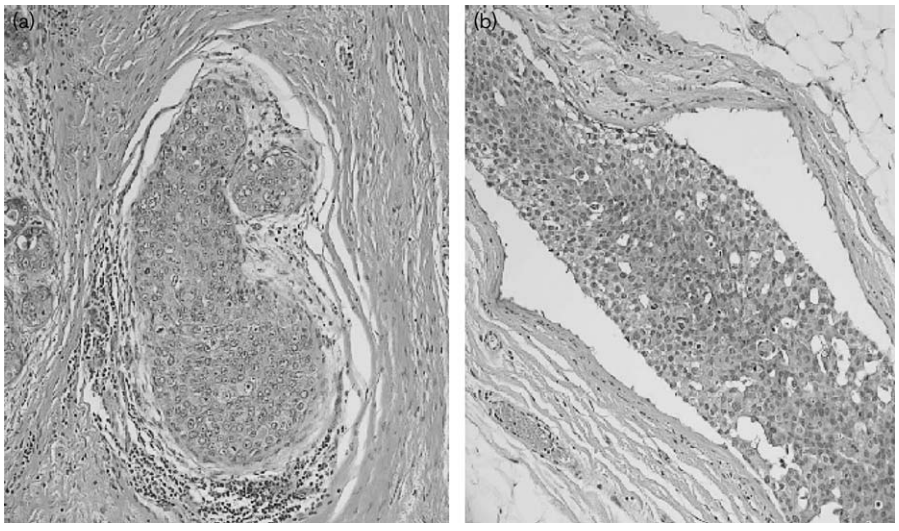
Ductal carcinoma *in situ* breast panel results

Breast biopsy before treatment (DCIS on initial biopsy):

Figure 1a: DCIS is negative (0% nuclear staining) for ER (6F11); negative (0% nuclear staining) for PR (IA6);

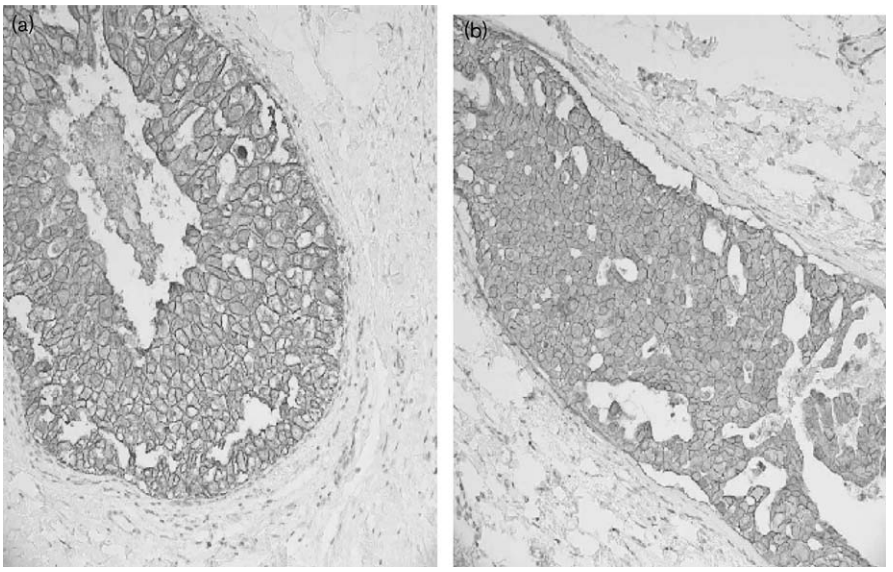
Figure 2a: HER-2 *neu* IHC (CB11) score 3+ membrane staining; Mib-1 35% nuclear staining (high).

Fig. 1



(a) Photomicrographs of sections of DCIS upon initial presentation stained by H&E (the invasive component is not depicted). (b) Photomicrographs of sections of DCIS upon recurrence stained by H&E. DCIS, ductal carcinoma *in situ*; H&E, hematoxylin and eosin.

