NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN:

TAMOXIFEN

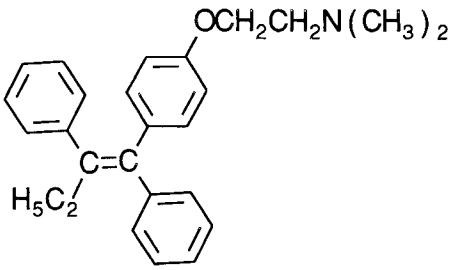
DRUG IDENTIFICATION

CAS Registry No.: 10540-29-1

CAS Name (9CI): (Z)-2-[4-(1,2-Diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethanamine

Synonyms: Tamoxifen Citrate
ICI-46,474
Nolvadex®

Structure:



EXECUTIVE SUMMARY

Tamoxifen is a nonsteroidal triphenylethylene derivative considered primarily a competitive estrogen antagonist in humans [reviewed in 1]. *In vitro* studies suggest that this effect results from direct estrogen receptor binding [reviewed in 2], which blocks estrogen activity, alters RNA transcription and decreases cell proliferation [reviewed in 3,4]; other antihormonal effects may include interference with gonadotrophin and prolactin release [5]. Possible non-hormonal effects, many mediated through cellular signal transduction pathways, include modulation of growth factor secretion (TGF-α, TGF-β, EGF, IGF-I) [*e.g.*, 6–11], inhibition of protein kinase C activity [12], calmodulin activity antagonism [13], inhibition of polyamine [14] and prostaglandin synthesis [15], inhibition of lipid peroxidation [*e.g.*, 16,17], inhibition of certain cytochrome P-450 activities [18], and alterations in membrane properties [19,20]. In some tissues, however, this agent may also have estrogenic effects (partial agonist) [1]. Tamoxifen citrate (Nolvadex®) is used for palliative endocrine treatment of advanced breast cancer and as adjuvant therapy to control micrometastatic relapse and new primaries in women surgically treated for early breast cancer (20–40 mg qd) [reviewed in 21]. It is currently in a large cooperative Phase III trial for prevention of breast cancer in high-risk women. Besides cancer chemoprevention, this trial is investigating other possible benefits of tamoxifen suggested by previous clinical trials—decreased cardiovascular morbidity and mortality, as well as prevention of osteoporosis in postmenopausal women.

Chemopreventive efficacy of the agent has been observed primarily in animal models of mammary gland cancer. In addition, comparable or synergistic efficacy has been observed in preclinical studies of tamoxifen/tamoxifen citrate in combination with other agents [*e.g.*, *N*-4-(hydroxyphenyl)retinamide (4-HPR)] at lower, less toxic doses. Tamoxifen and

4-HPR have some complementary chemopreventive activities (modulation of growth factors, inhibition of prostaglandin synthesis, decreased polyamine levels). For example, tamoxifen predominantly induces TGF-β1 and retinoids induce TGF-β2 and -β3; the combination may then achieve a greater response [8,22]. Based on these studies, clinical development of the combination was undertaken by NCI.

For further development of tamoxifen citrate as a single chemopreventive agent, additional preclinical toxicology should not be necessary. Although four recently published one-year studies of tamoxifen citrate in rats show significant dose-related incidences of hepatocellular carcinomas, no increases in liver cancer have been observed in six clinical adjuvant trials. In order to develop combination regimens, the CB has funded 90-day toxicity studies of tamoxifen citrate in combination with 4-HPR in dogs and rats. No synergistic toxicity was demonstrated in dogs; however, a NOEL was not achieved. The comparable study in rats is in progress.

NCI-funded clinical trials of tamoxifen citrate are summarized in Table I. No Phase I studies have been sponsored since a large amount of clinical data is already available in the literature and in ICI Pharma's NDA. Published results from six recent clinical adjuvant trials have shown mild adverse estrogenic effects, plus an endometrial cancer incidence two-fold higher than placebo or observation controls. The endometrial cancer risk appears to be similar to that for postmenopausal women on unopposed estrogen replacement therapy (ERT). However, recent results from a study of adjuvant tamoxifen treatment of breast cancer patients (NSABP-B14) and from the Netherlands Cancer Institute adjuvant trial suggest that the risk may actually be three-fold higher than matched controls.

A published Phase I trial investigated combinations of tamoxifen (20 mg qd) with 4-HPR (100–400 mg qd, three-day drug holiday/month) in metastatic breast cancer patients. After 2–14 months of treatment, adverse effects were attributed to disease progression, and the authors concluded that all combinations were safe and well-tolerated [23].

