Effect Modifiers of Low-Dose Tamoxifen in a Randomized Trial in Breast Noninvasive Disease

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ABSTRACT

Purpose: Low-dose tamoxifen halved recurrence after surgery in a phase III trial in breast noninvasive disease without increasing adverse events. We explored the effect of low-dose tamoxifen in clinically relevant subgroups, including menopausal status, estradiol levels, smoking, body mass index, and proliferation of baseline lesion.

Patients and Methods: Incidence of invasive breast cancer or ductal carcinoma *in situ* was the primary endpoint. HRs and interaction terms were estimated using Cox models.

Results: A favorable HR and 95% confidence interval (CI) could be demonstrated for postmenopausal status (HR = 0.30; 95% CI, 0.11–0.82 vs. HR = 0.73; 95% CI, 0.30–1.76 in premenopausal women; $P_{interaction} = 0.13$), women with estradiol less than 15.8 pg/mL, presence of menopausal symptoms at baseline, and never smoking ($P_{interaction} = 0.07$), although the interaction *P* value was >0.05 for all characteristics. Efficacy was similar in all body mass index categories. Tumors with Ki-67 above the median level of 10% had a greater benefit (HR = 0.27; 95% CI, 0.09–0.81) than those with Ki-67 ≤10% (HR = 1.58; 95% CI, 0.45–5.60; $P_{interaction} = 0.04$).

Conclusions: The efficacy of low-dose tamoxifen seems to be greater in postmenopausal women and in women with lower estradiol levels. Benefits appear to be larger also in women with menopausal symptoms, never smokers, and tumors with Ki-67 >10%. Our results by menopausal status provide important insight into low-dose tamoxifen personalized treatment, although caution is necessary given their exploratory nature. Observation of an improved response in tumors with Ki-67 >10% is consistent but the use of the marker in this setting is investigational.

See related commentary by Fabian, p. 3510

Introduction

The uptake of preventive therapy in women at high risk for breast cancer is very low despite strong evidence of efficacy, primarily because of the fear of adverse events (1–3). Women at high risk include subjects with genetic and reproductive risk factors as well as women with histologic diagnosis of breast intraepithelial neoplasia, which comprises atypical ductal hyperplasia (ADH), ductal carcinoma *in situ* (DCIS), and lobular carcinoma *in situ* (LCIS). This group of preinvasive disorders has the highest benefit from tamoxifen or anastrozole among high-risk women (4–9), but toxicity, especially menopausal symptoms, musculoskeletal adverse events, and serious adverse events such as endometrial cancer, venous thromboembolism, bone fractures, and stroke have largely hampered preventive therapy uptake.

These considerations prompted us to conduct a phase III deescalation trial of low-dose tamoxifen, otherwise defined as "babytam." Biomarker studies including a window of opportunity presurgical trial had shown that the minimal effective dose of tamoxifen was below 20 mg/day (10). In addition, a large observational study showed a highly significant reduction of recurrence with low-dose tamoxifen in women with high-risk DCIS (11). In the current trial, women with operated hormone-sensitive or unknown breast intraepithelial neoplasia were randomized to either low-dose tamoxifen, 5 mg/day or placebo for 3 years. A total of 500 women ages 75 or younger were included, and after a median follow-up of 5.1 years, low-dose tamoxifen significantly decreased recurrence by 52% and contralateral breast events by 75% (12). Serious adverse events, including endometrial cancer and venous thromboembolic events, and patient reported outcomes were not different between arms except for a slight increase in frequency of daily hot flashes on low-dose tamoxifen, consisting of less than one extra hot flash per day. Although the power of detecting rare serious adverse events was not high in our study, indirect comparison with 20 mg/day suggest that the risk of endometrial cancer and deep vein thrombosis was 2.5 lower with 5 mg/day of tamoxifen (12), providing a new treatment option in the management of breast intraepithelial neoplasia (13, 14).

To get further insight into the efficacy of low-dose tamoxifen toward a personalized preventive approach, and given the trend toward a higher efficacy of full-dose tamoxifen in postmenopausal women versus premenopausal women (or women ages <50 years) both in

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Trial registration: EudraCT Number: 2007-007740-10; ClinicalTrials.gov Identifier: NCT01357772. Registered 17 May 2011 - Retrospectively registered, https://clinicaltrials.gov/ct2/show/NCT01357772.

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Translational Relevance

An investigator initiated phase III trial showed that low-dose tamoxifen given at 5 mg/day for 3 years halved recurrence after surgery in breast noninvasive disease without increasing adverse events, thus representing a valid treatment option in women at risk for invasive breast cancer. In this study, we assessed whether benefits were greater in defined patient subgroups with a focus on menopausal status and symptoms. Our findings suggest that the efficacy of low-dose tamoxifen is greater in postmenopausal women and in women with lower estradiol levels. Benefits may also be larger in women with menopausal symptoms, in never smokers, and in tumors with Ki-67 >10%. Our results provide further insight into low-dose tamoxifen personalized treatment and open the door for an easy and safe preventive therapy in high-risk individuals.

the adjuvant (15) and preventive (4) setting, we assessed whether its benefits were greater in patient subgroups defined by menopause-related factors and biological plausibility.

