ARTICLES

A Randomized Trial of Low-Dose Tamoxifen on Breast Cancer Proliferation and Blood Estrogenic Biomarkers

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Background: **Tamoxifen reduces the risk of breast cancer in women at high risk for the disease but increases the risk for endometrial tumors and venous thromboembolisms, possibly in a dose-dependent fashion. We compared the effects of tamoxifen at 1 mg/day and 5 mg/day with those of the standard dose of 20 mg/day on breast cancer proliferation using a surrogate endpoint marker (Ki-67 expression) and blood biomarkers associated with breast cancer, cardiovascular disease, and bone fracture risk.** ***Methods:*** **We randomly assigned 120 women with estrogen receptor (ER)-positive breast cancer to tamoxifen at 1, 5, or 20 mg/day for 4 weeks. Expression of the tumor proliferation marker Ki-67 and of biomarkers of breast cancer (insulin-like growth factor-I, sex hormone-binding globulin), cardiovascular disease (cholesterol, triglycerides, ultrasensitive C-reactive protein, fibrinogen, antithrombin-III), and bone fracture (type I collagen C-telopeptide) risk were determined before (baseline) and after treatment. All levels were compared with those in two nonrandomized control groups (34 women with ER-negative breast cancer and 29 additional women with ER-positive breast cancer). Data were analyzed by analysis of covariance. All statistical tests were two-sided.** ***Results:*** **Expression of Ki-67 decreased in all three tamoxifen groups, with no difference in the magnitude of reduction among groups ($P = .81$). Relative to baseline, Ki-67 expression decreased by a median of 15.0% (95% confidence interval = 0.0% to 24.1%) among the tamoxifen groups but increased by 12.8% (95% confidence interval = 0.0% to 19.6%) among the nonrandomized control groups. Several blood biomarkers showed dose–response relationships with tamoxifen, including decreased insulin-like growth factor-I, increased sex hormone-binding globulin, and decreased low-density lipoprotein-cholesterol, ultrasensitive C-reactive protein, fibrinogen, and antithrombin-III levels.** ***Conclusions:*** **The effects on Ki-67 expression of lower doses of tamoxifen were comparable to those achieved with the standard dose, although the effects on blood biomarkers were variable. The effects of lower doses of tamoxifen should be assessed further in randomized trials. [J Natl Cancer Inst 2003;95:779–90]**

Tamoxifen, a selective estrogen receptor (ER) modulator, decreases mortality in patients with ER-positive breast cancer *(1)* and breast cancer incidence in healthy women at increased risk *(2)*. However, the partial estrogenic activity of tamoxifen is an important limiting factor in its clinical use. Indeed, tamoxifen

use has been associated with drug resistance in women with advanced disease *(3)* and with a detrimental trend in disease-free survival after treatment for more than 5 years in the adjuvant setting *(4)*. Although the estrogenic agonistic activity of tamoxifen reduces osteoporotic bone fractures *(5)*, tamoxifen has been associated with an increased risk of endometrial tumors *(1,5)*, including uterine sarcomas *(6)*, and with venous thromboembolic events *(5,7)*.

Several factors influence the complex antagonist/agonist effects of tamoxifen in humans, including differences in target tissue ER co-regulator recruitment *(8)*, endocrine milieu *(9,10)*, dose *(11)*, and duration of exposure *(4)*. For example, in the subgroups of premenopausal women *(5)* and women on hormone replacement therapy *(7)* assigned to tamoxifen or placebo, no difference in endometrial cancer and venous thromboembolic events was observed between arms, suggesting that tamoxifen pharmacodynamics may be influenced by the woman's endocrine environment. Furthermore, there is evidence that the endometrial cancer risk is time- and dose-dependent *(1,12)*, which provides the rationale for a reduction in an administered tamoxifen dose.

Although tamoxifen is a prototypical designer drug that binds to a specific molecular target, the ER, and follows saturation kinetics *(13,14)*, its early clinical development has been characterized by the search for the maximum tolerated dose rather than the optimal biologic dose. However, when a 20-mg/day dose of tamoxifen was compared with higher doses, their efficacies were similar in lowering recurrence of and mortality from breast cancer *(1)*. Furthermore, results from animal studies have suggested that reducing the tamoxifen dose to an equivalent of 1 mg/day

for humans did not affect its inhibitory activity on mammary tumor formation *(15)*. In line with these results, we have previously shown that treatment of healthy women with tamoxifen for 2 months at a dose of 10 mg/day or 10 mg every other day did not affect the drug activity on serum biomarkers of cardiovascular disease and may have a more favorable safety profile than 20 mg/day *(16,17)*. Whether these observations and results could also be obtained on breast cancer tissue biomarkers is unknown.

Studies of tamoxifen given to breast cancer patients in a preoperative setting (i.e., after diagnosis but before surgery) have demonstrated that a reduction in expression of the proliferative antigen Ki-67 predicts the clinical response to hormonal agents *(18–21)*. In the present study, we compared the activity of tamoxifen at 5 mg/day or 1 mg/day with that of the standard dose of 20 mg/day in breast cancer patients in a preoperative setting by using the change in breast cancer tissue expression of the proliferation antigen Ki-67 as a surrogate endpoint for the antitumor effect. Preoperative studies may help to improve the efficiency and reduce the costs of selecting new chemopreventive agents *(22)*. In addition, we measured several circulating biomarkers associated with risks for different diseases, including insulin-like growth factor-I (IGF-I) *(23)* and sex hormone-binding globulin (SHBG) *(24)* for pre- and postmenopausal breast cancer, respectively; cholesterol *(25)*, triglycerides *(26)*, fibrinogen *(27)*, and C-reactive protein *(28)* for coronary heart disease; fibrinogen and antithrombin-III for venous thromboembolic events *(29)*; and peptide-bound collagen type-I cross-linked C-telopeptide (C-telopeptide) for bone osteoporotic fractures *(30)*. These circulating biomarkers, of which IGF-I was the primary measure, are modulated by tamoxifen mainly as a result of its estrogenic activity on different target systems and are therefore suitable endpoints to assess dose–response relationships *(16,17)*.

SUBJECTS AND METHODS

Study Design

The study and all amendments during its conduct received approvals from the Institutional Review Board, and all subjects gave their written informed consent. The study was conducted from September 1, 1999, through August 31, 2001.

The aim of this study was to compare preoperative treatment with the standard dose of tamoxifen at 20 mg/day with treatment at either 5 mg/day or 1 mg/day for 4 weeks in a randomized, double-blind, three-arm trial of women with hormone-responsive breast cancer. Tumor biopsy specimens and blood samples were taken before the systemic treatment began and again at the time of surgery (i.e., end of the protocol) to analyze tissue Ki-67 expression and circulating biomarkers associated with estrogenic activity of tamoxifen.

When the trial was designed, ethical restrictions excluded a placebo arm because the proposed 5-week lag time from initial biopsy to surgery was longer than the actual 2-week period that was standard at the time. By the time the study was implemented, the waiting period at this hospital for breast cancer surgery had increased to approximately 5 weeks (because of logistics and patient referral), and the ethical committee approved the recruitment of women whose tumors were determined to be ER-negative at the time of the biopsy. The inclusion of these women (n = 34), who received no preoperative tamoxifen treatment, allowed us to monitor preoperative changes in

Ki-67 expression and in serum biomarkers to assess the effect of biopsy sampling and to assay variance. We also included 29 consecutive patients with ER-positive tumors who were screened and consented at the Institute and would have been eligible for this study but who either underwent surgery in a different hospital after an initial screening biopsy (n = 6) or were enrolled after the 120 women assigned to receive tamoxifen had been randomly assigned (n = 23). Although unstained histologic sections from the surgical specimens of six patients with ER-positive cancers were collected to assess tissue biomarkers, blood samples for circulating biomarkers were not available for several subjects in this ER-positive group because of logistic difficulties.

