

Cancer Therapy: Clinical**Effect of Tibolone on Breast Cancer Cell Proliferation in Postmenopausal ER+ Patients: Results from STEM Trial**

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Abstract Purpose: Tibolone is a selective tissue estrogenic activity regulator, approved for the treatment of vasomotor symptoms in postmenopausal women. We have done an exploratory, double-blind, randomized, placebo-controlled pilot trial to investigate the tissue-specific effects of 2.5 mg tibolone on breast cancer in postmenopausal women, in particular on tissue proliferation (STEM, Study of Tibolone Effects on Mamma carcinoma tissue).

Experimental Design: Postmenopausal women with initially stage I/II, estrogen receptor-positive (ER+) primary breast cancer, were randomly assigned to 14 days of placebo or 2.5 mg/d tibolone. Core biopsies of the primary tumor were obtained before and after treatment. Ki-67 and apoptosis index were analyzed in baseline and corresponding posttreatment specimen.

Results: Of 102 enrolled patients, 95 had evaluable data. Baseline characteristics were comparable between both treatment groups. Breast cancer cases are mainly invasive (99%), stage I or II (42% and 50% respectively), and ER+ (99%). Median intratumoral Ki-67 expression at baseline was 13.0% in the tibolone group and 17.8% in the placebo group, and decreased to 12.0% after 14 days of tibolone while increasing to 19.0% in the placebo group. This change from baseline was not significantly different between tibolone and placebo (Wilcoxon test; $P = 0.17$). A significant difference was observed between the treatment groups when the median change from baseline apoptosis index was compared between the treatment groups (tibolone, 0.0%; placebo, +0.3%; Wilcoxon test; $P = 0.031$). The incidence of adverse effects was comparable.

Conclusions: In ER+ breast tumors, 2.5 mg/d tibolone given for 14 days has no significant effect on tumor cell proliferation.

Vasomotor symptoms are a major side effect of adjuvant breast cancer treatment and it has been estimated that up to 96% of women who undergo chemotherapy or endocrine therapy suffer from hot flashes or night sweats (1). Although these symptoms are thought to result from systemic estrogen and progesterin deprivation and can effectively be treated by hormone replacement therapy in patients who are not taking

tamoxifen, such a therapeutic strategy is contraindicated, mainly because of a fear of the proliferative and tumor-promoting effects attributed to sex steroids (2). Unfortunately, the efficacy and safety of phytoestrogens as alternatives in the treatment of vasomotor symptoms is unproven and nonhormonal therapies with serotonin reuptake inhibitors and gabapentin are, at best, approximately half as effective as estrogen (3, 4). In addition, nonhormonal therapies have no effect on other postmenopausal symptoms such as bone loss or urogenital atrophy (5).

Tibolone (Livial) is a selective tissue estrogenic activity regulator, approved for the treatment of climacteric symptoms in postmenopausal women. Following oral administration, it is converted into three primary metabolites, of which the 3 α -hydroxymetabolite and the 3 β -hydroxymetabolite only bind to the estrogen receptor α , whereas the parent compound tibolone and its Δ^4 -isomer bind to the progesterone and androgen receptors. The metabolism is tissue specific and results in a highly favorable profile of effects: Although tibolone exerts estrogenic effects on bone, brain, and the urogenital system through its hydroxymetabolites, it does not seem to stimulate endometrium or to increase breast tissue proliferation (6). It has been proposed that the lack of an estrogenic effect on breast tissue may result from the inhibition of sulfatase and the stimulation of sulfotransferase in this tissue (7, 8). Indeed, clinical studies have shown that compared with women who

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use continuous combined hormone treatment, tibolone users experience considerably less breast tenderness and do not develop an increase in mammographic density (9). Breast safety studies in the 7,12-dimethylbenz(a)anthracene model have shown that tibolone is highly effective in reducing tumor growth in mice (10). In addition, tibolone inhibits the sulfatase enzyme and promotes apoptosis in normal as well as breast cancer cells, thus suggesting a local antiestrogenic effect (11). The observation that tibolone is also able to decrease the proliferation rate of normal and malignant breast epithelial cells *in vitro* warrants particular attention (12, 13, 14). Dowsett et al. (15) have recently shown that the nuclear antigen Ki-67, which is expressed in proliferating cells, is an appropriate end point in neoadjuvant models of response to long-term endocrine therapies.

