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Original article

Benefits and Risks of Hormonal therapy (Tamoxifen) in Women with Positive Hormones Receptors Breast Cancer

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Abstract

Aim: *We studied the benefits and risks of tamoxifen therapy in women with positive hormonal receptors breast cancer.* ***Methods:*** *A total of 170 women with breast cancer diagnosed between 2012 and 2013 at the National Oncology Institute, Sabratha, Libya were included. The demographic, clinicopathological data, treatment and follow-up were collected from the patient's files as well as data regarding the adverse event of therapy.* ***Results****: The mean age of the patients was 45.1 years (range 21-80 years). Of them, 127 patients had hormonal dependent tumour, and tamoxifen as hormonal therapy was given to 52.4% of them in a period of less than 5 years, while 22.3% of the patients were used tamoxifen for more than 5 years. Forty three patients had hormonal independent tumor and no hormonal therapy was given for those patients. The median follow-up duration was 47.2 months (range, 5-125 months). At the end of follow up period, 101 (59.4%) patients were alive, 60 (35.5%) had experienced disease recurrence, and 69 (40.6%) had died. Using Kaplan-Meier survival curves showed that a shorter survival was associated with hormonal receptors negative breast cancer patients who not received tamoxifen, While, patients with hormonal receptors positive tumors and received tamoxifen were associated with a longer survival ($p<0.0001$). Regarding the toxicity of tamoxifen therapy, the most frequent adverse events were sweating and hot flashes (22.4% and 14.7 % respectively) followed by weight gain, endometrial hyperplasia (11.8 % and 6.5 % respectively). No women experienced serious adverse events such as thromboembolic, cerebrovascular complications and endometrial carcinoma.* ***Conclusion****: Tamoxifen as hormonal therapy in patients with hormonal dependent breast cancer was associated with a longer overall survival as well as may be safe and valid.*

Keywords: *Hormone Receptors, Positive Breast Cancer, Tamoxifen Therapy, Benefits, Risks.*

Introduction

Globally, breast cancer is the most common invasive cancer in women. It is the most commonly diagnosed cancer with over two million cases were diagnosed in 2018 with 600.000 deaths [1.2]. Breast cancer is more common in developed countries and is more than 100 times more common in women than man [3,4]. Prognosis of breast cancer vary depending on the histology type, the stage of disease, and age of patients [5]. The five-year survival rates in developed countries are between 80 and 90% [6]. In developing countries, five-year survival rates are lower [3]. In the African countries and in Libya, management of breast cancer forms a big medical, social and economic issue. Breast cancer patients in those countries often present with younger age, premenopausal status, have early disease recurrence, late stage and are associated with poor survival [7-10].

Breast cancer is usually treated with surgery, which may be followed by chemotherapy and/ or radiotherapy or both [11]. Hormone receptor-positive cancers are often treated with hormone-blocking therapy over courses of several years [12].

Estrogen and progesterone are hormones that regulate the sex hormone-dependent organs such as the female reproductive system and the breast. Steroid hormones with growth factors drive the development, growth, and differentiation of breast epithelial tissue following activation of the nuclear estrogen (ER) and progesterone (PR) receptors and are also critical for breast cancer development and progression [13]. The removal of estrogen from the environment of the cells containing steroid receptors adversely affects the growth of these cells. This is the basis for hormonal therapy in breast cancer patients. Approximately 70%

of breast cancers are ER-positive, and most ER-positive breast cancers are also PR-positive [14].

Hormonal treatment slows or ceases the growth of hormone-dependent tumors by inhibiting ability of the body to yield hormones or by interfering with hormone action. Breast tumors that are hormone-independent do not respond to hormonal treatment. Hormonal treatment includes ovarian ablation, blocking the effects of estrogen, and blocking of estrogen production. Many types of drugs interfere with the ability of estrogens to stimulate the growth of breast cancer cells such as selective estrogen receptor modulators (SERMs). Examples of SERMs approved by the FDA are tamoxifen, raloxifene, and toremifene for treatment of breast cancer patients. Tamoxifen has been used for more than 3 decades to treat hormone-dependent breast cancer patients. Tamoxifen is metabolized by the cytochrome P450 (CYP2D6) into primary active metabolite (endoxifen) [15]. Tamoxifen plays an important role in the treatment of both premenopausal and post-menopausal patients with early and advanced breast cancer as well as for primary prevention in women with high risk of breast cancer [16,17].