Inclusion and Exclusion Criteria

Women were included in the study if they met the following criteria: palpable tumor less than 5 cm in diameter (by mammography or ultrasound) suitable for an adequate core biopsy, nonsuspicious lymph nodes and free of distant metastasis *(31)*, age 45 years or older, histologic confirmation of an ER-positive or progesterone receptor (PgR)-positive (i.e., at least 20% positive cells by immunohistochemistry) breast cancer, and performance status equal to zero (Southwest Oncology Group) *(32)*. Because tamoxifen is an effective treatment and preventive agent both in pre- and postmenopausal women *(1,2)*, both groups of women were included in the study. Postmenopausal status was defined as amenorrhea for at least 12 months or follicle-stimulating hormone levels above 40 IU/L in hysterectomized women aged younger than 50 years.

Women were excluded from the study if they met any of the following criteria: tumors that were candidates for neoadjuvant chemotherapy, previous chemotherapy or hormonal therapy for breast cancer, personal history of venous thromboembolic events, current anticoagulant therapy, abnormal (moderate or severe) hematologic and biochemical tests, retinal disorders, or active neurologic or psychiatric diseases or any medical condition that, at the physician's discretion, contraindicated tamoxifen use.

Study Procedures

Patients were usually screened at the Institute outpatient clinic by their reference physician within 1 week (mean ± standard deviation = 6 ± 2 days) to determine their eligibility for the study. Women were assessed clinically at the initial screening, 2 weeks before surgery, and the day before surgery. Blood samples (serum and plasma) for biomarker and drug concentration measurements were collected at baseline (i.e., at the initial screening and on the day of surgery between 7 AM and 9 AM and were stored as aliquots at –80 °C. The mean interval between the time the last drug was taken and the time the blood samples were drawn was approximately 24 hours.

A core-cut biopsy of the primary tumor was obtained using a 14-gauge needle at the initial screening, and a representative sample of the cancerous excision tissue was obtained at the time of surgery. Both samples were fixed in 10% neutral buffered formalin for 6–8 hours before being embedded in paraffin. Sections (4-μm thick) were cut and stained with hematoxylin and eosin. Additional consecutive serial sections were used for immunohistochemical experiments.

Adverse events in patients assigned to the tamoxifen arms were assessed at 2 and 4 weeks relative to starting tamoxifen

using the National Cancer Institute common toxicity criteria *(33)*. Hot flashes and vaginal discharge also were specifically recorded as drug-related symptoms.

Treatment Plan

If the tumor from the core-cut biopsy specimen expressed the ER and/or the PgR, the patient was randomly assigned to one of the following three arms: tamoxifen at 1 mg, 5 mg, or 20 mg per day for 4 weeks until the day before surgery. Forty patients were randomly assigned to each arm. Randomization was performed by telephone using permuted blocks of six with no stratification. Women were assigned on an individual basis to one of three tamoxifen dose groups. They remained on the same allocation throughout the study. A computer-generated randomization list was drawn up by the statistician and given to the data manager. Clinicians enrolling patients contacted the data manager via a centralized phone call to check eligibility. The data manager then allocated the next available number on entry into the trial, and each participant received her tablets directly from the clinician. The code was revealed to the researchers after recruitment, data collection, and laboratory analyses were complete. Study medications were prepared in packages containing weekly blisters. An initial dose of 20 mg was given to all patients in a separate blister, and participants were instructed to take it on the first day of treatment. Patient compliance was assessed by pill count. Tamoxifen was provided by Laboratori MAG, Milan, Italy (U.S. Food and Drug Administration drug master file number 6735).

The study was designed so that drug levels in patients who received the low-dose regimens could reach steady state within the treatment time period of 4 weeks. Because tamoxifen requires 4–6 weeks to reach steady-state levels *in vivo (34),* a loading dose of 20 mg was administered on day 1 to all 120 patients to avoid the possibility that any differences in the observed activities among the three doses were attributable to pharmacokinetics. A single dose of 20 mg gives a peak plasma level of approximately 20 ng/mL after 24 hours *(35),* which is twice as high as the expected steady-state drug concentration attainable with a dose of 1 mg/day *(36).* The initial 20-mg tamoxifen dose allowed steady-state drug levels to be reached within 4 weeks, even for patients randomly assigned to the lowest dose of tamoxifen.

Analytical Methods

Expression of ER, PgR, Ki-67, and HER2/neu was determined by immunohistochemistry, as previously described *(37).* Briefly, de-waxed tumor sections were pretreated with 3% hydrogen peroxide for 5 minutes to block endogenous peroxidase activity and were then treated with a solution of 0.001 *M* EDTA (pH 8.0) at 99 °C for 20 minutes to retrieve antigens. The tumor sections were then incubated with primary mouse monoclonal antibodies to ER (clone 1D5, 1 : 100 dilution), PgR (clone 1A6, 1 : 800 dilution), or Ki-67 (clone Mib-1, 1 : 200 dilution) or with rabbit polyclonal antibody to the HER2/neu protein (1 : 3200 dilution) (all obtained from Dako, Glostrup, Denmark) for 30 minutes at room temperature using an automatic immunostainer (Autostainer; Dako). The antibody–antigen complexes were subsequently treated with a high-sensitivity detection kit (EnVision Plus-HRP; Dako), according to the manufacturer's instructions. Peroxidase activity was developed with 3–3′-diaminobenzidine-copper sulfate (Sigma Chemical Co., St. Louis, MO) as the chromagen to obtain a brown-black end product. ER, PgR, and Ki-67 expression were all evaluated by the same experienced pathologist (G. Viale) who determined the percentage of immunoreactive cells from the total number of invasive neoplastic cells in the core biopsy specimens and from at least 2000 tumor cells randomly selected from the periphery of each of the invasive carcinomas in the surgical specimens. HER2/neu immunoreactivity was also evaluated by the same pathologist, who scored the tumor for the intensity of immunostaining, the completeness of cell membrane staining, and the percentage of immunoreactive neoplastic cells by using a four-tier scale (from 0 to 3+), as recommended *(38).* The pathologist was blinded to the treatment groups.