We have therefore studied tissue-specific effects of 14 days of 2.5 mg tibolone on breast cancer in postmenopausal women, in particular on the proliferation marker Ki-67 and on apoptosis, to support the hypothesis that tibolone has no adverse effects on malignant breast tissue.

Patients and Methods

Study design. The STEM (Study of Tibolone Effects on Mamma carcinoma tissue) study is a randomized, placebo-controlled, double-blind, multicenter, exploratory trial, in which postmenopausal women with initially stage I or II, estrogen receptor-positive (ER+) primary breast cancer were randomized 1:1 to receive a preoperative daily oral dose of 2.5 mg tibolone, or placebo for 14 days until surgery. Eligible patients had previously untreated, core-biopsy proven, invasive breast cancer without evidence of metastatic spread and any endocrine or enzyme modulator therapy was stopped at least 3 months before randomization.

Objectives. The aim of the trial was to confirm the safety of tibolone on breast tissue in women with early breast cancer as measured by the breast safety surrogate variables Ki-67 and apoptosis index. The primary trial objective was to compare changes in the expression of the proliferation marker Ki-67 label index in malignant breast tissue after treatment with tibolone or placebo for 14 days. Secondary objectives were the evaluation of treatment effects on tumor apoptosis index, on apoptosis-related proteins Bcl-2 and Bax, hormone receptors, hormone-

sensitive proteins, tumor vascularization, and angiogenesis. Moreover, endogenous estrogen levels and tibolone and tibolone metabolites in serum and breast tissue were measured. In addition, monitoring of (serious) adverse events, clinically significant abnormal laboratory values and summary statistics for vital signs was done to evaluate the overall safety of tibolone in women with early breast cancer. Although we here report the effect of tibolone, compared with placebo, on tumor proliferation and apoptosis and on overall safety in women with early breast cancer, other secondary end points will be presented elsewhere. The study was done in accordance with the ethical principles of the Declaration of Helsinki and was approved by the local institutional review board at all study sites. Only women who had given written voluntary informed consent were included into the trial. Block randomization was done per center to ensure that treatments were equally distributed in each of the participating centers. In total, 102 women were randomized in 14 sites in five countries between March 2003 and April 2005. Patient and tumor characteristics of women randomized into the tibolone and the placebo arm are depicted in Table 1.

Tumor assessments. Tumor samples were obtained by core biopsies at screening and after 14 days of treatment with study medication from the excised tumor at surgery. A minimum of three core biopsy samples was required per biopsy site to ensure a representative evaluation of proliferation and apoptosis. For each patient, Ki-67 and apoptosis index were analyzed, by immunohistochemistry, in both baseline and the corresponding posttreatment excision samples. Measurement of cell proliferation was done by using the MIB1 mouse monoclonal antibody to Ki-67 and counting the number of positive cells in a random sample of 1,000 cells. Apoptotic cells were identified by immunostaining using the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling method. The apoptotic index was expressed as a percentage of the total number of cells displaying apoptotic bodies in a random sample of 1,000 cells. The relative *Bcl-2* and *Bax* gene expression was determined by semiquantitative reverse transcription-PCR after extraction from snap-frozen tissue samples. Relative values were expressed as the ratio of specific transcripts/28S transcripts. All reverse transcription-PCR experiments were carried out at least thrice in duplicate on two different cDNA preparations.

Statistical measurements. For all breast tissue safety variables, descriptive summary statistics, number (*n*), median, mean, and SE were calculated for the intent-to-treat group. Subjects within this intent-to-treat group have received trial treatment (placebo or tibolone) and also have had a post-baseline assessment on Ki-67. The safety of tibolone versus placebo on the breast was explored by comparing the

Table 1. Baseline patient characteristics (intent-to-treat group)

