Journal of Cellular Biochemistry, Supplement 22:42–50 (1995)

Tamoxifen's Role in Chemoprevention of Breast Cancer: An Update

Susan G. Nayfield, MD, MSc

Community Oncology and Rehabilitation Branch, Division of Cancer Prevention and Control, National Cancer Institute, Bethesda, MD 20892

Abstract Tamoxifen is an oral antiestrogen first used in metastatic breast cancer in the early 1970s. Large clinical trials were initiated in the late 1970s and early 1980s to test the drug's role as adjuvant therapy in early stage breast cancer. Observations of marked decreases in the development of contralateral breast cancer among tamoxifen recipients suggested potential for the drug in chemoprevention of breast cancer, and a large clinical trial to test the efficacy of tamoxifen in prevention of invasive breast cancer among women at increased risk was implemented in the United States in 1992.

This paper reviews the rational for the clinical studies of tamoxifen as a chemopreventive agent for breast cancer and summarizes new information that has contributed to our understanding of tamoxifen's actions at the molecular and clinical levels. Current knowledge about the drug's mechanism of estrogenic and antiestrogenic action and its beneficial effects on blood lipids and bone metabolism will be presented. Recent research findings about DNA adduct formation and hepatic lesions, tamoxifen-associated gynecologic conditions, and the occurrence of second primary cancers in other organ systems will also be discussed.

Key words: Breast cancer, bone metabolism, chemoprevention, colorectal cancer, DNA adducts

During the late 1970s and early 1980s, large clinical trials were initiated to evaluate tamoxifen as adjuvant therapy following surgical resection of early stage breast cancer [1]. Observations of marked decreases in the development of new primary cancers in the contralateral breast of patients receiving tamoxifen compared to controls suggested potential for the drug in chemoprevention of breast cancer.

A large clinical trial to test the efficacy of tamoxifen in prevention of invasive breast cancer among women at increased risk for the disease, the NCI-sponsored Breast Cancer Prevention Trial (BCPT), was implemented in 1992 [2]. Experience with tamoxifen in breast cancer treatment and the rationale for the BCPT were presented in several review articles published around that time [3–5]. This paper summarizes new information that has contributed to our understanding of tamoxifen's actions at the molecular and clinical levels.

TAMOXIFEN PHARMACOLOGY

Tamoxifen is a triphenylethylene compound with both estrogenic and antiestrogenic actions. Oral medication is rapidly absorbed, 99% protein-bound, and eliminated by hepatic metabolism. Elimination of tamoxifen occurs in a biphasic pattern with an effective half-life of several days. Recently, Buzdar *et al.* [6] demonstrated that once-daily administration of 20 mg tamoxifen is bioequivalent to the standard 10 mg twice-daily regimen.

Address correspondence to Susan G. Nayfield, MD, MSc, Community Oncology and Rehabilitation Branch, Division of Cancer Prevention and Control, National Cancer Institute, Executive Plaza North, Suite 300, 9000 Rockville Pike, Bethesda, MD 20892

Metabolism and Formation of DNA-Adducts

Tamoxifen is metabolized in the liver by cytochrome P-450 (CYP) isoenzymes. Recent studies have explored metabolic functions associated with specific CYP isozymes. CYP1A1, CYP1A2, CYP2A2, CYP2A6, CYP2B6, CYP2D6, CYP2E1, and CYP3A4 metabolize tamoxifen to its major metabolite *N*-desmethyltamoxifen [7,8]. The highest rates of *N*-demethylation are associated with CYP2B6 and CYP3A4; however, CYP2B6 is expressed at low levels by most humans and is unlikely to play a major role in tamoxifen metabolism. Detectable levels of 4-hydroxytamoxifen were found in cells expressing human CYP2D6 and CYP2E1. Finally, an increase in micronuclei has been observed when cells expressing human CYP3A4, CYP2D6, and CYP2E1 were exposed to tamoxifen. The role of specific CYP isozymes in the formation of reactive intermediates and DNA adducts has not been clearly defined.

Reactive intermediates formed by CYP-dependent monooxygenases have been shown to bind irreversibly to liver microsomal proteins [9], while intermediates such as tamoxifen *N*-oxide epoxide appear to interact with DNA to produce covalent adducts, as demonstrated by ^{32}P postlabelling techniques [10]. Potter *et al.* [11] postulated that electrophilic (alkylating agent) intermediates which give rise to the DNA adducts are produced from tamoxifen by hepatic α-oxidation of its ethyl group. Observations of reduced genotoxicity (decreased DNA adduct formation and micronuclei induction) by deuterated [D_5-ethyl]-tamoxifen compared with tamoxifen implicates α-hydroxylation as a major pathway of tamoxifen activation to a reactive intermediate capable of DNA adduct formation [12].