Plasma IGF-I levels were determined by a chemiluminescent immunometric assay (Nichols Institute Diagnostics, San Juan, CA). The assay was performed on the automatic instrument LIAISON (Byk Sangtec Diagnostica, Dietzenbach, Germany). The sensitivity of the test was 0.8 nmol/L; intra- and interassay coefficients of variation of our in-house pooled serum control sample were 4.1% and 7.8%, respectively. Serum SHBG was measured using the AutoDELFIA SHBG kit from Wallac (Turku, Finland). The sensitivity of the test was 0.5 nmol/L. The precision of the assay, expressed as interassay coefficient of variation, was 3.1% and 4.1% for concentrations of 24.3 nmol/L and 50.3 nmol/L, which represent the average values in men and women, respectively. Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and antithrombin-III levels were measured in serum by enzymatic methods with a Hitachi 911 (Boehringer, Mannheim, Germany), a fully mechanized multichannel analyzer for routine clinical chemistry purposes. Measurements were taken according to the manufacturer's instructions. Total cholesterol, HDL cholesterol, and triglyceride determinations were based on enzymatic colorimetric methods, whereas antithrombin-III levels were determined by a kinetic colorimetric method. Low-density lipoprotein (LDL) cholesterol levels were obtained according to the Friedewald formula (LDL cholesterol = total cholesterol – HDL cholesterol – [triglycerides/5]) *(39).* Plasma fibrinogen was measured using the ELECTRA 1400C Automatic Coagulation Analyzer (Medical Laboratory Automation, Pleasantville, NY). This assay uses a photometric determination of blood clot formation. Serum concentration of ultrasensitive C-reactive protein (CRP) was determined by a two-site chemiluminescent enzyme immunometric assay (Diagnostic Products, Los Angeles, CA) designed for the IMMULITE automated analyzer (Diagnostic Products). The sensitivity of the assay is 0.01 mg/dL. The intra- and interassay coefficients of variation for ultrasensitive CRP levels were 3.6% and 3.9%, respectively. Serum C-telopeptide was measured with a chemiluminescent immunometric assay (Roche Diagnostics S.p.A, Monza, Italy) designed for the Elecsys automated analyzer (Roche Diagnostics S.p.A.). The sensitivity of the assay was 0.01 ng/mL. The intra- and interassay coefficients of variation of our in-house pooled serum control sample were 2.5% and 4.5% (mean = 0.557 ng/mL), respectively. With the exception of the lipid profile, fibrinogen, and antithrombin-III, which were determined on fresh specimens, pre- and post-treatment serum samples obtained from each subject were assayed at the same time to eliminate the effects of interassay variation.

Serum and tissue concentrations of tamoxifen and metabolites were measured using methods previously described *(40,41).* Briefly, a fluorescence detector revealed tamoxifen and its me-

tabolites after on-line conversion to fluorophors by UV light. The within-day precision of the assay for measuring tamoxifen and its metabolites for concentrations between 10 and 800 ng/mL was below 5.7%. The detection limit was 1 ng/mL, and the recovery of tamoxifen and its metabolites from human serum *(40,41)* and tissue samples *(36)* ranged from 100% to 108% and from 73% to 103%, respectively. The assay was modified to improve the separation and sensitivity of the highly potent metabolite, 4-hydroxytamoxifen, by decreasing the pre-column length from 30 mm to 8 mm and by changing the emission wavelength from 360 nm to 383 nm. All analyses were performed by one of the authors (E. R. Kisanga), who was blinded to the treatment allocations. The analyses included a random subgroup of 10 subjects from the ER-negative control group to detect any drug carryover from one sample analysis to the next.

Study Power and Statistical Analysis

This study was initially designed as a randomized, double-blind, three-arm study to detect differences in the percent reduction in Ki-67 expression (primary endpoint) in tumors from patients who received one of three tamoxifen doses. We anticipated the relative reduction in tumor-associated Ki-67 expression to be 40% in patients treated with 20 mg/day tamoxifen, 35% in those treated with 5 mg/day, and 10% in those treated with 1 mg/day. These reductions were anticipated on the basis of a previous study, which had suggested that, for patients treated with 20 mg/day tamoxifen for a median of 3 weeks, Ki-67 expression levels could be reduced by 40% relative to expression levels in those treated with placebo *(42)*. The 35% expression reduction in those treated with 5 mg/day was anticipated on the basis of our previous study, which showed that effects on the levels of serum cardiovascular markers and IGF-I were similar for patients treated with 10 mg of tamoxifen on alternate days or with 20 mg of tamoxifen per day for 2 months *(16)*. On the basis of a linear dose–response model that used doses of 0–5 mg, at which no change in Ki-67 expression was anticipated, we postulated a 10% reduction in Ki-67 expression levels in patients treated with 1 mg/day of tamoxifen. This level of anticipated reduction was justified because one study had shown the efficacy of a 1-mg equivalent human dose in a rat mammary tumor model *(15)*.

The level of plasma IGF-I was the secondary endpoint. We anticipated a dose–response relationship on its change and on that of most of the circulating biomarkers over the 4 weeks of treatment. Tumor size was recorded only as an exploratory measure because the treatment period was too short to detect a clinical effect and because of the lack of a standardized measurement of tumor size at baseline.

On the basis of archival data indicating that the standard deviation of the percent change in Ki-67 expression was approximately 40% after preoperative chemotherapy, a sample size of 40 subjects per arm was required for a study power of 90% with a 5% statistical significance level. However, subsequent studies available at the time of analysis showed that, using the most recent analytical methods, the initial assumptions were not applicable because baseline Ki-67 expression levels are three- to fourfold higher than we had anticipated, and the relative percent reduction achieved with tamoxifen or other selective ER modulators ranges from 15% to 20% *(43–45)*. On the basis of these new assumptions and the observed standard deviation of 0.67 in Ki-67 expression levels, and taking into account the 13% and

19% median proportional increase in Ki-67 in the nonrandomized ER-positive and ER-negative control groups, respectively, the *post hoc* power to assess a statistically significant difference at 5% between tamoxifen and control groups was 75%.

The study was analyzed using analysis of variance models for the baseline values and analysis of covariance models for the endpoint values of the biomarkers. For Ki-67, SHBG, triglycerides, fibrinogen, antithrombin-III, ultrasensitive CRP, and C-telopeptide, a logarithmic transformation was used to achieve normality. Residual plots were used to assess the validity of the assumptions of the models.

The randomized part of the study was initially analyzed separately, and then all five groups were analyzed together. The results are presented for the latter analysis because they provide more information and were not in conflict with the results of the randomized study. Four linear contrasts were used to compare the five treatment groups. The first was a contrast of the three randomized groups receiving tamoxifen with the two control groups. The second and third contrasts—the linear trend and the quadratic deviations from linearity—were used among only the three randomized groups receiving tamoxifen. The fourth contrast compared the ER-positive controls with the ER-negative controls to assess the consistency of the control groups. These contrasts were specified *a priori* and are consistent with the aims of the study. The most important contrasts were the linear contrast among the three tamoxifen groups and the comparison of the tamoxifen groups with the control groups. The former was used as the main test of the hypothesis under study, namely, whether there was a dose–response relationship on the change in tissue and serum biomarkers. The *P* values for the primary endpoint (Ki-67 expression) and the secondary endpoint (IGF-I levels) were not adjusted for multiple comparisons. *P* values for all other biomarkers were adjusted for multiple comparisons using the method proposed by Benjamini and Hochberg *(46)*. This method of adjustment controls a different error rate than the Bonferroni adjustment.

We analyzed expression of Ki-67 and the other biomarkers as the percent change after 4 weeks of treatment, because previous reports showed the relevance of such analysis *(43–45)*. Two thousand Bootstrap replications of the observed percent changes were selected, with replacement, and the median percent change was calculated for Ki-67 expression and blood biomarker levels for each of the three treatment groups and two control groups in each bootstrap replication. The 95% CIs were obtained from the distribution of the 2000 medians using the bias-corrected method. The distribution of values contains a large number of very small and very high percent changes that do not follow a normal distribution. All statistical analyses were carried out using S-plus 2000 (MathSoft Inc., Seattle, WA). All statistical tests were two-sided.