Variable	Tibolone (n = 46)		Placebo (n = 49)	
	Median	Mean (SE)	Median	Mean (SE)
Age (y)	66	64.8 (1.1)	65	65.1 (1.1)
Weight (kg)	71	70.6 (1.3)	70	70.2 (1.3)
Height (cm)	161	160.8 (1.2)	160	160.6 (1.2)
BMI (kg/m ²)	28	27.4 (0.5)	28	27.2 (0.5)
Age at last menses (y)	50	48.4 (0.8)	49	48.1 (0.8)
Time since menopause (y)	17.1	16.4 (1.4)	14.6	17.0 (1.4)
Frequency distribution	Tibolone (n = 46)		Placebo (n = 49)	
ER at screening (%)				
Positive	46 (100%)		49 (100%)	
Tumor stage, clinical (%)				
I	18 (39%)		21 (43%)	
II	24 (52%)		24 (49%)	
III	2 (4%)		3 (6%)	
IV	0 (0%)		1 (2%)	
Unknown	2 (4%)		0 (0%)	

Table 2. Changes in intratumoral Ki-67 protein expression in response to 14 d of treatment (intent-to-treat group)

	Baseline		Surgery		Change from baseline		P*
	Tibolone	Placebo	Tibolone	Placebo	Tibolone	Placebo	
Ki-67 (%)							0.170
<i>n</i>	46	49	46	49	46	49	
Median	13.0	17.8	12.0	19.0	-2.4	0.2	
Mean (SE)	18.2 (2.2)	21.6 (2.8)	16.3 (2.3)	22.0 (2.4)	-1.9 (2.1)	0.4 (1.2)	

NOTE: *n* = number of patients.

*Wilcoxon test on treatment differences for change from baseline.

postbaseline change from baseline measurements of Ki-67, apoptosis index, and the relative gene expression levels of *Bcl-2* and *Bax* by Wilcoxon tests. All analyses were done using SAS Version 8.2 or higher under Windows NT.

Results

Patients. The study cohort consisted of elderly (mean age ~ 65 years), postmenopausal women with a mean body mass index of slightly over 27 years, who suffered from breast cancer. There were no significant differences between the groups at baseline. At screening, tumor status in ~90% of patients was ER+ and also tumor staging was comparable between groups. Although at the time of randomization, all patients presented with stage I and II disease, the complete postoperative histologic evaluation of tumor size and lymph node involvement resulted in an upstaging in six patients. The main patient characteristics are presented in Table 1 for the intent-to-treat group.

Intratumoral Ki-67 protein expression in pretherapeutic and posttherapeutic breast cancer biopsies. Table 2 shows the intratumor levels of Ki-67 at baseline and after 14 days of trial treatment. The median baseline Ki-67 levels were 13.0% in the tibolone arm and 17.8% in the placebo arm, whereas median posttreatment levels ranged from 12.0% in the tibolone arm to 19.0% in the placebo arm. The difference between the treatment groups in changes from baseline in intratumoral Ki-67 expression of -2.4% in the tibolone group and of +0.2% in the placebo group was nonsignificant (Wilcoxon test, $P = 0.17$). The individual changes from baseline in Ki-67 are presented in Fig. 1.

Intratumoral apoptosis index and *Bcl-2* and *Bax* gene expression in response to tibolone. The intratumoral apoptosis index measured at baseline and after 14 days of treatment with 2.5 mg tibolone or placebo are shown in Table 3 for the intent-to-treat group. The median pretreatment apoptosis index in both the tibolone arm and the placebo arm was 1.4%, whereas the mean posttreatment apoptosis index was 1.6% in the tibolone arm, and 1.7% in the placebo arm. The resulting net changes in the median intratumoral apoptosis index were 0.0% in the tibolone group and 0.3% in the placebo group (Wilcoxon test on treatment differences $P = 0.03$).

Summary statistics of apoptosis-related proteins *Bcl-2* and *Bax* mRNA expression at baseline and surgery showed similar results (Table 3). Median values of the antiapoptotic marker *Bcl-2* and the proapoptotic marker *Bax* were not changed significantly by either tibolone or placebo (Wilcoxon test: *Bax*, $P = 0.739$; *Bcl-2*, $P = 0.642$).