Fig. 2



(a) Photomicrographs of sections of DCIS upon initial presentation stained by IHC for HER-2, score 3+ membrane receptor staining. (b) Photomicrographs of sections of DCIS upon recurrence stained by IHC for HER-2, score 2+ membrane receptor staining. DCIS, ductal carcinoma *in situ*; HER-2; human epithelial growth factor; IHC, immunohistochemistry.

Breast biopsy after treatment (recurrence):

Figure 1b: DCIS is negative (0% nuclear staining) for ER (6F11); negative (0% nuclear staining) for PR (IA6);

Figure 2b: HER-2 neu IHC (CB11) score 2+ membrane staining; Mib-1 25% nuclear staining (intermediate).

Comment: Positive and negative controls for all assays stained appropriately. Benign breast elements within the biopsy (internal controls) stained positive for ER and for PR.

Discussion

DCIS is the most common type of non-invasive breast cancer and it accounts for approximately 30% of all newly detected breast cancers in the US [1]. DCIS is characterized by proliferation of the epithelial cells that are confined within the mammary ducts. The malignant epithelial cells do not proliferate beyond the basement membrane of the adjacent breast tissue [4]. Traditionally, most patients with DCIS were treated with mastectomy; however, this treatment proved to be too invasive for most of them. Breast-conserving surgery followed by adjuvant radiation and tamoxifen for ER-positive disease is, therefore, the current recommended form of treatment. If left untreated, DCIS can progress to invasive breast cancer in 25–35% of women [7]. Risk factors for recurrence after breast-conserving therapy for DCIS include comedo-type histology, which constitutes 70% of DCIS. Many consist of lesions with HER-2 overexpression, epidermal growth factor receptor (EGFR) expression, absence of ER expression, high nuclear grade, and high proliferation rate [3,4,7]. Given the heterogeneity of the biologic potential of the DCIS lesions and the increased use of breast-conserving therapy for management, the identification of prognostic markers that reliably predict for local recurrence, progression to invasive cancer and resistance to therapy is needed. Various studies have attempted to identify correlations between the expression of biomarkers such as ER, HER-2 and EGFR, and the histopathological features of DCIS. About 75% of DCIS cases are ER-positive; ER expression is most common in low-grade lesions [15]. Approximately 40% of DCIS cases have been reported to express HER-2, most commonly seen in high-grade lesions [20]. HER-2 overexpression in DCIS is an indicator of poor clinical outcome [12,21]. It has been associated with expression of the *bcl-2* and *bcl-x*$_L$ genes, the two main genes of the *bcl-2* gene family that suppress tumor cell death/apoptosis [21]. In a series of 148 DCIS cases from one institution [15], 114 cases (77%) were ER-positive and 42 (28%) showed HER-2 overexpression. ER expression and HER-2 overexpression were reciprocally related in 128 cases (86%). All 74 low and intermediate nuclear-grade DCIS were ER-positive/HER-2-negative. In contrast, the high-grade DCIS lesions were more heterogeneous with regard to the patterns of ER and HER-2 expression. Among the high-grade lesions, 26 (35%) were ER-positive/HER-2-minus, 6 (8%) were ER-minus/HER-2-minus, 28 (38%) were ER-minus/HER-2-positive and 14 (19%) were ER-positive/HER-2-positive. Thus the coexpression of ER and HER-2 was seen only in high-grade DCIS [15].

These differences might help to identify high-risk DCIS cases. ER-minus/HER-2-positive cases, as in our featured case, account for 38% of all DCIS cases [15]. In our case, the MIB1 proliferation index of the DCIS component was also high. In invasive breast tumors, MIB1 index correlates with established methods for assessing tumor proliferation (S-phase fraction and mitotic index) and with the parameters of an aggressive phenotype of the tumor (bcl-2, p53, and cathepsin D) [22]. This can pose a high risk for recurrence despite optimal local treatment.