Patients and Methods

Subject and treatment

The study characteristics (EudraCT Number: 2007-007740-10; ClinicalTrials.gov Identifier: NCT01357772) and main clinical findings of the trial have recently been reported previously (12). The trial was approved in Italy by the Italian Medicines Agency (AIFA) and by the Ethical Committees for the Coordinating Center (E.O. Ospedali Galliera Ethical Committee) and the Participating Sites. The study was conducted in accordance with the Declaration of Helsinki and guidelines on Good Clinical Practice (ICH E6). All patients were informed of the objectives of the study and were invited to voluntarily participate. Patients who agreed to participate provided written consent before any study-specific procedure that could be withdrawn at any time without consequences for further treatment. Patients received a copy of their rights. The participant flow diagram is illustrated in the Supplementary Fig. S1. Briefly, women ages 75 or younger with Eastern Cooperative Oncology Group performance status ≤1 and excised hormone-sensitive [estrogen (ER) or progesterone receptor ≥1%] or unknown breast intraepithelial neoplasia, including ADH (20%), DCIS (70%), and LCIS (10%), were randomized to either low-dose tamoxifen, 5 mg/day or placebo for 3 years. Only women with high-grade or comedo/necrosis DCIS received 50 Gy adjuvant radiotherapy. Women received physical examination every 6 months and had an annual mammography and transvaginal ultrasounds for 3 years of treatment and 2 years of follow-up. Main exclusion criteria were: any prior invasive cancer, any tamoxifen contraindications, mental disorders, pregnancy, grade 2 or higher biochemical alterations, prior use of antiestrogens, current use of selective serotonin reuptake inhibitors. All breast events occurring during the trial were centrally adjudicated by a clinical committee. Treatment compliance was assessed by pill count. Adherence was defined at each study visit as the use of at least 85% of assigned pills of the 6-month study period. The primary endpoint was the incidence of invasive breast cancer or DCIS. Toxicity was assessed by the NCI-CTCAE version 3. Because menopausal symptoms are a major cause of treatment drop-out in tamoxifen trials (16, 17), patient reported menopausal symptoms at baseline were recorded by the Breast Cancer Prevention Trial Symptom Scale (18), which calculates scores on each 5-point Likert scale by averaging a number of items during the last 4 weeks before each semiannual visit, including vasomotor symptoms, bladder incontinence, sexual and vaginal problems, musculoskeletal pain/arthralgia, cognitive problems (forgetfulness, difficulty concentrating, and easily distracted) and weight gain. Menopause was defined as amenorrhea for at least 3 months and serum FSH above 45 mIU/mL and serum estradiol levels below 10 pg/mL. Six women originally classified as premenopausal women according to the interval from last menses were reclassified as postmenopausal according to hormone levels. Ki-67 labeling index was not mandatory in the protocol and was assessed in a subgroup of intraepithelial disorders at the entering institutions on a clinical basis and not performed centrally by IHC according to international recommendations and validation studies (19, 20) using the Mib-1 mAb (Dako).

Laboratory methods

The analyzes were performed in batches at a single laboratory at the European Institute of Oncology (Milan, Italy). We adopted an ultrasensitive RIA method (DSL4800, Immunotech) for the measurement of estradiol levels in postmenopausal women. The sensitivity of the assay is 2.2 pg/mL. The interassay coefficient of variation of our in-housed pooled serum sample (mean: 63.8 pg/mL; SD 7.2; 60 replicates) was 11.3%. For the determination of estradiol levels in premenopausal women, we adopted a chemiluminescent microparticle immunoassay technology designed for the ARCHITECT automated instrument (Abbott Diagnostics). The lower limit of detectability of the assay is <10 pg/mL. The interassay coefficient of variation of our in-housed pooled serum sample (mean: 75.1 pg/mL; SD 4.6; 10 replicates) was 6.1%. Blood collection according to the day since last menses in premenopausal was the following: days 1–7, $n = 33$, days 8–14, $n = 27$, days 15–21, $n = 28$, days 22–28, $n = 20$, days 29+, $n = 41$.

Statistical analysis

The cumulative incidences of invasive breast cancer and DCIS (primary endpoint) was estimated with the Kaplan–Meier method, which was also adopted to estimate treatment adherence by pill count at different follow-up times. All analyses included all randomized patients, according to the intention-to-treat principle. Censoring to last available follow-up visit was applied in the absence of a clinical event. Cox proportional hazards modeling was performed to calculate HRs in subgroups. Per protocol prespecified subgroup analyses were interval from diagnosis to study entry (≤12 vs. 13–60 months), ADH+DCIS versus LCIS and ER-positive versus unknown disease. The results of these subgroup analyses were reported previously (12). Additional unplanned subgroup analyses were based on menopause-related factors and biological plausibility consistent with the known effects of tamoxifen and included the following covariates: menopausal status, menopause-related symptoms according to Stanton and colleagues (18), baseline estradiol levels, body mass index (BMI), smoking status which is known to decrease tamoxifen adherence and efficacy in prevention trials (21, 22), Ki-67 labeling index of the primary intraepithelial neoplasm.