Overall patient safety. Treatment with 2.5 mg tibolone for 14 days was safe and well tolerated. No significant differences between tibolone and placebo were observed with respect to the incidence and type of adverse events (Table 4). The most frequent adverse events during tibolone treatment were postprocedural pain (13.7%), asthenia (10%), and pyrexia (8%). Two serious adverse events occurred, an ischemic stroke in the tibolone group and a postoperative wound infection in the placebo group. Tibolone treatment was only discontinued once (2%), in response to one serious adverse event (ischemic stroke). As expected, treatment with 2.5 mg tibolone for 14 days did not significantly alter blood pressure or heart rate (data not shown).

Discussion

It is estimated that ~70% of women who suffer from breast cancer are postmenopausal. The remaining 30% will often become postmenopausal as a result of their cancer treatment. Climacteric symptoms are thus an increasingly relevant side effect of antineoplastic strategies, and associated symptoms such as hot flashes and night sweats can considerably affect quality of life (16). Although hormone treatment effectively ameliorates vasomotor symptoms, its use is contraindicated in women with a history of breast cancer. These recommendations are mostly based on the proliferative effect of estrogens on tumor cells *in vitro*, but also on epidemiologic evidence for an increase in breast cancer incidence that is especially strong in estrogen/progestin-containing hormone replacement therapy formulations (17). At least three large trials have attempted to evaluate the safety of hormone replacement therapies in women with a history of breast cancer but none of them has been able to recruit sufficient patients, largely because of a fear of increased recurrence (18). A recently conducted meta-analysis of several smaller trials did not find an increase in breast cancer recurrence or in cancer-associated mortality with estrogen or estrogen progestin treatment, although one prospectively randomized trial was terminated early because of the finding that the treatment increased the risk of recurrence (19, 20). Thus far, no effective treatment for the alleviation of menopausal symptoms has been approved for women with breast cancer.

Tibolone is a synthetic steroid that is distinct from currently available hormone treatment because of its mode of action and by its favorable clinical profile (21). *In vitro*, tibolone and its Δ^4 -isomer have antiproliferative effects on normal breast cells and increase apoptosis in both normal and malignant breast