The role of α-hydroxytamoxifen as a reactive species is strongly supported by findings of Phillips *et al.* [13] who showed that α-hydroxytamoxifen gives rise to high levels of DNA adducts in cultured rat hepatocytes. This mechanism for activation is consistent with the lack of adduct formation found with tamoxifen analogues and other antiestrogens that undergo little phase I (oxidative) metabolism. In the mouse, inhibition of sulfotransferase activity by pentachlorophenol increases levels of several adduct fractions but decreases levels of other adducts [14]. This suggests that sulfation of tamoxifen reactive intermediates may be important in detoxification to non-electrophilic derivatives, but that an alternative pathway, insensitive to pentachlorophenol, also exists in this species.

Formation of tamoxifen-DNA adducts in rodent liver and associated morphologic changes in liver cells have been linked to the hepatic carcinogenesis observed in these species, particularly in the rat [15,16]. Pathak and Bodel [17] reported the formation of similar tamoxifen-DNA adducts by human and rat liver microsomal preparations. However, Martin *et al.* [18] found marked species differences in number of adducts: approximately 3,000 adducts/10^8 normal nucleotides in rats receiving the equivalent of 40 mg/kg/day compared to 15–45 adducts/10^8 normal nucleotides in patients chronically receiving standard doses of tamoxifen. In fact, adduct levels observed in livers from patients receiving tamoxifen did not differ from the levels of adducts observed in livers from human controls. This marked difference in adduct levels between rats and humans is consistent with the clinical observations that primary hepatic tumors rarely occur in breast cancer patients receiving tamoxifen despite their frequent occurrence in rats given the drug. Whether these findings relate to interspecies differences in the occurrence and/or inducibility of specific P-450 isozymes or in oxidative/conjugative metabolic pathways remains to be determined.

Interaction With the Estrogen Receptor

Inhibition of [^3H]estradiol binding to estrogen receptors by tamoxifen suggests that its antiestrogenic properties are due to competition with endogenous estradiol for estrogen receptor binding sites. Elucidation of the molecular structure of the estrogen receptor and specific functions of its structural domains has led to a better understanding of tamoxifen's estrogen antagonist and agonist activities [19]. Extracellular estradiol diffuses across the cell membrane and binds to the nuclear estrogen receptor. This leads to receptor dimerization and tight binding of the homodimer to its specific DNA target. Stabilization of estrogen receptor dimerization and its subsequent DNA binding, enhanced by estradiol, are followed by the transcription of targeted genes through unknown mechanisms.

In the estrogen receptor domain structure, the central region containing the DNA binding do-

main (DBD) separates two activation domains (AF-1 and AF-2). The AF-1 domain activates transcription when DNA is bound to the DBD. The AF-1 domain is constitutive, *i.e.*, stabilization of DNA binding is sufficient for AF-1 activation. Tamoxifen is able to stabilize receptor dimerization and DNA binding in a manner similar to estradiol, thus activating the AF-1 domain; its (weak) estrogen agonist activity has been attributed to this phenomenon. The AF-2 activation domain overlaps the adjacent hormone binding domain (HBD) and can only activate transcription when it is bound to estradiol or another estrogen agonist. Tamoxifen's estrogen antagonist activity has been attributed to competitive inhibition of estradiol-dependent activation of AF-2. Pure antiestrogens inhibit receptor dimerization and subsequent DNA binding or promote receptor degradation, thereby inhibiting activation of both AF-1 and AF-2.

The activities of AF-1 and AF-2 may also be mediated by cofactors which may be specific for the domains. A 160 kD estrogen receptor-associated protein (ERAP160) has been identified which exhibits estradiol-dependent binding to the HBD/AF-2 region and appears to mediate the ability of the receptor to activate transcription [20]. The ability of 4-hydroxytamoxifen, a metabolite of tamoxifen, to block interaction between ERAP160 and the estrogen receptor may explain tamoxifen's antiestrogenic effects in breast cancer. The ability of ERAP160 to bind to other members of the nuclear receptor family, specifically the retinoic acid receptors RARβ and RXRα, may explain the enhancement observed between tamoxifen and retinoids in animal models of carcinogen-induced mammary tumors.

METABOLIC EFFECTS

Cardiovascular Effects

Initial reports of lipid-lowering effects of tamoxifen have focused attention on potential reduction in morbidity and mortality due to cardiovascular disease. Comprehensive data on cardiovascular risk factors in postmenopausal women is available from the Wisconsin Tamoxifen Study of 140 patients with node-negative breast cancer randomized to receive tamoxifen 20 mg/day or placebo for a two-year period [21]; five year follow-up is now available for subgroups of participants who never received tamoxifen versus those who continued tamoxifen for a total of five years [22]. Statistically significant decreases from baseline values of serum cholesterol (approximately 12%) and low-density lipoprotein (LDL) cholesterol (approximately 20%) were observed during the first three to six months of tamoxifen therapy and appeared to stabilize at these lower levels for the duration of treatment. These findings are consistent with recent reports from other studies [23–25]. Women with higher baseline cholesterol levels had greater decreases with tamoxifen. Trends toward increased triglyceride levels and decreased high-density lipoprotein (HDL) cholesterol during the first year of tamoxifen treatment did not alter favorable ratios of total cholesterol to HDL cholesterol and of LDL to HDL cholesterol. After five years, levels of serum cholesterol, LDL cholesterol, and lipoprotein(a) were significantly lower among patients who continued tamoxifen throughout the follow-up period compared to those who never received tamoxifen. Decreases in fibrinogen among tamoxifen-treated patients were of borderline significance, and no significant differences were detected between baseline and five-year levels of triglycerides and HDL cholesterol [22]. Decreases in serum cholesterol were also maintained by the subgroup of tamoxifen-treated participants in the Scottish Adjuvant Trial who were re-randomized to continue tamoxifen for a total of five years; however, cholesterol lowering effects did not persist among patients re-randomized to stop therapy at two years [23].

