LETTER TO THE EDITOR

A Clinicopathologic Study on Patients with Endometrial Cancer after Adjuvant Tamoxifen Treatment for Breast Cancer: A Single Center Experience

To the Editor:

Tamoxifen (TAM) is a nonsteroidal, selective estrogen receptor (ER) modulator that has potent anti-estrogenic activity in the breast while displaying weak estrogen activity in the endometrium. It is the hormonal treatment of choice for postmenopausal breast cancer patients with positive ER over the past two decades and its use has been convincingly shown to improve the disease-free survival as well as overall survival (1).

One of the most significant and deleterious side effects of TAM treatment in postmenopausal women with breast cancer appears to be its proliferative effect on the endometrium. Overall endometrial pathologies, including hyperplasia, polyps, carcinoma and sarcoma have been identified in up to 36% of postmenopausal breast cancer TAM-treated patients. The frequency of these endometrial pathologies was found to be significantly higher among postmenopausal breast cancer TAM-treated patients, compared with postmenopausal breast cancer non-TAM-treated patients (2–4). It was also found to be more common among healthy women who receive preventive TAM treatment, compared with healthy non-TAM-treated women (5). The pathogenic mechanism for the development of TAM-associated malignant endometrial tumors has not yet been clearly defined.

The Stockholm Trial showed a continued divergence of the cumulative incidence curves of endometrial cancer for the TAM-treated and control groups even several years after cessation of TAM treatment (6). Many other reports published in recent years have demonstrated a significant positive association between longer duration of TAM treatment and the appearance of endometrial cancer. The relative risk (RR) for endometrial cancer, when compared with non-TAM-treated patients, with gradual increase in duration of TAM treatment, is increasing up to 60 consecutive months (7). It has been synonymously reported that endometrial pathologies are associated with high cumulative doses of TAM administered to postmenopausal breast cancer patients. Women who received 20 mg of TAM daily developed endometrial pathologies after longer periods of treatment compared to those who were treated with 40 mg of TAM daily (8).

During the last decade efforts have focused on attempting to identify cytokinetic or molecular events that correlate with the malignant potential of endometrial cancers. Several investigators have evaluated the expression of oncogenes and tumor suppressor genes. Furthermore indicators of cell proliferation have been evaluated.

Estrogen stimulates cellular proliferation regulated by the ER, whereas progesterone inhibits cellular growth and induces differentiation regulated by the progesterone receptor (PR). It is well recognized that ER and PR are important prognostic factors for endometrial carcinoma. High levels of ER and PR are directly correlated with a lower tumor grade, less myometrial invasion and a lower incidence of lymph node metastases (9).

Members of the human epidermal growth factor receptor family (HER) of receptor tyrosine kinases play a critical role in both development and oncogenesis. The latter is suggested by the frequent overexpression of HER-2, EGFR, HER-3 and HER-4 in a wide variety of tumors including breast, colorectal, ovarian, and non-small cell lung cancers. The biological activities of the HER family are exerted through various ligand-receptor and receptor-receptor interactions. One receptor that plays a central role in this signaling network is HER-2/Neu (erb2), which is considered the preferred heterodimerization partner for other members of the HER family and responsible for regulating cell growth and differentiation (10). Ki-67 is a marker of cell proliferation. It is expressed in the nucleus of cells that are actively undergoing cell proliferation (i.e., not in G0 or early G1) (11). The balance between cell proliferation...
and programmed cell death (apoptosis) is fundamental to the functioning of the menstrual cycle. It is increasingly believed that apoptosis and its control play an important, even pivotal, role in the development of endometrial cancer.

The aim of our retrospective study was to determine the immunocytochemical findings, i.e., the expression of ER and PR, cerbB-2 and Ki-67 on a small number of endometrial carcinomas developed in breast cancer patients during or after TAM treatment, and to describe the clinical findings and prognosis of these patients. Eleven TAM-related endometrial cancers were identified and analyzed retrospectively from the archives of the Department of Obstetrics and Gynecology of the University of Patras Medical School.

MATERIAL AND METHODS

Between May 1991 and December 2004, 114 women with histologically confirmed endometrial cancer were referred to our Department of Gynecologic Oncology. Among them, 11 cases of endometrial carcinomas were found in women with a history of breast carcinoma and adjuvant treatment with TAM. The patients were diagnosed with endometrial cancer, at least 12 months after cessation of TAM treatment for breast cancer.