Further subgroup analyses according to ipsilateral or contralateral recurrence were not considered appropriate given the very low number of events in each strata. We performed and showed tests for interaction for all potential effect modifiers adding the specific interaction term within the Cox models. Because the study was not designed primarily to evaluate the effect of the interaction, we decided to increase the type I error rate (i.e., the likelihood that the study will find an interaction when, in fact, there is no such interaction in the population), which has

the effect of reducing the type II error rate and thus increasing the power (1 minus the type II error rate) of the interaction test. Consequently, to identify potential effect modifiers of clinical interest, we adopted a cut-off *P* value for the interaction test equal to 0.20 (i.e., a <20% probability that there is no such interaction in the population). Also, we did not apply any adjustment for multiplicity due to the exploratory nature of the subgroups analyses. Results are shown with 95% confidence intervals (CI) and two-sided *P* values. We performed all analyses using STATA, version 14.2 (STATA).

Data availability statement

A. DeCensi and M. Puntoni had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Data may be shared upon request for collaborative studies.

Ethics approval and consent to participate

The trial was approved in Italy by the Italian Medicines Agency (AIFA) and by the local Ethical Committee for all enrolling sites. This

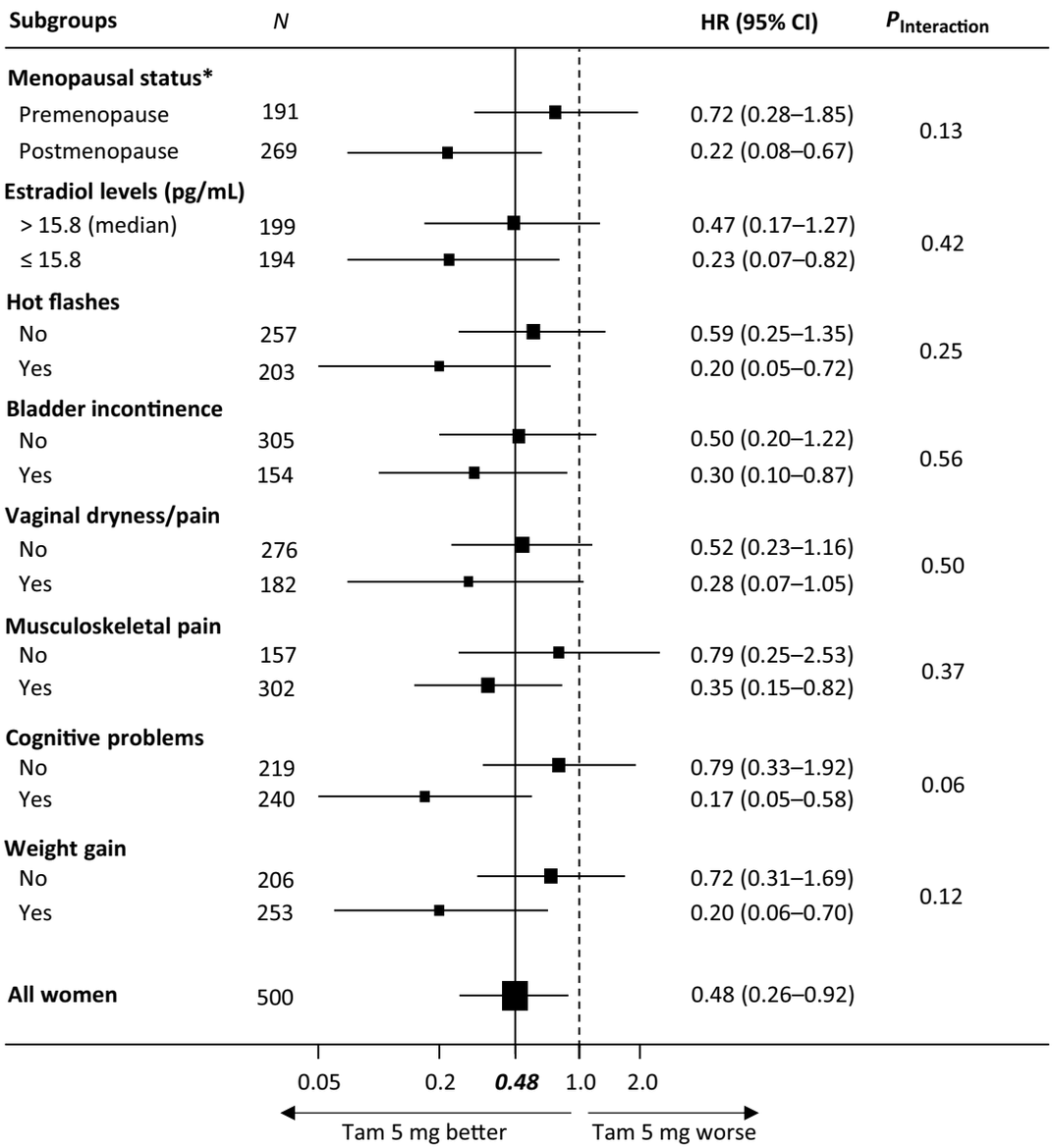

Figure 1.