Clinical benefits associated with tamoxifen's favorable impact on cardiovascular risk factors have been demonstrated within the context of two large randomized adjuvant therapy studies. Long-term follow-up of participants in the Stockholm Adjuvant Tamoxifen Trial through computer-based registry of hospital admissions and discharges has demonstrated a statistically significant reduction in hospital admissions due to cardiac disease among patients receiving tamoxifen compared to controls, with greater effect among patients receiving tamoxifen for five years compared to two years [26]. A significant decrease in fatal myocardial infarction associated with tamoxifen therapy has been reported among participants in the Scottish Adjuvant Tamoxifen Trial [27].

The mechanisms by which tamoxifen modulates cardiovascular risk factors are incompletely understood. While tamoxifen's effects resemble those of estrogen in some regards, qualitative and quantitative differences are reflected in greater decreases in LDL cholesterol, lipoprotein(a), and fibrinogen when tamoxifen is compared to estrogen, and by lack of changes in HDL cholesterol with tamoxifen [22]. Both tamoxifen and estradiol appear to protect LDL cholesterol from *in vitro* oxidative damage, possibly by stabilizing the phospholipid layer of the LDL particle [28]; tamoxifen's antioxidative activities may also protect cardiac microsomal membranes from oxidative damage and impede the development of atherosclerotic plaques [29]. Studies by Gylling *et al.* [30] suggest that tamoxifen may also inhibit cholesterol synthesis at the level of microsomal Δ^8-isomerase.

Bone Metabolism

Several recent studies have confirmed or expanded earlier findings suggesting estrogen agonist effects of tamoxifen on age-related changes in bone metabolism. Dual-photon absorptiometry studies of postmenopausal women participating in the Wisconsin Tamoxifen Study demonstrated a 0.61%/year increase in bone mineral density of the lumbar spine among those receiving tamoxifen compared to a 1.00%/year decrease observed among those receiving placebo [31]. Density of the radius, measured by single-photon absorptiometry, decreased similarly for patients in both groups. Prevention of bone loss at the femoral neck and lumbar spine by tamoxifen has also been demonstrated for women in the early menopausal period (within the first five years of menopause) [32]. In addition, predicted bone loss in the lumbar spine and femoral neck due to prednisolone administration may be prevented among patients receiving concurrent tamoxifen [33].

Long-term effects of tamoxifen on bone density are less well described. In contrast to the continued increase reported above, Kristensen *et al.* [34] found that lumbar spine bone mineral density in postmenopausal breast cancer patients on tamoxifen stabilized after increases during the first year of therapy, compared to continued decreases in the control group. Following an initial (minor) decrease during the first 12 months, bone mineral content of the forearm stabilized among tamoxifen-treated women compared to continued decline in controls. Stabilization of bone density in both the lumbar spine and forearm persisted through the remaining year of therapy; however, measures were not taken following cessation of tamoxifen. A small cross-sectional study of breast cancer patients receiving tamoxifen for at least five years showed only a nonsignificant trend toward higher bone mineral density in the femoral neck and lumbar spine by dual energy X-ray absorptiometry when compared to controls matched for age, stage, and date of diagnosis [35], raising questions regarding the duration of bone effects.

Serum and urinary markers for bone turnover also support a protective effect of tamoxifen on bone metabolism and suggest a beneficial effect, at least for the duration of therapy. Serum osteocalcin and alkaline phosphatase decreased significantly in women receiving tamoxifen compared to baseline values and to placebo-treated controls [31, 34]; significant decreases in urinary hydroxyproline and serum osteocalcin occurred by six months and persisted throughout treatment [32]. Histomorphometric studies on transiliac crest bone biopsies in post- and perimenopausal breast cancer patients receiving tamoxifen for a minimum of 15 months demonstrated significantly lower tissue-based bone formation rate and an increase in remodeling period compared to untreated controls [36]. Resorption cavity depth and area were significantly less for tamoxifen-treated women, and there was a tendency for increased connectedness in trabecular bone structure.

The mechanism of tamoxifen's effects on bone metabolism is largely unexplored, and the clinical significance of these changes remains to be determined. Long-term follow-up of patients participating in the Stockholm Adjuvant Tamoxifen Trial failed to show a difference in hospital admissions related to osteoporosis for patients receiving tamoxifen compared to those receiving no hormonal therapy [37].