Two trials of tamoxifen citrate as a single agent are in progress. A cooperative Phase III trial in women with risk for breast cancer equivalent to that at age 60 is being administered by the National Surgical Adjuvant Breast and Bowel Project (NSABP-P1). This trial is currently on hold. An NCI, DCPC-funded Phase II study (see Table I) is investigating tamoxifen citrate modulation of atypical hyperplasia, a risk factor for breast cancer development, in women previously treated for breast cancer or women who are first-degree relatives of breast cancer patients. Further development of tamoxifen citrate as a single agent will await results of these two trials.

In the interim, NCI is undertaking development of tamoxifen citrate in combination with 4-HPR to prevent breast cancer. This direction is supported by animal efficacy data in mammary glands, and takes advantage of the complementary mechanisms of the two agents. Three NCI-sponsored Phase II trials testing the combination of tamoxifen citrate with 4-HPR began this year. One CB-administered trial is evaluating tamoxifen citrate alone and in combination in DCIS patients when given in the period between diagnostic biopsy and definitive surgery. A significant aspect of this study is identifying intermediate biomarkers as potential surrogate endpoints for cancer chemoprevention trials. Two Phase II trials are being administered through the Clinical Oncology Program. One is a feasibility study of tissue sampling by three methods to assess proliferative intermediate biomarkers and TGF-β isoforms as drug effect measurements in women at high risk for breast cancer. The other trial will evaluate similar endpoints in areas of CIS and proliferation adjacent to breast cancer; combination treatment will take place between diagnostic biopsy and definitive surgery. Finally, a combination Phase III trial administered by the Eastern Cooperative Oncology Group (ECOG) is being designed to compare tamoxifen with tamoxifen plus 4-HPR as adjuvant therapy in women surgically treated for node-negative breast cancer.

ICI Pharma originally manufactured tamoxifen citrate as an oral formulation (10 mg capsule). Zeneca now holds proprietary rights to the drug; the possibility of a supply agreement must be considered.

PRECLINICAL EFFICACY STUDIES

Sufficient evidence of inhibition of mammary gland carcinogenesis by tamoxifen and tamoxifen citrate (oral formulation) has been obtained the CB. The agent appears to act during both the initiation and post-initiation phases of carcinogenesis. Tamoxifen (20 μg/rat, 3x/wk, sc) reduced mammary tumor multiplicity when administered beginning one week after the start of MNU exposure in the standard assay in young adult rats (50 days old).

Tamoxifen citrate in the diet (0.25 mg/kg, or *ca.* 0.022 nmol/kg-bw/day) reduced rat mammary tumors when given to mature rats (120 days old) beginning one week before MNU exposure; this may be a more appropriate model for adult human breast cancer [24]. Because of the chemopreventive evidence already available on the individual agent in published reports, NCI has concentrated on sponsoring studies of tamoxifen in combination with other agents to obtain similar or greater tumor inhibition at lower, less toxic doses. In completed studies, the dietary combination of 4-HPR with tamoxifen citrate (0.125 mg/kg diet, or *ca.* 0.011 nmol/kg-bw/day) showed synergistic activity against MNU-induced mammary carcinogenesis in older rats. Interestingly, subcutaneously administered tamoxifen plus dietary 4-HPR was even more effective in an adjuvant study using the induction of subsequent mammary carcinomas following surgical removal of the first cancer as the endpoint. In the standard MNU-induced rat mammary model, the cancer preventive effects of the three-agent combination of tamoxifen (0.2 mg/kg diet, or *ca.* 0.03 nmol/kg-bw/day), aminoglutethimide and progesterone were additive. In the same model, doses of tamoxifen citrate that were ineffective alone (0.04, 0.08 mg/kg diet, or *ca.* 0.004, 0.008 nmol/kg-bw/day) inhibited carcinogenesis in combination with DHEA, with carbenoxolone and DHEA, and with carbenoxolone and DFMO. Studies in progress are testing combinations of tamoxifen citrate with fumaric acid, oltipraz, or curcumin.

The majority of the efficacy against mammary carcinogenesis was demonstrated in early published studies of injectable tamoxifen by several laboratories. A number of studies showed inhibition of chemical (DMBA, MNU, EMS, DES)- and radiation (UV)-induced rat and virus-related mouse mammary carcinogenesis [*e.g.*, 25–30]; some studies have also demonstrated increased latency or decreased tumor size [*e.g.*, 31,32]. Interestingly, one of the major metabolites of tamoxifen (4-hydroxytamoxifen) with potent antiestrogen activity also inhibited DMBA-induced rat mammary carcinogenesis, although less effectively [33,34]. Agents which have inhibited carcinogen-induced mammary carcinogenesis in combination with tamoxifen in published studies include 4-HPR [24,35], 2-bromo-α-ergocryptine [36], 2-bromo-α-ergocryptine plus retinyl acetate [37,38], DFMO [31], and the vitamin D_3 analog Ro 24-5531 [39]. In addition, tamoxifen inhibited hamster kidney (17β-estradiol implant) [40], ovariectomized rat pituitary (DES) [27], and mouse cervix (20-methylcholanthrene) [41] carcinogenesis. Finally, a number of histological intermediate biomarkers have been modulated by the agent, including hyperplastic alveolar nodules in mouse mammary glands (infected with MMTV) [42], and hyperplastic nodules (induced by DEN, AAF, and partial hepatectomy) [43] and GGT-positive foci (induced by ethinyl estradiol) [44] in rat liver.