All 11 patients underwent a total abdominal hysterectomy with bilateral salpingo-oophorectomy. Lymph node sampling and cytologic test of the peritoneal fluid was performed in all patients.

Histopathology and Immunohistochemistry

All tissue specimens were stained with hematoxylin–eosin. Staging was determined using the surgical staging system for endometrial cancer established by the International Federation of Obstetrics and Gynecology (FIGO). Tumor histologic classification was performed using the criteria of the World Health Organization (WHO).

Formalin-fixed paraffin-embedded tissue sections, representative of the tumor in each case, were immunostained using the biotin-streptavidin peroxidase method. Monoclonal antibodies against ER, PR (Novocastra, Newcastle, United Kingdom), cerbB-2 (Biogenex, San Ramon, CA) and Ki-67 (Dako, Glostrup, Denmark) were used (Figs. 1–3). Microwave pretreatment was used to unmask epitopes. Diaminobenzidine was used as a chromogene. Sections were counterstained with hematoxylin. Tumors were scored by the proportion of tumor cells stained.

Statistical Analysis

Statistical analyses were performed using the SPSS-12 program for Windows. The chi-squared test was used to assess the association between categoric variables. The survival time was calculated from the date of initial surgery for endometrial cancer. The cumulative survivals were determined using the Kaplan–Meier product-limit method.

RESULTS

Clinical Findings

The clinical and treatment characteristics of the 11 endometrial cancer patients treated with TAM for their breast cancer are summarized in Tables 1 and 2. The median age at diagnosis of endometrial cancer was 65.8 years (range 49–80 years) and the median interval between diagnoses of two cancers was 104.2 months (range 12–251 months).

All patients had been treated with 20 mg of TAM daily. The median duration of TAM use for the cases was 104.2 months (range 12–251 months), with two patients having taken TAM for less than 24 months, one patient between 24 and 59 months, and 8 patients for more than 60 months. The median follow-up of this group was 50.1 months (range 9–109 months). None of the patients had been on estrogen replacement therapy.

Histopathologic Findings

The histopathologic findings of the TAM-related endometrial cancers are summarized in Table 3. The endometrial cancers included eight endometrioid adenocarcinomas, two adenosquamous carcinomas, and one papillary adenocarcinoma.

Immunohistochemical Findings

The immunohistochemical findings of the TAM-related endometrial cancers are summarized in Table 4. ER was positive in five and negative in six cases. PR was positive in five and negative in six cases. CerbB-2 was negative in all cases. Ki-67 expression was moderate to high in all cases.

Prognosis

The patients were followed from 9 to 109 months after surgery for the endometrial cancer, with a mean of 50.1 months.
One patient with stage Ib papillary adenocarcinoma died of endometrial cancer 28 months after surgery. She was ER negative, PR positive, cerbB-2 negative, Ki-67 > 10%. One patient with stage IIa endometrioid adenocarcinoma died of endometrial cancer 14 months after surgery. She was ER negative, PR negative, cerbB-2 negative, Ki-67 < 10%.

Findings of the TAM-related endometrial cancers are summarized in Table 5.

The 5-year cumulative endometrial carcinoma-specific survival in our patients was 72.9%.

The 5-year cumulative endometrial carcinoma-specific survivals for patients <60 years and ≥60 years
of ages at diagnosis of endometrial cancer were 100% and 62.5%, respectively. The 5-year cumulative endometrial carcinoma-specific survival for TAM treatment ≥60 months was 83.3%.

The 5-year cumulative endometrial carcinoma-specific survivals for patients treated with and without adjuvant therapy were 61.4% and 100%, respectively. We could not find any significant correlation between 5-year cumulative endometrial carcinoma-specific survival and expression of ER, PR, cerbB-2, and Ki-67, due to the small number of cases.

### DISCUSSION

A dose of 20 mg of TAM taken daily for 5 years has been shown to decrease the incidence of opposite-side breast cancer in 40% of the breast cancer women and increase the disease-free survival especially in postmenopausal women (12).