Tamoxifen has been associated with certain adverse effects. The most important event is the development of endometrial cancer; it is reported to occur at a rate from 2 to 7 times more often than in untreated women [18]. Tamoxifen use is also associated with an increased risk of deep venous thrombosis and pulmonary emboli, stroke, cataract, and depression [18,19]. The aim of this study was to investigate the benefits of hormonal therapy (tamoxifen) regarding the patient outcomes in women with hormone positive receptors breast cancer as well as the adverse events of tamoxifen therapy.

Materials and Methods

Clinicopathological data

The study group included 170 women with breast cancer diagnosed between 2012 and 2013 at the National Oncology Institute, Sabratha, Libya. The demographic and clinicopathological data were collected from the patient's files. The collected data included age, menopausal status, family history, hormonal status, histological type, tumor stage, histological grade, treatment, and follow up. Regarding collect data on the toxicity of hormonal therapy (Tamoxifen). In this context, data was also collected from the patient's files during follow-up. At each visit, data was collected on the following: menopausal like symptoms (sweating and hot flashes), nausea, vomiting, skin rashes, weight gain, any events of allergic reaction, changes in mood, eye disease, liver disease, gynecology related events (irregular period, vaginal discharge and/or itching, vaginal/uterine bleeding), and coagulation related events (deep vein thrombosis, stroke) The results of other investigations including ultrasound, CT, MRI and blood tests results were also collected by searching the patient's medical files. Follow-up was performed until Feb 2022 and included breast-abdomen ultrasound, chest X-ray every 3 months within the first 2 years of diagnosis and even 6 months within 3-5 years.

Tumour staging of breast carcinoma was evaluated according to TNM classification data [20]. Monoclonal antibody was used for determination of hormone receptors and interpretation was done with Allred score method [21].

Treatment and follow-up

Radical surgery was done for 151 patients. No therapeutic surgical intervention was done for 19 patients with metastasis at time of diagnosis (diagnosis with core biopsy). Adjuvant, neoadjuvant and palliative chemotherapy was given to 163 patients. No chemotherapy was given to 5 patients with early stage, and 2 patients were unfit to receive chemotherapy. Radiotherapy was given to node-positive patients (n=130). Hormonal treatment (tamoxifen at dose 20mg/day) was given to 127 patients with hormone receptor positive. The patients were followed-up until death or to the end of the observation period (Feb, 2022). The median follow-up duration was 47.2 months (range, 5-125 months). At the end of follow up period, 101 (59.4%) patients were alive, 60 (35.5%) had experienced disease recurrence, and 69 (40.6%) had died.

Statistical analysis

The variables of the material were grouped into logical classes and descriptive statistics calculated for the continuous variables using SPSS 26.0 for Windows (SPSS, Inc., Chicago, USA). Frequency tables were analysed using the Chi-square test to assess the significance of the correlation between the categorical variables. For survival analysis, Kaplan-Meier curves were plotted, and differences between the curves analyzed using the log-rank test. In all tests, values of $p< 0.05$ were regarded as statistically significant.

Ethical consideration

This study is part of the breast cancer studies, which have got permission from the local ethical committee at the National Cancer Institute at Sabratha, Libya.

Results

Demographic and clinicopathological features of patients

Demographic and clinicopathological features of patients are shown in Table 1. The mean age at diagnosis of the patients was 45.1 years (range 21-80 years). The majority of patients (64%) were aged less than 50 years and premenopausal (64% and 62.4; respectively) and 2. 8.2 percent of patients had a family history of breast cancer. The most frequent histological type was invasive ductal carcinoma (80%) and positive lymph nodes was reported in 130 patients. In term of stage of disease, 59 patients were early staged (1 and 2) and late stage (3 and 4) was reported in 111 patients. For hormonal status, 74.7% of patients had hormonal dependent tumor and tamoxifen as hormonal therapy was given to 52.4% of patients in period less than 5 years. while, 22.3 % of patients were used tamoxifen more than 5 years. 43 patients (25.3%) had hormonal independent tumor and no hormonal therapy was given for those patients.