PRECLINICAL SAFETY STUDIES

Safety The CB has funded only subchronic (90-day) toxicity studies of tamoxifen citrate at 0.4–32 mg/kg-bw/day (ig) alone and in combination with 4-HPR in two species [45]. In female Beagle dogs, no synergistic toxicity was observed with a capsule formulation of combined agents; however, a NOEL was not established. All tamoxifen-treated dogs exhibited enlarged vulvae, decreased serum albumin and estrogen, and histological lesions in the adrenal glands, ovaries, uterus, vagina and bone marrow. In female Sprague-Dawley rats, the highest dose of tamoxifen alone and in combination appeared to cause lesions in the adrenal glands, ovaries, uterus and vagina; however, the study is being repeated due to protocol deficiencies.

In CB-sponsored chemopreventive efficacy studies, significantly decreased weight gain has been observed at some doses of tamoxifen with and without 4-HPR. At doses of tamoxifen alone which did not affect weight (5 μg, 3x/week, sc), the reduction in tumor multiplicity (16%) retained significance, but the beneficial effect on latency was lost. In contrast, subtoxic doses of tamoxifen combined with 4-HPR have produced synergistic chemopreventive effects in rat mammary carcinogenesis models.

Three recently published one-year toxicology studies also found decreased weight gain (20–40%) at higher doses of tamoxifen citrate (2.8, 11.3, 22.6 and 45.2 mg/kg-bw/day, ig, or 5.0, 20.0, 40.1 and 80.2 nmol/kg-bw/day) in female rats [46–48]. Drug-related increases in osteoporosis, alopecia, seizures, general weakness, and liver tumors were also obtained. The incidence of hepatocellular carcinomas was 75–100% at the two highest doses and 10–44% at 11.3 mg/kg-bw/day [46,48,49]. Premalignant liver lesions were also increased, including the incidence of adenomas after one year (100%) and the incidence (100%), area and multiplicity (3.83/cm^2) of placental GST-positive foci after three months of treatment with 11.3 mg tamoxifen-

citrate/kg-bw/day [48]. Two-year carcinogenicity studies by the manufacturer produced similar results, with significantly greater incidences of hepatocellular carcinomas in rats (sex unknown) given doses of 5, 20, and 35 mg tamoxifen citrate/kg-bw/day (14%, 69%, and 69%, respectively) than in controls [49,50]. At doses comparable to the clinical doses, preliminary manufacturer's data suggested that tamoxifen promoted GGT-positive foci and increased hepatic carcinomas (11.5% *vs.* 1% in controls; significance not reported) in rats [4]; however, the data are unavailable for review. The neoplastic liver responses have been correlated with high levels of hepatic tamoxifen-DNA adducts [51,52], p53 mutations in 50% of hepatocellular carcinomas [53], karyotypic instability [54], and elevated cytochrome P-450, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase activities [55]. DNA adducts have also been observed in livers of tamoxifen-treated male rats, and female mice and hamsters [51,52,56]. The adducts appear to be formed from an epoxide metabolite [57]. In contrast, human breast cancer patients treated with tamoxifen show no increase in hepatic DNA adduct levels over controls [51], and no increases in liver cancer have been found in six large adjuvant therapy trials in women [4].

Tamoxifen acts as a partial estrogen agonist in rat uterus, increasing organ weight and RNA synthesis. Published reports have also shown increased expression of IGF-I and suppression of its binding protein [58], as well as induction of proto-oncogenes c-*fos* and *jun*-B expression in this tissue *in vivo* [59,60]. Protooncogene mRNA levels were elevated up to 10-fold 6–24 hours after tamoxifen injection (1 mg/kg-bw) [60]. c-*fos* transcripts were also increased by the agent or estrogen in human endometrial carcinomas transplanted into rats, demonstrating similar estrogenic effects in uterine tissue of rats and humans [61].

ADME Orally administered tamoxifen is well-absorbed and extensively metabolized in humans, mice, rats, dogs, and monkeys [48,62]. Following administration of 11.3 mg/kg-bw/day (ig) to female rats, the blood metabolite (*N*-desmethyltamoxifen and 4-hydroxytamoxifen) to drug ratio increased from 0.86 after 3 months to 1.4 after one year [48]. Elimination is biphasic, with a longer phase $t_{1/2}$ of 3–18 days [62]. Tamoxifen is excreted primarily in the feces.