Forest plot of the putative effect modifiers of low-dose tamoxifen with interaction terms. Menopausal status was adjusted for age, BMI, hot flashes, and smoking status at baseline ($P_{interaction} = 0.10$). Estradiol and menopausal symptoms were adjusted for age, menopausal status, BMI, and smoking status. HRs in subgroups are adjusted for age, BMI, menopausal, and smoking status at baseline. Please note that incomplete records were not included if there was missing or unknown data. Missing or unknown data: 33 (6.6%) for hot flashes; 34 (6.8%) for bladder control; musculoskeletal pain, and cognitive/weight problems; 35 (7%) for vaginal problems; 6 (1.2%) for smoking status; 23 (4.6%) for BMI.

study complies with the Declaration of Helsinki and guidelines on Good Clinical Practice (ICH E6). All patients are informed of the objectives of the study and are invited to voluntarily participate. Patients who agree to participate provide written consent before any study-specific procedure that can be withdrawn at any time without consequences for further treatment. Patients will receive a copy of their rights.

Results

The influence of menopause and menopause-related covariates on low-dose tamoxifen efficacy are depicted in **Fig. 1**. Menopausal status exhibited a borderline significant effect modification of low-dose tamoxifen after adjustment for age, BMI, hot flashes, and smoking status at baseline ($P_{interaction} = 0.13$). Similarly, after adjustment for age, menopausal status, BMI, and smoking status, the effect of low-dose tamoxifen was more pronounced in women with lower levels of estradiol at baseline as well as women with baseline menopausal symptoms, including hot flashes, bladder incontinence, vaginal problems, musculoskeletal pain, cognitive problems and particularly in women who reported cognitive problems and weight gain (low P values for interaction).

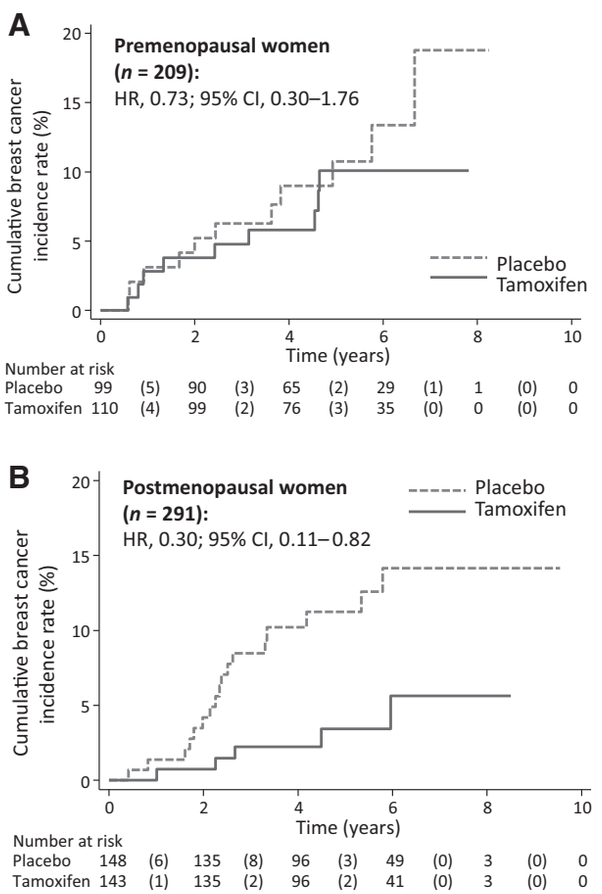

Figure 2.
Cumulative incidence of breast cancer by allocated arm and menopausal status. Premenopausal women are shown in **A** and postmenopausal women are shown in **B**. Numbers in parentheses represent the number of events (invasive breast cancer and DCIS) that occurred within each time interval by treatment arm.

Kaplan–Meier curves according to menopausal status are shown in **Fig. 2**. The unadjusted HR for low-dose tamoxifen on the primary endpoint were 0.73 (95% CI, 0.30–1.76) in premenopausal women ($n = 209$) and 0.30 (95% CI, 0.11–0.82) in postmenopausal ($n = 291$).

In a subgroup of women ($n = 406$), we measured estradiol levels at baseline. In **Fig. 3**, Kaplan–Meier curves of time to recurrence by median estradiol level (15.8 pg/mL) and treatment arm are shown. The unadjusted HRs for low-dose tamoxifen on the primary endpoint were 0.60 (95% CI, 0.23–1.55) in women with estradiol above the median ($n = 202$, mainly premenopausal women) and 0.23 (95% CI, 0.07–0.81) in women below the median level, mainly postmenopausal women ($n = 204$). Among postmenopausal women only, the effect of low-dose tamoxifen was equal (HR = 0.25) in women with median values below or above the median value of 11.7 pg/mL (not shown). The effect of low-dose tamoxifen on time to recurrence was similar according to BMI category (**Fig. 4**).