TOXICITY

General Symptomatology

In general, tamoxifen is well-tolerated with few side effects. Experience from adjuvant therapy clinical trials reflects a 5–10% dropout rate

[5]; however, the compliance rate among women in the British chemoprevention pilot study appears lower, approximately 70–75% at two years [38].

Hot flashes and other vasomotor symptoms are the most commonly reported side effect; approximately 15–20% of women receiving tamoxifen develop hot flashes attributable to the drug [38,39]. These symptoms appear more commonly among younger women [38], despite the exaggerated levels of estradiol and total estrogens reported among premenopausal patients receiving tamoxifen [40], suggesting that estrogen deficiency *per se* is not a major contributor to vasomotor symptoms in these patients. Although hormone replacement therapy is allowed for participants in the British prevention pilot study [41], its efficacy in controlling vasomotor symptoms among women receiving tamoxifen has not been reported. Low dose megestrol acetate may be effective in controlling hot flashes in many patients receiving tamoxifen [42]. However, the potential induction of cytochrome P-450 isozymes by hormonal medications, especially progestational agents, suggests that the impact of these drugs on tamoxifen metabolism should be explored carefully before routine use of hormonal supplements is undertaken among women receiving tamoxifen.

Endometrial Cancer and Other Uterine Effects

An increased risk of endometrial cancer associated with tamoxifen has been recognized since the late 1980s from both clinical and epidemiological studies. Follow-up of participants in the Stockholm Adjuvant Tamoxifen Trial by computer linkage to cancer registry records demonstrated a six-fold increase in endometrial cancer among women receiving 40 mg tamoxifen daily for two years compared to controls who did not receive tamoxifen [43]. However, second cancer information from other large, randomized adjuvant studies suggests that the risk of endometrial cancer associated with tamoxifen is lower, approximately two- to three-fold [5]. Prospective follow-up of patients in the NSABP B-14 adjuvant study found an annual hazard rate of 1.6 cases per 1,000 patient-years in the randomized, tamoxifen-treated group, giving a relative risk of 2.2 compared to population-based rates of endometrial cancer and of 2.3 compared to rates observed among participants in an NSABP surgical study who did not receive tamoxifen [44]. This is consistent with the risk suggested by a small case-control study by Hardell [45] and a nested case-control study performed through the population-based Netherlands Cancer Registry [46]. Whether endometrial cancers developing among patients receiving tamoxifen are of different histologies [47] or of higher histological grade [48] than those occurring among women not exposed to the drug remains to be determined.

A spectrum of endometrial abnormalities have been reported in patients receiving tamoxifen and have been attributed to the drug's estrogen agonist effects on postmenopausal endometrial tissue. Case control studies of breast cancer patients with postmenopausal bleeding by Neven *et al.* [49] demonstrated a statistically significant increase in risk of endocervical and endometrial polyps and hyperplastic uterine mucosa at hysteroscopy among patients receiving tamoxifen compared to controls (24-fold, 3.5-fold, and 5.2-fold, respectively). A second study among asymptomatic patients found a significantly increased risk of proliferative uterine mucosa among patients receiving tamoxifen compared to controls (2.9-fold) and confirmed the increased risk of endometrial polyps observed among the symptomatic patients. A cross-sectional hysteroscopic study of pre- and postmenopausal patients receiving tamoxifen for 6 to 36 months described normal endometrial mucosa in 50% of patients, endometrial and/or endocervical polyps in 28%, focal or diffuse hyperplasia in 17%, and neoplasia in 4% [50]; an increasing rate of endometrial hyperplasia or neoplasia was associated with increasing cumulative dose of tamoxifen. A small prospective study by the same researchers suggested that over 50% of patients receiving tamoxifen develop endometrial changes ranging from mucosal proliferation to endometrial polyps and adenocarcinoma by an average of 16 months from initiation of the drug [51].

Histological studies based on endometrial sampling have addressed similar issues of endometrial abnormalities associated with tamoxifen. A cross-sectional study of 38 postmenopausal women on tamoxifen for at least 12 months revealed endometrial abnormalities in 18%, ranging from simple hyperplasia without atypia to complex hyperplasia with atypia [52];

a prospective component of the study among asymptomatic women receiving tamoxifen, comprising 11 patients with both pre-treatment and on-treatment biopsies, reported the development of similar endometrial abnormalities in 27% of patients during the study period.

Screening for Uterine Pathology

Studies combining noninvasive imaging techniques with hysteroscopy and/or endometrial sampling have attempted to correlate findings on transvaginal ultrasonography with histologically defined endometrial changes. Uterine volume, as determined by ultrasound, is increased among women receiving tamoxifen compared to controls [53,54], and uterine enlargement is more frequent among tamoxifen-treated patients [55]. Increased endometrial thickness (>5 mm by ultrasound) has been reported in 85–90% of patients receiving tamoxifen for more than 12 months [53,56] and in 46%–62% of patients receiving tamoxifen for varying periods of time [54,57]; differences are statistically significant compared to control patients without hormonal therapy or to postmenopausal women from the general population.