Table 1. Demographic and Clinicopathological features of patients

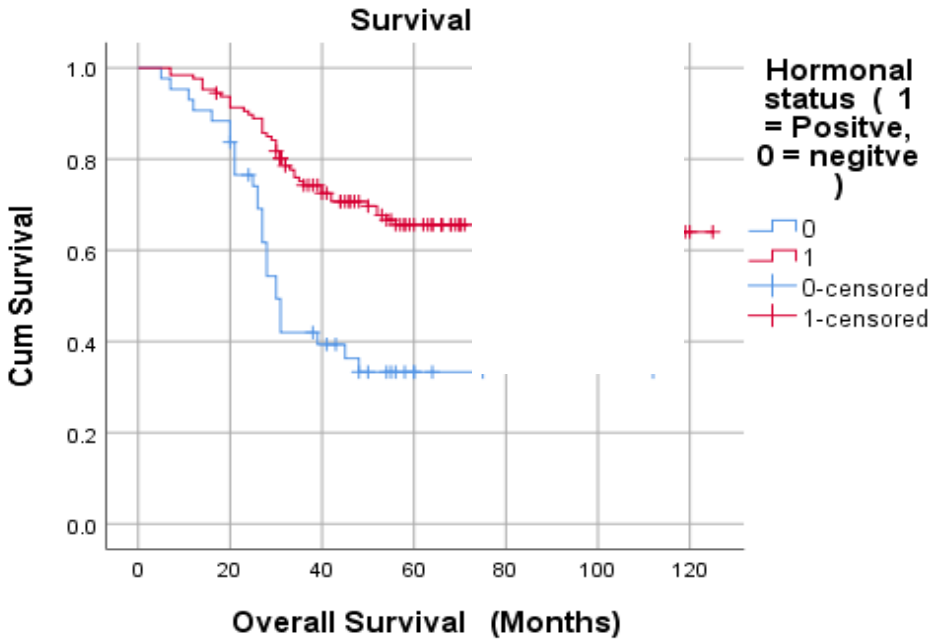
Features		Number of patients	Percent
Age (years)	<50	109	64.1
	≥50	61	35.9
Family history	Positive	14	8.2
	Negative	156	91.8
Menopausal status	Premenopausal	106	62.4
	Postmenopausal	64	37.6
Hormonal status	Positive	127	74.7
	Negative	43	25.3
Histological type	IDC	136	80.0
	Other types	34	20.0
Lymph node status	Positive	130	76.5
	Negative	40	23.5
Histological grade	Grade 1	22	12.9
	Grade 2	79	46.5
	Grade 3	69	40.6
Clinical stage	Early stage (1+2)	59	34.7
	Late stage (3+4)	111	65.3
Metastases at diagnosis	M0	151	88.8
	M1	19	11.2

Patients' outcome

Univariate survival analysis (survival rates) with hormone receptors expression and tamoxifen treatment is shown in Table 2. The survival rate was 58.0% in patients with positive hormone receptors and received tamoxifen as hormonal therapy. While 36.0% in patients with negative expression and did not receive hormonal therapy ($p < 0.0001$).

Patients' outcome using Kaplan-Meier survival curves for women who received tamoxifen as hormonal therapy and women who did not received tamoxifen are shown in Figure 1. A shorter survival was associated with hormonal receptors negative breast cancer patients who not received hormonal therapy. While, patients with hormonal receptors positive breast cancer and received tamoxifen were had the best overall survival ($p<0.0001$).

Figure 1. Overall survival according to analysis of hormone receptor expression in breast cancer (Kaplan-Meier curves). Women with hormone receptor positive breast cancer and received hormonal therapy (Tamoxifen) showed a longer survival than negative hormonal receptors



Adverse events of tamoxifen therapy

Side effects and adverse events in women with breast cancer patients treated with tamoxifen are shown in Table 3. The most frequent adverse events were sweating and hot flashes (22.4% and 14.7 % respectively) followed by weight gain, endometrial hyperplasia (11.8 % and 6,5 % respectively) and other events in decreasing frequency as shown in Table 2. In this study, no women experienced serious adverse events such as thromboembolic, cerebrovascular complications and endometria carcinoma.