Nevertheless, prolonged administration of TAM in breast cancer women in an adjuvant setting, is associated with an increased risk of endometrial cancer. The pathogenic mechanism for the development of TAM-associated malignant endometrial tumors has not been yet clearly defined. TAM may act as an initiator of carcinogenesis via estrogen agonistic activity in the endometrium. Perhaps TAM uses pathogenetic pathways similar to sporadic cancer (12,13). TAM may increase the proliferation of a subset of cells, thereby increasing the likelihood of mutations. Alternatively, it may promote the growth of cells that have already

### Table 3. Histopathologic Findings

| Stage | Case numbers | Percentage (%) |
|-------|--------------|----------------|
| I     | 7/11         | 63             |
| II    | 2/11         | 18             |
| III   | 1/11         | 9              |
| IV    | 1/11         | 9              |

### Table 4. Immunohistochemical Findings

| Stage | Grade | ER+ | PR+ | cerbB2+ | Ki-67 > 10% | Ki-67 < 10% |
|-------|-------|-----|-----|---------|------------|------------|
| I     | I     | 1   | 2   | 1       | 1          | 1          |
| II    | 1     | 2   | 3   | 0       | 0          | 0          |
| III   | 1     | 1   | 2   | 0       | 0          | 0          |
| IV    | I     | 1   | 0   | 1       | 1          | 1          |
|       | II    | 1   | 0   | 0       | 1          | 0          |
|       | III   | 0   | 0   | 0       | 0          | 0          |
|       | IV    | 0   | 0   | 0       | 0          | 0          |

### Table 5. Survival Data

| Case | Age | Stage | Grade | TAM use (months) | Follow-up (months) | Status |
|------|-----|-------|-------|------------------|-------------------|--------|
| 1    | 68  | Ia    | III   | 141              | 109               | NED    |
| 2    | 49  | Ia    | II    | 71               | 97                | NED    |
| 3    | 74  | Ib    | I     | 202              | 98                | NED    |
| 4    | 73  | Ib    | II    | 62               | 93                | NED    |
| 5    | 53  | IIIa  | III   | 94               | 62                | NED    |
| 6    | 60  | Ib    | II    | 112              | 28                | DOD    |
| 7    | 71  | Ila   | I     | 43               | 14                | DOD    |
| 8    | 78  | Ila   | II    | 23               | 15                | NED    |
| 9    | 58  | Ic    | III   | 12               | 12                | NED    |
| 10   | 80  | Ic    | I     | 135              | 9                 | NED    |
| 11   | 61  | IVa   | II    | 251              | 14                | NED    |

NED, no evidence of disease; DOD, death of disease.
sustained mutations. As a result of either (or both) possibilities, TAM exposure could lead to the production of a spectrum of mutations similar to that of sporadic endometrial cancer (14). Another study showed that carcinogenetic effect of TAM maybe due to genotoxic DNA damage (15).

The National Surgical Adjuvant Breast and Bowel Project calculated an annual hazard rate of 1.6 per 1000 women, giving an RR of 2.2 compared with population-based rates of endometrial cancer. Most of the detected cancers in this study were of low grade and low stage, with no differences in the stage, grade or histologic subtype of endometrial cancers found in TAM-treated patients, compared with non-TAM-treated patients (12). This led clinicians to hypothesize that TAM probably promotes an occult endometrial cancer much like continuous estrogen exposure.

However, in recent years other studies have linked TAM to poorly differentiated adenocarcinomas. The meta-analysis by the Early Breast Cancer Trialists’ Collaborative Group implies that TAM may be associated with an even higher incidence of endometrial cancer as well as a phenotypically more aggressive and more advanced cancer resulting in a higher mortality (16). Several investigators have reported that older women with endometrial cancer have frequently deep myometrial invasion and/or poorly differentiated histology (17).

The Stockholm Trial showed a continued divergence of the cumulative incidence curves of endometrial cancer for the TAM-treated and control groups even several years after cessation of TAM treatment (6). Many other reports published in recent years have demonstrated a significant positive association between longer duration of TAM treatment and the appearance of endometrial cancer. The RR for endometrial cancer, when compared with non-TAM-treated patients, with gradual increase in duration of TAM treatment, is increasing up to 60 consecutive months (7). Another study showed that stage III and stage IV endometrial cancers occurred more frequently in long-term (≥60 months) TAM users than in non-users (18). In our study one stage III patient received TAM for 53 months and another stage IV patient received TAM for 61 months. The median duration of TAM use was 104.2 months (range 12–251 months), with eight patients having taken TAM for more than 60 months. We could not find any significant correlation between the duration of TAM use and expression of ER, PR, cerbB-2, and Ki-67, due presumably to the small number of cases.