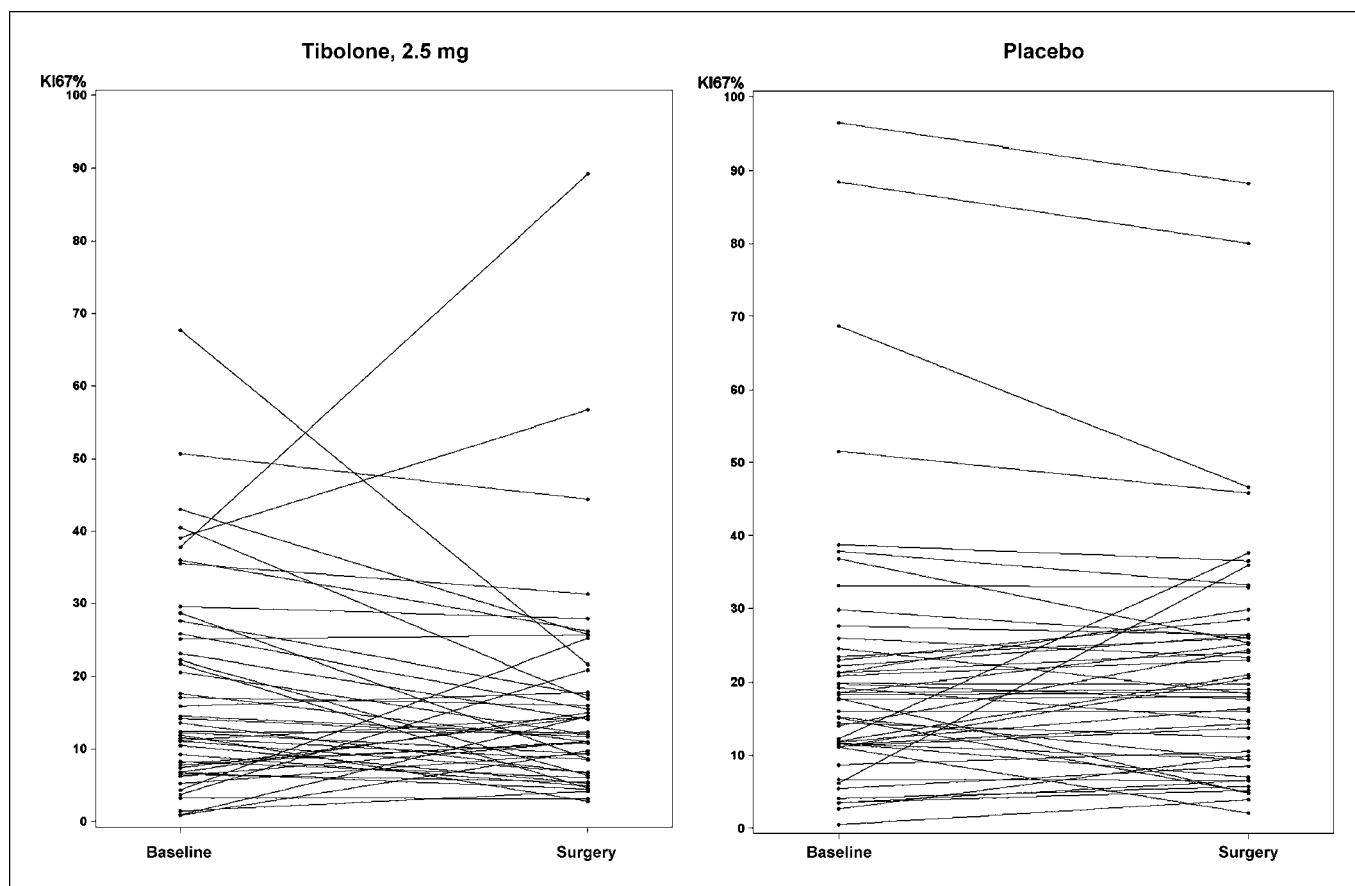


Fig. 1. Individual changes from baseline in Ki67 protein levels after 2 wk in the tibolone (left) and placebo (right) groups.

epithelium, presumably through decreased expression of the antiapoptotic proteins Bcl-2 and Bax (22). In addition, tibolone and its 3β-hydroxy metabolite and also the Δ⁴-isomer exert anti-invasive effects on breast cancer cell lines *in vitro*.

Two recent smaller trials have suggested that tibolone may indeed be safe in women with a history of breast cancer: An

observational study has recently found no increase in systemic recurrences or contralateral breast cancer in 156 breast cancer survivors who have received tibolone for 18 to 96 months when compared with placebo after completion of 5 years of tamoxifen (23). In addition, Kroiss et al. (24) have shown the efficacy of tibolone in the reduction of hot flushes in

Table 3. Changes in intratumoral apoptosis index, and relative *Bcl-2* and *Bax* gene expression in response to 14 d of treatment (intent-to-treat group)

	Baseline		Surgery		Change from baseline		P*
	Tibolone	Placebo	Tibolone	Placebo	Tibolone	Placebo	
Apoptosis index (%)							0.031
n	41	40	44	48	39	39	
Median	1.4	1.4	1.6	1.7	0	0.3	
Mean (SE)	1.5 (0.12)	1.5 (0.11)	1.6 (0.12)	1.7 (0.10)	0 (0.08)	0.3 (0.08)	
<i>Bcl-2</i> (%)							0.739
n	28	21	28	21	28	21	
Median	0.8	0.9	1	1	0.1	0.1	
Mean (SE)	0.8 (0.06)	0.9 (0.07)	1 (0.09)	1.1 (0.11)	0.2 (0.09)	0.3 (0.13)	
<i>Bax</i> (%)							0.642
n	28	21	28	21	28	21	
Median	0.6	0.6	0.7	0.7	0.1	0	
Mean (SE)	0.5 (0.04)	0.6 (0.04)	0.7 (0.08)	0.9 (0.15)	0.2 (0.08)	0.3 (0.15)	

NOTE: The relative *Bcl-2* and *Bax* gene expression is depicted as the ratios of the specific transcript/28S transcript.
*Wilcoxon test on treatment differences for change from baseline.