Table 2. Survival analysis of patients with hormonal therapy versus non hormonal therapy

Variables		Number of patients	Survival analysis			P- value
			Median survival (months)	Mean survival (months)	Survival rate (percent)	
All patients		170	46.50	52.34	59.4	
Hormonal therapy (tamoxifen)	Yes	127	55.00	57.94	66.9	<0.0001
	No	43	30.00	35.79	37.2	

Table 3. Adverse events in hormone positive breast cancer patients treated with Tamoxifen

Adverse events	Number of patients	Percent
Sweating	38	22.4
Hot flashes	25	14.7
Skin rashes	8	4.7
Nausea and vomiting	6	3.6
Allergic reaction	2	1.2

Depression	9	5.3
Blurred vision	4	2.4
Wight gain	20	11.8
Osteoporosis	5	2.9
Fatty liver	5	2.9
Irregular period	9	5.3
Vaginal discharge	8	4.7
Ovarian cyst	5	2.9
Uterine polyp	2	1.2
Uterine fibroid	4	2.4
Endometrial hyperplasia	11	6.5
Hepatic failure	0	0.0
Coagulations events	0	0.0
Endometrial carcinoma	0	0.0

Discussion

Breast cancer affects 1 in 8 women during their lifetime. About 2-4 of those cancers are positive for either the estrogen or the progesterone receptor, where estrogen and progesterone are the key drivers of carcinogenesis [22]. Hormonal therapy, which lowers estrogen levels and inhibits the growth of the cancer, remains the standard systemic treatment for hormone receptor positive breast cancer in the neoadjuvant, adjuvant, and metastatic settings as well as for primary prevention in women and high risk of breast cancer [16,17]. The balance between duration of hormonal therapy with the efficacy as well as adverse event is highly considered. After 5 years of tamoxifen, either continuation of tamoxifen or a switch to an aromatase inhibitor (AIs) for an additional 5 years is effective in reducing the risk of recurrence and to improve overall survival [23]. The absolute benefit of extended therapy is small and must be weighed against the potential side effects of serious complication such as venous thrombosis and endometrial cancer with tamoxifen and of osteoporotic fractures with the AIs [24,25].

In this study, 170 women with breast cancer diagnosed during 2012 and 2013 were investigated for the efficacy and toxicity of tamoxifen as hormonal therapy in patients with hormone positive breast cancer. The mean age the patients was 45.1 years and the majority of patients were aged less than 50 years as observed in this study. These results suggested that patients in Libya have low mean age at diagnosis showing that premenopausal cancers are more common than in Europe. These also confirm results of other studies in Libya [7-9]. The age pattern is identical with age of breast cancer patients in Africa or Middle and North Africa (MENA) region [7,9,10].

Libyan patients with breast cancer were presented with a high grade of malignancy such as a high-grade tumor, positive lymph nodes, and advanced stages as observed in this work. This results in agreements with other studies in Libya [7]. Some studies suggest that African American women with breast cancer are more likely to presents with advanced stage and more aggressive tumor at time of diagnosis than white women [26]. This can reflect the failure of medical care leading in delay of diagnosis and staging or differences in biology and /or genetic of the African American population.

In our study, 127 (74.7%) patients had hormonal dependent tumour and 43 (23.5%) were hormone independent. Tamoxifen as hormonal therapy was given to all hormone dependent tumour patients with variation in duration of treatment. The association between patients who received tamoxifen and patient's outcome was evaluated. The results suggested that overall survival was longer in patients who received tamoxifen than independent hormone patients ($p<0.0001$). These findings in the lines of other observations [22-27].

The adverse events of tamoxifen therapy also evaluated in this work and results showed that menopausal like symptoms such as sweating and hot flashes were the most frequent adverse events followed by weight gain, endometrial hyperplasia and other events in decreasing frequency. Moreover, serious adverse events such as thromboembolic, cerebrovascular complications and endometrial carcinoma were not reported in this study.

The toxicity of tamoxifen as hormonal therapy was investigating in numerous studies. Hot flashes are one of the most common side effects of tamoxifen, being reported in up to 40-

60% of patients undergoing therapy [28]. The selective serotonin reuptake inhibitors, reduce the occurrence of tamoxifen-related hot flashes by decreasing the conversion of tamoxifen to its most active metabolite [29]. However, strong cytochrome P450 2D6 (CYP2D6) inhibitors could adversely affect drug efficacy. Therefore, moderate CYP2D6 inhibitors (such as sertraline and duloxetine) are preferred over strong inhibitors (such as paroxetine and fluoxetine) for the treatment of hot flashes.