It has been reported that endometrial pathologies are associated with high cumulative doses of TAM administered to postmenopausal breast cancer patients. Women who received 20 mg of TAM daily developed endometrial pathologies after longer periods of treatment compared to those who were treated with 40 mg of TAM daily (8). In our study all patients received 20 mg of TAM daily.

It is well recognized that ER and PR are important prognostic factors in endometrial carcinoma. High levels of ER and PR are directly correlated with a lower tumor grade, less myometrial invasion, and a lower incidence of lymph node metastases. Patients with ER or/and PR-positive tumors have longer survival times compared to patients with ER and PR-negative tumors. Even patients with metastasis have an improved prognosis with receptor-positive tumors (9). In our study, two patients died because of the disease: one was ER negative-PR positive and the other was ER negative-PR negative.

It has been shown that ER and PR status is significantly related to survival, and demonstrated that when only one receptor could be obtained, PR provided the most helpful information for the majority of patients (19). In our study, we observed that all stage I patients were PR positive, but in more advanced stages most of them were PR negative. High expression of PR was significantly associated with low-grade tumors, which are associated with good prognosis, whereas low expression of PR is a feature of high-grade tumors. This supports previous findings and shows that reduced PR expression is associated with poorer prognosis tumor phenotypes (20,21). In our study we could not find any significant correlation between ER, PR, and grade, but again the number of cases was very small.

The HER family is a growth factor superfamily that includes cerbB1, B2, B3 and B4 receptor proteins. All four are cell-membrane proteins with tyrosine kinase activity (also named RTK). With no direct ligand identified to date, cerbB2 functions as a preferred partner for heterodimerization with other members of the HER family, and thus plays an important role in coordinating the complex ErbB signaling network that is responsible for regulating cell growth and differentiation. Overexpression of cerbB2 has been found to play a role in cellular transformation, oncogenesis and metastasis (10). Opinions in the literature regarding
the prognostic value of cerbB-2 overexpression in endometrial cancer are conflicting, and this molecular marker has been correlated with unfavorable prognosis in some studies (22,23), but not in others (24,25). Possible explanations of the lack of concordance in the prognostic value of cerbB-2 expression among the studies include differences in populations studied, techniques and antibodies used, or interpretation of results. The conflicting results reported in the literature about its possible prognostic role, the lack of independent prediction of patient outcome, the subjectivity in its measurement, and the concerns expressed regarding its reproducibility would minimize the potential role of cerbB-2 as a marker in the preoperative evaluation of patients with endometrial cancer. In our study we found cerbB-2 was negative in all cases.

Ki-67 is a marker of cell proliferation. It is expressed in the nucleus of cells that are actively undergoing cell proliferation (i.e., not in G0 or early G1) (11). However, reports in the literature about the ability of Ki-67 to predict extrauterine disease are conflicting. Ki-67 was significantly associated with prognosis, and it was an independent predictor of disease-related survival. Moderate to strong Ki-67 expression was associated, although not significantly, with extraterine spread of disease (26). In our study Ki-67 expression was moderate to high in all cases.

In summary, our findings suggest that PR may act as a protector in endometrial cancer after TAM treatment for breast carcinoma. However, we must recognize that the number of cases in this study was small, and further investigations are necessary to allow a more focused evaluation of molecular markers status, especially PR, as a prognosticator for endometrial cancer after TAM treatment.