Comparisons of histologic findings with ultrasonographic measurements of endometrial thickness support a positive correlation between abnormal thickness and histologic abnormalities. In Kedar's study [54] of postmenopausal women receiving tamoxifen, 39% had non-atrophic endometria on histologic examination; abnormalities included endometrial proliferation, atypical hyperplasia, polyps, and the presence of mitotic cells. Women with these histologic findings had a significantly thicker endometrial width on ultrasound, and the predictive value of an endometrial thickness of ≥8 mm for atypical hyperplasia or polyps was 100%. However, approximately half of the 43 tamoxifen-treated patients with endometrial thickness >5 mm evaluated by Lahti *et al.* [53] showed no pathologic findings at hysteroscopy or on endometrial biopsy. These figures do not take into account patients who could not undergo endometrial sampling or for whom pipelle sampling techniques yielded insufficient tissue for evaluation (over two-thirds of patients in one series [58]).

Measurement of endometrial thickness by transvaginal ultrasonography may be confounded by effects of tamoxifen on other uterine tissues. Endometrial polyps, occurring in about one-third of patients receiving tamoxifen [53], may appear as multiple sonolucent areas within the endometrial hyperechoic area. Small sonolucences in the proximal myometrium may produce a heterogeneous appearance suggestive of polyps or thickening [59]; this finding may occur more commonly than initially recognized [57] and may be associated with endometrial atrophy on histologic examination [59]. Methods for enhancing ultrasonographic evaluation of the endometrium in women receiving tamoxifen are clearly needed before this technology is used as routine surveillance for endometrial abnormalities among this group of patients. Similarly, the role of periodic endometrial sampling in endometrial surveillance among women on tamoxifen remains to be defined through randomized clinical studies.

Other Second Primary Cancers

In 1992, Arriagada *et al.* [60] reported a two-fold increase in adenocarcinomas (excluding contralateral breast cancers) among patients with early stage breast cancer who were receiving tamoxifen in the Stockholm Adjuvant Trial compared to controls. Most of this increase was attributed to endometrial cancers; however, an increase in adenocarcinomas of the gastrointestinal tract was also postulated. In an updated analysis of second primary cancers occurring among participants in this clinical trial, Rutqvist *et al.* [61] recently reported a nearly three-fold increase of gastrointestinal cancer among women receiving tamoxifen. Only when data from two other Scandinavian adjuvant studies was combined with that from the Stockholm Adjuvant Tamoxifen Trial was an association between tamoxifen and specific gastrointestinal sites (colorectal cancer and stomach cancer) suggested.

These reports are confounded by broad categories of tumor types and by other methodological and analytical issues. Prospective follow-up of patients in the NSABP B-14 adjuvant study found an annual hazard rate of 1.7 cases of colorectal cancer per 1,000 patient-years in the randomized, tamoxifen-treated group—not statistically different from the annual hazard rate of 1.6 cases per 1,000 patient-years in the placebo group [44]. Similarly, long-term follow-up of participants in other large randomized controlled

adjuvant therapy trials in the United States and Europe has failed to suggest a potential increase in any gastrointestinal cancers associated with tamoxifen [62]. Current clinical observations which do not support an association between tamoxifen and second primary gastrointestinal cancers are consistent with the negative findings of animal toxicology studies regarding non-hepatobiliary gastrointestinal tumors [63].

CONCLUSIONS

Tamoxifen's potential for chemoprevention of breast cancer is supported by demonstrated decreases in contralateral breast cancers among women receiving the drug as adjuvant therapy for early stage breast cancer. Its efficacy in breast cancer chemoprevention among women without a prior breast cancer diagnosis but at increased risk of disease is currently under evaluation in large, randomized, controlled clinical trials in the United States and abroad.

Challenges to further development of tamoxifen as a chemopreventive agent focus on the clinical acceptability of menopausal symptoms associated with its use, especially among younger women, and its long-term gynecologic effects including uterine cancer. While the addition of other hormonal therapy to tamoxifen (*e.g.*, progestational agents for contraception, control of menopausal symptoms, and potential prevention of endometrial cancer) is an attractive approach to these problems, further studies are needed to define safety and efficacy of hormone/drug combinations before widespread clinical application occurs.

The development and clinical testing of newer antiestrogens may give rise to compounds with activities similar to tamoxifen in breast tissue, bone metabolism, and lipid profiles, but with fewer and/or less serious side effects. Potential applications for the new antiestrogens and synergistic combinations of antiestrogens with other chemopreventive agents should be explored in prevention as well as in treatment of breast cancer.