RESULTS

Subject Characteristics

From September 1, 1999, through August 31, 2001, a total of 236 women were registered, and 221 were assessed to determine their eligibility for the study (Fig. 1). Of those women assessed, 38 were excluded primarily because no tumor tissue was obtained from the core biopsy, thus leaving 183 women available for analysis. The summary statistics for the subject demographics and tumor characteristics at baseline are presented in Table 1.

Fig. 1. Participant flow diagram.
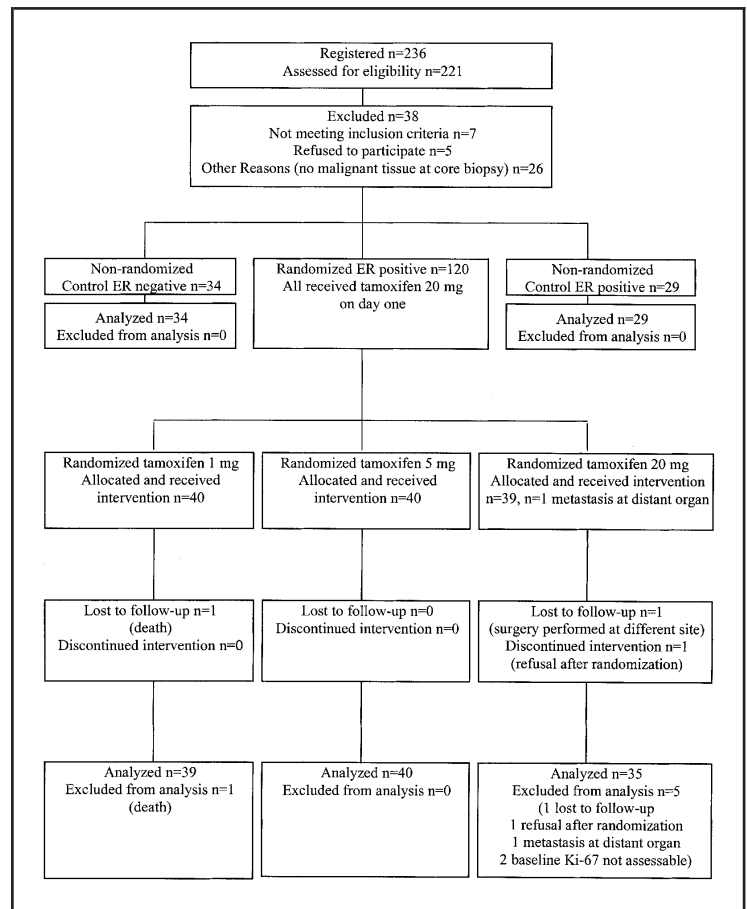
Table 1. Main subject and tumor characteristics at baseline according to treatment group (randomized to tamoxifen daily for 4 weeks or two nonrandomized control groups)*

Variable	Tamoxifen			Control	
	1 mg/day (n = 40)	5 mg/day (n = 40)	20 mg/day (n = 40)	ER-positive (n = 29)	ER-negative (n = 34)
Age, y	60 ± 11	61 ± 11	59 ± 10	63 ± 9	62 ± 8
Pre-/postmenopause, No.	10/30	11/29	10/30	3/26	6/28
Years since menopause	15 ± 10	15 ± 10	15 ± 12	17 ± 8	15 ± 12
Days since last period†	15 ± 12	18 ± 16	18 ± 11	16 ± 3	19 ± 2
BMI (kg/m²)	26.1 ± 3.7	26.5 ± 5.2	26.2 ± 4.9	28.2 ± 7.7	26.3 ± 4.0
Days from biopsy to surgery (baseline to endpoint)	34.7 ± 2.0	34.0 ± 2.4	35.0 ± 2.9	29.0 ± 11.4	33.8 ± 3.7
Days of tamoxifen treatment	27.3 ± 1.0	27.0 ± 1.7	27.5 ± 1.7		
Tumor size, cm	2.6 ± 0.9	2.7 ± 1.0	2.3 ± 0.8	2.8 ± 1.3	3.0 ± 1.0
Tumor grade (1/2/3/unknown), No.‡	5/29/3/3	10/26/2/2	11/28/1/0	3/26/1/0	0/25/8/1
% tumors with ER expression§	85.8 ± 13.8	83.4 ± 17.6	82.6 ± 17.8	87.0 ± 8.4	0.1 ± 0.3
% tumors with PgR expression§	55.4 ± 33.0	41.0 ± 34.8	42.1 ± 36.4	30.4 ± 31.5	0.1 ± 0.7
HER2 score (0/1/2/3/unknown)§	20/9/5/6/0	28/3/4/5/0	23/5/1/10/1	17/6/3/3/1	17/1/4/12/0

*Data are expressed as mean ± standard deviation. BMI = body mass index; ER = estrogen receptor; PgR = progesterone receptor.

†For premenopausal women.

‡Tumor grade was determined as described (53).

§Expression of ER, PgR, and HER2 was determined by immunohistochemistry, as described (37).

There were no statistically significant differences among the subjects randomly assigned to the three tamoxifen dose groups, although there was a slightly shorter length of time from biopsy to surgery in women in the nonrandomized ER-positive control group. The maximum tumor diameter, as assessed by mammography or ultrasound, was slightly smaller among those assigned to the 20 mg tamoxifen arm than among those in the other four groups (*P* = .06).

Baseline Biomarkers

Baseline values of the main biomarkers are presented in Table 2. There was no statistically significant difference in the baseline levels of any biomarker among women assigned to the three tamoxifen dose groups. The mean baseline level of Ki-67 expression was higher among women in the ER-negative control group than among women in any of the four ER-positive groups.

Table 2. Baseline tissue and circulating biomarker levels according to tamoxifen dose group and two nonrandomized control groups*

| Variable | Tamoxifen | | | Control | | P† |
	1 mg/day	5 mg/day	20 mg/day	ER-positive	ER-negative	
Ki-67, % positive	18.1 (14.2 to 22.1) n = 40	19.5 (13.9 to 25.1) n = 40	21.2 (16.3 to 26.0) n = 38	18.9 (14.6 to 23.1) n = 29	36.4 (29.0 to 43.8) n = 34	<.001‡
IGF-I, nmol/L	14.7 (12.2 to 17.1) n = 39	15.2 (13.4 to 17.1) n = 40	14.9 (12.7 to 17.1) n = 36	12.5 (10.1 to 14.9) n = 11	13.7 (12.1 to 15.3) n = 29	.630‡
SHBG, nmol/L	69.3 (56.0 to 82.5) n = 39	65.3 (52.5 to 78.1) n = 40	65.8 (53.0 to 78.5) n = 36	62.2 (42.8 to 81.5) n = 15	65.0 (51.4 to 78.6) n = 31	.930‡
Total cholesterol, mg/dL	217 (200 to 233) n = 37	225 (213 to 237) n = 37	231 (217 to 244) n = 39	222 (196 to 248) n = 14	240 (225 to 256) n = 31	.110
LDL cholesterol, mg/dL	132 (118 to 147) n = 37	139 (127 to 151) n = 37	145 (132 to 157) n = 39	135 (113 to 158) n = 14	156 (142 to 171) n = 31	.050‡
HDL cholesterol, mg/dL	62 (57 to 68) n = 37	66 (62 to 71) n = 37	64 (59 to 69) n = 39	64 (53 to 75) n = 14	61 (55 to 67) n = 31	.590
Triglycerides, mg/dL	110 (90 to 129) n = 37	99 (84 to 115) n = 37	108 (84 to 133) n = 39	113 (80 to 145) n = 14	116 (83 to 148) n = 31	.840‡
CRP, mg/dL	0.20 (0.13 to 0.27) n = 37	0.18 (0.11 to 0.25) n = 38	0.18 (0.12 to 0.24) n = 36	0.22 (0.03 to 0.41) n = 11	0.17 (0.12 to 0.22) n = 28	.980‡
Fibrinogen, mg/dL	315 (291 to 339) n = 38	287 (266 to 309) n = 37	288 (267 to 309) n = 38	328 (301 to 354) n = 16	308 (287 to 329) n = 30	.020‡
Antithrombin-III, % normal	92 (89 to 96) n = 36	91 (87 to 95) n = 35	93 (90 to 96) n = 39	105 (96 to 114) n = 14	94 (88 to 99) n = 27	<.001‡
C-Telopeptide, ng/mL	0.44 (0.36 to 0.52) n = 39	0.51 (0.43 to 0.59) n = 40	0.50 (0.41 to 0.59) n = 36	0.53 (0.35 to 0.71) n = 11	0.56 (0.45 to 0.67) n = 29	.130‡