REFERENCES

1. Early Breast Cancer Trials’ Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy: 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. Lancet 1992;339:1205.
2. Neven P, De Muylde Y, Van Belle Y, et al. Tamoxifen and the uterus and endometrium. Lancet 1989;2:375.
3. Coxen I, Rosen DJD, Shapiro J, et al. Endometrial changes with tamoxifen: comparison between tamoxifen-treated and non-treated asymptomatic breast cancer patients. Gynecol Oncol 1994;52:185-90.
4. Kedar RP, Bourne TH, Powels TJ, et al. Effects of Tamoxifen on uterus and ovaries of postmenopausal women in a randomized breast cancer prevention trial. Lancet 1994;343:1318–21.
5. Mauger G, Nardo LG, Campione C, Nardo F. Endometrial lesions after tamoxifen therapy in breast cancer women. Breast J 2001;7:240–44.
6. Rutqvist LE, Johansson H, Signomklao T, Johansson U, Fornander T, Wilking N. Adjuvant tamoxifen therapy for early stage breast cancer and second primary malignancies. J Natl Cancer Inst 1995;87:645–51.
7. Swerdlow AJ, Jones ME, for the British Tamoxifen Second Cancer Study Group. Tamoxifen treatment for breast cancer and risk of endometrial cancer: A case control study. J Natl Cancer Inst 2005;97:375–84.
8. Cohen I, Perel E, Tepper R, et al. Dose-dependent effect of tamoxifen therapy on endometrial pathologies in postmenopausal breast cancer patients. Breast Cancer Res Treat 1999;53:25–62.
9. Rose PG. Endometrial carcinoma. N Engl J Med 1996;335:640–49.
10. Casalini P, Iorio MV, Gamozzi E, Menard S. Role of HER receptors family in development and differentiation. J Cell Physiol 2004;200:343–50.
11. Key G, Becker MH, Baron B, et al. New Ki-67 equivalent murine monoclonal antibodies (MIB 1–3) generated against bacterially expressed parts of the Ki-67 cDNA containing three 62 base pair repetitive elements encoding for the Ki-67 epitope. Lab Invest 1993;68:629–36.
12. Fisher B, Costantino JP, Redmond CK, Fisher ER, Wickerham DL, Cronin WM. Endometrial cancer in tamoxifen treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. J Natl Cancer Inst 1994;86:527–37.
13. Mourits MJ, Ten Hoor KA, van der Zee AG, Willemse PH, de Vries EG, Hollema H. The effects of tamoxifen on proliferation and steroid receptor expression in postmenopausal endometrium. J Clin Pathol 2002;55:514–9.
14. Prasad M, Wang H, Douglas W, Barakat R, Ellenson LH. Molecular genetic characterization of tamoxifen-associated endometrial cancer. Gynecol Oncol 2005;96:25–31.
15. Kim SY, Suzuki N, Laxmi YR, Shibutani S. Genotoxic mechanism of tamoxifen in developing endometrial cancer. Drug Metab Rev 2004;36:199–218.
16. The Early Breast Cancer Trials’ Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomized trials. Lancet 1998;351:1451–67.
17. Muniyara AJ, Waggone S, Yamada D, Rotmensch J, Connell PP. Age as a prognostic factor for recurrence in patients with endometrial carcinoma. Gynecol Oncol 2000;79:79–85.
18. Bergman L, Beelen MLR, Galle MPW, et al. Risk and prognosis of endometrial cancer after tamoxifen for breast cancer. Lancet 2000;356:881–7.
19. Palmer DC, Muir IM, Alexander AL, Cauchi M, Bennett RC, Quinn MA. The prognostic importance of steroid receptors in endometrial carcinoma. Obstet Gynecol 1988;72:388–93.
20. Fukuda K, Mori M, Uchiyama M, Iwaki K, Iwasaka T, Sugimori H. Prognostic significance of progesterone receptor immuno-
histochemistry in endometrial carcinoma. Gynecol Oncol 1998;69:220–25.

21. Nyholm HCJ, Lielsen AL, Lyndrup J, Norup P, Thorpe SM. Biochemical and immunohistochemical estrogen and progesterone receptors in adenomatous hyperplasia and endometrial carcinoma: correlations with stage and other clinicopathologic features. Am J Obstet Gynecol 1992;167:1334–42.

22. Hetzel DJ, Wilson TO, Keeney GL, Roche PC, Cha SS, Podratz KC. HER-2/neu expression: a major prognostic factor in endometrial cancer. Gynecol Oncol 1992;47:179–85.

23. Saffari B, Jones LA, el-Naggar A, Felix JC, George J, Press MF. Amplification and overexpression of HER-2/neu (c-erbB-2) in endometrial cancers: correlation with overall survival. Cancer Res 1995;55:5693–8.

24. Pisani AL, Barbuto DA, Chen D, Ramos L, Lagasse LD, Karlan BY. HER-2/neu, p53, and DNA analyses as prognosticators for survival in endometrial carcinoma. Obstet Gynecol 1995;85:729–34.

25. Lukes AS, Kohler MF, Pieper CF, et al. Multivariable analysis of DNA ploidy, p53, and HER-2/neu as prognostic factors in endometrial cancer. Cancer 1994;73:2380–5.

26. Mariani A, Sebo T, Katzmann J, et al. Pretreatment assessment of prognostic indicators in endometrial cancer. Am J Obstet Gynecol 2000;182:1535–43.