Treatment efficacy according to smoking status is illustrated in **Fig. 5**. Never smokers ($n = 323$) had a tendency to a greater benefit

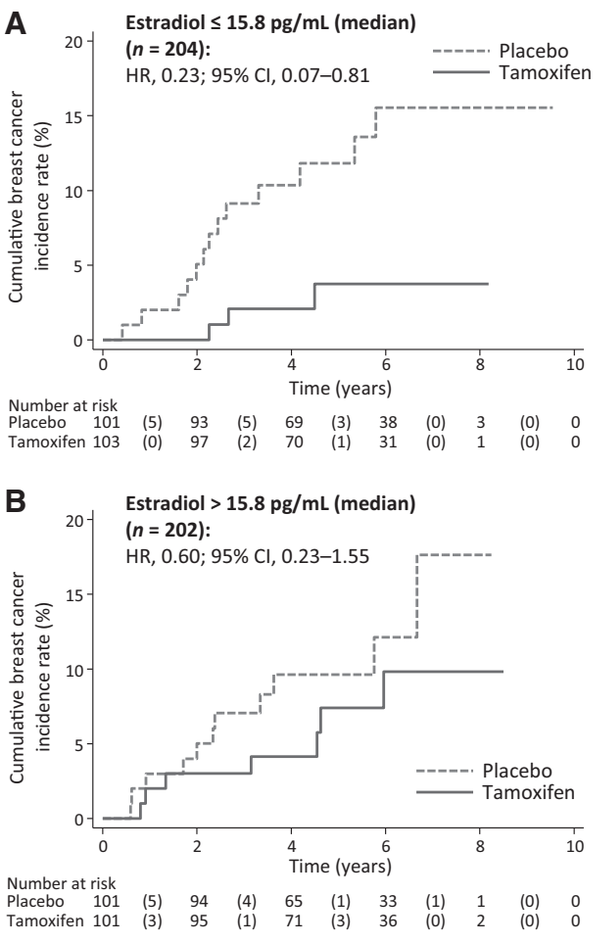

Figure 3.
Cumulative incidence of breast cancer by allocated arm and baseline estradiol level. Women with baseline estradiol levels below (**A**) and above (**B**) the median (18.5 pg/mL) are shown. Numbers in parentheses represent the number of events (invasive breast cancer and DCIS) that occurred within each time interval by treatment arm.

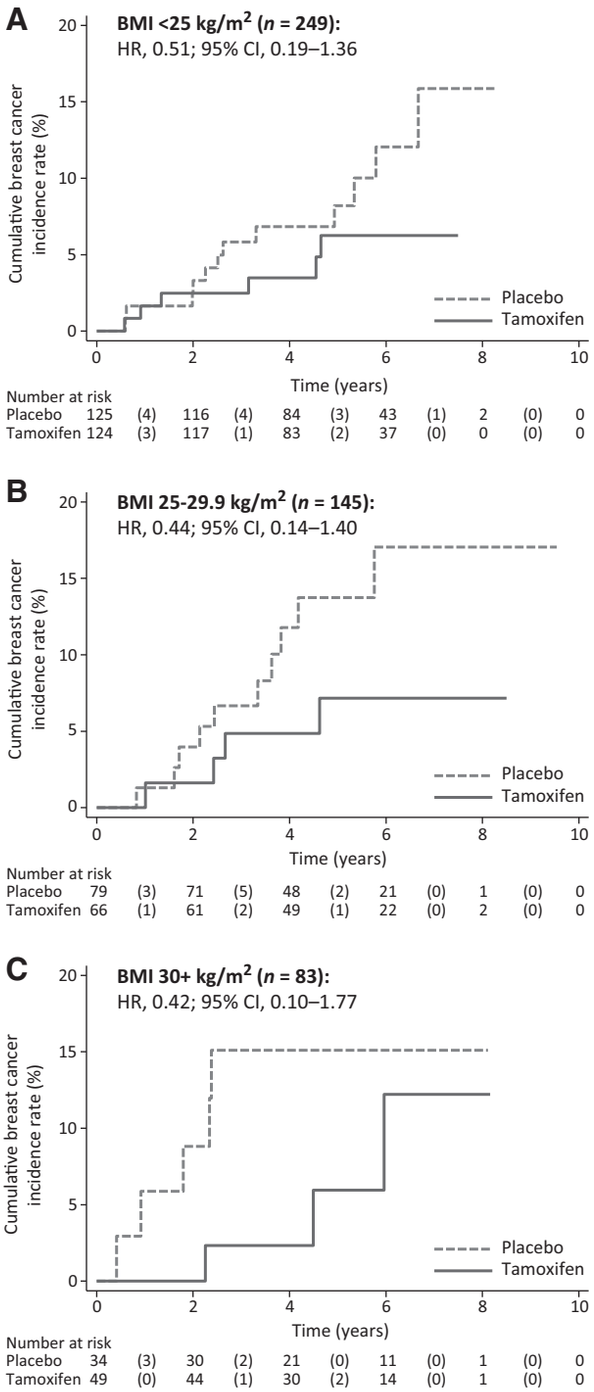

Figure 4.
Cumulative incidence of breast cancer by allocated arm and baseline BMI. Baseline normal weight (**A**), overweight (**B**), and obese women (**C**) are shown. Numbers in parentheses represent the number of events (invasive breast cancer and DCIS) that occurred within each time interval by treatment arm.