*Data are expressed as mean with 95% confidence intervals. ER = estrogen receptor; IGF-I = insulin-like growth factor-I; SHBG = sex hormone-binding globulin; LDL cholesterol = low-density lipoprotein cholesterol; HDL cholesterol = high-density lipoprotein cholesterol; CRP = ultrasensitive C-reactive protein; C-Telopeptide = peptide-bound collagen type-I cross-linked C-Telopeptide.

†P value for the difference among the five groups. There were no statistically significant differences when considering only the three randomized groups (one-way analysis of variance model).

‡Denotes the use of a log transform of the variable.

Compared with Ki-67 expression levels (i.e., the percentage of immunoreactive cells) in the ER-negative control group, the mean Ki-67 expression level was 46% lower (95% CI = 27% to 60%) in the ER-positive control group, 49% lower (95% CI = 33% to 62%) in the 1-mg/day tamoxifen group, 53% lower (95% CI = 37% to 64%) in the 5-mg/day tamoxifen group, and 45% lower (95% CI = 27% to 59%) in the 20-mg/day tamoxifen group. LDL cholesterol levels were slightly higher among subjects in the ER-negative control group, and fibrinogen and antithrombin-III levels were higher among subjects in the ER-positive control group than among subjects in the tamoxifen dose groups.

Endpoint Tissue Biomarkers

The post-treatment biomarker values after 4 weeks are shown in Table 3. Expression of Ki-67 was decreased in the surgical tumor specimens from subjects in the tamoxifen dose groups, although there was no evidence of a dose–response relationship among the groups (P = .81). By contrast, expression of Ki-67 was increased in the surgical tumor specimens from subjects in the nonrandomized control groups. The post-treatment values of Ki-67 expression were statistically significantly lower among subjects in the three tamoxifen dose groups than among those in the two nonrandomized control groups (P<.001) (Table 3).

We next determined the median percent change in Ki-67 expression from baseline (Fig. 2, A). Relative to baseline, tumor cell Ki-67 expression levels decreased to a similar degree among subjects in the three tamoxifen dose groups, although the 95% CIs included zero. The median percent change in Ki-67 expression was –14.0% (95% CI = –38.8% to 0.0%) for subjects in the 1-mg/day tamoxifen group, –11.7% (95% CI = –32.0% to 8.5%) for those in the 5-mg/day group, and –15.6% (95% CI =

–44.5% to 14.1%) for those in the 20-mg/day group. Relative to baseline levels, tumor cell Ki-67 expression increased among subjects in the nonrandomized control groups, although the 95% CIs also included zero. The median percent change in Ki-67 expression was 18.6% (95% CI = –3.3% to 33.0%) among subjects in the ER-positive control group and 12.7% (95% CI = 0.0% to 19.6%) among subjects in ER-negative control group. We compared the pooled results from the three tamoxifen dose groups with the pooled results from the two control groups. Ki-67 expression decreased by a median of 15.0% (95% CI = 0.0% to 24.1%) from baseline among those who received tamoxifen but increased by a median of 12.8% (95% CI = 0.0% to 19.6%) among control subjects. Compared with the control groups, the mean percent change in Ki-67 expression was 32.4% lower (95% CI = 18.6% to 49.9%) in the three tamoxifen groups.

Among the subjects in the tamoxifen groups, postmenopausal women had statistically significantly lower post-treatment Ki-67 expression levels than premenopausal women (the mean percent reduction was 36%, 95% CI = 20% to 49%; P<.001), but menopausal status had no influence on the effect of tamoxifen on endpoint Ki-67 expression levels (P = .49 for the interaction). Likewise, HER-2/neu expression had no influence on the effect of tamoxifen on the endpoint Ki-67 expression levels (P = .47 for the interaction).

The mean (± standard deviation) tumor diameter (in centimeters) at surgery was 2.28 ± 1.0, 2.19 ± 1.2, 1.70 ± 0.6, 2.40 ± 1.1, and 2.53 ± 1.6 for those in the 1-mg/day tamoxifen, 5-mg/day tamoxifen, 20-mg/day tamoxifen, ER-positive control, and ER-negative control groups, respectively. The difference in tumor size among the three tamoxifen groups was not statistically significant (P = .28, adjusted for baseline tumor diameter).

Table 3. Endpoint tissue and circulating biomarker levels according to tamoxifen dose group after 4 weeks of tamoxifen treatment or observation*

| Variable | Tamoxifen | | | | Control | | | |
	1 mg/day	5 mg/day	20 mg/day	P†	ER-positive	ER-negative	P‡	P§
Ki-67 labeling, % positive	15.1 (11.1 to 19.0) n = 39	16.2 (11.4 to 21.0) n = 40	14.0 (10.5 to 17.4) n = 37	.812‖	25.8 (18.2 to 33.5) n = 29	42.9 (34.5 to 51.4) n = 34	<.001	.168
IGF-I, nmol/L	13.8 (11.6 to 16.0) n = 39	11.9 (10.3 to 13.5) n = 40	10.4 (8.9 to 11.8) n = 36	<.001‖	11.6 (10.1 to 13.2) n = 11	13.7 (12.0 to 15.4) n = 29	<.001	.340
SHBG, nmol/L	70.0 (57.0 to 83.0) n = 39	70.9 (60.2 to 81.7) n = 40	84.3 (68.7 to 99.8) n = 36	.013‖	47.4 (27.5 to 67.3) n = 11	62.3 (46.8 to 77.7) n = 30	<.001	.908
Total cholesterol, mg/dL	196 (180 to 211) n = 38	193 (182 to 204) n = 40	196 (183 to 209) n = 37	.130	220 (200 to 240) n = 11	223 (201 to 244) n = 30	.018	.908
LDL cholesterol, mg/dL	120 (105 to 134) n = 38	117 (108 to 126) n = 38	116 (105 to 127) n = 36	.029‖	136 (112 to 159) n = 11	141 (122 to 160) n = 30	.055	.908
HDL cholesterol, mg/dL	55 (50 to 60) n = 38	55 (51 to 59) n = 40	54 (50 to 59) n = 37	.663	57 (45 to 69) n = 11	56 (49 to 62) n = 30	.223	.908§
Triglycerides, mg/dL	107 (88 to 126) n = 38	110 (94 to 125) n = 38	118 (94 to 141) n = 36	.174‖	137 (83 to 192) n = 11	130 (87 to 174) n = 30	.587	.908
CRP, mg/dL	0.17 (0.10 to 0.24) n = 37	0.12 (0.07 to 0.17) n = 38	0.10 (0.05 to 0.15) n = 36	.007‖	0.21 (0.04 to 0.38) n = 11	0.21 (0.12 to 0.30) n = 28	.008	.908
Fibrinogen, mg/dL	279 (256 to 302) n = 38	243 (221 to 265) n = 40	232 (214 to 249) n = 37	.004‖	303 (270 to 337) n = 11	309 (287 to 331) n = 30	<.001	.908
Antithrombin-III, % normal	85 (79 to 91) n = 38	80 (75 to 84) n = 40	77 (73 to 81) n = 36	.029‖	100 (92 to 108) n = 10	88 (83 to 93) n = 30	<.001	.871
C-Telopeptide, ng/mL	0.55 (0.44 to 0.66) n = 39	0.60 (0.50 to 0.70) n = 40	0.51 (0.43 to 0.59) n = 36	.140‖	0.63 (0.41 to 0.85) n = 11	0.71 (0.58 to 0.84) n = 29	.223	.908