The relative risk of venous thromboembolism is increased by a factor of 1–3 in older women receiving tamoxifen [30]. The risk seems to be further pronounced when therapy is extended to 10 years from 5 in the adjuvant setting [31].

Tamoxifen has been associated with a risk for both endometrial cancer and uterine sarcoma that is increased by a factor of 2.3; however, the absolute annual risk of endometrial cancer remains low at 1.2 per 1000 patient/years ([32]. The elevated risk of cancer persists as long as the patient takes tamoxifen and declines after treatment discontinuation [33]. The following recommendations are in place for surveillance of uterine cancer in women taking tamoxifen [33].

Conclusion

Tamxifen as hormonal therapy in patients with hormonal dependent breast cancer was associated with a longer overall survival. Sweating and hot flashes were the most frequent adverse events. No women experienced serious adverse events such as thromboembolic, cerebrovascular complications and endometrial carcinoma.

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References

1. McGuire A, Brown JA, Malone C, McLaughlin R, Kerin MJ (May 2015). "Effects of age on the detection and management of breast cancer". Cancers. 7 (2): 908–29.
2. Cancer. World Health Organization. 12 September 2018. Retrieved 16 July 2020.
3. World Cancer Report 2014. World Health Organization. 2014. pp. Chapter 5.2. ISBN 978-92-832-0429-9.
4. Male Breast Cancer Treatment". National Cancer Institute. 2014. Archived from the original on 4 July 2014. Retrieved 29 June 2014.
5. Breast Cancer Treatment (PDQ®)". NCI. 26 June 2014. Archived from the original on 5 July 2014. Retrieved 29 June 2014.
6. Cancer Survival in England: Patients Diagnosed 2007–2011 and followed up to 2012" (PDF). Office for National Statistics. 29 October 2013. Archived (PDF) from the original on 29 November 2014. Retrieved 29 June 2014.
7. Ermiah E, Buhmeida A, Khaled B, Abdalla F, Salem N, Pyrhönen S, Collan Y. Prognostic value of bcl-2 expression among women with breast cancer in Libya. Tumor Biol 2013; 34:1569-1578.
8. Stapleton JM, Mullan PB, Dey S, Hablas A, Gaafar R, Seifeldin IA, Banerjee M, Soliman AS: Patient-mediated factors predicting early- and late-stage presentation of breast cancer in Egypt. Psycho-oncology 2011, 20(5):532-537.
9. El Mistiri M, Verdecchia A, Rashid I, El Sahli N, El Mangush M, Federico M: Cancer incidence in eastern Libya: the first report from the Benghazi Cancer Registry, 2003. Int. J. Cancer 2007, 120(2):392-397.
10. Abulkhair O, Saghir N, Sedky L, Saadedin A, Elzahwary H, Siddiqui N, Al Saleh M, Geara F, Birido N, Al-Eissa N et al: Modification and implementation of NCCN guidelines on breast cancer in the Middle East and North Africa region. J Natl Compr Canc Netw 2010, 8 Suppl 3:S8-S15.
11. Saini KS, Taylor C, Ramirez AJ, Palmieri C, Gunnarsson U, Schmoll HJ, et al. (April 2012). "Role of the multidisciplinary team in breast cancer management: results from a large international survey involving 39 countries". Annals of Oncology. 23 (4): 853–9.
12. Burstein HJ, Temin S, Anderson H, Buchholz TA, Davidson NE, Gelmon KE, et al. (July 2014). "Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: american society of clinical oncology clinical practice guideline focused update". Journal of Clinical Oncology. 32 (21): 2255–69.
13. Joshi PA, Jackson HW, Beristain AG, Di Grappa MA, Mote PA, Clarke CL, Stingl J, Waterhouse PD, Khokka R. Progesterone induces adult mammary stem cell expansion. Nature 2010; 465: 803- 807.
14. Freedman RA, Winer EP. Adjuvant therapy for postmenopusal women with endocrine-sensetive breast cancer. Breast 2010; 19: 69-75.
15. Desta Z, Ward BA, Soukhova NV, Flockhart DA. Comprehensive evaluation of tamoxifen sequental biotrasnsformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. J Pharmacol ExpTher 2004; 310: 1062-1675.
16. Beslija S, Bonneterre j, Burstein JH, Cocquy V, Gnant M, Heinemann V, Jassem J, Ko stler WJ, Krainer M, Menard S, Petit T, Petruzelka L, Possinger K, Schmid P, Stadtmauer E,