REFERENCES

1. Early Breast Cancer Trialists' Collaborative Group: Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. Lancet 339:1–15, 71–85, 1992.
2. Kramer BS, Brawley OW, Nayfield S, Johnson K, Greenwald P, Ford LG: NCI studies in primary prevention of breast and prostate cancer. Cancer Res Therapy Control 3:203–211, 1993.
3. Buckley MMT, Goa KL: Tamoxifen. A reappraisal of its pharmacodynamic and pharmacokinetic properties, and therapeutic use. Drugs 37:451–490, 1989.
4. Jordan VC, Murphy CS: Endocrine pharmacology of antiestrogens as antitumor agents. Endocr Rev 11: 578–610, 1990.
5. Nayfield SG, Karp JE, Ford LG, Dorr AF, Kramer BS: Potential role of tamoxifen in prevention of breast cancer. J Natl Cancer Inst 83:1450–1459, 1991.
6. Buzdar AU, Hortobagyi GN, Frye D, Ho D, Booser DJ, Valero V, Holmes FA, Birmingham BK, Bui K, Yeh C, Plourde PV: Bioequivalence of 20-mg once-daily tamoxifen relative to 10-mg twice-daily tamoxifen regimens for breast cancer. J Clin Oncol 12:50–54, 1994.
7. Styles JA, Davies A, Lim CK, De Matteis F, Staley LA, White INH, Yuan Z-X, Smith LL: Genotoxicity of tamoxifen, tamoxifen epoxide and toremifene in human lymphoblastoid cells containing human cytochrome P-450. Carcinogenesis 15:5–9, 1994.
8. Gibbs AH, De Matteis F, Wolf CR, Henderson C, Yuan Z-X, Styles JA, Davies A, Smith LL, White INH: Metabolism and covalent binding of [^{14}C]tamoxifen by human and rat CYP isoenzymes (abstract). Toxicologist 14:403, 1994.
9. Mani C, Kupfer D: Cytochrome P-450 mediated action and irreversible binding of the antiestrogen tamoxifen to proteins in rat and human liver: Possible involvement of the flavin-containing monooxygenases in tamoxifen activation. Cancer Res 52:1360–1363, 1991.
10. Pongracz K, Pathak DN, Bodell WJ: Investigation of DNA adduct formation by tamoxifen metabolites using ^{32}P postlabelling. Proc Am Assoc Cancer Res 35:137, 1994.
11. Potter GA, McCague R, Jarman M: A mechanistic hypothesis for DNA adduct formation by tamoxifen following hepatic oxidative metabolism. Carcinogenesis 15:439–442, 1994.
12. Phillips DA, Potter GA, Horton MN, Hewer A, Crofton-Sleeigh C, Jarman M, Venitt S: Reduced genotoxicity of [D_5-ethyl]-tamoxifen implicates alpha-hydroxylation of the ethyl group as a major pathway of tamoxifen activation to a liver carcinogen. Carcinogenesis 15:1487–1492, 1994.
13. Phillips DH, Carmichael PL, Hewer A, Cole KJ, Poon GK: α-Hydroxytamoxifen, a metabolite of tamoxifen with exceptionally high DNA-binding activity in rat hepatocytes. Cancer Res 54:5518–5522, 1994.
14. Randerath K, Bi J, Mabon N, Sriram P, Moorthy B: Strong intensification of mouse hepatic tamoxifen DNA adduct formation by pretreatment with the sulfotransferase inhibitor and ubiquitous environmental pollutant pentachlorophenol. Carcinogenesis 15:797–800, 1994.