*The P values for the primary endpoint (Ki-67) and the secondary endpoint (IGF-I) have not been adjusted for multiple comparisons. An adjustment for multiple comparisons has been made for the P values for all other biomarkers using the method proposed by Benjamini et al. *(41)*. Data are expressed as mean with 95% confidence intervals. ER = estrogen receptor; IGF-I = insulin-like growth factor-I; SHBG = sex hormone-binding globulin; LDL cholesterol = low-density lipoprotein cholesterol; HDL cholesterol = high-density lipoprotein cholesterol; CRP = ultrasensitive C-reactive protein; C-Telopeptide = peptide-bound collagen type-I cross-linked C-Telopeptide. The P values are all extracted from an analysis of covariance model adjusting for the baseline value of the response variable.

†P value for the linear trend among the three tamoxifen groups.

‡P value for the linear contrast between the three tamoxifen groups and the two control groups.

§P value for the linear contrast between the two control groups.

‖Denotes log transform of the response variable and its baseline value to satisfy the assumption of normality required in the analysis of covariance model.

However, the effect of tamoxifen on tumor size at surgery was different, depending on the tumor diameter at baseline. Compared with tumor size among subjects in the control groups, tamoxifen had a greater effect on tumors larger than 2.5 cm at baseline than it did on smaller tumors at baseline (P = .02 for the interaction).

Endpoint Blood Biomarkers

The effect of the tamoxifen dose on circulating biomarkers at 4 weeks is shown in Table 3. The post-treatment levels of IGF-I followed a highly statistically significant linear dose–response relationship, with the greatest decrease in levels observed in women who received the highest dose. The median percent change from baseline in IGF-I levels was –5.7% (95% CI = –12.3% to 2.4%) for women in the 1-mg/day tamoxifen group, –21.8% (95% CI = –35.6% to –15.2%) for those in the 5-mg/day group, and –34.8% (95% CI = –39.1% to –24.8%) for those in the 20-mg/day group (Fig. 2, B). For subjects in the nonrandomized ER-positive and ER-negative control groups, the median IGF-I percent change from baseline was –6.8% (95% CI = –13.5% to 4.7%) and –0.2% (95% CI = –2.2% to 8.8%), respectively.

We detected a statistically significant linear dose–response relationship for several serum biomarkers, including increased SHBG, decreased LDL cholesterol, decreased ultrasensitive CRP, decreased fibrinogen, and decreased antithrombin-III levels (Table 3). We detected no statistically significant dose–response relationship for total cholesterol, HDL cholesterol, triglycerides, and C-telopeptide (Table 3). Similar results regarding dose–response relationships were evident when the data were expressed as median percent change (and interquartile range) of baseline levels (Fig. 3). When data from the nonrandomized control groups were compared with data from the tamoxifen dose groups and included in the analysis, all post-treatment biomarker levels were statistically significantly different, with the exception of LDL cholesterol, HDL cholesterol, triglycerides, and C-telopeptide (Table 3).

Drug and Metabolite Concentrations

We next measured serum and breast cancer tissue tamoxifen and metabolite concentrations for women in the three tamoxifen dose groups (Table 4). Dose-concentration relationships were detected. Breast cancer tissue concentrations of tamoxifen and its metabolites were 2–15 times higher than the corresponding serum levels. Even at the lowest tamoxifen dose, the tissue concentrations of the metabolite 4-hydroxytamoxifen were approximately 4 ng/g. The concentration of tamoxifen and its metabolites in one subject in the 1-mg/day, three subjects in the 5-mg/day, and two subjects in 20-mg/day groups were below the level of detection. With the exception of one subject assigned to the 1-mg/day and one assigned to the 5-mg/day groups who did not return any drug blisters, all subjects exhibited greater than 93% compliance by pill count.

Adverse Events

The numbers of subjects reporting adverse events, all of which were determined to be grade 1, were nine, 12, and 11 in those assigned to the 1-mg/day, 5-mg/day, and 20-mg/day
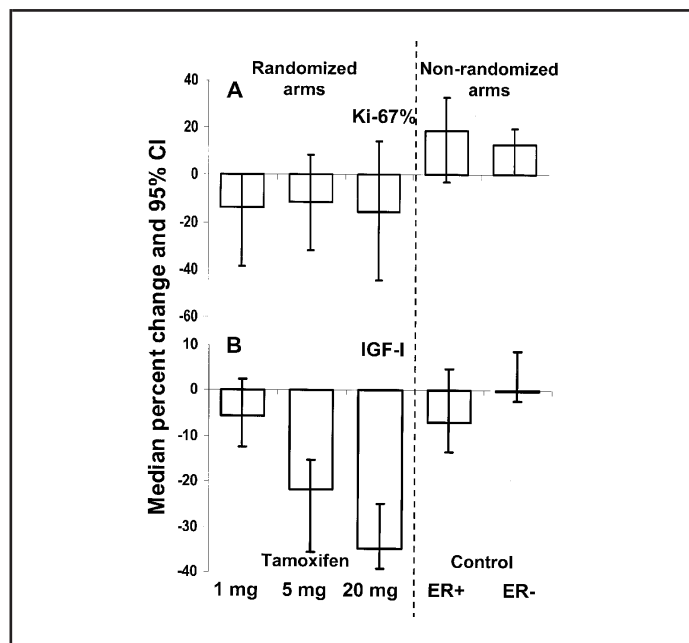
Fig. 2. A) Baseline and endpoint Ki-67 expression levels were determined by immunohistochemistry on tissue sections from either patients randomly assigned to receive 1 mg/day, 5 mg/day, or 20 mg/day tamoxifen for 4 weeks or control patients who had either estrogen receptor (ER)-positive or ER-negative tumors, as described *(33)*. The percentage of immunoreactive cells was counted from the total number of invasive neoplastic cells in baseline core biopsy specimens or from at least 2000 tumor cells randomly selected from the periphery of invasive carcinomas collected at surgery (endpoint). Data are presented as median percent change with 95% confidence interval (CI) of Ki-67 expression from baseline to endpoint surgery. B) Baseline and endpoint plasma insulin-like growth factor-I (IGF-I) levels were determined by a chemiluminescent immunometric assay. Data are presented as median percent change with 95% CI in insulin-like growth factor-I levels from baseline to endpoint.
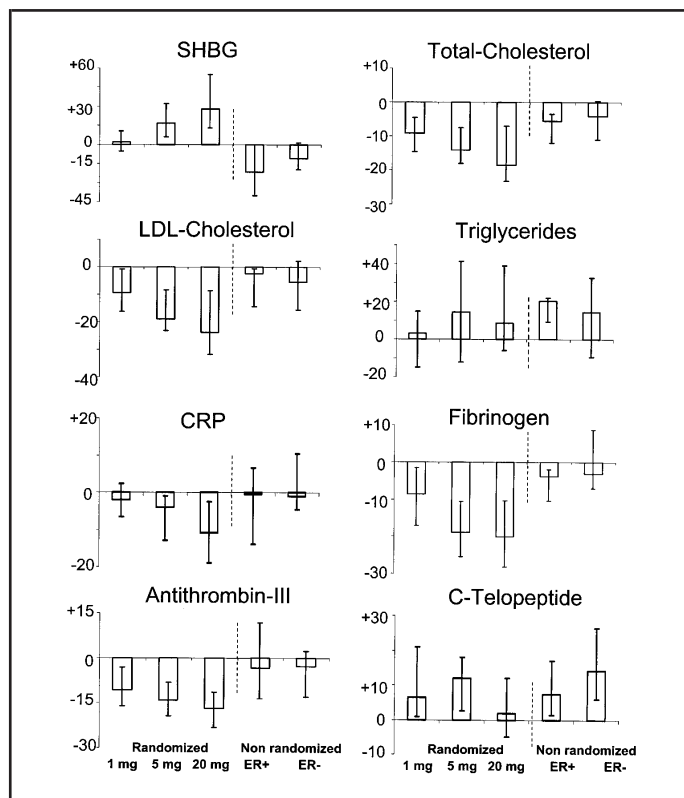