The identification of high-risk subtypes of DCIS based on biological markers is required. ER-negative DCIS, in particular, poses a treatment challenge. A treatment strategy is greatly needed for DCIS tumors with a high propensity for recurrence and progression. A preclinical study of DCIS xenografts, expressing both HER-2 and EGFR, examined whether the effect of inhibiting EGFR [with the orally active, selective tyrosine kinase inhibitor (TKI) ZD1839] or HER-2 (with a humanized monoclonal antibody directed against an epitope on the extracellular domain of c-erbB2, trastuzumab) would result in decreased proliferation and increased apoptosis in DCIS [23]. DCIS tissues from 18 women with DCIS were implanted into 16–20 athymic nude mice per experiment. Two weeks after the implantation, antibody treatments began and xenografts were removed on days 14, 21 and 28. The study observed that EGFR–TKI treatment decreased cell proliferation and increased apoptosis in EGFR-positive DCIS, whereas treatment of HER-2-positive DCIS with HER-2 antibody did not inhibit proliferation. The study suggests that an orally active and selective EGFR–TKI like ZD1839 might have the potential to be used as adjuvant therapy in DCIS, but raised doubts against the use of trastuzumab in HER-2-positive DCIS [23]. In this case, the patient developed DCIS recurrence while receiving trastuzumab. Although the addition of trastuzumab to traditional adjuvant chemotherapy results in a 50% reduction in the risk of recurrence in early-stage invasive breast cancer over-expressing HER-2 [18,19], the role of trastuzumab has not been determined in DCIS and needs further evaluation. Other potential therapeutic targets in DCIS can include the inhibition of cyclooxygenase type 2 (COX-2). COX-2 expression is upregulated in in-situ breast cancer and is associated with surrogate markers of an aggressive DCIS phenotype, including nonestrogen-regulated signaling pathways [24]. Moreover, some evidence points to the possibility that COX-2-related HER-2 and vascular endothelial growth factor (VEGF) over-expression might mediate the carcinogenic effects of COX-2 [25]. More specifically, overexpression of COX-2 has been shown to correlate with the overexpression of VEGF and enhanced tumor angiogenesis [26]. In a retrospective study on archival samples of 187 cases with DCIS, COX-2 expression using IHC was determined very clearly in 87% of the cases. COX-2 expression was associated with higher nuclear grade and ER negativity. Moreover, COX-2 positivity was significantly higher in HER-2-positive DCIS (82%) than in HER-2 negative tumors (48%, $P < 0.0001$) [27]. In another study of 49 patients with DCIS, a significant positive correlation was observed between COX-2 IHC, VEGF score and HER-2 expression. The more invasive breast cancers expressed a higher percentage of COX-2. This suggests that COX-2 might be directly correlated with a contemporaneous increase in VEGF and HER-2 expression in DCIS [28]. COX-2, being highly expressed in DCIS, might therefore play a part in the molecular pathway implicated in the progression of breast cancer and may provide a rationale for targeting COX-2 in DCIS [28]. Indeed, the use of nonsteroidal antiinflammatory drugs has been suggested to have a role in protecting against breast cancer [29]. The reported cardiovascular risk with the use of COX-2 inhibitors has, however, dampened any enthusiasm for the use of these drugs in preclinical conditions [30]. Other suggested potential target pathways include the expression of the protooncogene c-KIT (CD117), which was reported in about 50% of DCIS. This protooncogene might define a subset of poorly differentiated, HER-2-positive cases with decreased expression of steroid hormone receptors, comedonecrosis, and a solid growth pattern [31]. The implications of c-KIT and HER-2 coexpression in DCIS should be further evaluated [31].

In summary, we presented a case of a patient with invasive breast cancer and DCIS, who developed a likely recurrent DCIS, possibly with new foci, while receiving trastuzumab. DCIS is a heterogeneous spectrum of lesions, varying in morphology, clinical presentation and prognosis. Differential biomarker expressions in DCIS should be taken into consideration when designing treatment strategies. DCIS might be better defined by its distinct biological background, which should, in turn, impact upon patient-management decisions similar to the approaches used in invasive breast cancer. More studies need to be conducted to determine appropriate therapies for high-risk DCIS, including ER-negative/HER-2-positive tumors. Trastuzumab might not halt the progression of such tumors; however, it needs to be evaluated further. Integration of translational studies into clinical trials is required.

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