CLINICAL SAFETY: PHASE I STUDIES

Drug Effect Measurement An easily measured valid drug effect has not been demonstrated in published trials. Variable effects on circulating estradiol, estrone, FSH, LH, insulin-like growth factor I (IGF-I), and antithrombin III levels have been observed between premenopausal and postmenopausal women [4,63]. Other estrogenic effects, such as decreased low density lipoprotein (20%), may take 3–6 months to develop [reviewed in 4]. In one Phase II trial which began recently, the effect of tamoxifen plus 4-HPR on tissue TGF-β isoforms is being assessed. Alternatively, increased serum sex hormone-binding globulin appeared to be consistently related to tamoxifen intake in all women [64–66].

Safety No Phase I trials of tamoxifen have been sponsored by NCI because of the extensive published clinical trial data. Interim results are available from an NCI-sponsored Phase II trial in patients with atypical hyperplasia. Of the dropouts (27%), a few more in the tamoxifen group reported hot flashes and memory loss/insomnia; the only case of CIS occurred in the placebo group.

In published reports, the most frequently reported side effects (15–20% incidence) from clinical doses of tamoxifen are mild and appear to be from estrogen blockade, *e.g.*, nausea, gastrointestinal (GI) disturbances, rapid pulse, and hot flashes [reviewed in 4]. Menstrual irregularities have been observed in premenopausal women at the same doses. Antithrombin III activity is decreased in postmenopausal patients, but rarely to clinically relevant levels in patients without prior history of clotting disorders [67]. It should be noted that no change in the incidence of thromboembolic disease has been observed in the Stockholm trial of adjuvant tamoxifen therapy (40 mg qd) after 5 years [68]. Ocular damage (retinal changes, keratopathy, optic neuritis) has occurred at high doses [reviewed in 4]; some recent reports show a 6% incidence of such damage at the chemopreventive trial dose of 20 mg qd [reviewed in 69,70]. Other effects seen in cancer patients during tamoxifen chemotherapy are difficult to differentiate from the disease process itself (thrombosis, hypercalcemia, flare reaction) [21] and are probably not clinically relevant for chemoprevention trials.

Cumulative evidence from six recent trials of adjuvant tamoxifen therapy (20 mg qd) in women with early breast cancer (Nolvadex Adjuvant Trial, Scottish, Copenhagen, Toronto Edmonton, ECOG, and NSABP-B14) show no large differences in second primary cancer incidence by site (except breast cancer, which decreased 35%) compared with placebo or observation groups [reviewed in

4]. No increase in liver cancer has been observed, even though two of these trials involve continuous administration of the agent for at least five years. In contrast, endometrial cancer incidence in these six adjuvant trials was two-fold higher than control, probably due to tamoxifen's partial estrogen agonist effects in the uterus. This rate is similar to that of postmenopausal women on unopposed ERT [71,72], and appears to be related to total dose (median: 29 g). Recently, however, data from the NSABP-B14 trial [73–75] and the Netherlands Cancer Institute [76] suggested that tamoxifen actually increases the risk of endometrial cancer three-fold after five years of adjuvant treatment of breast cancer patients. There were also more deaths than expected from endometrial cancer in the NSABP-B14 trial [74,75]. The NCI has instructed institutions involved in the Phase III chemoprevention trial (NSABP-B1, see **CLINICAL EFFICACY**) to rewrite consent forms to reflect this information, and will also require yearly endometrial biopsies in all participants [77]. Endometrial changes observed in tamoxifen-treated breast cancer patients have included hyperplasia, dysplasia, fibroids, polyps and sarcoma [78–82].

The effect of tamoxifen on the histopathologic grade of uterine cancers is uncertain. A retrospective study of Connecticut breast cancer patients reported that adjuvant tamoxifen therapy (40 mg qd; mean, 4.2 years) produced a significantly larger proportion of high-grade endometrioid carcinomas (67.7%) than patients who were not treated with tamoxifen and had received unopposed ERT (23.7%) [83]. In contrast, an interim report from the Stockholm Adjuvant Tamoxifen Trial (40 mg qd; mean, 2.2 years) stated that the majority (94%) of the endometrial cancers were low grade [84]; NSABP-B14 also recently reported that the distribution of endometrial cancers by stage and grade did not differ between the placebo and tamoxifen groups [75]. The addition of progestin to adjuvant tamoxifen has been suggested to counteract the endometrial estrogenic stimulus [85]. For example, long-term use of combined oral contraceptives (estradiol with any dose of progestin) reduces the risk of endometrial cancer compared with women who have never used them [86]. However, the progestin may also interfere with the potential benefit of tamoxifen to breast cancer risk [87,88].