Fig. 3. Baseline and endpoint levels of various risk biomarkers for breast cancer and cardiovascular disease were determined from serum or plasma collected either from patients randomly assigned to receive 1 mg/day, 5 mg/day, or 20 mg/day tamoxifen for 4 weeks or from control patients who had either estrogen receptor (ER)-positive or ER-negative tumors. Data are expressed as median percent change, with interquartile range, of serum biomarkers from baseline to endpoint surgery. SHBG = sex hormone-binding globulin; LDL = low-density lipoprotein; CRP = ultrasensitive C-reactive protein; C-Telopeptide = peptide-bound collagen type-I cross-linked C-telopeptide.

tamoxifen groups, respectively. Of the anticipated drug effects, hot flashes were recorded in 32%, 36%, and 50% of the subjects and vaginal discharge in 26%, 22%, and 47% of the subjects in the 1-mg/day, 5-mg/day, and 20-mg/day tamoxifen groups, respectively. The difference between the groups was not statistically significant for either adverse event.

DISCUSSION

Historically, drugs have often been marketed at what were later recognized as excessive doses, namely, doses on the plateau phase of the dose–response curve for the desired effect. Our results suggest that tamoxifen does not escape this rule, notwithstanding evidence of an antitumor effect through ER binding that follows saturation kinetics *(13,14)* and the similar efficacies between 20 mg/day and higher doses in adjuvant treatment trials *(1)*. However, in the human endometrium, a dose–response relationship has been associated with proliferative disorders *(47)* and cancer *(12)*, including rare cases of uterine sarcoma *(6)*. These considerations led us to propose a dose reduction as a reasonable attempt to decrease tamoxifen-associated toxicity, while retaining the drug activity.

In the present study, we showed that the effects of tamoxifen on breast cancer cell proliferation, as assessed by the change in the percentage of cells expressing Ki-67, were comparable among women who received a daily tamoxifen dose of 1 mg, 5 mg, or 20 mg administered for 4 weeks. Ki-67 expression is a proliferation index that is increasingly being used as a surrogate

biomarker of antitumor efficacy in ER-positive breast cancer *(18–21)*. By contrast, dose–response relationships were observed for several circulating biomarkers of breast cancer and cardiovascular disease risk, including IGF-I, SHBG, LDL cholesterol, ultrasensitive CRP, fibrinogen, and antithrombin-III. These results suggest that a reduced tamoxifen dose retains antiproliferative activity, at least in a short-term study, and potentially diminishes the favorable and unfavorable estrogenic effects of tamoxifen.

Because our study was designed assuming a dose–response effect on Ki-67 change, the power to detect equivalence in Ki-67 expression among the doses is limited. However, the difference, if any, in tumor proliferation as assessed by changes in Ki-67 expression among the three doses is likely to be marginal and of little clinical significance. Admittedly, whether proliferation was reduced in all three tamoxifen arms is unknown because the CIs included zero, and thus the reductions did not reach statistical significance. Differences in some study characteristics, such as interval from biopsy to surgery, tumor diameter from the treatment subjects, and lack of random assignment of control subjects, also limit appropriate comparisons. It is interesting to note that there was no evidence of a dose–response effect on decreasing tumor size, although this measure was not a defined study endpoint. Consequently, our data strongly suggest that the standard tamoxifen dose of 20 mg/day may be more than the dose

Table 4. Serum and breast cancer tissue concentrations of tamoxifen and metabolites after 4 weeks of treatment*

Variable	Tamoxifen			P_{trend}†
	1 mg/day	5 mg/day	20 mg/day	
Serum tamoxifen, ng/mL	15.8 (6.2 to 25.4) n = 39	29.0 (17.2 to 40.8) n = 37	72.7 (58.8 to 86.6) n = 36	<.001
Tissue tamoxifen, ng/g	97.4 (76.6 to 118.2) n = 26	291.6 (209.3 to 373.9) n = 21	814.8 (512.9 to 1116.7) n = 20	<.001
Serum N-desmethyltamoxifen, ng/mL	23.5 (10.3 to 36.7) n = 39	41.9 (24.1 to 59.7) n = 37	110.9 (87.9 to 133.9) n = 36	<.001
Tissue N-desmethyltamoxifen, ng/g	93.1 (69.9 to 116.3) n = 26	273.1 (168.6 to 377.6) n = 21	961.8 (514.5 to 1409.1) n = 20	<.001
Serum 4-hydroxytamoxifen, ng/mL	0.92 (0.38 to 1.46) n = 39	1.36 (0.92 to 1.80) n = 37	2.88 (2.34 to 3.42) n = 36	<.001
Tissue 4-hydroxytamoxifen, ng/g	3.88 (0.19 to 7.57) n = 26	8.47 (2.60 to 14.34) n = 21	23.70 (12.06 to 35.34) n = 20	.002

*Data are expressed as mean value with 95% confidence intervals.

†P value for the linear trend among dose groups. All of the values below the measurable levels have been scored as the lowest measurable level.

needed for maximal antiproliferative activity. A similar conclusion was made in a study in which 10 mg/day or 20 mg/day had similar effects on cell proliferation in women with benign breast disease who were candidates for excision biopsy (48). Our results do not confirm previous preclinical findings of a partial stimulatory effect of low concentrations of tamoxifen in breast cancer cells grown in culture (49).