Stockler M, Van Belle S, Vogel C, Wilcken N, Wiltschke C, Zielinski C, Zwierzina H for the Central European Coopoerative Oncolgy Group (CECOG). Third consensus on medical tratment of metastatic breast cancer. Ann Oncol 2009; 20:1771-1785.
17. National Comprehensive Cancer Network (NCCN) guidelines, Breast cancer Version 2019.
18. Fisher B, costantino JP, Wickerham DL, Cecchini RS, Cronin WM, Robidoux A, Bevers TB, Kavanah MT, Atkins JN, Margolese RG, Runowicz CD, James JM, Ford LG, Wolmark N. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. J Natl Inst 2005; 97:1652-1662.
19. Vogel VG, costantino JP, Wickerham DL, , Cronin WM, Cecchini RS, Atkins JN, Bevers TB, Fehrenbacher L, Pajon ER Jr, Wade JL 3rd, Robidoux A, Margolese RG, James J, Lippman SM, Runowicz CD, Ganz PA, Reis SE, McCaskill-Stevens W, Ford LG, Jordan VC, Wolmark N. Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease oucome: the NSABP Study of Tamoxifen and Raloxifene (STAR) p-2 trial. JAMA 2006; 295: 2727-2741.
20. AJCC: 5th Edition AJCC Cancer Staging Manual. In : Lippincott-Raven; 1997.
21. DC Allred, JM Harvey, M Berardo, GM Clark. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 1998; 11: 155-68.
22. Howlader N, Altekruse SF, Li CI, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. J Natl Cancer Inst. 2014;106:pii. doi: 10.1093/jnci/dju055. dju055.
23. Davies C, Pan H, Godwin J, et al.on behalf of the ATLAS Collaborative Group Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor–positive breast cancer: ATLAS, a randomised trial. Lancet. 2013;381:805–16.
24. Davies C, Godwin J, Gray R, et al.on behalf of the Early Breast Cancer Trialists' Collaborative Group Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet. 2011;378:771–84. doi: 10.1016/S0140-6736(11)60993-8.
25. Braithwaite RS, Chlebowski RT, Lau J, George S, Hess R, Col NF. Meta-analysis of vascular and neoplastic events associated with tamoxifen. J Gen Intern Med. 2003;18:937–47. doi: 10.1046/j.1525-1497.2003.20724.
26. Adams SA, Butler WM, Fulton J. Racial disparities in breast cancer mortality in a multiethenic chort in Southeast. Cancer 2012; 118: 2693-2699.
27. Early Breast Cancer Trialists' Collaborative Group Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet. 2005;365:1687–717.
28. Day R. Quality of life and tamoxifen in a breast cancer prevention trial: a summary of findings from the NSABP P-1 study. National Surgical Adjuvant Breast and Bowel Project. Ann N Y Acad Sci. 2001;949:143–50.
29. Goetz MP, Rae JM, Suman VJ, et al. Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. J Clin Oncol. 2005;23:9312–18.
30. Davies C, Godwin J, Gray R, et al.on behalf of the Early Breast Cancer Trialists' Collaborative Group Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet. 2011;378:771–84.
31. Davies C, Pan H, Godwin J, et al.on behalf of the ATLAS Collaborative Group Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor–positive breast cancer: ATLAS, a randomised trial. Lancet. 2013;381:805–16.
32. Braithwaite RS, Chlebowski RT, Lau J, George S, Hess R, Col NF. Meta-analysis of vascular and neoplastic events associated with tamoxifen. J Gen Intern Med. 2003;18:937–47.
33. Committee on Practice Bulletins–Gynecology ACOG practice bulletin no. 126: management of gynecologic issues in women with breast cancer. Obstet Gynecol. 2012;119:666–82.