15. Han X, Liehr J: Induction of covalent adducts in rodents by tamoxifen. Cancer Res 52:1360–1363, 1992.
16. Sargent LM, Dragan YP, Bahnub N, Wiley JE, Sattler CA, Schroeder P, Sattler GL, Jordan VC, Pitot HC: Tamoxifen induces hepatic aneuploidy and mitotic spindle disruption after a single *in vivo* administration to female Sprague-Dawley rats. Cancer Res 54: 3357–3360, 1994.
17. Pathak DN, Bodell WJ: DNA adduct formation by tamoxifen with rat and human liver microsomal activation systems. Carcinogenesis 15:529–532, 1994.
18. Martin EA, Rich KJ, Heydon R, White INH, Woods KL, Powles TJ, Smith LL: A comparison of ^{32}P-post-labelled tamoxifen liver DNA adducts in rats, mice and breast cancer patients. Toxicologist 14:405, 1994.
19. Brown M: Estrogen receptor molecular biology. Hematol Oncol Clin North Am 8:101–112, 1994.
20. Halachmi S, Marden E, Martin G, MacKay H, Abbondanza C, Brown M: Estrogen receptor-associated proteins: Possible mediators of hormone-induced transcription. Science 264:1455–1458, 1994.
21. Love RR, Wiebe DA, Newcomb PA, Cameron L, Leventhal H, Jordan VC, Feyzi J, DeMets DL: Effects of tamoxifen on cardiovascular risk factors in postmenopausal women. Ann Intern Med 115:860–864, 1991.
22. Love RR, Wiebe DA, Feyzi JM, Newcomb PA, Chappell RJ: Effects of tamoxifen on cardiovascular risk factors in postmenopausal women after 5 years of treatment. J Natl Cancer Inst 86:1534–1539, 1994.
23. Dewar JA, Horobin JM, Preece PE, Tavendale R, Tunstall-Pedoe H, Wood RAB: Long term effects of tamoxifen on blood lipid values in breast cancer. Br Med J 305:225–226, 1992.
24. Thangaraju M, Kumar K, Gandhirajan R, Sachdanadam P: Effect of tamoxifen on plasma lipids and lipoproteins in postmenopausal women with breast cancer. Cancer 73:659–663, 1994.
25. Dnistrian AM, Schwartz MK, Greenberg EJ, Smith CA, Schwartz DC: Effect of tamoxifen on serum cholesterol and lipoproteins during chemohormonal therapy. Clinica Chimica Acta 223:43–52, 1993.
26. Rutqvist LE, Mattsson A for the Stockholm Breast Cancer Study Group: Cardiac and thromboembolic morbidity among postmenopausal women with early-stage breast cancer in a randomized trial of adjuvant tamoxifen. J Natl Cancer Inst 85:1398–1406, 1993.
27. McDonald CC, Stewart HJ for the Scottish Breast Cancer Committee: Fatal myocardial infarctions in the Scottish adjuvant tamoxifen trial. Br Med J 303: 435–437, 1991.
28. Wiseman H, Paganga G, Rice-Evans C, Halliwell B: Protective actions of tamoxifen and 4-hydroxytamoxifen against oxidative damage to human low-density lipoproteins: A mechanism accounting for the cardioprotective action of tamoxifen? Biochem J 292:635–638, 1993.
29. Wiseman H, Cannon M, Arnstein HRV, Halliwell B: Tamoxifen inhibits lipid peroxidation in cardiac microsomes. Comparison with liver microsomes and potential relevance to the cardiovascular benefits associated with cancer prevention and treatment by tamoxifen. Biochem Pharmacol 45:1851–1859, 1993.
30. Gylling H, Mäntylä E, Miettinen TA: Tamoxifen decreases serum cholesterol by inhibiting cholesterol synthesis. Atherosclerosis 96:245–247, 1992.
31. Love RR, Mazess RB, Barden HS, Epstein S, Newcomb PA, Jordan VC, Carbone PP, DeMets DL: Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. N Engl J Med 326:852–856, 1992.
32. Ward RL, Morgan G, Dalley D, Kelly PJ: Tamoxifen reduces bone turnover and prevents lumbar spine and proximal femoral bone loss in early postmenopausal women. Bone Mineral 22:87–94, 1993.
33. Fentiman IS, Saad Z, Caleffi M, Chaudary MA, Fogelman I: Tamoxifen protects against steroid-induced bone loss. Eur J Cancer 28:684–685, 1992.
34. Kristensen B, Ejlertsen B, Dalgaard P, Larsen L, Holmegaard SN, Transbol I, Mouridsen HT: Tamoxifen and bone metabolism in postmenopausal low-risk breast cancer patients: A randomized study. J Clin Oncol 12:992–997, 1994.
35. Neal AJ, Evans K, Hoskin PJ: Does long-term administration of tamoxifen affect bone mineral density? Eur J Cancer 29A:1971–1973, 1993.
36. Wright CDP, Garrahan NJ, Stanton M, Gazet J-C, Mansell RE, Compston JE: Effect of long-term tamoxifen therapy on cancellous bone remodeling and structure in women with breast cancer. J Bone Mineral Res 9:153–159, 1994.
37. Fornander T, Rutqvist LE, Cedermark B, Glas U, Mattsson A, Skoog L, Somell A, Theve T, Wilking N, Askergren J, Rotstein S, Hjalmar M-L, Perbeck J: Adjuvant tamoxifen in early-stage breast cancer: Effects on intercurrent morbidity and mortality. J Clin Oncol 9:1740–1748, 1991.
38. Powles TJ, Tillyer CR, Jones AL, Ashley SE, Treleaven J, Davey JB, McKinna JA: Prevention of breast cancer with tamoxifen—an update on the Royal Marsden Hospital pilot programme. Eur J Cancer 26:680–684, 1990.
39. Fisher B, Costantino J, Redmond C, Poisson R, Bowman D, Couture J, Dimitrov NV, Wolmark N, Wickerham DL, Fisher ER, Margolese R, Robidoux A, Shibata H, Terz J, Paterson AHG, Feldman MI, Farrar W, Evans J, Lickley HL, Ketner M, and others: A randomized clinical trial evaluating tamoxifen in the treatment of patients with node-negative breast cancer who have estrogen-receptor-positive tumors. N Engl J Med 320:479–484, 1989.
40. Jordan VC, Fritz NF, Langan-Fahey S, Thompson M, Tormey DC: Alteration of endocrine parameters in premenopausal women with breast cancer during long-term adjuvant therapy with tamoxifen as the single agent. J Natl Cancer Inst 83:1488–1491, 1991.
41. Powles TJ: Tamoxifen and estrogen replacement. Lancet 336:48, 1990.
42. Loprinzi CL, Michalak JC, Quella SK, O'Fallon JR,

Hatfield AK, Oesterling JE: Placebo controlled clinical trial of megestrol acetate for ameliorating hot flashes in both men and women: A North Central Cancer Treatment Group study. Proc Am Soc Clin Oncol 13:432, 1994.