A Phase I trial of 20 mg tamoxifen qd in combination with increasing doses of 4-HPR has been published [23]. In consecutive cohorts of three metastatic breast cancer patients each, the levels of 4-HPR were 100, 200, 300, and 400 mg qd with a three-day drug holiday per month. Duration of treatment ranged from 2–14 months, with a total of 6 patients receiving ≥6 months of therapy. Adverse effects (anemia, altered hepatic enzymes) were felt to be due to progressive disease, and all combinations were safe and well-tolerated. No ophthalmic or dermatological effects were observed.

ADME In published studies, serum levels peak 4–8 hours after a single dose of tamoxifen [reviewed in 4]. The plasma elimination $t_{½}$ is 7 days due to significant protein binding, and steady-state concentrations are reached after at least 4 weeks of treatment. Tamoxifen is extensively metabolized after oral administration. The principal metabolite, *N*-desmethyltamoxifen, has a longer plasma $t_{½}$ of 14–24 days, reaching steady-state in 8 weeks; 4-hydroxytamoxifen, a minor plasma metabolite, has a much shorter $t_{½}$ than the parent drug [reviewed in 4,50,89]. Since AUC_{0-24} (2,794 and 2,698 ng·hr/ml, respectively) and C_{max} (162 and 147 ng/ml, respectively) are essentially the same after 20 mg qd or 10 mg bid, the two tamoxifen citrate dosing regimens are considered bioequivalent [89].

Once steady-state is achieved, the metabolites of tamoxifen remain stable for up to 7 years of treatment [90]. Tissue levels are 10–60-fold higher than serum levels [66]. The principal metabolite, *N*-desmethyltamoxifen, has low estrogen receptor binding affinity. 4-Hydroxytamoxifen has stronger receptor binding affinity than the parent compound, but it is present in serum and tissue at 100-fold lower concentrations than tamoxifen [reviewed in 21,66]. The clinical response may be due to the combined effects of tamoxifen and 4-hydroxytamoxifen. It is uncertain whether the potential for uterine toxicity is related to tissue levels of the latter.

Approximately 65% of a 20 mg tamoxifen dose is excreted from the body over a period of two weeks, primarily in the feces [50]. The majority (60%) of the drug eliminated by this route is polar metabolites or conjugates.

CLINICAL EFFICACY: PHASE II/III STUDIES

A cooperative Phase III trial (funded by NCI, National Heart, Lung, and Blood Institute, and National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases) is assessing 20 mg tamoxifen citrate qd for 5 years as a breast cancer chemopreventive drug in a population of women >35 years of age with relative risks equivalent to that of 60 year-old women [70,91]. Risk is calculated from family history, benign biopsy,

atypical hyperplasia or previously excised LCIS, nulliparity, late age at first live birth, and/or early onset of menarche according to the method of Gail and co-workers [92]. The trial, known as the Breast Cancer Prevention Trial, began in June 1992, and is being administered as part of the National Surgical Adjuvant Breast and Bowel Project (NSABP-P1) and is currently on clinical hold. As of March 1994, 10,883 of the 16,000 target population had been accrued in the U.S. and Canada [93]. The evaluable trial endpoints include breast cancer, osteoporosis, cardiovascular events, other cancers, and all causes of death [91]. It should be noted that seven of eight published clinical trials involving 10,000 women with resected breast cancer have demonstrated a 40% decrease in second primary cancers in the contralateral breast on adjuvant tamoxifen treatment [reviewed in 4].

Several international Phase III trials of breast cancer prevention by tamoxifen are also in progress. Under the auspices of the U.K. Coordinating Committee on Cancer Research, the Cancer Research Campaign and the Imperial Cancer Research Fund, a feasibility study was expanded into a pilot trial (4 years total) involving 435 women aged 30–69 years with a family history of breast cancer [94]. Compliance was high, and the frequency of side effects was similar for 20 mg tamoxifen or placebo qd except for a significant increase in hot flashes in the treatment group. A multicenter trial has been approved and is recruiting 15,000 women; however, accrual was restricted to women >40 years old with a greater than 4-fold risk of breast cancer [69]. The same protocol has been in progress for a year in Australia, and sites in Switzerland, France, the Netherlands, Austria, Germany and Spain are expected to join soon, potentially resulting in a much larger cohort [95]. Finally, a study in Italy was funded in September 1992 by the Italian League for the Fight Against Cancer and several research grants [96]. As of April 1993, 801 hysterectomized women (n=20,000) aged 45–65 years without prior breast cancer had been randomized to 20 mg tamoxifen or placebo daily for 5 years [69,95–97].

One NCI, DCPC-funded Phase II trial (Dr. J. Ward, University of Utah) is evaluating tamoxifen-induced modulation of moderate to severe atypical hyperplasia on fine-needle aspirates; atypical hyperplasia is considered to be a risk factor for breast cancer development. Subjects eligible for histological assessment are women with previously treated (surgery/radiation) unilateral breast cancer (<2 cm) or CIS, or who are first-degree postmenopausal relatives of breast cancer patients. As of July 1993, 64 women had been randomized to placebo or tamoxifen.