We observed dose-concentration relationships for measurements of tamoxifen and its metabolites in serum and cancer tissue. The high tamoxifen concentration in breast tissue confirmed its preferential accumulation there, with the drug and metabolite concentrations being 2–15 times those of the corresponding serum level. Importantly, with a tamoxifen dose of 1 mg/day, levels of 4-hydroxytamoxifen in tissue were approximately 10–20 times greater than the 50% inhibitory concentration range observed in different MCF-7 breast cancer cell clones after 48–72 hours, namely, 0.5–5 n*M* or 0.2–2 ng/mL (50). Although caution is necessary when extrapolating *in vitro* inhibitory concentrations to clinically effective levels because of the known variability in both systems, our findings suggest that we achieved the target concentration of the desired effect in humans even with the lowest dose, and they support the hypothesis that the currently used dose of 20 mg/day may lead to an excess accumulation of tamoxifen in the target tissues. Given the dose-related carcinogenicity of tamoxifen in animal models (51), excess drug accumulation could at least in part explain the negative endometrial effects of tamoxifen in humans. This contention is further supported by the known prolonged half-life of tamoxifen and its metabolite N-desmethyltamoxifen after attainment of steady-state levels (approximately 1 and 2 weeks, respectively) (52), which may allow administration on a weekly basis. Overall, our results provide strong rationale for additional phase I and II biomarker trials of tamoxifen to define its optimal biologic dose and schedule, particularly in a prevention setting.

The lack of a dose–response relationship in Ki-67 expression cannot be attributed to a lower-than-anticipated effect of the 20 mg/day dose because the median percent reduction in Ki-67 expression, by 15% from baseline, is comparable with that seen in the recent studies (43–45) using tamoxifen or other selective ER modulators in a preoperative setting. Moreover, *post hoc* calculations showed that, despite the high variability associated with changes in Ki-67 expression, our study had adequate power to detect a 30% difference in Ki-67 expression between the

tamoxifen dose and control groups. The increase in Ki-67 expression in the nonrandomized control groups, which is in line with what was seen in previous studies (43–45), may be explained by the fact that the baseline value was derived from a biopsy specimen taken from the core of the tumor, an area that contains few proliferating cells, and not from the tumor periphery, where the proliferative activity of the tumor is higher (53). An alternative explanation is that there is a proliferative boost, secondary to the collection of the core biopsy, resulting from the local release of growth and/or healing factors.

In contrast with there being no difference in the median percent change in Ki-67 expression among tamoxifen doses, the majority of circulating biomarkers followed a linear dose–response relationship. Some biomarkers, such as SHBG and, to a lesser extent, IGF-I, are under estrogen regulation; SHBG, in particular, is a sensitive and rapidly changing marker of the agonistic effects of selective ER modulators (54,55). Although the clinical implications of the dose–response effects are unclear, the results suggest that tamoxifen at 1 mg/day might be associated with lower estrogenic effects at target systems than tamoxifen at 20 mg/day. The findings are consistent with the dose-related pattern of tamoxifen estrogenicity observed in liver cancer cells, in which high concentrations are fully agonistic and low concentrations (i.e., <1 μ*M*) are predominantly antagonistic to SHBG gene expression (56). Thus, we speculate that a lower dose of tamoxifen may have diminished prothrombotic effects, as suggested by the lack of modulation of antithrombin-III, and possibly a lower stimulatory effect on the endometrium than a higher dose of tamoxifen. A clinical trial assessing the effect of low doses of tamoxifen on endometrial proliferation is underway.

Conversely, the marginal decline in IGF-I levels and the lack of an increase in SHBG levels with the lowest tamoxifen dose may result in a loss of the benefit mediated by these biomarkers in the breast (23,24). However, the contribution of a decrease in IGF-I levels and an increase in SHBG levels to the preventive and therapeutic efficacy of tamoxifen is presently unknown. Likewise, the lack of effect of tamoxifen at 1 mg/day on LDL cholesterol, ultrasensitive CRP, and fibrinogen might be associated with a lower benefit in reducing coronary heart disease. Although early adjuvant studies with tamoxifen at 30–40 mg/day have shown statistically significant reductions of coronary heart disease (57,58), no cardiovascular benefit has been ob-

served with 20 mg/day of tamoxifen in large primary prevention trials *(2,59)*. The reason for these differences is unclear, but one potential explanation may be that tamoxifen exerts cardioprotective activity only above a certain dose. However, in a primary prevention setting, where the cardinal rule is "First, do no harm," attempts to reduce risks outweigh attempts to maintain benefits.

There are several limitations to our study. First, the lack of a randomized control group limits the comparison between those who received tamoxifen and those who did not. However, subject and tumor demography as well as baseline Ki-67 levels in the ER-positive control group and the vast majority of baseline circulating biomarkers in the ER-negative control group were similar to those in the randomized groups. These findings provide little evidence for a selection bias in the nonrandomized control groups. In addition, the type of control groups did not materially affect the comparison among the three randomized doses, which was the primary aim of the study. Second, the administration of 20 mg on day 1 for all 120 randomly assigned subjects slightly attenuated the differences in the cumulative dose of drug received among the three dose groups over the 4 weeks, with the total tamoxifen dose in the 1 mg group being 3.3 and 12 times lower than that in the 5-mg and 20-mg dose groups, respectively. Yet, the cumulative dose reductions relative to 20 mg/day remain substantial, particularly when projected to the currently recommended period of 5 years of tamoxifen treatment. Third, the mean serum concentration attained with 20 mg/day was approximately 25%–40% lower than that attained in a previous study after 2–3 months of tamoxifen administration *(17)*, thus suggesting that steady-state levels of tamoxifen were not attained after 4 weeks in the subjects assigned to receive 20 mg/day. However, the breast cancer tissue concentrations were comparable with those observed in previous studies *(60)* and show that tamoxifen and the metabolites measured are concentrated approximately 10 times in breast cancer tissue during a 20-mg/day regimen. Also, the reductions in levels of circulating biomarkers with 20 mg/day were similar to those found in our previous study *(16)* at the same dose administered for 2 months, where a higher serum tamoxifen concentration was achieved, suggesting attainment of plateau over a certain drug concentration threshold.

New hormonal agents with improved therapeutic indexes, including novel selective ER modulators such as raloxifene, ER downregulators such as fulvestrant, and aromatase inhibitors such as anastrozole, letrozole, or exemestane *(61)* are actively being sought for treatment and prevention of breast cancer. Preliminary results with anastrozole look promising and possibly better than those obtained with tamoxifen *(10)*, although the compelling, extensive, and long-term data available make tamoxifen still the standard therapy for women with ER-positive breast cancer *(62)*. Our results support the notion that a reasonable approach to improving the risk-to-benefit ratio associated with tamoxifen is a dose reduction. Further clinical studies addressing this issue, both in treatment and prevention settings, are warranted. From a public health perspective, inexpensive drugs such as tamoxifen offer advantages in pharmacoeconomic terms, where drug cost is an important factor *(63)*. This approach may facilitate the assessment of chemoprevention as a useful strategy to reduce cancer risk, particularly in populations where medicinal costs represent an important consideration.

In summary, we found that reducing the tamoxifen dose to 1 mg/day retains its antiproliferative activity on breast cancer, as assessed by Ki-67 expression, without substantially modulating the levels of most of the analyzed circulating biomarkers of breast cancer and cardiovascular disease risk. A lower dose may improve the therapeutic index of tamoxifen. Indeed, studies comparing lower tamoxifen doses in trials with more definitive clinical endpoints are warranted.

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