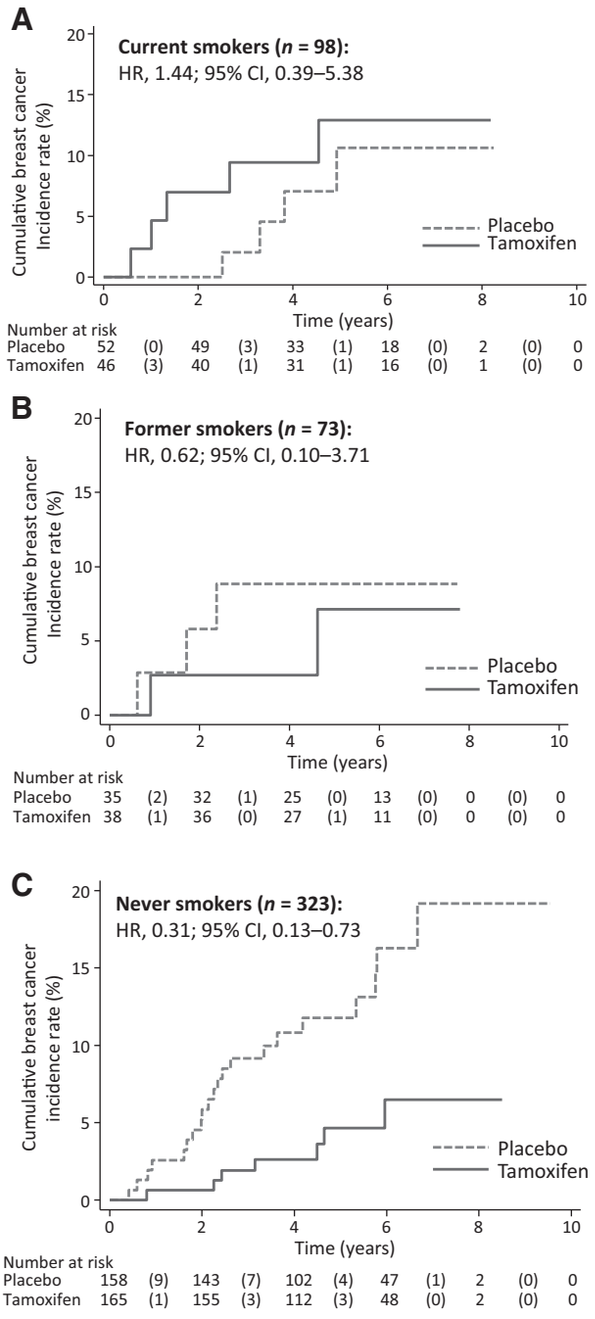

Figure 5.
Cumulative incidence of breast cancer by allocated arm and smoking status. Current smokers (**A**), former smokers (**B**), and never smokers (**C**) are shown. ($P_{interaction} = 0.07$). Numbers in parentheses represent the number of events (invasive breast cancer and DCIS) that occurred within each time interval by treatment arm.

from low-dose tamoxifen (HR = 0.31; 95% CI, 0.13–0.73) than former smokers ($n = 73$; HR = 0.62; 95% CI, 0.10–3.71) and current smokers ($n = 98$; HR = 1.44; 95% CI, 0.39–5.38, $P_{interaction} = 0.07$, adjusted for adherence = 0.11). Smokers were half premenopausal (49%) and half postmenopausal (51%), so there is no confounding effect of menopausal status to explain the trend toward a lower tamoxifen efficacy in smokers. Smoking habit also modified treatment adherence. Adjusting for age, BMI, and menopausal status at baseline, the risk of

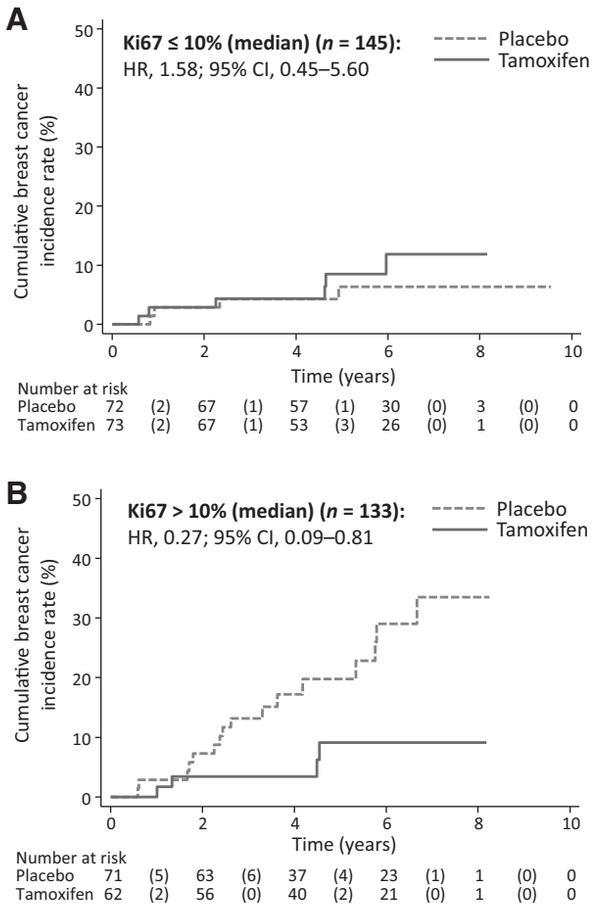

Figure 6.