Development of 4-HPR combined with tamoxifen as a breast cancer chemoprevention strategy is a priority at NCI. The CB recently funded a Phase II trial (Dr. K. Dhingra, University of Texas, M.D. Anderson Cancer Center) which women with DCIS or breast carcinoma are administered the combination or individual agents between diagnostic core biopsy and definitive surgery. A significant aspect of this study is modulation of histological lesions and other types of intermediate biomarkers, such as nuclear morphometry, DNA ploidy, PCNA, and Ki-67. In a related U.K. study, the Ki-67 labelling index was significantly lower in biopsies from women newly diagnosed with breast tumors and treated with a loading dose of 160 mg tamoxifen qd, followed by 20 mg qd until surgery [98]. Also, the drug (0.1 μM) has been shown to significantly decrease BrdU incorporation and Ki-67 expression in human breast cancer MCF-7 cells *in vitro* [99].

Other NCI programs are also contributing to the development of tamoxifen plus 4-HPR for breast cancer chemoprevention. The Clinical Oncology Program is conducting a Phase II trial (Dr. J. O'Shaughnessy, DCT) in a breast cancer cohort evaluating modulation of intermediate biomarkers in DCIS and proliferative lesions adjacent to the malignant lesion. A second Phase II trial (Dr. J. O'Shaughnessy) administered under the same program involves women at increased risk for breast cancer due to LCIS or surgically treated DCIS, atypical hyperplasia with a first-degree breast cancer relative, and other familial risk [91]. Mammography-guided core biopsy, nipple aspiration, and four-quadrant fine-needle aspiration biopsy are being compared as methods of obtaining tissue for assessment of TGF-β isoforms as a drug effect measurement and of proliferative endpoints as intermediate biomarkers. Adverse effects on the endometrium are also being evaluated. Finally, the Eastern Cooperative Oncology Group (ECOG) under the Cooperative Clinical Oncology Program is assessing tamoxifen *vs* tamoxifen plus 4-HPR as adjuvant treatments in a Phase III trial (Dr. M.A. Cobleigh, Rush-Presbyterian-St. Luke's Medical Center) in women surgically treated for node-negative breast cancer.

PHARMACODYNAMICS

Preclinical studies suggest that doses of tamoxifen below 20 mg qd may retain efficacy against

human breast carcinogenesis. In CB-funded studies in female rats, the six-month NOEL of 0.25 mg/kg diet/day (*ca.* 0.02 nmol/kg-bw/day) approximates the tumor inhibitory dose in the MNU-induced mammary cancer model. The clinically effective dose of 20 mg qd (0.6 nmol/kg-bw) is approximately thirty-fold higher; thus, lower doses may retain efficacy in future trials, especially when combined with 4-HPR.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

A measurement to demonstrate drug effect needs to be identified and evaluated. Some antiestrogen-related biochemical alterations appear to be variable, and differ between premenopausal and postmenopausal women. However, consistent increases in serum sex hormone-binding globulin have been demonstrated in all women receiving adjuvant tamoxifen therapy [64–66]. This serum measurement should be investigated in future clinical trials. In a Phase II trial which began recently, the effect of tamoxifen plus 4-HPR on TGF-β isoforms is being assessed as a tissue drug measurement.

Safety Issues

Endometrial cancer increased two- to three-fold in adjuvant trials of tamoxifen after surgery for early stage breast cancer. This may be acceptable to women at high risk for breast cancer; however, there is some evidence to suggest the endometrial cancers seen with tamoxifen are of higher grade. Evaluation of the risk of these endometrial lesions versus the benefits of tamoxifen on breast cancer incidence, osteoporosis and cardiovascular disease needs to continue. In all subsequent long-term trials, consideration will be given to investigating biomarkers of endometrial cancer (*e.g.*, c-*fos*, *jun*-B and IGF-I expression) and the mechanism of tamoxifen activity in this tissue. It should be noted, however, that the effects of tamoxifen on the uterus are complex; it has been used successfully to treat endometrial cancer in some cases, and inhibits growth of some endometrial cancer cell lines and primary cultures [reviewed in 100]. A group in the NSABP-B1 trial will have annual endometrial biopsies to evaluate the estrogenic effects of tamoxifen citrate.

An approach to consider is the use of tamoxifen in combination with a progestin to decrease the proliferative effect on the uterus. In rats, this protocol inhibited induction of c-*fos*, but also reversed the antitumor effects of tamoxifen in DMBA-induced mammary glands. Also, studies of ERT with progestin replacement suggest a slightly higher risk of breast cancer compared with ERT alone [101,102]. However, because of the differences in receptors and responses across species, a clinical trial involving a tamoxifen plus progestin arm should be considered; the endpoints would include endometrial biopsies and evaluation of proliferative indices and oncogene expression.

