The actual benefit of tamoxifen treatment (1), irrespective of estrogen receptor (ER) status. Such findings are challenged by the observation that tamoxifen treatment was unable to reduce breast cancer incidence in BRCA1 mutation carriers participating in the National Surgical Adjuvant Breast and Bowel Project breast cancer prevention trial P1 (NSABP-P1) (2), despite the possibility that negative NSABP-P1 results could be caused by late initiation of chemopreventive treatment. Although the NSABP observations may be explained by the prevalence of an ER-α-negative phenotype in BRCA1-related breast cancers, several biologic hypotheses have been provided to justify the protective effect of tamoxifen treatment (1).

Tumors arising in BRCA1 mutation carriers seem to reveal a unique pattern of ER negativity—that is, they express ER-β more frequently than do sporadic tumors (3). Whether ER-β protein expression is associated with responsiveness to tamoxifen is still a controversial issue. However, the initial finding of an association between ER-β protein expression and clinical outcome (4) may be supported by a retrospective analysis we performed in a series of 246 post-menopausal patients with primary lymph node-positive, ER-positive (determined by ligand binding assay) invasive breast cancer who had received tamoxifen for at least 2 years. ER-β protein expression in the tumors of these patients was inversely associated with 6-year relapse when analyzed as either a continuous variable (Table 1; \( P = .003 \), univariate analysis) or when using a total immunohistochemistry (IHC) score, which combines the positive cell fraction with staining intensity (hazard ratio [HR] for low versus high IHC score = 3.36, 95% confidence interval [CI] = 1.32 to 8.58, \( P = .011 \); HR for intermediate versus high IHC score = 2.35, 95% CI = 1.00 to 5.50, \( P = .049 \); adjusted for tumor size and lymph node involvement). Such an association, which was maintained in the presence of other variables, i.e., tumor size, number of positive lymph nodes, and ER-α (Table 1; \( P = .004 \), multivariable analysis), might in part explain the efficacy of tamoxifen in BRCA1-related, ER-α-negative and ER-β-positive cancers. Moreover, recent data (5) indicate that, after prolonged estradiol deprivation (following treatment with anti-estrogens), estradiol itself stimulates apoptosis in breast can-

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**Table 1. Univariate and multivariable analysis of 6-year relapse***

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)†</td>
<td>( P ) value‡</td>
</tr>
<tr>
<td></td>
<td>HR (95% CI)†</td>
<td>( P ) value‡</td>
</tr>
<tr>
<td>Tumor size (&gt;2 cm vs. ≤2 cm)</td>
<td>2.17 (1.28 to 3.68)</td>
<td>.01</td>
</tr>
<tr>
<td>No. of positive lymph nodes (&gt;3 vs. ≤3)</td>
<td>3.30 (1.98 to 5.51)</td>
<td>.001</td>
</tr>
<tr>
<td>ER-α+</td>
<td>1.59 (1.28 to 2.00)</td>
<td>.038</td>
</tr>
<tr>
<td>ER-β+</td>
<td>1.92 (1.54 to 2.37)</td>
<td>.003</td>
</tr>
</tbody>
</table>

*Relapse is defined as the occurrence of the first adverse event (i.e., local-regional, distant, or contralateral metastasis) from the date of surgery. The prognostic role of the different variables on relapse was evaluated singularly (univariate) or jointly (multivariable) by fitting a Cox proportional hazards regression model. The interaction between estrogen receptor (ER)-β and ER-α was first investigated in a bivariate fashion using a Cox proportional hazards regression model that included the main effect and its interaction term. Because no statistically significant first-order interaction term was identified, only tumor size, number of positive lymph nodes, ER-α and ER-β were included in the final model. CI = confidence interval; HR = hazard ratio.

†The HR for relapse compares two categories for each variable. For continuous variables, such as ER-α and ER-β, we used an approach already adopted (7) to illustrate how to interpret the HR, i.e., specific values were selected corresponding to a low (10%) or to a high (60%) fraction of cells expressing ER isoforms so that the HR for relapse compares the cause-specific hazard for patients with tumors with 10% positive cells versus those with tumors with 60% positive cells.

‡These variables were analyzed on a continuous scale. Formalin-fixed tissue sections (4 μm) were subject either to microwave antigen retrieval in 10 mM sodium citrate (pH 6.0) followed by overnight incubation at 4 °C with 1D5 monoclonal antibody (1:100 dilution; Dako AS, Glostrup, Denmark) for the determination of ER-α expression or to pressure cooker antigen retrieval in 10 mM sodium citrate (pH 6.0) followed by overnight incubation at 4 °C with the rabbit polyclonal MYEB (1:600 dilution) (4) for the determination of ER-β expression. Tissue sections were then processed with immunoperoxidase staining (Vectorstain ABC Kit; Vector Laboratories, Burlingame, CA). The fraction of positive tumor cells was defined as the percent ratio of the number of positive cells to the total number of cells (a total of 1000–3000 tumor cells were scored).

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**Re: Tamoxifen May Be an Effective Treatment for BRCA1-Related Breast Cancer Irrespective of Estrogen Receptor Status**

The actual benefit of tamoxifen treatment in patients carrying BRCA1 mutations remains an open question due to contrasting clinical results. In fact, the outcome of retrospective analyses suggesting a protective effect of tamoxifen in preventing contralateral tumors was recently supported in a cohort of Ashkenazi Jewish breast cancer patients by the observation of a reduced risk of death following adjuvant tamoxifen-
cer cells. Such a finding could represent another possible explanation for ER-β involvement in determining the protective effect of tamoxifen in BRCA1-related tumors because, in cells of neuronal origin, estradiol stimulates proliferation when the prominent receptor isofrom is ER-α, but drives cells to activate Fas-mediated apoptosis when the major receptor isofrom is ER-β (6). An evaluation of ER-β protein expression in a comparable series of BRCA1-related breast cancers from patients who received tamoxifen or were subject to other treatment modalities may provide information to validate our preliminary findings that BRCA1-related tumors have prevalent ER-β protein expression and that ER-β may have a role in determining the effect of tamoxifen in such tumors.

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NOTES

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