Cumulative incidence of breast cancer by allocated arm and baseline Ki-67. Ki-67 ≤10% (median value; **A**) and Ki-67 >10% (**B**) are shown ($P_{interaction}$ = 0.04). Numbers in parentheses represent the number of events (invasive breast cancer and DCIS) that occurred within each time interval by treatment arm.

nonadherence was higher in current smokers compared with former/never smokers in the low-dose tamoxifen arm (HR = 0.53; 95% CI, 0.32–0.88), but not in the placebo arm (HR = 0.92; 95% CI, 0.55–1.54; $P_{interaction}$ = 0.13; Supplementary Fig. S2).

Measurement of the proliferation index Ki-67 in the primary lesions was available in a subgroup of 262 cases, 66% in DCIS, 36% in ADH, and 27% in LCIS. The effect of low-dose tamoxifen on the primary endpoint was significantly modified by Ki-67 ($P_{interaction}$ = 0.04; **Fig. 6**). Tumors with Ki-67 above the median level of 10% (n = 133) had a greater benefit from low-dose tamoxifen (HR = 0.27; 95% CI, 0.09–0.81) than those with Ki-67 ≤10% (n = 145, HR = 1.58; 95% CI, 0.45–5.60).

Discussion

Our exploratory study suggests that the efficacy of low-dose tamoxifen on the primary endpoint (invasive breast cancer or DCIS) is greater in postmenopausal women as well as in women with lower estradiol levels at baseline, in women with menopausal symptoms, in never smokers, and in tumors with Ki-67 above 10%.

In the worldwide overview of adjuvant trials (15), there was a significant trend toward a greater efficacy of tamoxifen on recurrence in women ages 55 or older compared with younger women. Likewise, in the NSABP-P1 prevention trial, there was a trend toward a greater risk reduction in women ages ≥60 years compared with women ages 50–59 years and women ages <49 years (1, 23). Conversely, in the IBIS-I trial, the efficacy of full-dose tamoxifen was not greater in women ages >50 years (24). Importantly, toxicity was much greater in postmenopausal women in the NSABP-P1 trial (1, 23), where tamoxifen pharmacodynamics tends to switch toward agonistic effects at target organs under low estrogen levels (25–27). In our trial, there was no association between menopausal status or age and low-dose tamoxifen on adverse events (12).

In our trial, the numerically and borderline statistically significant higher efficacy of low-dose tamoxifen in postmenopausal women and in women with menopausal symptoms may be explained by the different levels of circulating estrogens. All menopausal symptoms analyzed in the Stanton and colleagues questionnaire (18) can indeed be elucidated by the estrogen drop at menopause (28). Conversely, several reports have shown the levels of estradiol in premenopausal women on 20 mg of tamoxifen to be increased by 200%–300% (29–31), which might attenuate its antitumor effect by overcoming the levels of active metabolites endoxifen and 4OH-tamoxifen (32). In our trial, the levels of estradiol in premenopausal women increased by only 30% at 1 year and returned to baseline after 3 years (33), in line with prior studies of 5 mg/day, where the increase of circulating estradiol in premenopausal women was much lower than with the full dose (34). Our hypothesis is that low levels of active tamoxifen metabolites are still sufficient to inhibit low circulating estradiol levels in postmenopausal women or women with menopausal/hypoestrogenic symptoms, whereas in premenopausal women low-dose tamoxifen might be less effective because of the moderate increase in estrogen levels. Measurements of tamoxifen and metabolites and CYP2D6 genotype are currently underway in our trial to shed lights into this important issue. Interestingly, a dose reduction of tamoxifen from 20 to 10 mg daily resulted in halving of endoxifen levels and a significant subjective improvement of hot flashes during treatment (35), in line with our findings of only a mild increase in hot flashes frequency with low-dose tamoxifen versus placebo (12). Given the excellent efficacy and toxicity profile of low-dose tamoxifen in postmenopausal women, a primary prevention trial versus an aromatase inhibitor to define the most acceptable agent in terms of efficacy and safety/tolerability is warranted. Aromatase inhibitors may well be more effective than tamoxifen (36) but their tolerability and long-term compliance remains an important issue both in treatment and prevention setting (37).

Importantly, BMI did not influence low-dose tamoxifen efficacy, consistent with prior studies at 20 mg/day and in contrast with aromatase inhibitors (38, 39). This is reassuring as one might theoretically expect a lower efficacy of a low dose in overweight/obese women who represent a large fraction of the population with breast neoplasms in Western countries.