In independent, long-term preclinical assays in rats, tamoxifen citrate appears to be a liver carcinogen. According to one review [4], the manufacturer found this to occur at the clinical dose (data unavailable at this time). The estrogenic antagonist and agonist effects of tamoxifen are species- and tissue-specific, so it is difficult to apply toxicologic data to humans. For example, mice and dogs show a full estrogenic response; in rats and humans, the predominantly antiestrogenic effect of tamoxifen is accompanied by partial estrogen activity in some tissues. Although the rat would appear to be a valid model, some data suggest that estrogen receptors differ quantitatively and qualitatively between rat and human hepatocytes [103]. Furthermore, a recent comparative metabolic study reported that rat liver microsomes produced at least five-fold greater levels of tamoxifen epoxides than human liver microsomes [104]. The increased levels of the potentially reactive epoxide metabolite produced in the rat may be responsible for the liver cancer observed in this species. In humans, no increase in liver cancer has been observed in six clinical adjuvant trials. However, the negative results of these studies have been critized since the studies failed to specifically examine liver cancer incidence and provided only limited information on patients treated longer than 10 years [105].

Pharmacodynamics Issues

The difference in the major metabolite between humans and other species makes extrapolation of preclinical toxicology and efficacy data to humans difficult. *N*-desmethyltamoxifen is the major metabolite in humans [106], and 4-hydroxytamoxifen is the major metabolite in dogs, mice, rats and monkeys [62,107]. 4-Hydroxytamoxifen, a more potent antiestrogen than the parent, is the principal form

in rodent tissues with high drug concentrations (liver, uterus, plasma) [108,109]; it also has partial estrogenic effects in the uterus [110]. Thus, while this metabolite has been shown to inhibit rat mammary carcinogenesis, it also induces uterine c-*fos* expression [59,60]. The latter effect can be blocked by the progestin medroxyprogesterone [59], inhibiting induction of c-*fos*, an early response gene involved in transcriptional activation. Unfortunately, the progestin may also negate the beneficial effect of tamoxifen on breast cancer risk; even intermittent administration of progesterone (cycle: 1 week on, 3 weeks off) reverses the antitumor effects of tamoxifen in DMBA-induced rats [87].

Regulatory Issues

The CB has sponsored only 90-day subchronic toxicity studies of tamoxifen alone and in combination with 4-HPR in two species. Dependable results are available for the dog only; the rat study is in progress. Chronic toxicity, carcinogenicity and reproductive/developmental toxicity study results may be cross-referenced in ICI Pharma's NDA. For the new Phase II trial of the combination of tamoxifen and 4-HPR, the CB will cross file DCT's IND.

Intermediate Biomarker Issues

A significant aspect of the proposed Phase II trial of tamoxifen alone and in combination with 4-HPR is the assessment of intermediate biomarkers as surrogate trial endpoints, especially those measured quantitatively and reproducibly by techniques such as computer-assisted image cytometry. Possible biomarkers include DCIS and other histological lesions, DNA ploidy, nuclear morphometry, PCNA, and Ki-67. Genetic markers may also be evaluated; a published study has demonstrated that breast carcinoma and DCIS tissue from women presurgically treated with tamoxifen (30 mg qd for a mean of 3 weeks) had lower oncogene expression than untreated patients [111].

Supply and Formulation Issues

Nolvadex as 10 mg tablets was originally produced by ICI Pharma (Wilmington, DE); however, the proprietary rights are now held by Zeneca, International. Arrangements will need to be made with Zeneca for sufficient drug and placebo for additional chemoprevention trials.

Clinical Studies Issues

Further development of tamoxifen citrate as a single agent will await results of the ongoing Phase II and III trials. In the interim, tamoxifen citrate in combination with 4-HPR will be developed for prevention of breast cancer. Under several programs at NCI, three Phase II trials and one Phase III trial testing the combination of tamoxifen citrate with 4-HPR will begin this year. This direction is supported by animal efficacy data in mammary gland, and takes advantage of the complementary mechanisms of the two agents. Rat studies have demonstrated an appreciable inhibitory effect when tamoxifen citrate was combined with 4-HPR at individually ineffective doses; thus, lower doses of each agent may ameliorate the adverse effects while retaining chemopreventive efficacy. In the current CB-administered Phase II trial, a daily regimen of 20 mg tamoxifen citrate plus 200 mg 4-HPR is being compared with the individual agents and placebo in DCIS patients in the period between diagnostic biopsy and definitive surgery. A significant aspect of this short-term (2–4 weeks) study is the identification of intermediate biomarkers as potential surrogate endpoints for cancer chemoprevention trials. The toxicity and efficacy of doses below 20 mg tamoxifen in combination with 4-HPR may be assessed in future Phase II trials of longer duration.