Table 4. Adverse events (all subjects treated group)

	Tibolone (n = 51)	Placebo (n = 51)
Adverse events, total	17 (33%)	14 (28%)
Postprocedural pain	7 (14%)	4 (8%)
Asthenia	5 (10%)	5 (10%)
Pyrexia	4 (8%)	2 (4%)
Nausea	3 (6%)	1 (2%)
Dizziness	3 (6%)	0 (0%)
Headache	3 (6%)	0 (0%)
Serious adverse events, total	1 (2%)	1 (2%)
Ischemic stroke	1 (2%)	0 (0%)
Postoperative wound infection	0 (0%)	1 (2%)
Serious adverse event leading to discontinuation	1 (2%)	0 (0%)
Death	0 (0%)	0 (0%)

tamoxifen-treated breast cancer patients. Although only 70 patients were enrolled into the trial, none of the women in the tibolone arm experienced a recurrence during the 12 months of treatment and no endometrial stimulation was observed in the tibolone/tamoxifen arm.

The favorable therapeutic profile has led to the design of the "Livial Intervention following Breast Cancer; Efficacy, Recurrence, and Tolerability Endpoints" (LIBERATE) study, an international multicenter trial that has recruited >3,000 patients and that is expected to report a first analysis by end of 2007. The aim of the study is to show noninferiority of tibolone compared with placebo in respect to breast cancer recurrence and to show that tibolone is safe and effective in women with breast cancer and vasomotor symptoms. Recently, however, the use of tibolone has been associated with an increased relative risk for the development of breast cancer in a large cross-sectional cohort trial (25). Although it should be noted that in this study only 88 of the 7,140 women who developed breast cancer during the observational period had exclusively received tibolone as hormone treatment, this unexpected observation has prompted further investigation of an explanation for this finding. It can, at least partly, be explained by the widespread practice of "preferential prescribing": Women with a history of breast cancer or chronic breast disease and women from high breast cancer risk families are more likely to receive tibolone for alleviation of their climacteric symptoms than estrogen-progestin combinations because of the perceived "breast safety" of tibolone by many physicians (26, 27).

We have studied the effects of tibolone on malignant breast tissue in an *in vivo* model that has been suggested to parallel long-term endocrine effects: Dowsett et al. (15) have recently

shown that changes in intratumoral Ki67 protein expression under neoadjuvant endocrine therapy can correspond to the disease-free survival in the large adjuvant ATAC Trial. Ki67 is therefore now increasingly used as an end point in neoadjuvant studies, and our observation of essentially unchanged Ki67 levels in biopsies from the placebo group that were taken 2 weeks apart underscore the appropriateness of this approach (28). Our results are also somewhat support observations by Valdivia et al. (29), who even showed that a 12-month treatment with tibolone leads to a reduction in Ki67 expression and to a stimulation of apoptosis, albeit in normal breast tissue of postmenopausal women. Although, in contrast to their findings, we did not observe an increase in intratumoral apoptosis by tibolone, we did find a nominal but insignificant reduction in Ki67. The attenuated effect of tibolone that is seen in tumor tissue can at least in part be explained by the fact that whereas in normal breast tissue the effect of tibolone is measured in more or less homogenous tissues, in malignant breast tumors the situation is considerably more complex: Core biopsies can contain considerably different fractions of tumor cells and tumor stroma, and the biological activity of tumor cells also varies from patient to patient, which could have somewhat weakened the effect of the compound. We have, however, no explanation for the fact that 14 days of placebo result in a significant increase of apoptosis.

Taken together, the STEM trial has shown that tibolone, a commonly prescribed hormone replacement therapy, given for 14 days in postmenopausal women with ER+ breast cancer, has no adverse effect on the expression of the proliferation marker Ki-67 and on surrogate variables of apoptosis in malignant epithelium. To our knowledge, this is the first clinical trial that has investigated the proliferative and apoptotic effects of a hormone replacement therapy in breast cancer patients in the preoperative setting. If the still ongoing LIBERATE trial indeed shows that tibolone given for 5 years has no adverse effects on tumor recurrence, our results would complement previous findings by the IMPACT trial and thus provide further evidence for the usefulness of short-term neoadjuvant *in vivo* assays. Preoperative *in vivo* studies such as the one presented here might thus potentially allow us to predict the long-term outcomes of novel endocrine therapies in a small, safe, and short *in vivo* setting, and eventually help to avoid unnecessarily lengthy and considerably more expensive adjuvant trials in the future.

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