Interestingly, in our study, low-dose tamoxifen efficacy was influenced by smoking status at two levels, adherence and smoking. First, current smoking decreased adherence in women taking tamoxifen but not in women taking placebo. While the influence of smoking in decreasing compliance has previously been described in the NSABP-P1 trial (21), the selective effect on adherence just in the active arm of our double-blind placebo-controlled trial is novel and not easy to explain. Smoking may induce a "chemical rash" in the participants taking low-dose tamoxifen which leads to a selective tamoxifen withdrawal compared with smokers taking placebo. A second level

of effect modification induced by smoking habit was the approximately 70% reduction of recurrence noted in never smokers which was blunted in current smokers. This finding is in line with the results of the NSABP-P1 trial, where smoking was both a risk factor for breast cancer and an inhibitory factor of tamoxifen efficacy compared with former or never smokers (22). In their analysis, Land and colleagues (22) attributed this decreased efficacy mainly to the loss of adherence. Our results indicate that the trend toward a lower efficacy of low-dose tamoxifen in current smokers is at least partially independent of treatment adherence and suggest an additional hypothesis, that is, smoking attenuates low-dose tamoxifen efficacy due to direct and indirect mechanisms on the ER pathway, which is activated by tobacco smoking condensate through different metal estrogens (40, 41). While further confirmatory studies are necessary, our findings strengthen the importance of counseling women receiving tamoxifen to quit smoking to avoid loss of efficacy.

Our results suggest that tumors with higher proliferation and recurrence rate tend to respond well to low-dose tamoxifen, with over 70% risk reduction. It is possible that the tumor requires a minimum threshold of proliferation to be sensitive to the drug (42), or that a short period of observation is not sufficient to demonstrate a difference in events in women with low Ki-67 levels. Caution is necessary in interpreting our results given the lower number of observations and the investigational nature of this biomarker in the setting of noninvasive breast cancer which is not a standard practice and has not been validated by international guidelines.

Conclusions

In conclusion, low-dose tamoxifen given for 3 years is effective in women with preinvasive disease and provides a valid and potentially safer alternative to full-dose tamoxifen, as indicated by the new ASCO guidelines for breast cancer risk reduction (13). There was a tendency to a large benefit in postmenopausal women and in women with menopausal symptoms which might be explained by the presence of low circulating estrogens being effectively counteracted by low drug levels. BMI did not influence low-dose tamoxifen effect. Current smoking may decrease both adherence and efficacy to low-dose tamoxifen, possibly because of an activation of the ER pathway by tobacco smoking condensate. Subjects with higher tumor proliferation had a significant benefit to low-dose tamoxifen. Although biologically plausible our subgroup analyses were unplanned and based on small numbers, so findings should be taken with caution. Given the excellent efficacy and toxicity profile, however, a prevention trial in postmenopausal women comparing low-dose tamoxifen with an aromatase inhibitor is warranted.

Authors' Disclosures

H. Johansson reports grants from Italian Ministry of Health, Italian Association for Cancer Research (AIRC), and Italian League against Cancer and other from Italian Ministry of Health during the conduct of the study. A. Guerrieri-Gonzaga reports grants from Italian Association for Cancer Research and Italian League Against Cancer and other from Italian Ministry of Health during the conduct of the study. L. Cortesi reports personal fees from AstraZeneca, MSD, Pfizer, and Novartis outside the submitted work. M. Lazzeroni reports grants from Italian Association for Cancer Research and Italian League Against Cancer and other from Italian Ministry of Health during the conduct of the study. D. Serrano reports grants from AIRC and LILT, and other from Italian Ministry of Health during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

A. DeCensi: Conceptualization, resources, supervision, funding acquisition, investigation, visualization, methodology, writing–original draft. **M. Puntoni:** Conceptualization, data curation, software, formal analysis, supervision, methodology, writing–original draft. **H. Johansson:** Resources, data curation, investigation, methodology, writing–original draft. **A. Guerrieri-Gonzaga:** Conceptualization, data curation, writing–original draft. **S. Caviglia:** Data curation, project administration. **F. Avino:** Resources, investigation, writing–review and editing. **L. Cortesi:** Resources, investigation, writing–review and editing. **A. Ponti:** Resources, investigation, writing–review and editing. **M.G. Pacquola:** Resources, investigation, writing–review and editing. **F. Falcini:** Resources, investigation, writing–review and editing. **M. Gulisano:** Resources, investigation, writing–review and editing. **M. Digennaro:** Resources, investigation, writing–review and editing. **A. Cariello:** Resources, investigation, writing–review and editing. **K. Cagossi:** Resources, investigation, writing–review and editing. **G. Pinotti:** Resources, investigation, writing–review and editing. **M. Lazzeroni:** Resources, investigation, writing–review and editing. **D. Serrano:** Resources, investigation, writing–review and editing. **I.M. Briata:** Data curation, project administration. **T. Buttiron Webber:** Data curation, project administration. **L. Boni:** Conceptualization, data curation, software, formal analysis, supervision, methodology, writing–original draft. **B. Bonanni:** Conceptualization, resources, supervision, investigation, methodology, writing–original draft.

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