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Table I. Clinical Trials of Tamoxifen Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose titration, efficacy, intermediate biomarkers)					
UO1-CA-53807 Effect of Tamoxifen on Proliferative Breast Disease (Dr. John Ward, Univ. of Utah) 2/91–1/96	Breast	Women with aspirate-proven atypical hyperplasia who were previously treated for unilateral breast cancer or CIS or 1° relatives of breast cancer patients 120 patients	Oral 20 mg qd for 3 years	Efficacy: Regression of atypical hyperplasia on serial fine needle aspiration cytology	Study in progress; 64 women randomized
New Study Phase II Clinical Trial of *N*-(4-Hydroxyphenyl)retinamide (4-HPR) and Tamoxifen in Breast Neoplasia, Administration During the Period Between Diagnostic Core Biopsy and Definitive Surgery Dr. Kapil Dhingra, Univ. of Texas, M.D. Anderson Cancer Center 9/94–	Breast	Women with mammogram highly suspicious for DCIS 100 patients	Oral 200 mg 4-HPR + 20 mg tamoxifen citrate qd; or 200 mg 4-HPR qd; or 20 mg tamoxifen citrate qd; or placebo between core biopsy and surgical excision (2–4 weeks)	Intermediate biomarkers (*e.g.*, nuclear polymorphism, DCIS grade, ploidy, PCNA, Ki-67, S-phase fraction)	Modulation of DCIS and other intermediate biomarkers

Table I. Clinical Trials of Tamoxifen Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose titration, efficacy, intermediate biomarkers) ***(continued)***					
NCI 94C-0056D Pilot Chemoprevention Study of Tamoxifen and Fenretinide in Subjects at High Risk for Developing Invasive Breast Cancer (Dr. Joyce O'Shaughnessy, Clinical Oncology Program, DCT, NCI) 1994 IND 40,294	Breast	Women at increased risk for breast cancer from LCIS or resected DCIS, atypical hyperplasia with 1° relative, familial risk pattern 25 women	Oral 20 mg tamoxifen citrate qd during months 2–24 + 200 mg 4-HPR qd on days 1–25 during months 1–4 2 years	Drug effect measurement: TGF-β isoforms Intermediate biomarkers: Proliferation (PCNA, Ki-67) Safety: Endometrial biopsy and transvaginal ultrasound, ophthalmic assessment	Feasibility study of tissue sampling by guided needle biopsy, nipple aspiration and 4-quadrant fine needle aspiration for biomarker and drug effect measurements
New Study A Pilot Trial of Tamoxifen and 4-HPR (4-*N*-(Hydroxy)phenyl retinamide) in Patients with Newly Diagnosed Breast Cancer (Dr. Joyce O'Shaughnessy, Clinical Oncology Program, DCT, NCI, at University of Maryland) 1994 IND 40,294	Breast	Women with newly diagnosed DCIS or breast cancer 20 women	Oral 20 mg tamoxifen citrate + 200 mg 4-HPR qd between diagnostic biopsy and surgical excision (<25 days)	Drug effect measurement: Tissue TGF-β isoforms Intermediate biomarkers: Proliferation (cyclin D1 and E, MIB-1, EGFR, IGF-1), histological, and genetic (p53, *erb*B-2)	Study of biomarker modulation in areas of proliferation and CIS adjacent to cancer

Table I. Clinical Trials of Tamoxifen Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase III (Efficacy, intermediate biomarkers)					
UO1-CA-37377 Randomized, Placebo-controlled Clinical Trial to Determine the Worth of Tamoxifen for Preventing Breast Cancer (National Surgical Adjuvant Breast and Bowel Project) 5/92–	Breast	Women >60 years old, or 35–59 years old with risk equivalent to 60 years old 16,000 women	Oral 20 mg qd for 5 years	Efficacy: Breast cancer incidence and mortality, heart disease incidence and mortality, bone fracture rate	Study risk assessments in progress; more than half of study population accrued Published reports: [75,90,92]
ECOG EB-193 Phase III Double-blind, Placebo-controlled, Prospective Randomized Comparison of Adjuvant Therapy with Tamoxifen *vs* Tamoxifen and Fenretinide in Postmenopausal Women with Involved Axillary Lymph Nodes and Positive Receptors (Dr. Melody A. Cobleigh, Rush-Presbyterian-St. Luke's Medical Center) 1994 IND 40,294	Breast	Patients >65 years with surgically treated, node-positive, receptor-positive breast cancer 1,500 women	Oral 20 mg tamoxifen citrate qd a.m., or 20 mg tamoxifen citrate qd a.m. + 400 mg 4-HPR qd p.m. (3-day 4-HPR holiday/month) for 5 years	Survival, disease free survival; safety	Protocol under review

TAMOXIFEN CLINICAL DEVELOPMENT