43. Fornander T, Rutqvist LE, Cedermark B, Glas U, Mattson A, Silversward JD, Skoog L, Somell A, Theve T, Wilking N, Askergren J, Hjolmar ML: Adjuvant tamoxifen in early breast cancer: Occurrence of new primary cancers. Lancet 2:117–120, 1989.
44. Fisher B, Costantino JP, Redmond CK, Fisher ER, Wickerham DL, Cronin W, Other NSABP Contributors: Endometrial cancer in tamoxifen-treated breast cancer patients: Findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. J Natl Cancer Inst 86:527–537, 1994.
45. Hardell L: Pelvic irradiation and tamoxifen as risk factors for carcinoma of corpus uteri. Lancet 2:1432, 1988.
46. van Leeuwen FE, Benraadt J, Coebergh JWW, Kiemeney LALM, Gimbrère CHF, Otter R, Schouten LJ, Damhuis RAM, Bontenbal M, Diepenhorst FW, van den Belt-Dusebout AW, van Tinteren H: Risk of endometrial cancer after tamoxifen treatment of breast cancer. Lancet 343:448–452, 1994.
47. Dallenbach-Hellweg G: Endometrial carcinomas. Curr Top Pathol 85:1–34, 1992.
48. Magriples U, Naftolin F, Schwartz PE, Carcangiu ML: High-grade endometrial carcinoma in tamoxifen-treated breast cancer patients. J Clin Oncol 11:485–490, 1993.
49. Neven P, De Muylder X, Van Belle Y, Vanderick G, De Muylder E: Tamoxifen and the uterus and endometrium. Lancet 2:375, 1989.
50. De Muylder X, Neven P, De Somer M, Van Belle Y, De Muylder Y: Endometrial lesions in patients undergoing tamoxifen therapy. Int J Gyneacol Obstet 36:127–130, 1991.
51. Neven P, De Muylder X, Van Belle Y, Vanderick G, De Muylder E: Hysteroscopic follow-up during tamoxifen treatment. Eur J Obstet Gynecol Reprod Biol 35:235–238, 1990.
52. Gal D, Kopel S, Bashevkin M, Lebowicz J, Lev R, Tancer ML: Oncogenic potential of tamoxifen on endometria of postmenopausal women with breast cancer—Preliminary report. Gynecol Oncol 42:120–123, 1991.
53. Lahti E, Blanco G, Kauppila A, Apaja-Sarkkinen M, Taskinen PJ, Laatikainen T: Endometrial changes in postmenopausal breast cancer patients receiving tamoxifen. Obstet Gynecol 81:660–664, 1993.
54. Kedar RP, Bourne TH, Powles TJ, Collins WP, Ashley SE, Cosgrove DO, Campbell S: Effects of tamoxifen on uterus and ovaries of postmenopausal women in a randomised breast cancer prevention trial. Lancet 343:1318–1321, 1994.
55. Rayter Z, Gazet J-C, Shepherd J, Trott PA, Fisher C, Svenson WE, Ford HT, A'Hern R: Gynaecological cytology and pelvic ultrasonography in patients with breast cancer taking tamoxifen compared with controls. Eur J Surg Oncol 20:134–140, 1994.
56. Uziely B, Lewin A, Brufman G, Dorembus D, Mor-Yosef S: The effect of tamoxifen on the endometrium. Breast Cancer Res Treat 26:101–105, 1993.
57. Ciatto S, Cecchini S, Bonardi R, Grazzini G: Ultrasonography surveillance of endometrium in breast cancer patients on adjuvant tamoxifen. Lancet 344:60, 1994.
58. Cohen I, Rosen DJ, Shapira J, Cordoba M, Gilboa S, Altaras MM, Yigael D, Beyth Y: Endometrial changes in postmenopausal women treated with tamoxifen for breast cancer. Br J Obstet Gynaecol 100:567–570, 1993.
59. Goldstein SR: Unusual ultrasonographic appearance of the uterus in patients receiving tamoxifen. Am J Obstet Gynecol 170:447–451, 1994.
60. Arriagada R, Rutqvist LE, and the Stockholm Breast Cancer Group: Adjuvant systemic therapy in early breast cancer and incidence of new primary malignancies. Proc Am Soc Clin Oncol 11:52, 1992.
61. Rutqvist LE, Johnasson H, Signomklao T, Johnasson U, Fornander T, Wilking N, Theve T: Adjuvant tamoxifen in early stage breast cancer and second primary malignancies. Int J Oncol 5 (Suppl):304–395, 1994.
62. Zeneca Pharmaceuticals Group: Data presented at the United States Food and Drug Administration Oncology Drugs Advisory Committee Meeting, Bethesda, MD, June 7, 1994.
63. Greaves P, Goonetilleke R, Nunn G, Topham J, Orton T: Two-year carcinogenicity study of tamoxifen in Adderly Park Wistar-derived rats. Cancer Res 53: 3919–3924, 1993.